The Role of *Alnus viridis ssp. cripsa* (Ait.) Pursh (green alder) In Boreal Jack Pine Forests in Southeastern Manitoba

by

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The role of green alders (*Alnus viridis* spp. *crispa* (Ait.) Pursh) in boreal jack pine forests in southeastern Manitoba.

Thesis Abstract

I compared understorey communities under *Alnus viridis* ssp. *crispa* (green alder) and *Corylus cornuta* (beaked hazel) in two boreal jack pine forests. There was no difference in inorganic soil nitrogen, but alder plots had lower species richness at Star Lake and higher evenness in the Sandilands. I incubated chopsticks and litterbags containing natural litter assemblages underneath *A. crispa* and *C. cornuta* in the field, and litterbags containing artificial litter mixes in a dark growth chamber. There was no significant difference between treatments for litterbags or chopsticks in the field, nor between mixes with or without alder in the growth chamber.

Thesis Introduction and Objectives

What role do species play in their community? It is one of the most basic, and also one of the most interesting and complex questions community ecologists can ask. Some species are antagonists in their community, hindering the growth of other species through the production and release of allellopathic chemicals. Still some other species can ameliorate harsh conditions, creating more favorable conditions for their community. Nitrogen (N) fixers enrich their environment by shunting N from the atmospheric N_2 pool to the soil, increasing the amount of N available for their non-fixing neighbours. This principle is well known in agriculture, where the rotation of legume crops to revive overworked agricultural fields has long been practiced. Nitrogen-fixers are also important in natural systems, especially in areas where the plant community is N-limited, such as the boreal forest. Nitrogen limitation in the boreal forest is due to the confluence of a number of factors including the retardation of decomposition by cold soil temperatures and the composition of the boreal litter layer, which contains many species with highly recalcitrant litter including the tough leathery leaves of ericoid species and the waxy and resinous needles from conifer species.

Green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) is a N-fixing shrub species that is common in the boreal forest. Alders can access the N fixed by their endosymbiont *Frankia*, using it in the formation of new leaf tissue, and adding it to the soil pool when their leaves are dropped. The addition of alder tissue changes the conditions of the soil environment by increasing the amount of N and by adding highly decomposable tissue to the litter layer. The N liberated from the senesced alder tissue can be used by neighbouring plants, affecting the growth of other members of the community. Alder

litter may also affect decomposition dynamics by increasing the overall quality of the litter layer.

The objective of this project was to investigate the role of *Alnus viridis* ssp. *crispa* (green alder) in boreal forest plant communities. The first chapter of my thesis deals with the effects of alders on the understorey, and the second chapter investigates the effect of alder on decomposition.

Chapter 1

Alnus viridis ssp. *crispa* (Ait.) Pursh (green alder) exerts a weak effect on understorey community properties in boreal jack pine forests in Southeastern Manitoba.

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Abstract

Alders have the ability to enrich their local pool of nitrogen (N) by contributing tissues that contain fixed N to the litter layer. As the alder litter decomposes, the fixed N is liberated from the tissues and becomes available for uptake by other plants. This study explores the effects of the presence of green alders on their associated understorey communities. Plots were established under green alders (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) and reference shrubs (beaked hazel: *Corylus cornuta* Marsh.) in two boreal jack pine forests - one in the Sandilands Provincial Forest and the other near Star Lake. I created two hypotheses to explain the effects of alders on their associated understorey communities – the Fertilizer Effects Hypothesis (FEH) and the Diversity Curve Hypothesis (DCH). The FEH is based on fertilizer experiments, which have shown that understorey communities respond predictably to increased nutrient levels by increasing total cover and decreasing diversity, species richness and evenness. The FEH predicts that if alders are acting as natural fertilizers, their associated understorey communities should show similar effects to those observed in artificial fertilization studies. My study offers no evidence to support this hypothesis. The DCH relates community attributes, such as diversity and species richness, to the concentration of nitrogen (N) in the soil. My data offers minimal evidence to support this model. Alder plots had lower species richness (13.6 vs. 15.1 spp./m^2, p=0.01) in Star Lake, and higher evenness in the Sandilands (evenness index E6: 0.687 vs. 0.646, p=0.08). The weakness of the

observed effects may be largely attributed to the weakness of the N-enrichment by alders. There was no significant increase in inorganic soil N (∆inorganic N) in alder plots at either the Sandilands (∆inorganic N=0.7 mgN/kg soil, p=0.58) or Star Lake (∆inorganic N=1.7 mgN/kg soil, p=0.18). There was an increase in pH under the green alders at Star Lake (4.07 alder v 3.91 reference, p=0.03), which may indicate the inhibition of the nitrification process in these alder plots.

1. Introduction

1.1. Alders

Alders (genus *Alnus*) are a taxon of deciduous trees and shrubs. Unlike the other members of the birch family (Betulaceae), alders have developed a relationship with *Frankia*, a group of actinomycetes with the ability access atmospheric nitrogen (N_2). Most plant species can only access nitrogen (N) in its inorganic forms, i.e., ammonium (NH_4^+) and nitrate (NO_3^-), which are often scarce in terrestrial systems. Dinitrogen is highly abundant in the atmosphere but very few organisms are able to metabolize N_2. There are some prokaryotic species though, including *Frankia*, that have evolved the ability to produce nitrogenase, an enzyme that converts N_2 to ammonia (NH_3) in a reduction reaction known as nitrogen fixation.

Frankia invade the alders' roots, triggering the formation of nodules that house the bacteria and become the site of N-fixation (Dalton and Naylor 1975, Torrey 1978, Berry and Sunell 1990). The nodules are formed through the modification of lateral roots, reaching up to 5 cm long and forming dense clusters (Baker and Seling 1984). The nodules maintain high concentrations of fixed N that can be accessed by the host and incorporated into new tissues (Ekblad and Huss-Danell 1995). The ability to exploit N-fixation is especially beneficial when inorganic nitrogen (inorganic N) is scarce. Under these conditions, individuals with the ability to fix N can bypass the inorganic N pool and acquire the N necessary for growth and maintenance from the abundant N_2 pool.

Although the ability to utilize the atmospheric N_2 pool can confer an advantage, this facility is only exploited when energetically beneficial. The process of fixation requires that a large amount of the energy gained from photosynthesis be sacrificed to

support the N-fixation pathway instead of being used in the creation of new tissues (Bormann and Gordon 1984). The cost of N-fixation is 16 ATP to fix one molecule of N_2 (Huss-Danell 1990) or 22.8 g of glucose to fix 1.0 g of N (Rastetter et al. 2001). Due to the large energy requirements of this pathway, fixation is only switched on when nitrogen is in such low supply that the costs of fixation are less than the costs of uptake (Rastetter et al. 2001).

Despite the energetic requirements of N-fixation, alders can assimilate a substantial amount of N from fixation and in some cases the N in alder tissues is derived primarily from fixed nitrogen (Myrold and Huss-Danell 2003). In a study comparing values produced by two different ^{15}N protocols, Domenach et al. (1989) estimated the percentage of N derived from fixation (Ndfa) as 97-110% in *Alnus glutinosa* Gaert. (black alder) and 75% in *Alnus incana* (L.) Moench (grey alder). Another study in southern Quebec estimated aboveground Ndfa as 68% in *A. glutinosa* (Coté and Camire 1984). It is important to note that these species – *A. glutinosa* and *A. incana* - are both tree type alders. Shrub type alders, such as *Alnus viridis* ssp. *crispa* (green alder), growing under a closed canopy likely have lower Ndfa than the tree type alders. The N-fixation pathway is supported by the energy gained via photosynthesis and under a dense canopy, low light would limit photosynthesis, which would in turn limit N-fixation (Wurtz 1995).

1.2. Alders in the Community

The presence of N-fixing species in a community affects the competitive balance between the members, with N-fixing individuals at an advantage over their non-N-fixing neighbours when N is limiting and at a disadvantage when N is plentiful (Pennings et al. 2005). While neighbouring individuals are suppressed by insufficient access to N, which is needed in large supply for the creation of new tissues, N-fixers can tap into the abundant N_2 pool and continue to grow. As the N-fixers grow, they shift the balance of species in the community by occupying spaces that could have been used by other species in their strata and casting shade on shorter species. Although N-fixers may negatively affect other species through light competition, N-fixers can also positively affect members of their community by sharing fixed N with their neighbours (Ekblad and Huss-Danell 1995).

It has been well documented that alders can increase the amount of N available to their neighbours by shunting N from the atmospheric N_2 pool to the soil N pool (Hobbie 1992, Ekblad and Huss-Danell 1995, Stottlemeyer et al. 1995, Wurtz 1995, Vogel and Gower 1998, Hu et al. 2001, Rothe and Binkley 2001). Isotopic measurements can be used to track N derived from fixation, with fixed-N showing a greater proportion of ^{15}N. Hu et al. (2001) found a substantial increase in ^{15}N and biogenic silica, indicative of diatoms, in Alaskan sediments during the expansion of *Alnus* in the Holocene. Closer to my study sites, Vogel and Gower (1998) found elevated levels of ^{15}N in jack pine needles from stands with green alder in the understory of boreal jack pine forests near Thompson, MB and Prince Albert, SK.

Unlike legume species, alders do not leak N-rich exudates through their roots (Huss-Danell 1990). Nitrogen enrichment by alders occurs primarily via the decomposition of tissues containing fixed N, especially leaf litter (Torrey 1978). *Alnus incana* (grey alder) has been shown to store significantly more nitrogen derived from fixation (Ndfa) in its leaf and stem tissue than in its root tissue, with the exception of nodule tissue, which has the highest Ndfa in the host organism (Ekblad and Huss-Danell 1995). When leaves or woody tissues constructed from fixed N are shed from the parent plant and decompose, the N from the tissue is added to the soil pool where it becomes available for uptake.

This mechanism fits Lawton's (1994) definition of allogenic ecosystem engineering. Alders perform the role of ecosystem engineers by changing "the availability (quality, quantity, distribution) of resources utilized by other taxa… by transforming living or non-living materials from one physical state to another" (Lawton 1994 p. 372). As allogenic engineers, alders are very important pioneers in primary succession (Hu et al. 2001), and important members of early successional forest stages (Clein and Schimel 1995).

Alders can contribute a substantial amount of N to the soil pool. *Alnus viridis ssp. crispa* (green alder), a shrub type alder, in northwest Alaska were estimated to contribute 2.1-5.2 $gN/m^2/y$ to the soil N pool (Rhoades et al. 2001). Another study estimated N-fixation by green alders in southwestern Alaska at 15.7 $gN/m^2/y$ (Silvester 1983). These estimates fall within the low to mid range stated by Torrey (1978) who reported N contributions by green alder as 4.0-36.2 $gN/m^2/y$. This rate is on par with

Alnus rubra Bong. (red alder), a tree type alder, which was estimated to contribute 14.0-30.0 $gN/m^2/y$ (Torrey 1978).

Although these examples are from early successional communities, alders are also found in older communities. Alders are common in boreal forests, where inorganic nitrogen is limited by to the retardation of decomposition processes by the cold climate (Bonan and Shugart 1989, Näsholm and Persson 2001, Nordin et al. 2005, Turkington et al. 2002). Although alders are common in boreal forests, they are rarely the dominant shrub species. One might expect alders to be more common in these areas considering the advantage N-fixation confers under inorganic N-limited conditions. There are many possible reasons for the failure of alders to dominate the shrub community in boreal forests. The distribution and growth of alders may be strongly controlled by other factors, including light availability. Light is required in high supply to fuel the process of N-fixation, but is often low in boreal forests where dense canopies filter out much of the light (Turkington et al. 2002). It is also possible that the ability to access an alternative source of N does not offer alders a unique advantage; i.e., that other boreal species have the ability to harness alternative N-sources, such as the organic N pool.

Although the common perception had been that organic N (ON) is unavailable to plants, this tenet has been challenged (Näsholm et al. 1998, Näsholm and Persson 2001, Nordin et al. 2001, Persson and Näsholm 2001, Persson et al. 2003, Schimel and Bennett 2004, Chapman et al. 2006, Kranabetter et al. 2007). Näsholm et al. (1998) found that organic N can be taken up directly by plants, especially by those with ericoid mycorrhiza (EM) such as *Vaccinium myrtillus* and by ectomycorrhizal (ECM) trees such as *Pinus sylvestris* and *Picea abies*. Mycorrhizal associations are particularly common

in boreal forests. In fact, boreal communities are dominated by plant species with ericoid mycorrhizae and ecto-mycorrhizae (Näsholm and Persson 2001, Nordin et al. 2001). Even plants without mycorrhizal associations have been shown to utilize organic N (Näsholm and Persson 2001, Nordin et al. 2001, Persson and Näsholm 2001). Some plants have been shown to prefer ON over inorganic N, such as *Eriophorum vaginatum* L., an arctic sedge (Chapin et al. 1993).

Not only have many species been shown to use ON, but these species have also been shown to utilize a broad suite of amino acids. Organic N transporters show little preference among amino acids, and many of the individual amino acid symporters that have been identified in plants show broad specificity for substrates (Näsholm and Persson 2001).

In systems with very low availability of inorganic nitrogen (inorganic N), as would be found in the boreal forest, organic nitrogen (ON) uptake could be both common and significant (Schimel and Bennett 2004). Unlike the N-fixation pathway, the ON uptake system is not inhibited by high concentrations of inorganic N (Näsholm and Persson 2001), suggesting that ON uptake may be more than a secondary alternative to the uptake of inorganic nitrogen. It isn't surprising that ON uptake is expected to be high in boreal systems. There is strong selective pressure in N-poor environments for species with alternative strategies to access nitrogen (Chapman et al. 2006). If most boreal species are able to use an alternative N-acquisition strategy, the alders' ability to utilize fixed N would not confer a unique advantage.

1.3. Previous Studies and Approaches

1.3.1. Direct Methodologies

Many studies have been conducted in the past to assess the effects of alders in North American forests. Unfortunately there are many problems with attempting to quantify N-enrichment by alders. Firstly, there is a wide range of methodologies and parameters to consider: N-fixation rates, litter input of fixed N, differences in the soil concentrations of the various forms of inorganic and organic N, as well as the flux in these soil components. Additionally, these parameters can be difficult to measure accurately.

One of the most common rate-based techniques is the acetylene reduction assay (ARA), but its estimates of fixed N inputs are greatly affected by small changes in the reduction ratio, which varies on spatial and temporal scales (Bormann and Gordon 1984, Anderson et al. 2004). There is debate about the value of C_2H_2:N_2, with new data pointing to 4:1 rather than the traditional value of 3:1 (Anderson et al. 2004). The ratio was found to vary by up to three orders of magnitude depending on the experimental conditions, leading to the recommendation that the specific ratios be determined for each system and the caution that "assessments of ecosystem N cycling based on ARA may require re-examination" (Anderson et al. 2004 p. 110).

Another common methodology for tracking N in the ecosystem is an isotopic abundance comparison using ^{15}N. Binkley et al. (1985) discuss some of the problems associated with ^{15}N abundance methods, emphasizing the importance of an accurate index. Uptake rates vary between species and calibrations from different species may produce inaccurate estimates of ^{15}N uptake. They suggest that the most accurate index

would come from a non-N-fixing isoline of the species of interest but this situation is not particularly common.

Measuring N-inputs from decomposing litter is problematic largely due to the difficulty in measuring belowground tissues (Wells and Eissenstat 2001, Schimel and Bennett 2004). Although contributions from above ground tissues are fairly easy to measure, the task of measuring contributions from belowground organs is difficult to say the least, especially with regards to fine roots. Fine roots turn over very quickly and the contribution of N by shed root tissues is likely high (Wells and Eissenstat 2001, Gartner and Cardon 2004).

Soil-based techniques are highly influenced by the heterogeneity of inorganic N under natural conditions. Soil N is strongly heterogeneous even on very fine spatial scales (Schimel and Bennett 2004) and may be briefly influenced by disturbances such as animal activity, including urination. Traditionally the magnitude of N addition by alders has been investigated using direct techniques that attempt to measure the various pools of soil nitrogen (Silvester 1983, Torrey 1978, Rhoades et al. 2001) but N flux may be a better predictor of the amount of N available to plants than total concentration (Näsholm and Persson 2001, Schimel and Bennett 2004).

1.3.2. Indirect Methodologies

Some researchers have used indirect methods to assess N enrichment by targeting the effects of alders on the growth of other species. The target species in these studies were generally tree species, including various species of conifers and deciduous trees (Binkley 1983, Binkley et al. 1985, Binkley et al. 1992, Vogel and

Gower 1998). In boreal forests in Northern Manitoba and Saskatchewan, Vogel and Gower (1998) found that *Pinus banskiana* Lamb. (jack pine) growing with *Alnus viridis* ssp. *crispa* had greater basal area and leaf area index than trees growing in stands without the presence of green alder.

Extensive work has been carried out on the west coast of North America on *Alnus rubra* (Bormann and Gordon 1984, Anderson et al. 2004). Particular attention has been paid to its effects on *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) in mixed canopies (Binkley 1983, Binkley et al. 1985, Binkley et al. 1992). Their findings have shown that the effects of alder on their associated species largely depended on site fertility. Binkley (1983) compared *P. menziesii* growing with and without *A. rubra* at a fertile and an infertile site. At the infertile site (Mt. Benson, British Columbia) *A. rubra* increased the diameter of associated *P. menziesii*, despite a lack of change in basal area. Conversely, at a fertile site (Skykomish, Washington) alder-associated Douglas fir had both decreased diameter and basal area. In a later study, Binkley et al. (1992) found that *P. menziesii* growing with alder in infertile stands had greater biomass and aboveground net primary production (ANPP) than adjacent stands without alder. Conversely association with alder was linked to lower biomass and ANPP of Douglas fir at a fertile stand.

Although many studies have investigated the effect of alders on tree species, largely due to the efforts of forestry-related research, none investigate the effect of alders on their associated understorey communities. If alders are adding enough N to their local systems to affect the growth of individuals in the tree strata, it seems likely that they should also affect the growth of individuals in the lower strata. Any effects on

the growth of individuals will alter the structure of the understorey community, including diversity and species richness.

1.4. The Role of Alders in their Communities

The composition (i.e., species membership) and structure (i.e., species abundance and distribution) of the community will be affected by the identity of species in the species pool and the environmental conditions that control the abundance of those species, including N availability. If alders affect the local abundance of nitrogen, then they should also affect the survival and growth of individuals in their associated understorey communities. The effects of nutrient enrichment on the understorey community should manifest themselves in the health and abundance of individuals and consequently, in the structure of the community as a whole.

1.4.1. Fertilizer Effect Hypothesis

If alders are enriching their local soil pool with fixed nitrogen, then alders should affect their understorey communities in the same way as artificial N fertilization. Extensive research has been conducted in the past to investigate the effect of artificial nutrient addition on community structure, especially in open communities (i.e., areas with high canopy openness) such as grasslands. These and other studies have shown that nutrient addition causes predictable changes in community structure, including decreases in species richness and diversity, and increases in total cover (Bardgett and

Shine 1999, Gough et al. 2000, Rajeniemi 2002, Pennings et al. 2005, Clark et al. 2007).

Although generalization from the extensive work done in grassland systems to my boreal forests sites may be unwise due to their difference in nutrient status, data from fertilization studies conducted in boreal forests agree with the results of the grassland studies. In a fertilization study in two boreal meadows in southwestern Yukon, the addition of N:P:K (35:10:5) fertilzer caused lower species richness and evenness (E_{var}) in the understorey (Turkington et al. 2002). Fertilization also shifted the balance between functional categories, increasing the proportion of herbaceous dicots and grasses, and decreasing the proportion of woody species.

The changes to the components and structure of the understorey community occur as the competitive ranking of the species is altered. When an accessible form of N becomes available, some species will acquire and utilize N faster than others. In the absence of other limiting factors, the superior competitors are able to increase their growth rates relative to the other members of the understorey (Nordin et al. 1998). This results in higher cover and increased dominance by the better competitors, and in decreased cover and potentially extirpation of less competitive species. One of the mechanisms hypothesized to drive this change is a shift from belowground competition for nutrients such as nitrogen, to aboveground competition for light as shoot growth and shading increases (Pennings et al. 2005). This mechanism assumes that the response to ameliorating nutrient limitation will manifest itself as an increase in productivity, i.e.,, an increase in total cover. While this holds true in open areas, such as grasslands and forest meadows, this effect may not manifest itself in closed areas (i.e., areas with low

canopy openness) where light is also a limiting factor for growth in the understorey. In areas where low light restricts plant growth, such as under a dense shrub and/or tree canopy, there may be a shift in the composition and abundance of the members of the community without a change in total cover.

Species Effect

The response of an individual to fertilization will be affected by the morphology of its species, with some species showing a highly consistent response to fertilization. Grasses commonly increase in response to fertilization, while ericaceous species decrease or do not respond at all (Nordin et al. 1998). Suding et al. (2005) assessed the response of 967 species to fertilization in an attempt to elucidate traits typical to species that increase or decrease in response to fertilization. They identified typical increasers as being (1) non-legumes, (2) monocots with runners and (3) members of the tallest strata of the understorey layer.

Some species show more consistent responses to fertilization than others. In a review of 31 N-fertilization experiments in the continental US and Alaska, Penning et al. (2005) found evidence to suggest that 10 of the 20 species assessed were responding in a consistent and predictable fashion to the addition of N across multiple experiments and environments. In a fertilization experiment in a boreal forest in southwestern Yukon, several species showed predictable responses to nutrient enrichment (Turkington et al. 2002). After 10 years *Festuca altaica* Trin. (northern rough fescue), *Mertensia paniculata* (Ait.) G. Don (tall lungwort), *Epilobium angustifolium* L. (fireweed), *Achillea millefolium* L. (common yarrow) increased cover while *Linnaea borealis* (twinflower),

Lupinus arcticus S. Wats. (arctic lupine) and *Arctostaphylos uva-ursi* (common bearberry) decreased cover. In certain cases the effects took multiple growing seasons to become evident and some of the species did not show a response until 4-5 years after the experiment started, including *Achillea millefolium* and *E. angustifolium*.

Regional Regulating Factors

The effects of nutrient enrichment on diversity are greatly affected by the specific environmental conditions of the system. In the previously referenced review, Penning et al. (2005) found that 4 of 20 species, including *Achillea millefolium*, respond consistently to N-fertilization but only when other factors, including annual net primary production (ANPP) and community composition, were considered in conjunction with fertilization. On a similar geographic scale, Clark et al. (2007) found that regional temperature and soil cation exchange capacity (CEC) were the most important environmental variables determining the effects of nutrient enrichment on understorey community attributes, including diversity and species richness. Apart from the abundance of C_4 species, initial community attributes were found to be largely unimportant in determining the effect of fertilization on diversity. Ultimately they identify four factors – CEC, regional temperature, C_4 abundance and the change in production following fertilization – that explained 56% of the variation in community response. Greater loss of diversity was observed when CEC and regional temperature were low, and when C_4 abundance and the change in production following fertilization were high (Clark et al. 2007).

The regulating factors discussed by Clark and colleages (2007) and Penning et al. (2005) are important determinants of community responses among geographically

removed areas. They have not been included in the finalized Fertilizer Effects Hypothesis because the goal of my model is to determine whether a single factor (N-input by alders) is an important determinant within a small area.

Finalized Fertilizer Effects Hypothesis

My Fertilizer Effect Hypothesis predicts that if alders are adding N to the local pool, similar effects to those observed in fertilizer studies should be seen in understories associated with alder relative to understories associated with other non-N-fixing shrubs; i.e., alder-associated understorey communities should have higher total cover and lower species richness, evenness and diversity than understories associated with non-N-fixing shrub species. The explanatory variable used by this model is the magnitude of N-enrichment (ΔN), which is expected to show a positive correlation with the magnitude of the diversity difference (i.e., the difference between alder and non-alder understories). The model predicts that the effect of ΔN on community attributes is unidirectional; it will always take the form of decreasing species richness, evenness and diversity and increasing total cover.

1.4.2. Diversity Curve Hypothesis

My Diversity Curve Hypothesis (DCH) and its corresponding mathematical model attempt to explain the effects of N enrichment on biodiversity over a broad spectrum of N availability. The DCH is intended to explain the changes to the structure of the understorey community along a gradient of N availability when all other factors are constant. This model is a gross over simplification of natural systems and a

consideration of many other factors, especially light availability, would be required to adequately model the true dynamics in nature. Nonetheless I believe that this model serves as an interesting and promising starting point for the development of a more complex and realistic model.

The Fertilizer Effects Hypothesis predicts a unidirectional response by the community, but many biological responses show a hump-shaped relationship with environmental factors on a broad scale. For example, the species richness of a community at different levels of productivity follows a hump-shaped curve (Grime 1973). Since productivity is correlated with the availability of nitrogen (Rajaniemi 2002, Turkington et al. 2002), species richness should show a similar relationship with N availability. This led me to predict that the relationship between the availability of N and measures of biodiversity (i.e., species richness, evenness and diversity) may also follow a hump-shaped curve.

The Diversity Curve Model attempts to explain the effects of natural N enrichment by alders at various levels of baseline N (i.e., the [N] in the absence of N enrichment by alder litter) on the following community attributes: total cover, species richness, evenness and diversity. Most of the parameters (species richness, diversity and evenness) are expected to show a hump-shaped relationship with nitrogen. The exception to the hump-shape rule is total cover, which is expected to show a positive, unidirectional relationship with available nitrogen (Rajaniemi 2002, Turkington et al. 2002).

Species Richness

Species richness is expected to exhibit a hump-shaped relationship with the availability of nitrogen (N_i), similar to its relationship with productivity (Grime 1973), since [N] should be correlated with productivity (Rajaniemi 2002, Turkington et al. 2002). Species richness is expected to reach a maximum at some level of N availability (N_Ω), and then fall of to either side of that maximum as community membership is restricted by either nutrient limitation ($N_i<N_\Omega$) or competition ($N_i>N_\Omega$).

At the low end of the N availability spectrum, extremely low [N] should limit membership within the community to those species that are able to tolerate extreme N-limitation. Only a subset of the species in the species bank will have the ability to withstand the limited conditions and thus species richness will be low. As [N] increases, N limitation gradually lifts, easing off the suppression of intolerant species. Species richness increases as the less tolerant species are released from their suppressed state and can establish a presence in the community. At some point, species richness will reach a maximum as the extreme tolerators are joined by the less tolerant species. The [N] at which species richness peaks is referred to as N_Ω in my mathematical model. As N increases past N_Ω, species richness will decrease as the tolerators (i.e., the species that persisted in the area due to their ability to tolerate low N) are replaced by competitors (i.e., species that outcompete their neighbours in the uptake of soil N). Thus at the high end of the spectrum, species richness will also be low, but due to competitive effects rather than nutrient limitation. In its most extreme form, this model is the transition from a monoculture composed of the species with the highest tolerance

for N-limitation, through an assemblage of tolerators and competitors, to a monoculture composed of the species with the best competitive ability.

Evenness

Eveness describes the degree of heterogenerity in the proportional abundance values of all the members of a community, where a system with similar abundances for all the members has high evenness and a system with disparous abundances has low evenness. Evenness is expected to show the reciprocal trend to species richness. The extreme form of the model is a transition from a monoculture of the top tolerator species, through a species rich community of tolerators and competitors, to a monoculture of the top competitor species.

At the lowest N availability, both species richness and total cover are low and there should be very little competition for space and resources, and evenness among the surviving species should be high. As N availability and species richness increase, new species establish themselves in the community according to their lower limits of N tolerance. Species enter the community at low abundance and gradually increase their cover as the level of nitrogen availability (N_i) moves away from the lethal ends and into the species' optimal range. As more species enter the community, they establish themselves at different abundances according to their tolerance and competitive abilities. Some species will have higher tolerance of the low nutrient status and will be able to increase their growth relative to the other members of the community. These species will establish themselves at a relatively higher cover than the other species. Some species will establish themselves at intermediate cover but the majority of

species will establish themselves at low cover (Hughes 1986). As the number of low-cover species increases and the disparity in the abundances of the dominant and subordinate species increase, the evenness in the community decreases. The lowest evenness should occur at N_{Ω}, where adequate [N] allows the largest suite of species to coexist at a wide range of abundances. As N_i increases past N_{Ω}, the dynamics of the community become controlled by competition for nitrogen. Species that are unable to win N are excluded by superior competitors, and the best competitors assume dominance in the community. The exclusion of marginalized species and dominance by a few species translate into higher evenness.

The extreme form of the model is a transition from a monoculture, to a diverse community and back to a monoculture. The theory states that evenness will be high at the ends of the spectrum, i.e. at the monoculture states. Evenness index E6 yields a value of infinity for a monoculture (species A p_i=1.0) (see example 1.1. and section 3.2.1. for details on the E6 formula), with lower values for any community with more than one species present.

Example 1.1. $E6 = (N2-1)/(N1-1)$

$= ((1-D)^{-1}-1)/(e^{H}-1)$

$= (((\sum p_i^2)^{-1}) - 1)/((e^{(\Sigma\ pi\ ln\ pi)}) -1)$

$= (((1^2)^{-1}) - 1)/((e^{(1ln1)}) -1)$

$= (((1)^{-1})-1)/((e^{(0)})-1)$

$= (1-1)/(1-1)$

$= 0/0$

$= \infty$

Diversity

Diversity is a function of both species richness and evenness, although the relative importance of these factors varies among diversity indices (Lloyd and Ghelardi 1964, Mouillot and Lepretre 2000). The Shannon-Weiner (H) and Gini-Simpson (D) diversity indices are the most commonly used methodologies. Both indices respond to an increase in species richness by increasing the value for diversity. The algorithms assign each species a diversity credit based on its abundance. The more species that are present, the more diversity credits that are summed for the community. Thus, in general there is a positive correlation between diversity and species richness (Lloyd and Ghelardi 1964). There is however, a major discrepancy among the diversity indices in the weighting of evenness (i.e., the relative proportion of species in the community). The Shannon-Weiner (H) index assigns more weight to species with moderate cover (30-40% cover), while the Gini-Simpson index (D) assigns exponentially more weight to more abundant species (approaching 100% cover) (Fig 1.1).

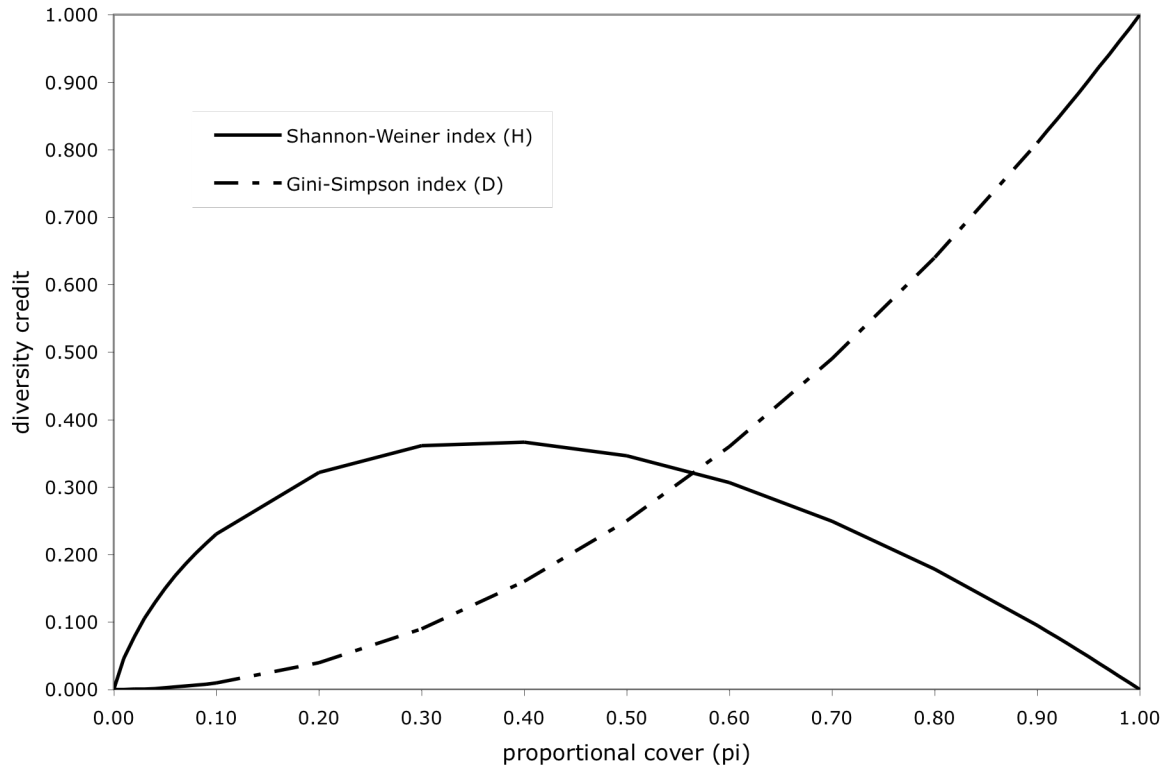


Fig 1.1. A comparison of the weighting or various p_i values by the Shannon-Weiner (H) and the Gini-Simpson (D) diversity indices. Where $H=-\sum p_i \ln p_i$, and $D=1-(\sum p_i^2)$.

Although I have included both diversity measures in my analysis, I will primarily follow the assertions of the Shannon-Weiner concept of diversity. With this bias to H, diversity should trend in a manner similar to species richness. Diversity should be low at the low end of N-availability, where only a few exceptionally tolerant species can survive extreme N-limitation. Diversity should then peak as more species enter the community as N-availability increases and N-stress is alleviated, and then should decline past N_Ω as species richness declines due to the exclusion of tolerators by superior competitors.

Mathematical Expression of the Diversity Curve Hypothesis

The hump-shaped curve detailed by the Diversity Curve Hypothesis can be described as a parabola, which is produced by a square root function. The simplest equation that describes a parabola is $y=x^2$. In this study, the explanatory variable (x) is the baseline amount of nitrogen available for plant uptake (N_i). The multiple response variables (y) in this model are expected to show proportional relationships (i.e., species richness $\propto$ diversity $\propto$ evenness^{-1}) and they can be referred to with one general parameter (B_i).

The basic formula ($y=x^2$) can be modified to manipulate the shape of the curve. The general equation for a horizontal parabola is described with the following equation (Equation 1):

Equation 1: $y = a(x-h)^2 + k$

In this general equation, the modifier "a" controls the width of the parabola as well as its vertical orientation. A fractional modifier (i.e., $|a|<1.0$, e.g. $a=0.5$) will narrow the curve, while a modifier whose absolute value is greater than 1.0 will widen the curve. Changing the width of the curve shifts the positions of the two x-intercepts. In this study, the x-intercepts represent the upper and lower lethal limits of N availability; i.e., the [N] at which $B_i=0$. I have no information to suggest any particular values for the x-intercepts (lethal [N] limits) in my study. As such, I have not assigned a numerical value to "a" and have left the "a" term in the final equation as an unknown. Regardless of the absolute value of "a", its sign must be negative. A positive value for "a" leads to a bowl

shaped curve, while a negative value will produce the hump-shaped curve expected for the relationship between N_i and the diversity measures.

In the Diversity Curve Model, the values on the x-axis represent nitrogen (N_i). Since the concentration of any substance, including nitrogen, will always be positive, the curve must be constrained to the positive portion of the x-axis. In the parabolic equation, the h term determines the position of the apex on the x-axis. A positive value for h will move the curve to the left, while a negative value will move the curve to the right. In the N-enrichment model, the curve must be moved to the right to be centered around N_Ω, thus in this model, $h = -N_\Omega$ in the absence of N-enrichment by alders.

The final modifier term (k) determines the position of the apex on the y-axis. A positive value for k moves the curve upwards, while a negative value moves the curve downwards. In this study, the peak of the curve corresponds to peak diversity, which has been designated as B_{max} and thus $k = B_{max}$ in this model.

In the following equation, $y= B_i$, $a=-a$, $x=N_i$, $h=N_\Omega$ and $k=B_{max}$ (Equation 2). This equation describes the basic hump-shaped relationship between the amount of N available for plant uptake and measures of understorey diversity, without the inclusion of N-enrichment by alders (Fig 1.2).

Equation 2: $B_i = -a(N_i-N_\Omega)^2 + B_{max}$

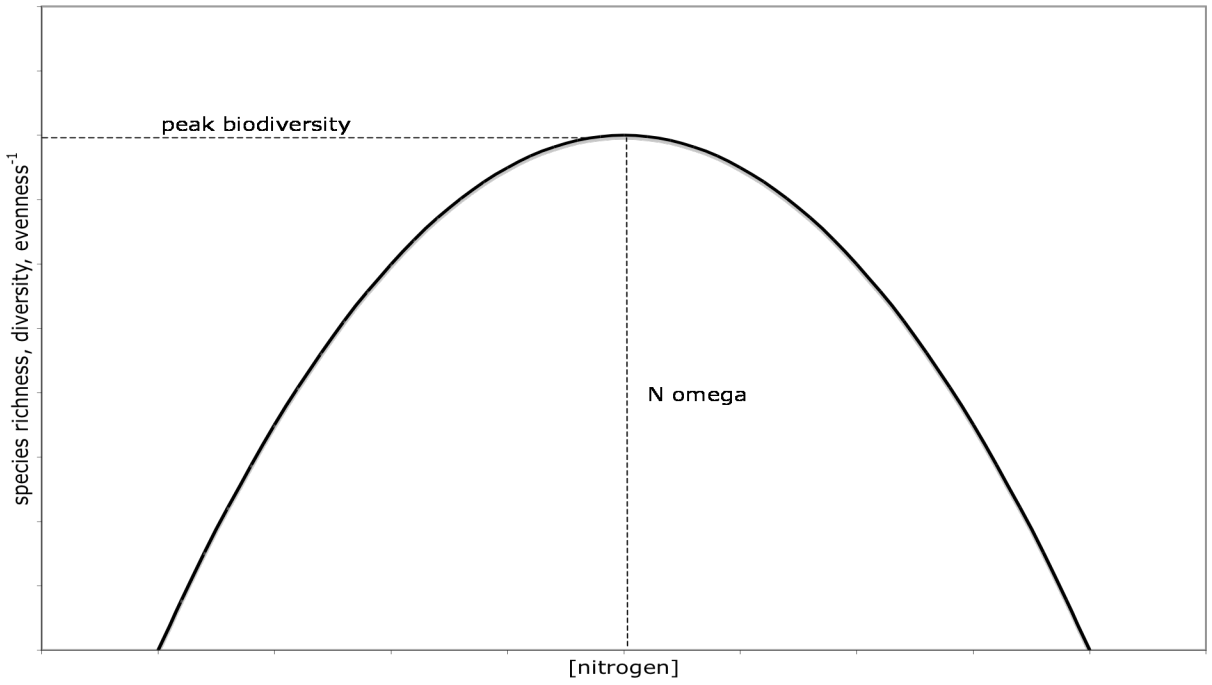


Fig 1.2. Graphical expression of the Diversity Curve Hypothesis, including the description of a hump-shaped curve where the general equation ($y=-(x-n)^2-b$) has been expressed as species richness = $-(N_i - N_\Omega)^2$ - peak diversity.

The equation can be modified to describe the effects of N-fixing alders on the structure of the understorey through the addition of fixed N (N_f), which shifts the position of the curve along the x-axis, pushing it to the left. The factor that controls the position of the parabola on the x-axis and that must be modified to represent nutrient, is h. The h term has already been defined as $k=-N_\Omega$ in the absence of N-enrichment, but can be redefined as $k=-N_\Omega+N_f$ to include N derived from fixation (N_f) (Equation 3).

Equation 3: $B_i = -a(N_i-N_\Omega+N_f)^2 + B_{max}$

If N-fixation proceeded at equal rates across all levels of N, then the contribution of fixed N would remain constant across the N spectrum at its maximum (N_{max}).

However as N increases and approaches N_I, the fixation pathway is inhibited by inorganic N and light availability and eventually becomes inactive (Markham and Zekveld 2008). This effect can be modeled to determine the value for N_f when $N_i \leq N_I$.

At the lowest end of the N spectrum, fixation proceeds uninhibited by [N] and the contribution of N by fixation is maximized at its potential for the given system ($N_f = N_{max}$). As N_i approaches N_I, increasing [N] inhibits the activity of the N-fixation pathway and the contribution of fixed N decreases ($N_f < N_{max}$) and eventually reaches zero. The level of N that triggers the onset of inhibition will be referred to as N_{99}, which is defined as the N at which fixation rates decrease to 99% of the potential rate. As N_i increases, it eventually reaches a concentration that inhibits the fixation pathway completely and there is no contribution of fixed N to the soil pool ($N_f = 0$). These parameters (N_i, N_I, N_{99} and N_{max}) can be combined to describe the inhibition of the fixation pathway in this model by creating a factor that calculates a decrease in N_{max} to zero between N_{99} and N_I (Equation 4).

Equation 4: $N_f = N_{max} \times (\sqrt{(N_I - N_i)}/\sqrt{(N_I - N_{99})})$ when $N_{99} \leq N_i \leq N_I$

The equation above describes the values for N_f when $N_{99} \leq N_i \leq N_I$. The modifying factor $(\sqrt{(N_I - N_i)}/\sqrt{(N_I - N_{99})})$ ranges from 0 to 1 and its multiplication with N_{max} results in a decrease of N_f, the magnitude of which increases as N_i approaches N_I. When $N_i \geq N_{99}$ and $N_i \leq N_I$, fixation decreases below its maximal rate ($N_f < N_{max}$) and gradually reaches zero when $N_i = N_I$. Beyond these values fixation either occurs at it maximum potential rate (i.e., $N_f = N_{max}$ when $N_i < N_{99}$) or not at all (i.e., $N_f = 0$ when $N_i \geq N_I$). Thus,

$N_f = N_{max}$ when $N_i < N_{99}$

$N_f = N_{max} \times (\sqrt{(N_l - N_i)}/\sqrt{(N_l - N_{99})})$ when $N_{99} \leq N_i \leq N_l$

$N_f = 0$ when $N_i \geq N_l$

Modeling the Effects of Alder in the Diversity Curve Model

The foundation of the Diversity Curve Hypothesis is the hump-shaped relationship between the amount of N available for plant uptake and community attributes, i.e., species richness, evenness and diversity (Fig 1.3a). It is important to note that this only applies to those parameters that exhibit a hump-shaped response; i.e., species richness, evenness and diversity but not productivity.

The next step in the development of the model was the transposition of the curve to illustrate the expected effect of N-enrichment on understorey diversity. Nitrogen enrichment shifts the position of the curve on the x-axis ([N]), moving the curve to the left (Fig 1.3b). The transposed curve exists on different sides (above or below) of the original curve on either side of N_Ω, resulting in the formation of two regions or phases, each of which has different predictions. The model predicts that alder plots should have (a) higher species richness and diversity, but lower evenness than the surrounding community when $N_i < N_\Omega$ (phase I), and (b) lower species richness and diversity, but higher evenness than the surrounding community when $N_i > N_\Omega$ (phase II).

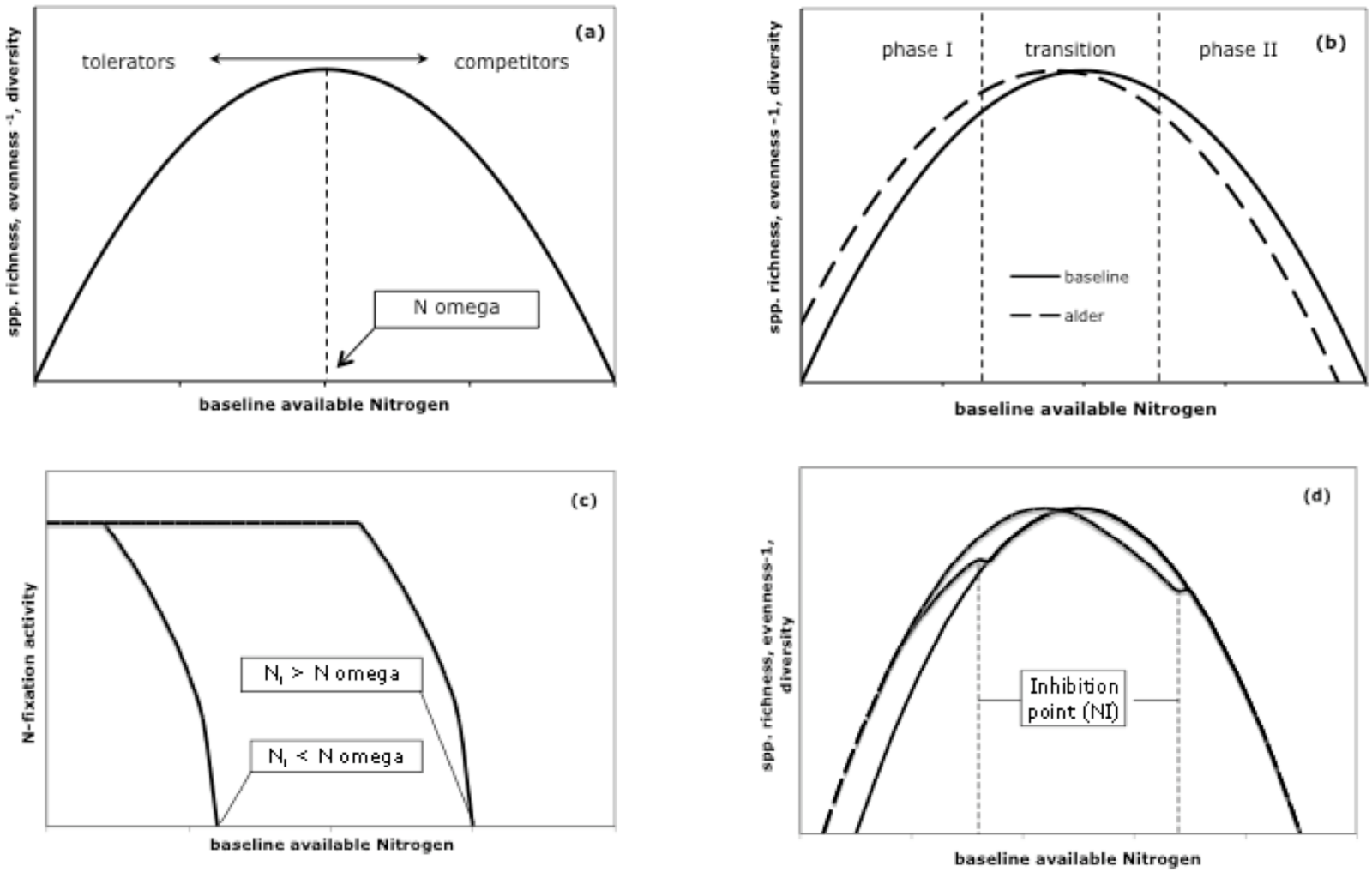


Fig 1.3. The Diversity Curve Model: a proposed model for the effect of N-enrichment by N-fixing alders on understorey community structure. (a) the effect of [N] on community attributes, (b) the effect of general nutrient enrichment on community attributes, (c) the effect of [N] on the activity of the N-fixation pathway, and (d) the finalized proposed Diversity Curve Model.

Between the two phases, there is a transition zone in which there is no significant difference between the biodiversity parameters in the alder and reference communities. The transition between the two phases occurs at the apex of the curve. To either side of the transition, the diversity difference (ΔB_i) between alder and reference plots decreases in magnitude as N_i approaches N_Ω. As the diversity difference decreases, the statistical significance of the difference also decreases. When $N_i \approx N_\Omega$ the diversity difference becomes negligible and eventually becomes zero. The boundaries of the transition zone are defined as the N_i at which the diversity difference becomes statistically insignificant. Within this zone the model predicts that there will be no difference between the biodiversity parameters in alder and reference plots.

At this stage of development, the model assumes that N-enrichment occurs equally over the entire [N] spectrum, i.e., without the suppression of the N-fixation pathway by high nitrogen availability. The next step in the development of the Diversity Curve Model was to include the effects of baseline [N] on the activity of the N-fixation pathway. The activity of the N-fixation pathway is regulated by the availability of N, shutting down entirely when inorganic N is in high supply (Gentili and Huss-Dannell 2003). Nitrogen-fixation is expected to be highest at low [N] and will eventually cease as higher [N] inhibits the fixation pathway (Fig 1.3c). The [N] at which the inhibition of the N-fixation pathways occurs shall be referred to as N_I from this point forward. The inhibition point may fall (a) before peak diversity ($N_I < N_\Omega$) or (b) past peak diversity ($N_I > N_\Omega$), and the position of N_I will likely differ depending on the nature of each specific community. If the N_i is below the inhibition point ($N_i < N_I$) then the N-fixation pathway should be active, but if N_i falls past the inhibitions point ($N_i > N_I$) then the N-fixation

pathway should be inactive and no N-enrichment should occur. In the absence of N-enrichment, there is no transposition of the curve, and there should be no diversity difference between understorey communities associated with alder and those associated with other non-fixing shrubs. The finalized Diversity Curve model (Fig 1.3d) illustrates the expected effect of alders on understorey diversity with the incorporation of an inhibition of the N-fixation pathway.

The Diversity Curve Model predicts that N-enrichment by *Alnus viridis ssp. crispa* should cause an increase in total cover at all N_i, and either (a) increased species richness and diversity, but decreased evenness (phase I: $N_i < N_\Omega$), (b) no change in species richness, diversity or evenness (transition zone: $N_i \approx N_\Omega$), or (b) decreased species richness and diversity, but increased evenness (phase II: $N_i > N_\Omega$). From a mathematical perspective, the model can be described as the transposition of a diversity curve with the inclusion of an extinction of the effect when N-fixation is inhibited by inorganic nitrogen.

1.4.3. Model Summary

The two models – The Fertilizer Effect Hypothesis and The Diversity Curve Hypothesis – predict different effects of N-enrichment by alders on the understorey community structure. The predictions of the models and their phases, where applicable, have been tabulated below (Table 1.1).

Table 1.1. Expected effects on understorey attributes of N-enrichment by N-fixing alders as predicted by the Fertilizer Effect and Diversity Curve Hypotheses. Higher indicates a value that is predicted to be larger in the alder plots than the reference plots, lower indicates a value that is predicted to be smaller in the alder plots than in the reference plots.

	Fertilizer Effect	Diversity Curve		
		Phase I	Transition	Phase II
Total cover	increase	increase	increase	increase
Diversity	decrease	increase	no change	decrease
Species richness	decrease	increase	no change	decrease
Evenness	decrease	decrease	no change	increase

1.5. Project Objectives

The purpose of this study is to determine the effects of *Alnus viridis* ssp. *crispa* (Ait.) Pursh (green alder) on understorey communities in two boreal jack pine forest sites with the assumption that *A. crispa* is adding fixed nitrogen to the local soil N pool. The study compares the understorey communities in plots underneath *A.crispa* to plots underneath *Corylus cornuta* Marsh. (beaked hazel), a similar but non-N-fixing shrub species, in two boreal jack pine forests in Southeastern Manitoba.

2. Methods

2.1. The Study Sites

I selected two jack pine forests that I knew to have large populations of *Alnus viridis* ssp. *crispa*, one in the Sandilands Provincial Forest (49º24'N, 96º16'W) and the other near Star Lake (49º44'N, 95º15'W) in the Whiteshell region. Both sites are located towards the southern edge of boreal forest in southeastern Manitoba (plot maps: Fig 1.4, Fig 1.5). Although the soil is derived from glacial outwash at both sites, there are some differences in the soil conditions between the two sites. The Sandilands has a much higher proportion of sand in the soil, and Star Lake has a higher proportion of coarse rock as well as organic matter. Both sites are part of provincial nature reserves (Sandilands Provincial Forest and Whiteshell Provincial Park).

The site at the Sandilands was located at the southwestern portion of the forest stand (GPS coordinates in Appendix Table A1). The local trails are well used by skiers in the winter and ATVers in the summer. Despite the frequent use of the trails in the area, there is very little disturbance to the forest adjacent to the trails. There is a gentle consistent slope to the site leading down into a slightly boggy area, which was avoided during sampling due to its unique nature. The understorey vegetation is largely herbaceous, and the moderate shrub stratum is composed largely of *Corylus cornuta* with sporadic *Alnus viridis ssp. crispa*.

The site at Star Lake is located in the southwestern portion of Whiteshell Provincial Park (GPS coordinates in Appendix Table A1). The site is located along the road to the field station for the University of Manitoba's Department of Geology. There are numerous cottages in the surrounding area, and the local trails are frequently used

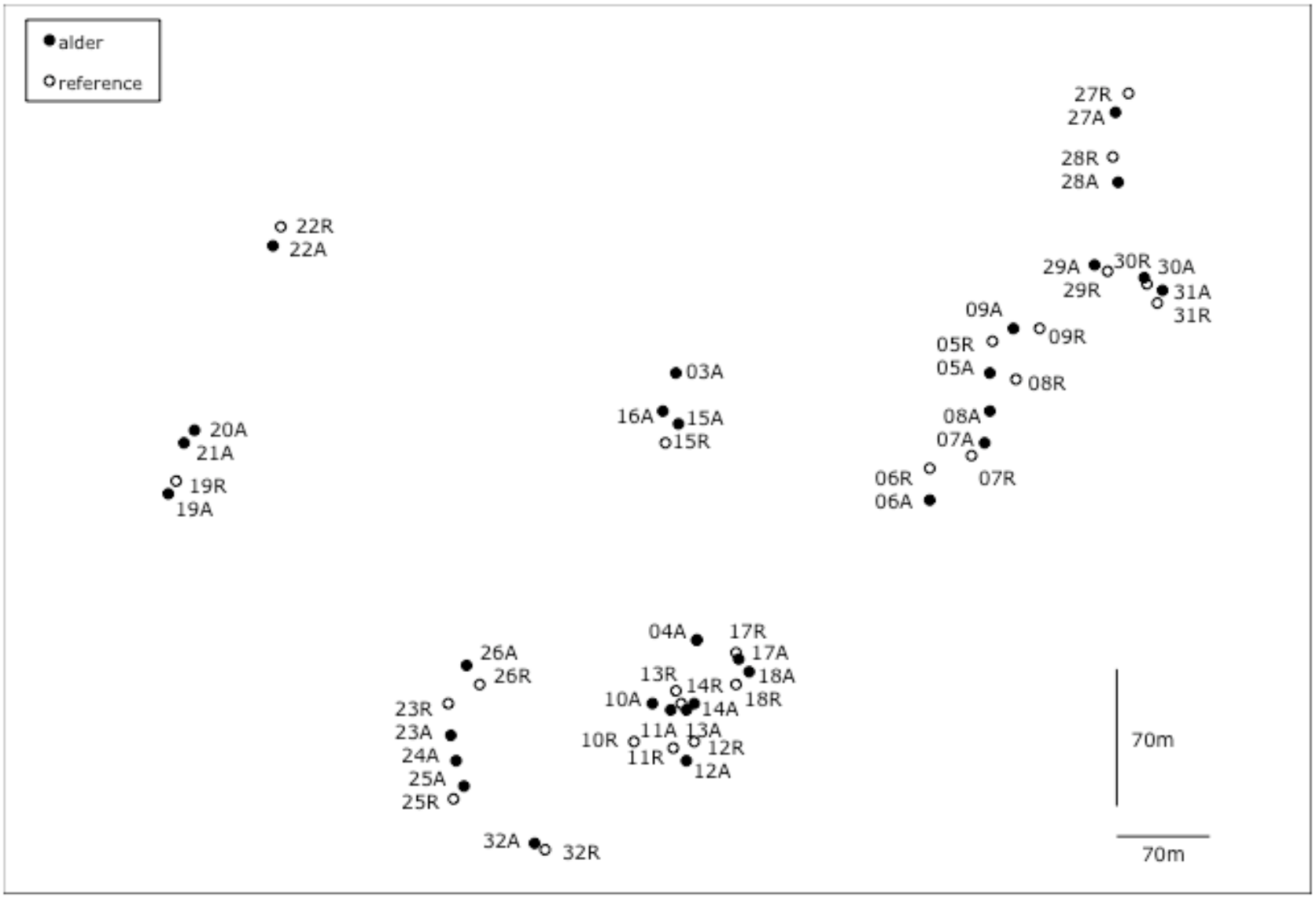


Fig 1.4. A map of the sampling locations in a study of the effects of green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) on understorey communities in a boreal jack pine forest at the Sandilands Provincial Forest (N49.24 W96.16) in Southeastern Manitoba.

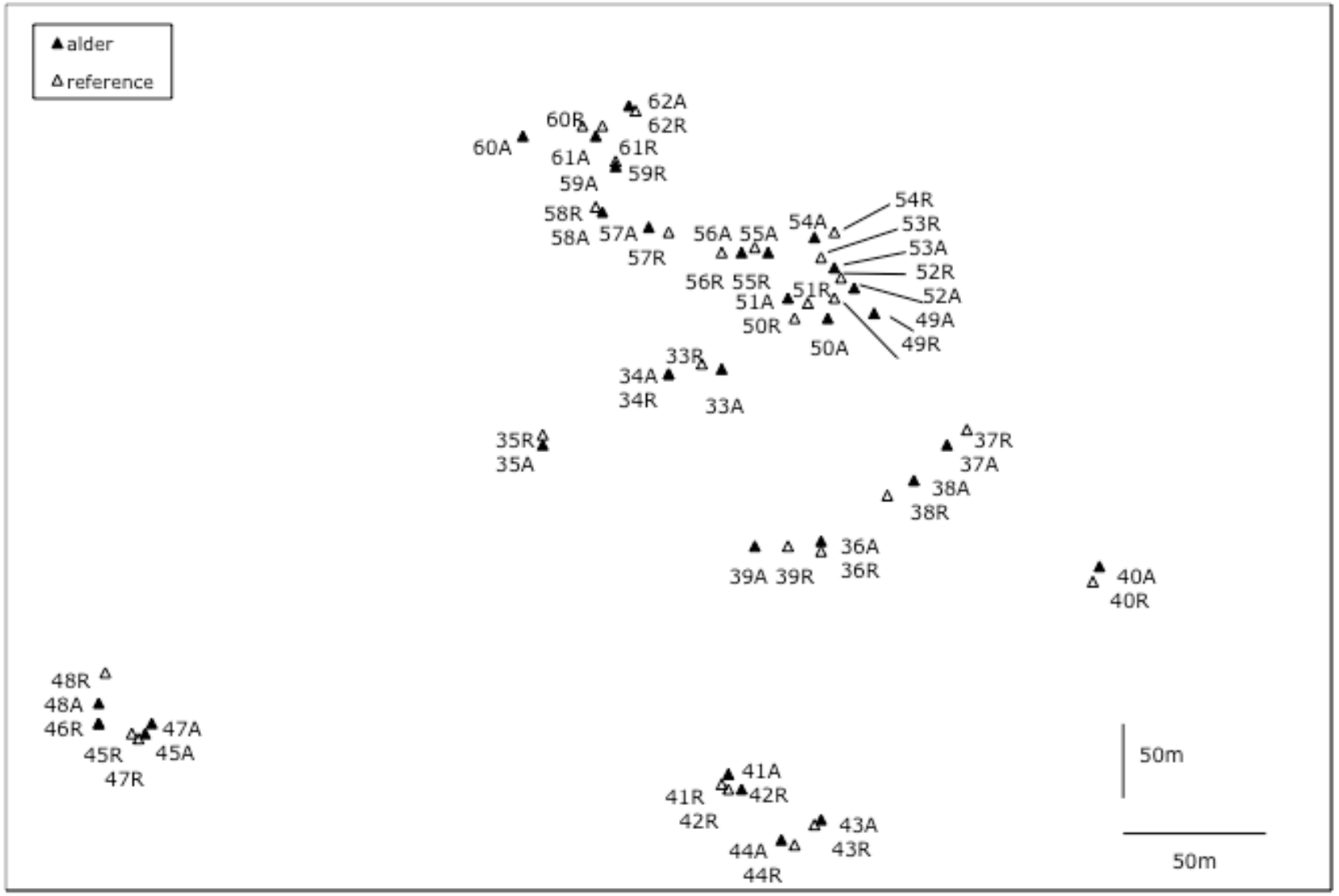


Fig 1.5. A map of the sampling locations in a study of the effects of green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) on understorey communities in a boreal jack pine forest near Star Lake (N49.44 W95.15) in Southeastern Manitoba.

by hikers and off-road ATV enthusiasts. There is a modest amount of anthropogenic disturbance in the forest site, largely by mushroom and berry pickers. Several rights-of-way have been cut through the forest for Hydro lines, but I avoided sampling these areas due to their unique and artificial properties. The topography in the area is somewhat patchy, with low-lying boggy areas interspersed through the forest. These areas were also avoided during sampling. The understorey vegetation in the surrounding forest is largely ericaceous, indicative of N-poor soils (Nordin et al. 2001), with a moderately dense shrub stratum composed largely of beaked hazel and green alder.

2.2. Sampling Design

The sites were sampled during the midsummer period (June 13 - July 13) of 2008. At each site, I scouted the population for *Alnus viridis* ssp. *crispa* (green alder) and selected thirty individuals that were (a) healthy (i.e., with green foliage and no signs of disease), (b) mature (at least 1.5 m tall), and (c) not affected by unusual environmental conditions (i.e., in localized depressions or on a game trail). Each of the green alders were paired with a reference shrub of similar size, with the same stipulations for inclusion as outlined above (i.e., healthy, mature, typical). *Corylus cornuta* (beaked hazel) was chosen as the reference species due to its abundant presence in the shrub strata at both sites, as well as its similar morphology to alders. Green alder and beaked hazel are both tall, deciduous shrubs from the birch family (Betulaceae). Unlike alders, hazels have not developed a relationship with N-fixing

symbionts and their only source of N should be inorganic N in the soil pool. The alder-hazel pairs were selected to maximize similarity within the pair, with respect to environmental factors such as topography and light, as well as to biotic factors such as total cover by shrubs and trees. The reference shrubs were located a minimum distance of 2 m apart and a maximum distance of 20 m from their alder partner.

Three nested quadrats were set up at each shrub. The understorey plot (1 m x 1 m) was placed just outside of the alder root crown or the base of the beaked hazel. Exact quadrat placement was adjusted to avoid disturbances such as deer trails, and to maximize environmental similarity within shrub pairs with respect to slope, aspect and both shrub and canopy cover. All understorey species (<1 m tall) were identified and visually assessed for aerial cover (%). The majority of the plant community could be confidently identified to the species level, but the graminoids were not flowering and showed too few identifying features to confidently identify them past the level of sedges or grasses. The shrub layer was assessed similar to the understorey, with percent aerial cover visually estimated in a 2 m x 2 m quadrat for all woody species >1 m tall and <10 cm circumference at breast height (CBH). The tree canopy was sampled using 5 m x 5 m quadrats. Although this quadrat size is smaller than what is conventionally used for tree sampling, the goal was to obtain a point estimate of the most important trees affecting the corresponding understorey plot. All trees in the quadrat that were >1 m tall and >10 cm CBH were identified to species and measured for circumference at breast height.

Canopy openness was measured for each plot using a photograph of the canopy taken by a fish eye lens. The image was processed using Gap Light Analyzer (GLA)

2.0, which estimates total canopy cover with a resolution of 0.01% (Frazer et al. 1999). A surface soil sample was taken from of each plot at the time of the understorey sampling. The LFH layer was scraped away from a 10 cm x 10 cm area and mineral soil was collected from the top 5 cm. Samples were air dried and passed through a 2 mm sieve. Soil inorganic N (ammonium and nitrate) was determined by micro diffusion method (Khan et al. 2000). The alder enrichment effect was quantified as the change in the concentration of inorganic nitrogen (ΔIN) in the soil where $\Delta IN = [\text{inorganic N}]_{\text{alder plot}} - [\text{inorganic N}]_{\text{reference plot}}$. Soil pH was measured using a saturated paste of soil and 0.01M CaCl (Kalra and Maynard 1991).

3. Analysis

In the analysis T-tests were performed using S-plus and post-hoc power analysis was performed using G*Power 3.0.10 (Faul et al. 2007).

3.1. Univariate Analysis

Means were calculated from the plot data for each of the four treatments: Sandilands alder, Sandilands reference, Star Lake alder and Star Lake reference. The sites were analyzed separately because preliminary assessment showed that the differences between the sites were much greater than the differences between the alder and reference plots within the sites.

The raw cover values for each understorey (1 m x 1 m plots) and shrub (2 m x 2 m plots) species were subjected to paired, 2-sided T-tests. Although there is some debate about the robustness of T-tests to zero values (Quinn and Keough 2002), its almost universal use and the lack of a better alternative makes T-tests the most appropriate for this data. The abundance data was used to determine the frequency index (FI) for each of the species in the community (Gleason 1920). The difference in species frequency between alder and reference plots were assessed for significance by χ^2 tests (Formula 1.2) where the expected frequency for each species was the average of the alder and reference groups at each site.

Formula 1.2: $\chi^2 = \Sigma((\text{observed} - \text{expected})^2/\text{expected})$

$= ((\text{alder-average})^2/\text{average}) + ((\text{reference-average})^2/\text{average})$

Understorey species were lumped according to functional group (mosses, ferns and allies, graminoids, herbaceous, ericoid, low-growing woody species, and seedlings and saplings) and their individual species cover values were summed to produce an estimate of total cover by the functional group. Total cover was calculated by summing the cover for all species encountered and often surpassed 100% cover due to overlapping leaves.

Basal area (BA) was calculated for each species from the CBH measurements of the trees in the 5 m x 5 m plots (Formula 1.3). Species were grouped to determine coniferous, deciduous and total basal area. Tree density (# trees/5 m x 5 m plot) was also calculated for each plot according to species and group.

Formula 1.3: $BA = (\Sigma(CBH^2/4\pi))/\text{plot area}$

3.2. Community Attributes

Understorey species were grouped according to functional group to produce aerial cover values for each group. The ericoid group was defined as non-coniferous species with thick, leathery leaves that are kept over multiple growing seasons. The differences in the group values between the treatments at each site were compared with paired, 2-sided T-tests. The level of significance for ecological methods was set at α=0.10. The high α value was chosen to account for the high levels of variation found in ecological measures.

Understorey species were ranked according to both cover and frequency. The ranked species were used to construct rank abundance curves. In Whittaker's original description (1965), abundance can be represented by many parameters, including aerial cover and frequency. I constructed rank versus cover curves for each plot type, which can be used to assess the evenness of the community (Fonseca and Ganade 1996, Chiarucci et al. 1999). A horizontal linear relationship indicates a community with high evenness, and downward deviation indicates a decrease in evenness.

Rank versus frequency curves, can be used to assess the homogeneity of the biotic community. Consider a perfectly homogenous system; i.e., a system in which species are distributed regularly and equally. In such a system, all members should be captured with equal frequency by a random sampling of the system, and a plot of rank versus frequency would yield a horizontal linear relationship. Conversely, consider a system that has sporadic patches of distinct vegetation. We would say that such a system is heterogeneous because of the patches of locally rare species. A random sampling of the area should capture the patches and the species within them infrequently. The resulting data set would have many species with very low frequency values (FI), creating a long right-hand tail in a plot of rank versus frequency.

The ranked frequency values were also used to construct Raunkiaer J-curves, which illustrate the number of species in categories based on frequency (Gleason 1929). According to Raunkiaer's Law of Frequency, the typical curve should have a J shape, with a peak at the lowest frequency category (0-20%) and another peak at the highest frequency group (81-100%).

3.2.1. Quantitative Community Attributes and Indices

Diversity measures were calculated from the plot data and in certain cases, the average site data for each treatment as well (Sandilands alder plots, Sandilands reference plots, Star Lake alder plots, and Star Lake reference plots). Measures calculated from the plot data were subjected to paired, 2-tailed T-tests. I performed regression analysis on the plot values for the community indices (see below) against the environmental parameters (N, canopy openness, pH) to produce coefficients of determination (R^2).

Species Richness

Species richness was expressed using three statistics: observed plot and site species richness (s), Kreb's jackknife estimate of site species richness (S) and effective species richness (N1). Observed species richness (s) was calculated for both the understorey plots and the sites as a whole. The observed plot species richness indicates the mean number of species captured by each 1 m x 1 m understorey plot. The observed site species richness indicates the total number of species encountered in all of the plots belonging to a given treatment (i.e., alder or reference) at a single site.

Although the observed species richness (s) provides a useful index, it will only provide an accurate description of the total number of species present in a community if every species was captured by the sampling technique. This assumption is rarely met in reality; most sampling techniques will not find every species present in a community, producing an underestimate of species richness. To compensate for this, I used Kreb's jackknife technique to estimate the true number of species present (Krebs 1999). This

method estimates the number of missed species by using a correction term that incorporates the number of quadrats sampled and the number of unique species (i.e., species that are captured in only one quadrat) encountered (Formula 1.4). The jackknife formula can only be applied to the site averages; it is not appropriate for plot species richness because it does not calculate a meaningful value for individual plots (S=s).

Formula 1.4: $S = s + ((n-1)/n)/k$

where: S = jackknife estimate of species richness
s = observed number of species in n quadrats
n = total number of quadrats sampled
k = number of unique species

Effective species richness (N1) is a mathematical interpretation of the absolute cover values for each species (Hill 1973). The calculated value is meant to indicate how many species are “effective” members of the community (Formula 1.5), and will always be less than the observed species richness (N1 < s).

Formula 1.5: $N1 = e^{H}$ where H = Shannon-Weiner diversity index

Since N1 is a simple transformation of H, this index does not provide any additional information beyond what is provided by the Shannon-Weiner diversity index. I have included N1 in the analysis because it is an interesting way to consider the

membership in a community. Rather than reporting the absolute number of species in the community (i.e., observed plot species richness), N1 assigns relative weights to each species to determine how many species are actually effectual members of the community.

Evenness

Evenness indices are meant to describe the variation in the relative proportion of the constituents of the community. There are many mathematical strategies to quantify the evenness of communities. I have included three common measures of evenness (E6, E_Q and E_{var}) as well as a simplistic index that I developed to describe the proportion of trace species in the community. The first index, evenness index E6, combines information taken from both the effective species richness (N1) and the entropy of order 2 (N2) (Formula 1.6) (Kvalseth 1991). A re-articulation of this index shows that it is actually a simultaneous expression of both the Gini-Simpson (D) and the Shannon-Weiner (H) indices.

Formula 1.6: $E6 = (N2-1)/(N1-1)$ where: $N1=e^H$, $N2=(\Sigma p_i^2)^{-1}=G^{-1}$, $G=1-D$

$= ((G)^{-1}-1)/(e^H-1)$

$= ((1-D)^{-1}-1)/(e^H-1)$

Smith and Wilson (1996) recommend two other measures of evenness, E_Q (Formula 1.7) and E_{var} (Formula 1.8). The algorithms are far more complex than the

formula for E6, producing values that are perhaps difficult to interpret in the context of the data.

Formula 1.7: $E_Q = -2/\pi \arctan(b')$

where b = the slope of the log abundance on the rank abundance, fitted by least-squares regression

Formula 1.8: $E_{var} = 1-2/\pi \arctan\{\sum(\ln(x_s)-\sum\ln(x_t)/S)^2/s\}$

I calculated a simplistic index (Ptr) to quantify the proportion of trace species in the plots and in the sites (Formula 1.9) (Essery 2003). Trace species were defined as those species present in the community at a level of or below 1.0% cover. The proportion of trace species is then defined as the number of trace species divided by the total number of species.

Formula 1.9: $Ptr = tr/s$ where tr = number of trace species

Diversity

The formulas for most diversity indices work with proportional (p_i) cover values. The absolute cover values (%) were converted into proportional values by dividing the individual cover values by the total cover.

Diversity has become a very commonly used term, especially with widespread concerns about increasing rates of species losses. Ironically, despite the concerns about decreasing biodiversity, there is very little understanding of what the term diversity actually means. Diversity is not an absolute parameter - it cannot be physically or directly measured - and does not have a single concrete mathematical definition. The quantification of diversity is actually quite subjective. Diversity is a function of many factors, including the total number of species present (species richness) as well as the absolute and relative abundance at which the species are present (evenness) (Lloyd and Ghelardi 1964, Chiarucci et al. 1999, Mouillot and Lepretre 2000).

Many different indices have been developed to quantify diversity, each with their own bias. One of the major discrepancies between quantification strategies is whether greater diversity credit should be assigned to species with intermediate cover or to species with high cover. The treatment of rare species is also an important defining feature of the various indices. Some methods assign a relatively greater amount of diversity credit to rare species than other methods. The two diversity measures employed here reflect this difference (Fig 1.1). The Shannon-Weiner index (H) incorporates a logarithmic term, which increases the contribution of small p_i values (Formula 1.10) (Legendre and Legendre 1998).

Formula 1.10: $H = \sum p_i \ln p_i$

The upper limit of the Shannon-Weiner index is reached when all species exist at equal abundance in the community (Lloyd and Ghelardi 1964).

Conversely, the Gini-Simpson index (D) incorporates a squaring function, and is thus biased against small p_i values (Formula 1.11) (Legendre and Legendre 1998).

Formula 1.11: $D = 1 - (\sum p_i^2)$

3.2.2. Hypothesis and Model Assessment

The observed data was used to assess the accuracy of the predictions by the Fertilizer Effects Hypothesis (FEH) and the Diversity Curve Hypothesis and Model. The FEH required the quantification of the magnitude of N-enrichment (ΔN), which is used as the predictor variable for the effect of alders on understorey diversity. The magnitude of N-enrichment (ΔN) is defined as the difference in [N] between the alder and reference plots (Formula 1.12).

Formula 1.12: $\Delta N = [N]_{alder} - [N]_{reference}$

3.3. Multivariate Analysis

Multivariate analysis was used to assess both the plot and the site data. There are numerous multivariate methodologies, each with their own biases. A common feature of the methodologies is that they reduce complex interactions that exist in a multidimensional space to express them in a simplified format in a two dimensional space.

The first step of multivariate analysis converts the raw data matrix (n x p) into an association matrix, which describes the similarity of the units (n) in each of the variable (p) dimensions (Legendre and Legendre 1998, Quinn and Keough 2002). The algorithm then assesses the association matrix (n x n) and the suite of variables to determine which variables are most important in discriminating between the units. The algorithm manipulates the variables such that the amount of variation explained in two dimensions (i.e., on two axes) is maximized. Depending on the methodology, the variables may be assigned numerical weights (eigenvalues) that indicate the strength of their association with each of the first two axes. The data is then expressed such that the first axis explains the greatest amount of variation in the data, and the second axis explains the second greatest amount of variation. The ordination diagram positions the units according to their weights on the first two axes, creating four quadrants, which will be referred to using standard labeling (I: +,+, II: +,-, III: -,-, IV:-,+) where coordinates refer to an object's position on the x and y axes (x,y). Although the ordination diagram resembles a typical x-y graph, the axes on the ordination diagram do not represent dependent (response) and independent (explanatory) variables, and are referred to only as axis 1 and axis 2.

The ordination diagram indicates the positions of the units (i.e., plots) in variable space (i.e., species space) (Legendre and Legendre 1998, Quinn and Keough 2002). Within the space, the proximity of units indicates their similarity. Units that are located close together are similar, and units that are located far apart are dissimilar. In this study, a consistent separation of alder and reference plots would indicate a difference in overall community composition between the two groups. The relationship between the

plots may also provide useful information. The plot pairs create vectors that represent trajectories. The presence of consistent direction and magnitude of the plot trajectories would indicate a predictable shift in the community in response to N-enrichment by green alders.

Multivariate ordination can be performed on any parameter, qualitative or quantitative, including binary parameters such as presence/absence of species. I used both binary and abundance matrices in the analysis of the understorey community data. The ordinations extract different information about the community. The ordination of the presence/absence data only considers which species are present in the community, with no consideration to their abundance. The ordination of the abundance matrix considers both the presence of each species in the community as well as the level at which each species exists in the community.

Binary Data

The binary (presence/absence) matrix was subjected to principle coordinates analysis (PCoA) using Sorensen's metric. A drawback of performing a binary PCoA is that it does not provide species scores, i.e., it does not provide information on the relative importance of the various species in positioning the plots throughout species space. This methodology is best used in conjunction with other analyses.

Due to the limited amount of information contained in binary matrices, the separation of plots in this ordination speaks only to the membership of species at each plot, but not to the relative abundance of the species. As such, the ordination only reflects the species richness of an area, but is inherently incapable of assessing the

evenness component of diversity. This also restricts the amount of information contained in the plot trajectories. A consistency of trajectories would indicate a difference in the membership of the community, i.e., the species richness, but not the structure (i.e., distribution and abundance and distribution) within the community, i.e., the evenness and diversity.

Abundance Data

I used multiple multivariate techniques to assess the understorey abundance data, and evaluated the individual plot positions as well as the trajectories formed by the plot pairs. While the ordination of binary data only provides information about the presence of species in the community (i.e. species richness), the ordination of abundance data provides information on the relative quantities of the members (i.e. evenness and diversity) in addition to simple membership. Thus in the ordinations of the abundance data, consistent plot trajectories would reflect a difference in both the presence and the abundance of species in the community, and thus reflect species richness, evenness and diversity.

The major disadvantage of the analysis of abundance matrices, especially those produced from percent cover measures, is that they generally contain numerous zero values due to the patchy distribution of species in plots (Warton 1995). Multivariate methods, like univariate ones, are highly sensitive to zero values. Shared zero values mislead the ordination algorithms, causing them to attribute greater similarity to plots with the shared absence of species. The distance metrics attribute similarity to points with common zero values; i.e., the association measures consider plots with the shared

absence of a species to be similar. Although shared absences may in fact be important, an overwhelming number of zero-zero associations may obscure any trends that exist among the species that are present. This dilemma becomes more problematic as the number of rare species increases.

In a preliminary analysis, I attempted to minimize the effects of zero values in the abundance matrix using five mathematical transformations – edited, log, square root, cube root and fourth root (Legendre and Legendre 1998, Quinn and Keough 2002, Warton 2005). I compared the ordinations of the raw data to those produced from transformed matrices. Although my primary focus was the positioning of the plots in species space, I also considered the amount of variation explained on the first two axes.

The edited transformation eliminated all unique species from the data set, and was the only conversion that decreases the number of zero values in the data set. Although the conversion increased the amount of variance explained by the first two axes, the benefits of editing the data likely do not outweigh the consequences of removing species from the data set (Quinn and Keough 2002). The presence of rare species may be an important and informative indicator. The other transformations (log and root) attempt to mitigate the presence of zero values by linearizing the data rather than remove the zero values. The root transformations were largely unsuccessful at separating the plots and often caused a substantial decrease in the amount of variation explained by the first two ordination axes. Although the log transformation ($\log_{10}$(cover +1)) often decreased the amount of variation explained by the first two axes, it was the most successful transformation at separating the plots in species space.

I performed numerous analyses with various combinations of association measures (correlation, covariance) and analytical strategies (principle components analysis (PCA), correspondence analysis (CA)). One of the benefits of PCA over CA is that PCA produces species vectors that indicate the direction and magnitude of the association of each species with the first two axes. This provides information on the relative importance of each variable (i.e., species) in the separation of units (i.e., plots).

The preliminary analysis showed that the PCA of the covariance matrix from the log-transformed data was the most effective at separating the plots in species space. Although many analyses were performed, I will only present the results from the PCA of the covariance matrix from the log-transformed data.

4. Results

The data was initially assessed in two groups, alder and reference plots, with no separation by site. However preliminary analysis made it immediately apparent that the differences between the sites were far greater than the differences between the treatments and would mask any trends that existed within either site. The data was then broken into four groups on the basis of both plot type and site, and each site was assessed independently of the other.

4.1. Environmental Parameters

There was no increase in inorganic N in the alder plots relative to the reference plots at either the Sandilands (ΔIN=0.73 mg N/kg soil, p=0.58) or Star Lake (ΔIN=1.71 mg N/kg soil, p=0.18) (Table 1.2). There was a moderate increase in pH in the alder plots at Star Lake (4.07 alder v 3.91 reference, p=0.06).

During plot selection I attempted to select shrub pairs such that environmental variation was minimized. Despite my efforts there were a number of statistically significant differences between environmental conditions at alder and reference plots (Table 1.2). Shrub cover was greater in alder plots at the Sandilands (39.8% alder v 30.2% reference, p=0.01), largely due to the additional contribution from beaked hazel (9.5% cover) in the alder plots (Table 1.3). There was no difference in total tree density or in either of its components. Total basal area was greater in alder plots (38.5 m^2/ha alder v 25.0 m^2/ha reference, p=0.02), with no significant difference in the deciduous component, but significantly higher coniferous basal area, all of which is solely

Table 1.2. Environmental parameters describing understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. Sample size is 30 for all treatments. Tree density measured as number of trees per 5m x 5m quadrat. P-values were calculated using 2 sided, paired T-tests. Bold values indicate a significant difference (***α=0.01, **α=0.05, *α=0.10). A dash (-) indicates a species that was not encountered in any plots belonging to that treatment. Variation has been indicated with 95% confidence intervals (±).

	Sandilands			Star Lake		
	Alder	Reference	p	Alder	Reference	p
Abiotic factors						
Soil N (mg N/kg DW soil)	8.67 ± 2.51	7.95 ± 2.35	0.58	8.23 ± 1.84	6.52 ± 1.83	0.18
pH	5.14 ± 0.19	5.02 ± 0.26	0.36	**4.07 ± 0.12**	**3.91 ± 0.15**	**0.06***
Biotic factors						
Total shrub cover (%)	**39.8 ± 6.7**	**30.2 ± 6.1**	**0.01*****	20.4 ± 4.5	19.2 ± 4.3	0.48
Alnus viridis ssp. crispa	**22.8**	**-**	**0.00*****	**17.7**	**-**	**0.00*****
Corylus cornuta	**9.5**	**27.7**	**0.00*****	**1.8**	**18.7**	**0.00*****
Total tree density (stems/quadrat)	2.6 ± 0.64	2.1 ± 0.57	0.17	1.4 ± 0.35	1.4 ± 0.35	0.90
deciduous	1.0	0.9	0.69	0.3	0.2	0.36
coniferous	1.6	1.2	0.14	1.0	1.2	0.23
Total tree basal area (m^2/ha)	**38.5 ± 8.6**	**25.0 ± 7.3**	**0.02****	29.9 ± 8.5	25.8 ± 7.5	0.44
deciduous	6.5	5.8	0.68	3.0	0.4	0.20
coniferous	**32.0**	**19.1**	**0.02****	26.9	25.4	0.76
Average circumference (cm)						
Pinus banksiana	74.6 ± 7.9	64.9 ± 9.8	0.12	85.0 ±10.7	84.8 ± 9.4	0.98
Populus tremuloides	43.1 ± 10.0	37.3 ± 11.4	0.43	65.4 ± 478.4	-	-
Canopy openness (%)	17.3 ± 1.2	18.1 ± 1.5	0.19	**18.9 ± 1.4**	**17.2 ± 1.2**	**0.00*****

Table 1.3. The aerial cover (%) and frequency (%) and rank within these categories, of shrub species growing with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. Sample size is 30 for all treatments. P-values for aerial cover from 2-sided, paired T-tests. P-values for frequency from χ^2 tests on count data. Bold values indicate a significant difference (***α=0.01, **α=0.05, *α=0.10). A dash (-) indicates that the species was not encountered.

	Aerial cover					Frequency				
	Absolute (%)			Rank		Absolute (%)			Rank	
Species	Alder	Ref	p	Alder	Ref	Alder	Ref	p	Alder	Ref
Sandilands	**39.8**	**30.2**	**0.01*** **							
Alnus viridis ssp. crispa	**22.8**	**-**	**0.00*** **	1	-	**100.0**	**-**	**0.00*** **	1	-
Amelanchier alnifolia	1.6	1.9	0.73	5	2	**40.0**	**26.7**	**0.10***	3	2
Betula papyrifera	0.7	-	0.33	7	-	**3.3**	**-**	**0.07***	7	-
Corylus cornuta	**9.5**	**27.7**	**0.00*** **	2	1	**50.0**	**100.0**	**0.00*** **	2	1
Pinus banksiana	0.0	-	0.33	9	-	**3.3**	**-**	**0.07***	7	-
Populus tremuloides	**1.2**	**0.3**	**0.10***	6	3	**23.3**	**6.7**	**0.00*** **	5	3
Prunus pensylvanica	1.2	0.1	0.14	6	5	**10.0**	**3.3**	**0.07***	6	4
Rosa acicularis	0.3	0.2	0.80	8	4	3.3	6.7	0.29	7	3
Salix scouleriana	**2.4**	**0.1**	**0.02****	3	5	**26.7**	**6.7**	**0.00*** **	4	3
Star Lake	20.4	19.2	0.48							
Alnus viridis ssp. crispa	**17.7**	**-**	**0.00*** **	1	-	**100.0**	**-**	**0.00*** **	1	-
Amelanchier alnifolia	0.5	0.4	0.66	3	2	10.0	6.7	0.41	3	2
Betula papyrifera	-	0.2	0.33	-	3	**-**	**3.3**	**0.07***	-	3
Corylus cornuta	**1.8**	**18.7**	**0.00*** **	2	1	**26.7**	**100.0**	**0.00*** **	2	1
Populus balsamea	0.1	-	0.33	5	-	**3.3**		**0.07***	5	-
Prunus pensylvanica	0.3	-	0.16	4	-	**6.7**	**-**	**0.01*** **	4	-

attributed to *Pinus banksiana* (jack pine) (Table 1.4). Despite the differences in the basal area, there was no significant difference in canopy openness.

Total shrub cover showed no significant difference at Star Lake, and alder and hazel were present at similar levels in their respective treatments. There was no difference with respect to tree density or basal area, or any of their components, but canopy openness was significantly higher in the alder plots (18.9% alder v 17.2% reference, $p<0.01$).

4.2. Species-Level Response

Few species showed a significant difference in cover or frequency between alder and reference plots at either the Sandilands (Table 1.5) or at Star Lake (Table 1.6). Although ten species (n=10) showed significant differences between the treatments, no species showed significant trends in both sites. Of these ten species, five are known or suspected to use an alternate N-source, such as atmospheric N_2 (N-fixers) or organic N. There were only three species in the community that have the ability to fix N_2: *Alnus viridis* ssp. *crispa*, *Pleurozium schreberi* (big red stem feather moss) and *Lathyrus venosus* (veined pea vine), a N-fixing legume. The *L. venosus* was more abundant in alder plots at the Sandilands, and *Pleurozium schreberi* was less abundant in alder plots at the Sandilands. Neither species showed a significant difference in cover at the Star Lake site.

Most of the species that showed significant differences with respect to cover did not show differences in frequency. *Lycopodium complanatum* (ground cedar) was more

Table 1.4. Basal area (m^2/ha) and density (number of trees per 5m x 5m quadrat) in plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. Sample size is 30 for all treatments. P-values were calculated for absolute cover using 2 sided, paired T-tests. Bold values indicate a significant difference at α = 0.05. A dash (-) indicates that the species was not encountered in any plots belonging to the respective group.

	Sandilands						Star Lake					
	Basal area			Density			Basal area			Density		
Species	Alder	Ref	p	Alder	Ref	p	Alder	Ref	p	Alder	Ref	p
Deciduous	6.5	5.8	0.68	1.00	0.90	0.69	3.0	0.4	0.20	0.33	0.17	0.36
Acer spicatum	-	-	-	-	-	-	0.0	-	0.33	0.03	-	0.33
Betula papyrifera	0.0	0.1	0.52	0.03	0.03	1.00	1.8	0.4	0.40	0.23	0.13	0.54
Populus tremuloides	6.4	5.6	0.63	0.83	0.83	1.00	1.2	-	0.29	0.07	0.00	0.16
Prunus pensylvanica	0.0	-	0.33	0.03	-	0.33	-	-	-	-	-	-
Quercus macrocarpa	-	0.1	0.33	-	0.03	0.33	-	-	-	-	-	-
Salix scouleriana	0.0	-	0.33	0.10	-	0.33	-	0.0	0.33	0.00	0.03	0.33
Coniferous	**32.0**	**19.1**	**0.02**	1.60	1.20	0.14	26.9	25.4	0.76	1.03	1.23	0.23
Abies balsamea	-	-	-	-	-	-	0.0	0.0	0.89	0.03	0.03	1.00
Picea mariana	-	-	-	-	-	-	3.2	0.5	0.40	0.07	0.20	0.25
Pinus banksiana	**32.0**	**19.1**	**0.02**	1.60	1.20	0.14	23.7	24.9	0.75	0.93	1.00	0.66
Total	**38.5**	**25.0**	**0.02**	2.60	2.10	0.17	29.9	25.8	0.44	1.37	1.40	0.90

Table 1.5. The aerial cover (%) and frequency (%) of understorey species growing with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in a boreal jack pine forest at the Sandilands in Southeastern Manitoba. P-values for aerial cover from 2-sided, paired T-tests. P-values for frequency from χ^2 tests on count data. Bold values indicate a significant difference (***α=0.01, **α=0.05, *α=0.10). A dash (-) indicates that the species was not encountered.

	Aerial cover					Frequency				
	Absolute (%)			Rank		Absolute (%)			Rank	
Species	Alder	Ref	p	Alder	Ref	Alder	Ref	p	Alder	Ref
Mosses	7.7	12.6	0.13							
Brachythecium velutinum	0.0	0.0	0.18	39	39	10.0	3.3	0.32	17	22
Dicranum spp.	0.0	0.2	0.33	40	32	3.3	6.7	0.56	19	21
Eurhynchium pulchellum	1.4	0.8	0.23	20	23	56.7	56.7	1.00	6	7
Hylocomium splendens	0.6	0.7	0.93	27	24	13.3	26.7	0.25	16	15
Moss spp.	0.2	0.1	0.59	33	36	3.3	6.7	0.56	19	21
Pleurozium schreberi	**5.0**	**10.5**	**0.06***	7	2	80.0	80.0	1.00	4	3
Polytrichum juniperum	0.3	0.0	0.38	30	38	3.3	3.3	1.00	19	22
Ptilium crista-castrensis	0.1	0.3	0.24	38	29	3.3	13.3	0.18	19	19
Ferns and Allies	17.5	13.6	0.36							
Lycopodium complanatum	**2.7**	**0.3**	**0.10***	10	28	**20.0**	**3.3**	**0.06***	14	22
Pteridium aquilinum	14.7	13.2	0.71	1	1	50.0	53.3	0.86	7	8
Graminoids	11.0	10.2	0.61							
Grass spp.	0.3	0.2	0.50	31	33	10.0	10.0	1.00	17	20
Sedge spp.	10.7	10.0	0.67	4	3	96.7	100.0	0.90	2	1
Herbaceous	31.9	28.3	0.29							
Anemone quinquefolia	4.1	4.0	0.94	8	8	86.7	80.0	0.78	3	3
Antennaria canadensis	-	0.0	0.33	-	38	-	3.3	0.32	-	22
Aralia nudicaulis	5.8	5.8	0.98	6	7	43.3	33.3	0.53	8	13
Aster spp.	0.0	-	0.33	41	-	3.3	-	0.32	19	-
Clintonia borealis	0.2	0.2	1.00	33	31	3.3	3.3	1.00	19	22
Epilobium angustifolium	1.9	3.0	0.41	16	10	30.0	30.0	1.00	11	14
Fragaria vesca	2.1	1.4	0.54	14	21	26.7	23.3	0.80	12	16
Galium septentrionale	0.2	0.3	0.47	34	28	23.3	20.0	0.78	13	17
Galium triflorum	1.2	0.6	0.11	22	25	43.3	33.3	0.53	8	13
Heuchera richardsonii	-	0.0	0.33	-	38	-	3.3	0.32	-	22
Lathyrus venosus	**3.9**	**1.8**	**0.04****	9	18	50.0	43.3	0.71	7	11
Lithospermum canescens	0.1	0.1	0.73	37	34	10.0	3.3	0.32	17	22
Maianthemum canadense	8.8	8.8	0.97	5	5	100.0	96.7	0.90	1	2
Melampyrum lineare	0.1	0.1	0.87	36	35	10.0	10.0	1.00	17	20
Mertensia paniculata	0.8	0.3	0.36	25	30	20.0	13.3	0.53	14	19

Table 1.5. cont.

	Aerial cover					Frequency				
	Absolute (%)			Rank		Absolute (%)			Rank	
Species	Alder	Ref	p	Alder	Ref	Alder	Ref	p	Alder	Ref
Herbaceous Cont.										
Taraxacum officinale	-	0.2	0.17	-	33	-	6.7	0.16	-	21
Thalictrum venulosum	2.6	1.6	0.38	11	19	26.7	16.7	0.41	12	18
Trientalis borealis	0.1	0.1	1.00	38	37	6.7	3.3	0.56	18	22
Viola spp.	0.0	-	0.33	40	-	3.3	-	0.32	19	-
Ericoid	**8.6**	**12.3**	**0.10***							
Arctostaphylos uva-ursi	0.1	0.1	1.00	38	37	3.3	3.3	1.00	19	22
Chimaphila umbellata	1.3	1.9	0.47	21	16	26.7	23.3	0.80	12	16
Gaultheria procumbens	1.6	2.7	0.34	17	12	33.3	46.7	0.41	10	10
Linnaea borealis	2.4	1.9	0.30	12	15	23.3	30.0	0.62	13	14
Pyrola secunda	2.1	1.8	0.71	13	17	60.0	60.0	1.00	5	6
Vaccinium angustifolium	**1.1**	**3.9**	**0.01*****	23	9	43.3	66.7	0.22	8	5
Vaccinium myrtilloides	0.1	0.1	1.00	38	37	3.3	3.3	1.00	19	22
Low-growing Woody species	29.1	24.6	0.31							
Cornus canadensis	0.8	0.2	0.21	24	33	6.7	3.3	0.56	18	22
Diervilla lonicera	13.7	9.1	0.11	2	4	26.7	26.7	1.00	12	15
Juniperus communis	0.1	-	0.33	35	-	3.3	-	0.32	19	-
Lonicera dioica	0.0	0.9	0.31	40	22	3.3	6.7	0.56	19	21
Rosa acicularis	2.0	2.0	1.00	15	14	40.0	50.0	0.56	9	9
Rubus idaeus	10.7	8.7	0.38	3	6	80.0	70.0	0.65	4	4
Rubus pubescens	0.0	0.3	0.33	40	28	3.3	3.3	1.00	19	22
Spiraea alba	-	0.5	0.19	-	26	**-**	**10.0**	**0.08***	-	20
Symphoricarpos occidentalis	1.5	2.7	0.36	18	11	30.0	23.3	0.62	11	16
Viburnum edule	0.0	-	0.33	40	-	3.3	-	0.32	19	-
Viburnum rafinesquianum	0.1	0.2	0.61	35	31	3.3	3.3	1.00	19	22
Seedlings and Saplings	3.3	6.7	0.21							
Abies balsamea	0.0	-	0.33	41	-	3.3	-	0.32	19	-
Alnus viridis ssp. crispa★	0.0	-	0.33	40	-	3.3	-	0.32	19	-
Amelanchier alnifolia	0.7	1.5	0.37	26	20	26.7	40.0	0.37	12	12
Corylus cornuta★	1.4	2.6	0.35	19	13	30.0	33.3	0.82	11	13
Pinus banksiana	-	1.8	0.33	-	17	-	3.3	0.32	-	22
Populus tremuloides	0.3	0.4	0.53	32	27	16.7	20.0	0.76	15	17
Prunus pensylvanica	0.4	0.3	0.62	28	29	16.7	20.0	0.76	15	17
Salix scouleriana	0.4	-	0.17	29	-	6.7	-	0.16	18	-

★Plots were defined by mature individuals of these species. Tabulated values represent cover by seedlings and saplings in the 1m x 1m quadrat.

Table 1.6. The aerial cover (%) and frequency (%) of understorey species growing with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in a boreal jack pine forest at Star Lake in Southeastern Manitoba. P-values for cover from 2-sided, paired T-tests. P-values for frequency from χ^2 tests on count data. Bold values indicate a significant difference (***α=0.01, **α=0.05, *α=0.10). A dash (-) indicates an absent species.

	Aerial cover					Frequency				
	Absolute (%)			Rank		Absolute (%)			Rank	
Species	Alder	Ref	p	Alder	Ref	Alder	Ref	p	Alder	Ref
Lichen	0.2	0.1	0.40	34	39	13.3	10.0	0.71	16	20
Mosses	15.4	16.3	0.77							
Dicranum spp.	1.8	1.2	0.48	14	20	26.7	33.3	0.64	12	13
Eurhynchium pulchellum	1.5	1.1	0.44	17	21	56.7	53.3	0.86	6	8
Hylocomium splendens	-	0.1	0.18	-	38	-	6.7	0.16	-	21
Moss spp.	0.4	0.3	0.96	29	30	23.3	13.3	0.37	13	19
Pleurozium schreberi	11.6	12.9	0.67	3	3	76.7	80.0	0.88	4	3
Polytrichum juniperum	0.0	-	0.33	40	-	3.3	-	0.32	19	-
Ptilium crista-castrensis	0.1	0.7	0.20	38	26	3.3	10.0	0.32	19	20
Ferns and Allies	24.5	23.1	0.69							
Equisetum arvense	0.2	0.2	1.00	33	35	3.3	3.3	1.00	19	22
Lycopodium clavatum	0.8	0.3	0.59	23	31	3.3	3.3	1.00	19	22
Lycopodium complanatum	7.1	6.3	0.66	5	7	40.0	43.3	0.84	10	11
Lycopodium obscurum	0.1	-	0.33	38	-	3.3	-	0.32	19	-
Pteridium aquilinum	16.3	16.3	1.00	2	2	50.0	56.7	0.72	7	7
Graminoids	6.6	7.4	0.62							
Grass spp.	0.1	0.2	0.33	38	35	3.3	3.3	1.00	19	22
Sedge spp.	6.5	7.3	0.66	6	5	76.7	86.7	0.67	4	2
Herbaceous	25.5	27.4	0.57							
Achillea millefolium	-	0.2	0.20	-	33	-	6.7	0.16	-	21
Anemone quinquefolia	1.3	0.7	0.19	19	26	50.0	50.0	1.00	7	9
Arenaria lateriflora	0.2	0.1	0.75	33	36	6.7	6.7	1.00	18	21
Aralia nudicaulis	10.1	7.8	0.38	4	4	43.3	56.7	0.47	9	7
Aster spp.	0.1	0.3	0.25	36	32	6.7	13.3	0.41	18	19
Clintonia borealis	0.1	0.2	0.66	35	34	3.3	3.3	1.00	19	22
Epilobium angustifolium	0.2	0.2	0.85	32	34	10.0	13.3	0.71	17	19
Fragaria vesca	0.0	0.1	0.23	40	37	3.3	10.0	0.32	19	20
Fragaria virginiana	**1.8**	**4.6**	**0.05****	15	8	30.0	43.3	0.39	11	11
Galium septentrionale	**0.7**	**1.4**	**0.03****	25	18	23.3	33.3	0.47	13	13
Galium triflorum	0.0	1.2	0.21	40	20	3.3	6.7	0.56	19	21
Lathyrus venosus	1.0	0.8	0.58	21	25	23.3	30.0	0.62	13	14

Table 1.6. Cont.

	Aerial cover					Frequency				
	Absolute (%)			Rank		Absolute (%)			Rank	
Species	Alder	Ref	p	Alder	Ref	Alder	Ref	p	Alder	Ref
Herbaceous cont.										
Maianthemum canadense	5.9	6.9	0.40	7	6	93.3	96.7	0.89	1	1
Melampyrum lineare	1.0	0.8	0.65	20	23	46.7	63.3	0.38	8	5
Mertensia paniculata	0.7	0.8	0.94	24	24	23.3	23.3	1.00	13	16
Trientalis borealis	**2.4**	**1.2**	**0.10***	11	19	40.0	33.3	0.67	10	13
Viola rugulosa	0.1	-	0.30	37	-	6.7	-	0.16	18	-
Ericoid	16.3	15.5	0.76							
Arctostaphylos uva-ursi	1.7	1.8	0.87	16	16	23.3	16.7	0.56	13	18
Chimaphila umbellata	0.3	0.1	0.34	31	37	16.7	6.7	0.26	15	21
Gaultheria procumbens	2.1	2.0	0.95	13	14	50.0	70.0	0.32	7	4
Rhododendron groenlandicum	0.2	1.7	0.22	33	17	3.3	6.7	0.56	19	21
Linnaea borealis	4.6	3.2	0.32	9	11	63.3	60.0	0.87	5	6
Oxycoccus microcarpus	0.0	0.0	0.39	41	41	6.7	3.3	0.56	18	22
Potentilla tridentata	-	0.0	0.33	-	42	-	3.3	0.32	-	22
Pyrola secunda	0.4	0.7	0.57	27	27	20.0	40.0	0.16	14	12
Vaccinium angustifolium	5.6	3.6	0.11	8	10	90.0	80.0	0.67	2	3
Vaccinium myrtilloides	1.5	2.3	0.37	18	13	30.0	53.3	0.16	11	8
Low woody species	38.2	37.9	0.94							
Cornus canadensis	30.6	28.4	0.52	1	1	83.3	80.0	0.89	3	3
Diervilla lonicera	3.9	4.2	0.86	10	9	43.3	56.7	0.47	9	7
Juniperus communis	0.7	1.7	0.24	26	17	13.3	6.7	0.41	16	21
Lonicera dioica	0.0	0.5	0.36	40	29	3.3	3.3	1.00	19	22
Rosa acicularis	0.9	0.5	0.47	22	28	20.0	26.7	0.59	14	15
Rubus idaeus	1.5	1.9	0.72	18	15	16.7	20.0	0.76	15	17
Rubus pubescens	0.3	-	0.33	30	-	3.3	-	0.32	19	-
Symphoricarpos occidentalis	0.2	0.7	0.27	33	26	3.3	6.7	0.56	19	21
Unknown woody species	0.2	0.1	0.59	33	40	3.3	3.3	1.00	19	22
Seedlings and Saplings	3.7	4.2	0.75							
Acer spicatum	0.1	-	0.33	35	-	3.3	-	0.32	19	-
Alnus viridis ssp. crispa★	**2.2**	**-**	**0.04****	12	-	**20.0**	**-**	**0.01*****	14	-
Amelanchier alnifolia	0.9	1.1	0.75	22	22	26.7	26.7	1.00	12	15
Betula papyrifera	-	0.1	0.33	-	38	-	3.3	0.32	-	22
Corylus cornuta★	**0.4**	**3.0**	**0.02****	28	12	**6.7**	**46.7**	**0.00*****	18	10
Populus tremuloides	0.1	-	0.33	38	-	3.3	-	0.32	19	-
Prunus pensylvanica	0.1	-	0.18	39	-	6.7	-	0.16	18	-

★Plots were defined by mature individuals of these species. Tabulated values represent cover by seedlings and saplings in the 1m x 1m quadrat.

frequently associated with alder than with hazel. *Spirea alba* (narrow-leaved meadowsweet) was only found in the reference plots at the Sandilands, but due to its low cover value did not show any significant difference with respect to aerial cover.

I constructed species-area curves from my data in order to determine whether the sampling effort had captured the full richness of the area (Fig 1.6). I encountered 57 species in the Sandilands, with 52 in the alder plots and 50 in the reference plots. The curves had not completely leveled off by the 30^{th} sample, indicating that we most likely captured the majority of the species present in the community but also missed many (Fig 1.6a). The jackknife estimates of the true species richness for the site predicted that the true species richness should be approximately 69 species in the alder communities and 67 in the reference communities – a discrepancy of 17 species between the observed and projected values in each plot type.

A total of 58 species were captured at Star Lake, with 54 present in the alder plots and 50 in the reference plots. The species area curves show a similar degree of leveling off to the Sandilands curves (Fig 1.6b), suggesting that the sampling likely missed many species in Star Lake as well. The jackknife estimate of species richness for the alder community was 71 species, a discrepancy of 17 species. The estimate for the reference communities was 62 species, indicating that 12 species were missed.

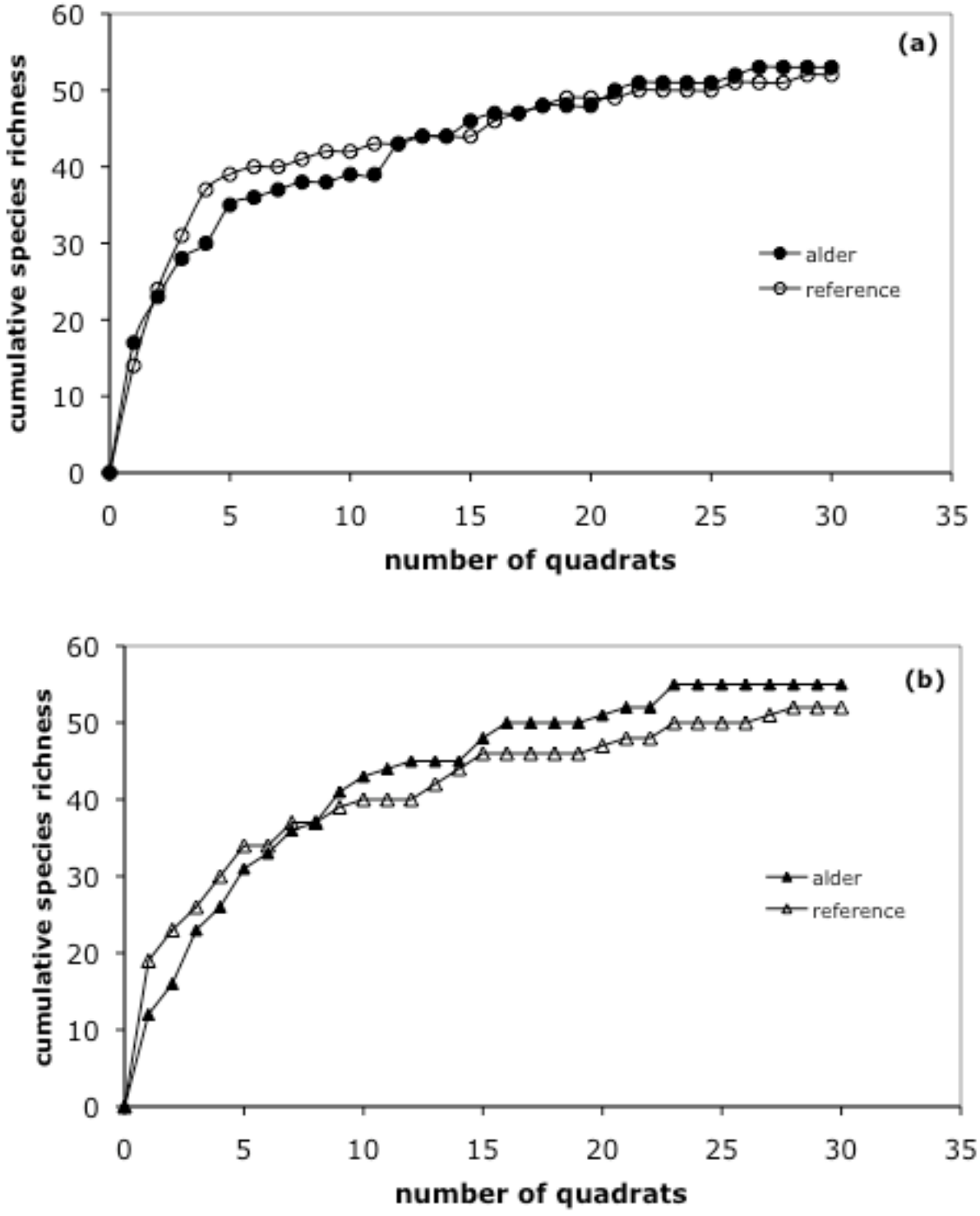


Fig 1.6. Species-area curves for understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in boreal jack pine forests at (a) the Sandilands Provincial Forest and (b) Star Lake in Southeastern Manitoba. Closed symbols indicate alder plots. Open symbols indicate reference plots.

4.3. Community-Level Response

The structure of the dominant community members was largely similar between treatments at each site (Table 1.7). The highest degree of variation was in the membership and rank of the top five most abundant species in the two treatments in the Sandilands. Two of the five most abundant species at the Sandilands maintained their respective ranks between both treatments, with *Pteridium aquilinum* (bracken fern) maintaining its position as the top species and *Maianthemum canadense* (wild lily-of-the-valley) holding the fifth rank. Both *Diervilla lonicera* (bush honeysuckle) and the sedge species ranked higher in the alder plots than in the reference plots.

Table 1.7. The top five most abundant (% aerial cover) and frequent species in understorey plots with and without green alder (*Alnus viridis ssp. crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba.

		Abundance	Frequency
Sandilands	**alder**	*Pteridium aquilinum* (14.7%)	*Maianthemum canadense* (100.0%)
		Diervilla lonicera (13.7%)	Sedge spp. (96.7%)
		Rubus idaeus (10.7%)	*Anemone quinquefolia* (86.7%)
		Sedge spp. (10.7%)	*Pleurozium schreberi* (80.0%)
		Maianthemum canadense (8.8%)	*Rubus idaeus* (80.0%)
	reference	*Pteridium aquilinum* (12.3%)	Sedge spp. (100.0%)
		Pleurozium schreberi (10.5%)	*Maianthemum canadense* (96.7%)
		Sedge spp. (10.0%)	*Anemone quinquefolia* (80.0%)
		Diervilla lonicera (9.1%)	*Pleurozium schreberi* (80.0%)
		Maianthemum canadense (8.8%)	*Rubus idaeus* (70.0%)
Star Lake	**alder**	*Cornus canadensis* (30.6%)	*Maianthemum canadense* (93.3%)
		Pteridium aquilinum (16.3%)	*Vaccinium angustifolium* (90.0%)
		Pleurozium schreberi (11.6%)	*Cornus canadensis* (83.3%)
		Aralia nudicaulis (10.1%)	Sedge spp. (76.7%)
		Lycopodium complanatum (7.1%)	*Pleurozium schreberi* (76.7%)
	reference	*Cornus canadensis* (28.4%)	*Maianthemum canadense* (96.7%)
		Pteridium aquilinum (16.3%)	Sedge spp. (76.7%)
		Pleurozium schreberi (12.9%)	*Cornus canadensis* (80.0%)
		Aralia nudicaulis (7.8%)	*Pleurozium schreberi* (80.0%)
		Sedge spp. (7.3%)	*Vaccinium angustifolium* (80.0%)

The membership of the five most frequent species was consistent between plot types in the Sandilands. Even the ranking was consistent with the exception of the alternation of *Maianthemum canadense* and the sedge as the two most frequent species.

The four most abundant species at Star Lake showed complete consistency in membership and rank. The fifth rank switched between *Lycopodium complanatum* in alder plots and the sedge species in the reference plots. The five most frequent species at Star Lake showed consistent membership, but the ranking of the species showed less consistency. Only *Maianthemum canadense* and *Cornus canadensis* (bunchberry) maintained their spots as the first and third ranked species respectively. *Cornus canadensis* shared the third rank in with *Pleurozium schreberi* and *Vaccinium angustifolium* at 80.0% frequency.

The rank-cover curves were very similar between treatments at both sites (Fig 1.7a,b). The most pronounced deviation is a slight kink in the reference curve in the lower ranks in the Sandilands. There was a marked difference between the two sites in the lowest ranks. The top-ranked species in Star Lake had much higher cover (~30% cover) than the top-ranked species in the Sandilands (~15% cover).

The frequency-cover curves for the alder and reference communities were similar at the Sandilands (Fig 1.7c), with a slight offset in the lower ranks. The reference curve was smoother in the low ranks than the alder curve, which had some brief plateaus. There was a pronounced difference between the curves at Star Lake in the middle ranks (Fig 1.7d). The alder curve was less linear than the reference curve. Between rank 6 and 25,

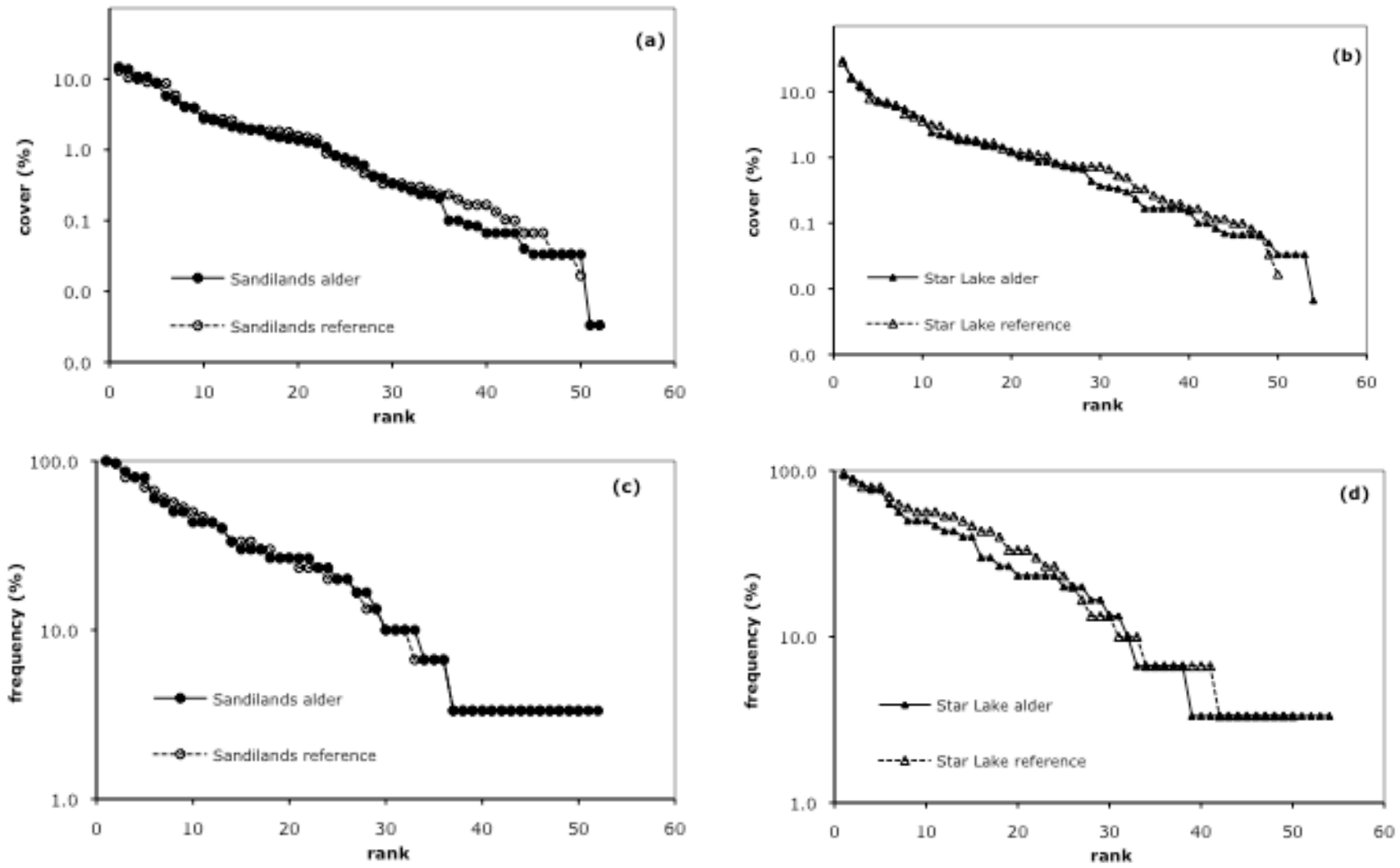


Fig. 1.7. Rank versus cover (a,b) and rank versus frequency (c,d) od species in understorey plots with and without green alders (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in boreal jack pine forests at the Sandilands Provincial Forest and near Star Lake in Southeastern Manitoba.

each rank in the alder plots was associated with a lower frequency value than in the reference plots.

In general, all of the treatments conform to Raunkiaer's J-curve. The curves were very similar between treatments at the Sandilands, although there was some discrepancy at the higher end of the curve (Fig 1.8a,b). The alder plots show a deeper dip in the 60.0-79.9% category and a higher recovery in the 80-100% category than is seen in the reference curve.

4.3.1. Quantitative Community Attributes and Indices

There were no statistically significant differences in total cover or the proportion of trace species at either site (Table 1.8). Alder plots had higher evenness in the Sandilands (E6: 0.687 alder v 0.646 reference, p=0.08) and lower species richness at Star Lake (13.6 spp/m^2 alder v 15.1 spp/m^2 reference, p=0.01). There was no significant difference between treatments for either the Shannon-Weiner (H) or the Gini-Simpson (D) diversity indices at either site. The p(β)-values from the post hoc analysis were all very low ($p \geq 0.37$), with the obvious exception of those associated with the parameters that showed significant difference with respect to α.

In order to assess whether the observed data adequately describes the community, I calculated running means for total cover, plot species richness, the Shannon-Weiner diversity index (H) and evenness index (E6) for each of the four treatments (Fig 1.9a-d). Plots were randomly added to the cumulative total to prevent the intrusion of geographical effects. All of the curves show leveling off by the 30th sample, indicating that our sampling effort should have yielded reasonably accurate

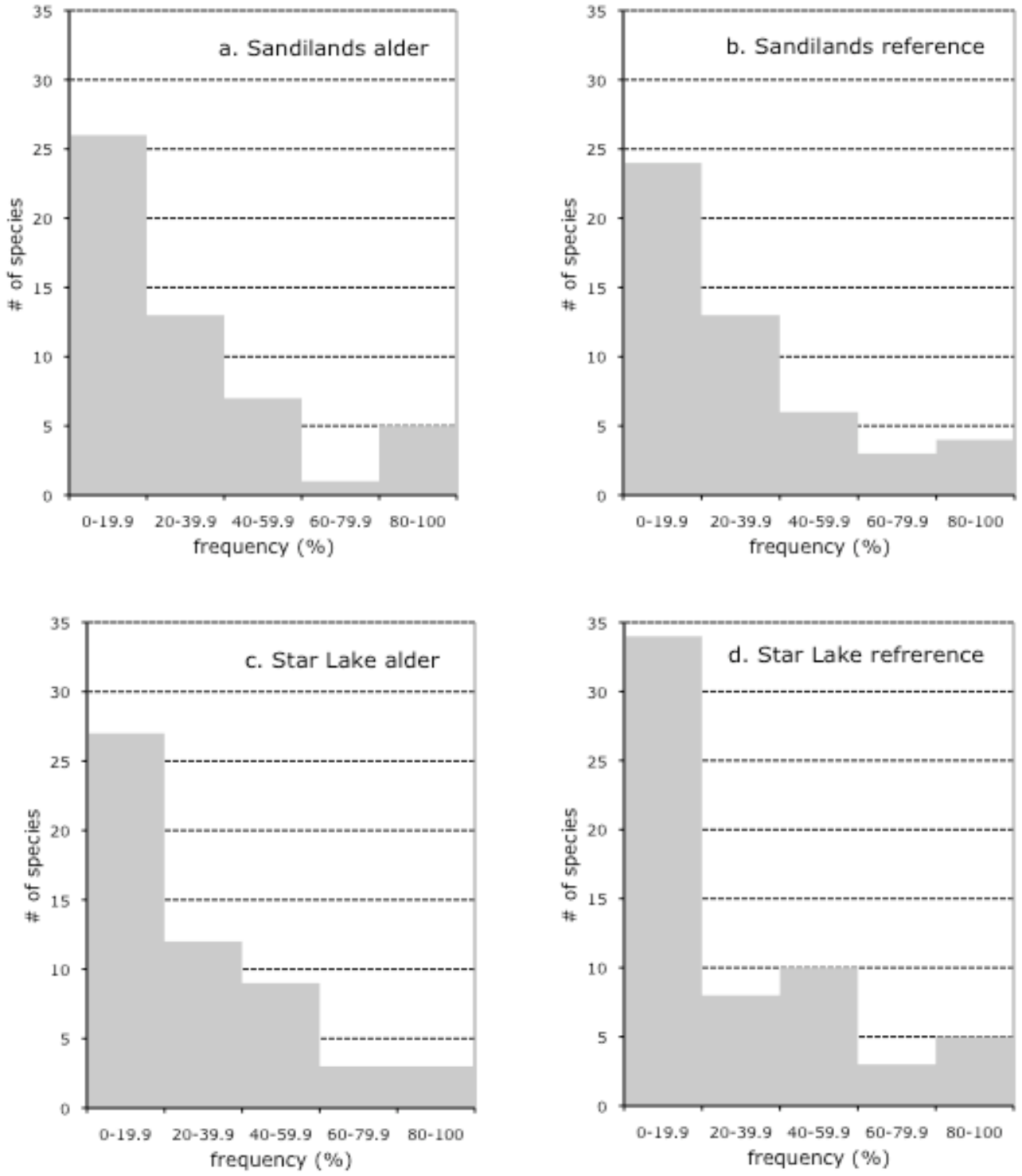


Fig 1.8. Raunkiaer J-curves (frequency v. number of species) for species in understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests at the Sandilands Provincial Forest and near Star Lake in Southeastern Manitoba.

Table 1.8. Measures of community composition for understorey plots with and without green alder (*Alnus viridis ssp. crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. Sample size is 30 for all treatments. P(α) values were determined by 2 tailed, paired T-tests. P(β) values were determined using post-hoc power analysis. Asterisks indicate significant differences (***α=0.01, **α=0.05, *α=0.10).

	Sandilands				Star Lake			
Diversity measure	Alder	Reference	p(α)	p(β)	Alder	Reference	p(α)	p(β)
Total cover	109.0	108.2	0.89	0.93	130.3	131.9	0.84	0.91
Plot species richness (s/m^2)	13.3	13.3	1.00	0.95	**13.6**	**15.1**	**0.01*** **	**0.02** **
Effective species richness (N1)	7.48	6.96	0.24	0.45	7.29	7.84	0.17	0.37
Proportion of trace species (Ptr)	0.225	0.237	0.67	0.85	0.225	0.241	0.53	0.77
Shannon-Weiner index (H)	1.96	1.91	0.48	0.73	1.95	2.02	0.19	0.45
Gini-Simpson index (D)	0.792	0.780	0.32	0.77	0.787	0.795	0.61	0.83
Evenness (E6)	**0.687**	**0.646**	**0.08***	0.19	0.674	0.649	0.28	0.55
Evenness (EQ)	**0.678**	**0.658**	**0.08***	**0.01*** **	0.676	0.668	0.50	0.43
Evenness (Evar)	**23.1**	**20.0**	**0.09***	**0.02** **	22.1	23.0	0.58	0.57

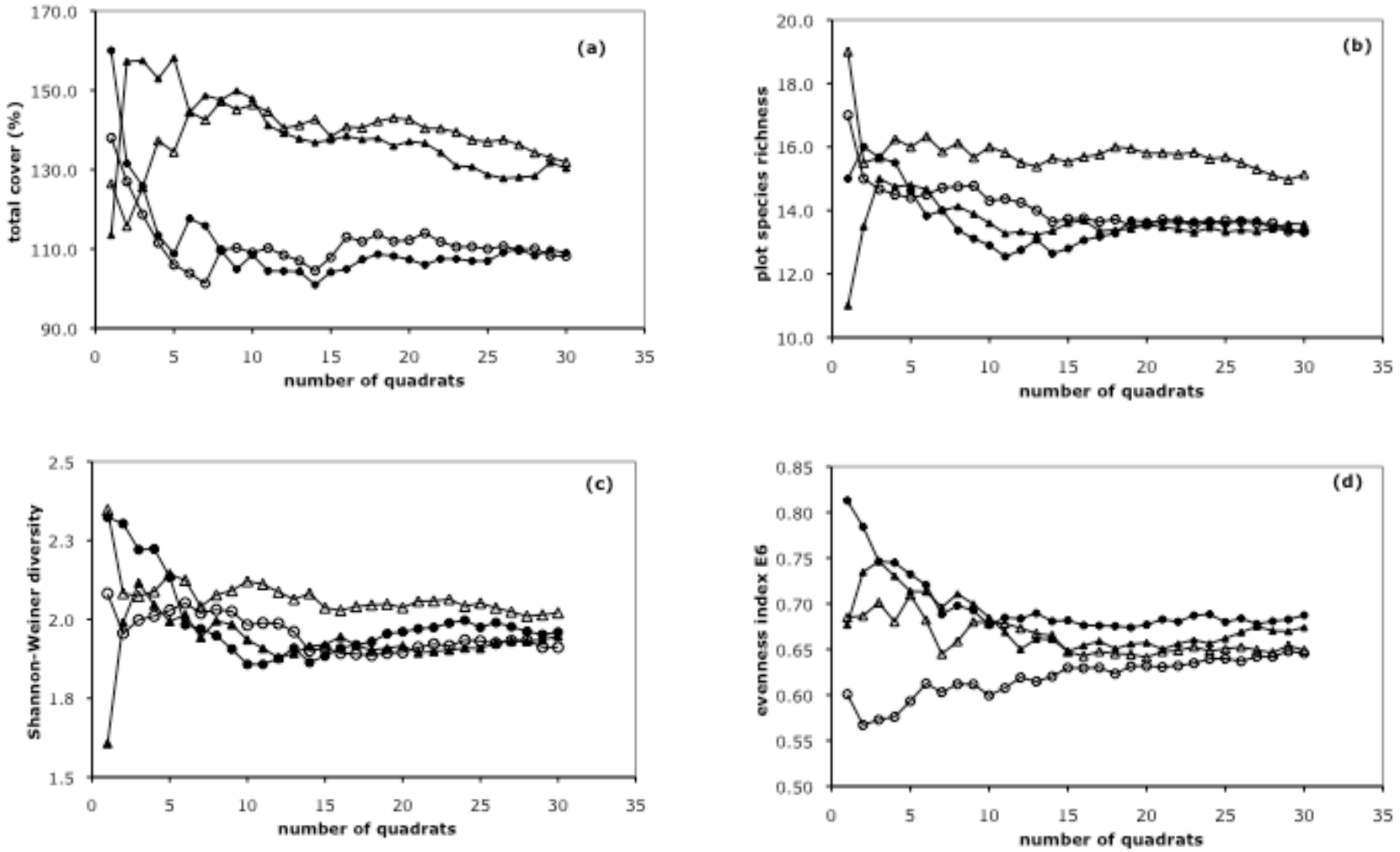


Fig 1.9. Running means for (a) total cover, (b) plot species richness, (c) Shannon-Weiner diversity index, and (d) evenness index E6 in understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests at the Sandilands Provincial Forest and near Star Lake in Southeastern Manitoba. Circles indicate Sandilands. Triangles indicate Star Lake. Open symbols indicate alder plots. Closed symbols indicate reference plots.

estimates of these parameters and that further sampling would likely not have improved our estimation of the means to any considerable extent.

None of the community parameters showed a correlation with inorganic N at the plot level at either the Sandilands (Fig 1.10) or Star Lake (Fig 1.11) (Table 1.9). There was also no correlation ($R^2 \leq 0.10$) between the other environmental parameters (canopy openness and pH) and the community attributes (Table 1.9). The environmental parameter that showed the greatest correlation with the community measures was canopy openness (average $R^2=0.05$), while inorganic N (average $R^2=0.02$) and pH (average $R^2=0.01$) showed even lower correlations.

Table 1.9. Correlation (R^2) between environmental parameters and community attributes and indices in understorey plots in two boreal jack pine forests at the Sandilands (Sand) and Star Lake (Star) in Southeastern Manitoba.

	Inorganic nitrogen		Canopy openness		pH	
	Sand	Star	Sand	Star	Sand	Star
Total cover	0.00	0.01	0.10	0.02	0.02	0.00
Plot species richness	0.00	0.04	0.03	0.05	0.00	0.04
Shannon-Weiner (H)	0.02	0.02	0.04	0.08	0.00	0.03
Gini-Simpson (D)	0.02	0.00	0.06	0.08	0.01	0.01
E6	0.01	0.01	0.04	0.06	0.00	0.00
Ptr	0.08	0.03	0.02	0.02	0.00	0.00

There was also no correlation between inorganic N and the community attributes at the site level. When the site values for the community measures were plotted against inorganic N, two of the measures – total cover and evenness - showed parallel trajectories between both sites (Fig 1.12a,d). Parallel trajectories indicate a similar response at both sites, while perpendicular trajectories indicate that the response at the sites is idiosyncratic. Overlapping alder and reference points would indicate no response. The total cover trajectories were similar in both size and direction (Fig 1.12a).

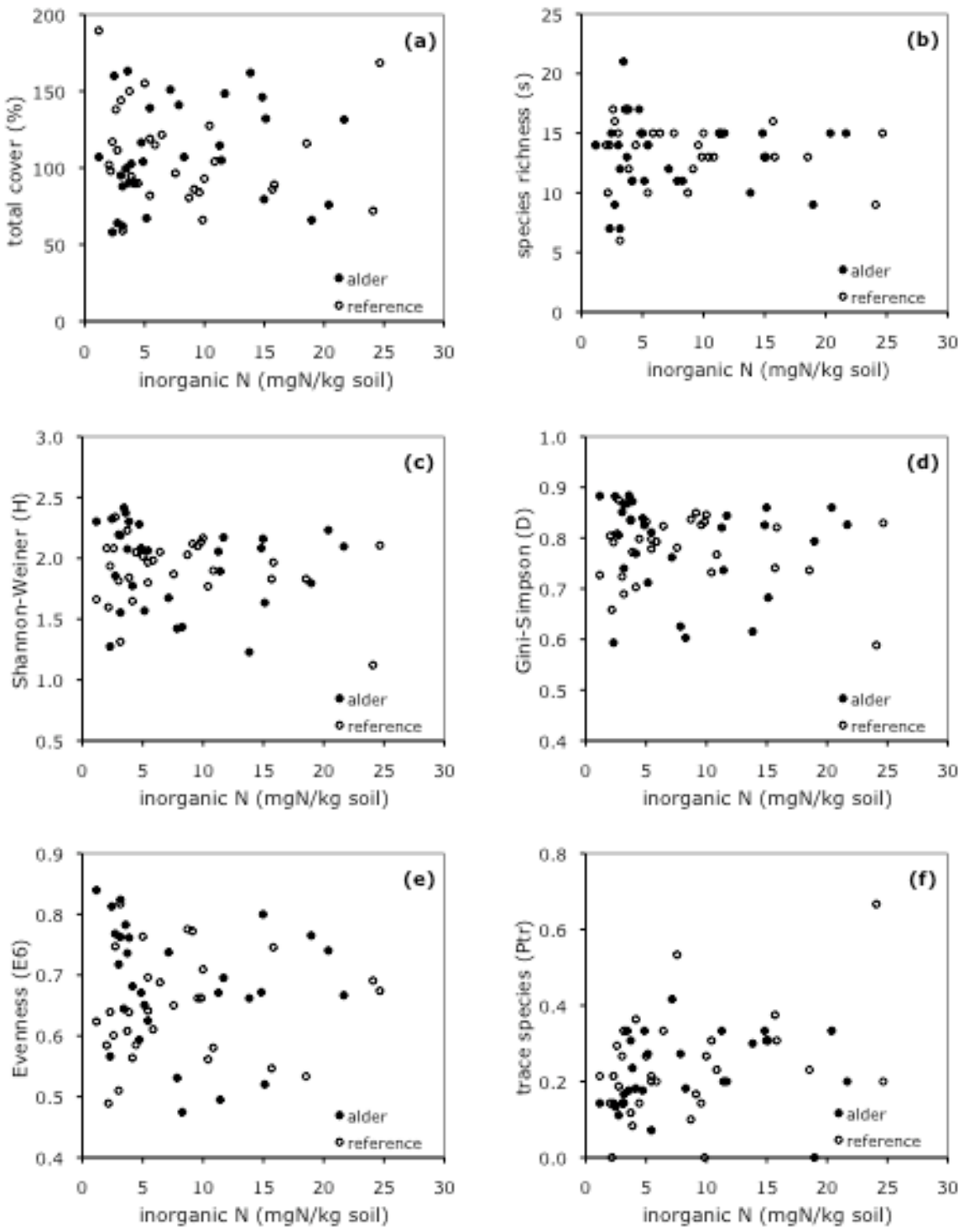


Fig 1.10. Inorganic nitrogen v. community measures in understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in a boreal jack pine forest at the Sandilands Provincial Forest in Southeastern Manitoba.

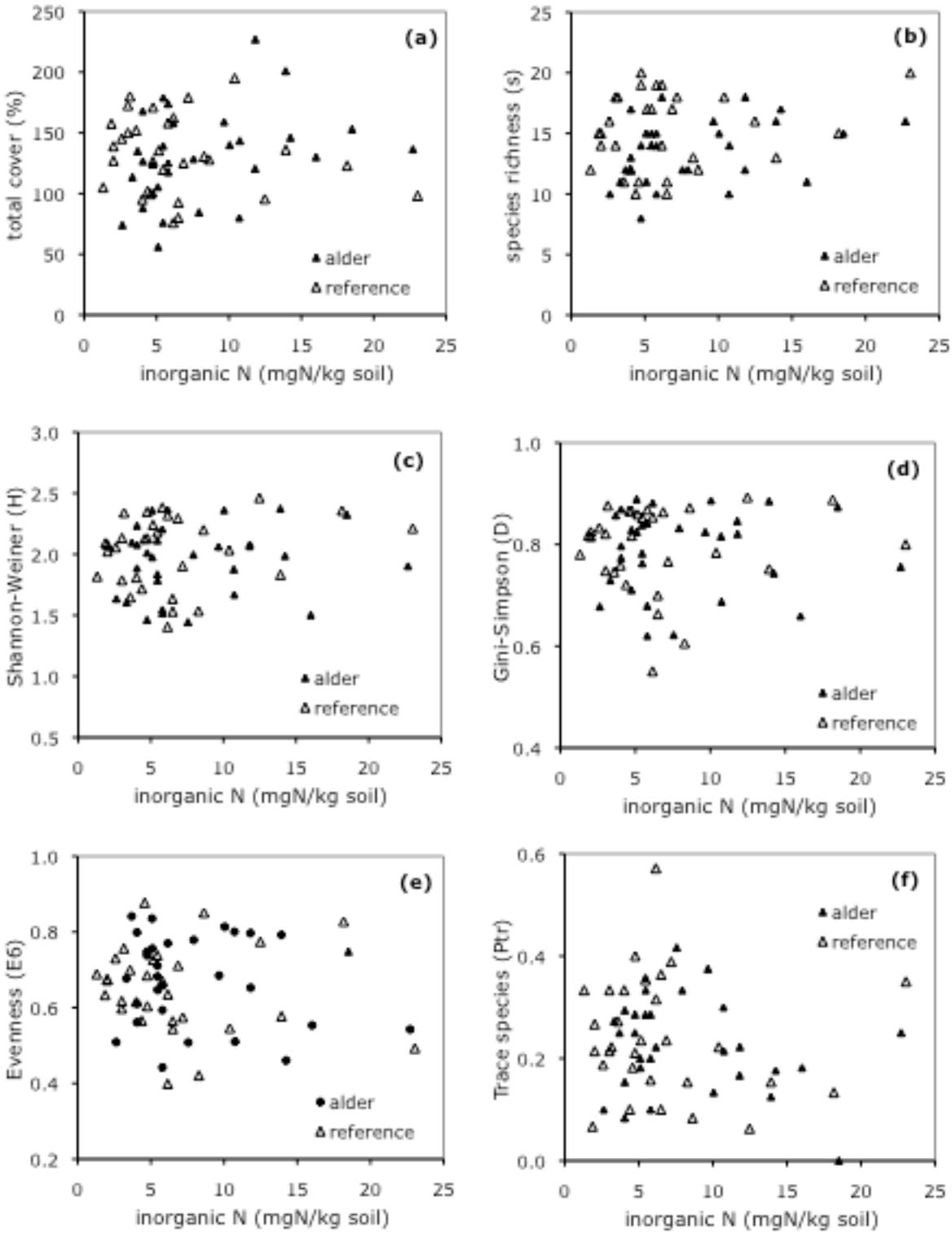


Fig 1.11. Inorganic nitrogen v. community measures in understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in a boreal jack pine forest near Star Lake in Southeastern Manitoba.

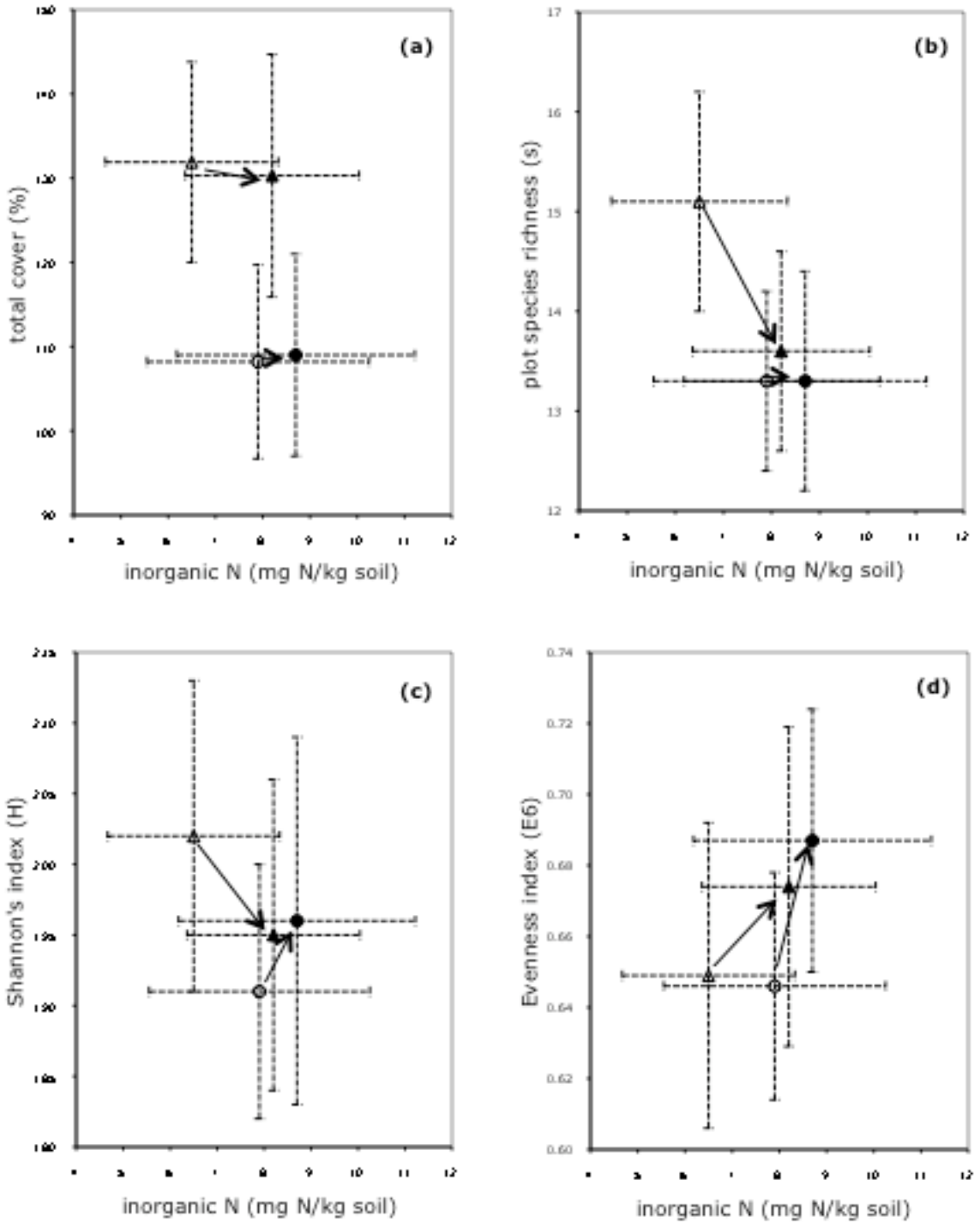


Fig 1.12. Inorganic nitrogen and (a) total cover, (b) plot species richness, (c) Shannon-Weiner diversity index, and (d) evenness index E6 in understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. Circles: Sandilands. Triangles: Star Lake. Closed: alder. Open: reference. Error bars indicate 95% CIs. Arrows indicate trajectories.

The shift occurred along the x-axis but not on the y-axis, indicating a change in inorganic N without a change in total cover at both sites. The trajectories for plot species richness and the Shannon-Weiner diversity index (H) were largely perpendicular, indicating an idiosyncratic effect. The evenness index trajectories showed strong movement along the y-axis, indicating a shift in both evenness and inorganic N.

4.3.2. Multivariate Analysis

Ordination of Binary Matrix

The first two axes of the PCoA explained 18.92% of the total variation in the Sandilands communities and 19.18% of the total variation in the Star Lake communities. The ordination of the binary data showed small plot groups with parallel or converging trends, but did not reveal a consistent separation of alder and reference plots at either site (Fig 1.13, Fig 1.14). There was no correlation between the multivariate distance and the physical distance within plot pairs at either the Sandilands (R^2=0.01) or Star Lake (R^2=0.03), suggesting that the difference in ordination is not a simple reflection of distance in physical space.

The ordination of the Sandilands data showed little consistency in trajectory direction from the reference to alder plot, with 17 of the 30 plot pairs showing trajectories to the left of the ordination space when alder is present (Fig 1.13). The ordination of the plots at Star Lake showed a greater deal of similarity with regards to trajectory direction, with two thirds of the plot pairs trending towards the right when alder is present (Fig 1.14). There were also two large groups that show a convergence of

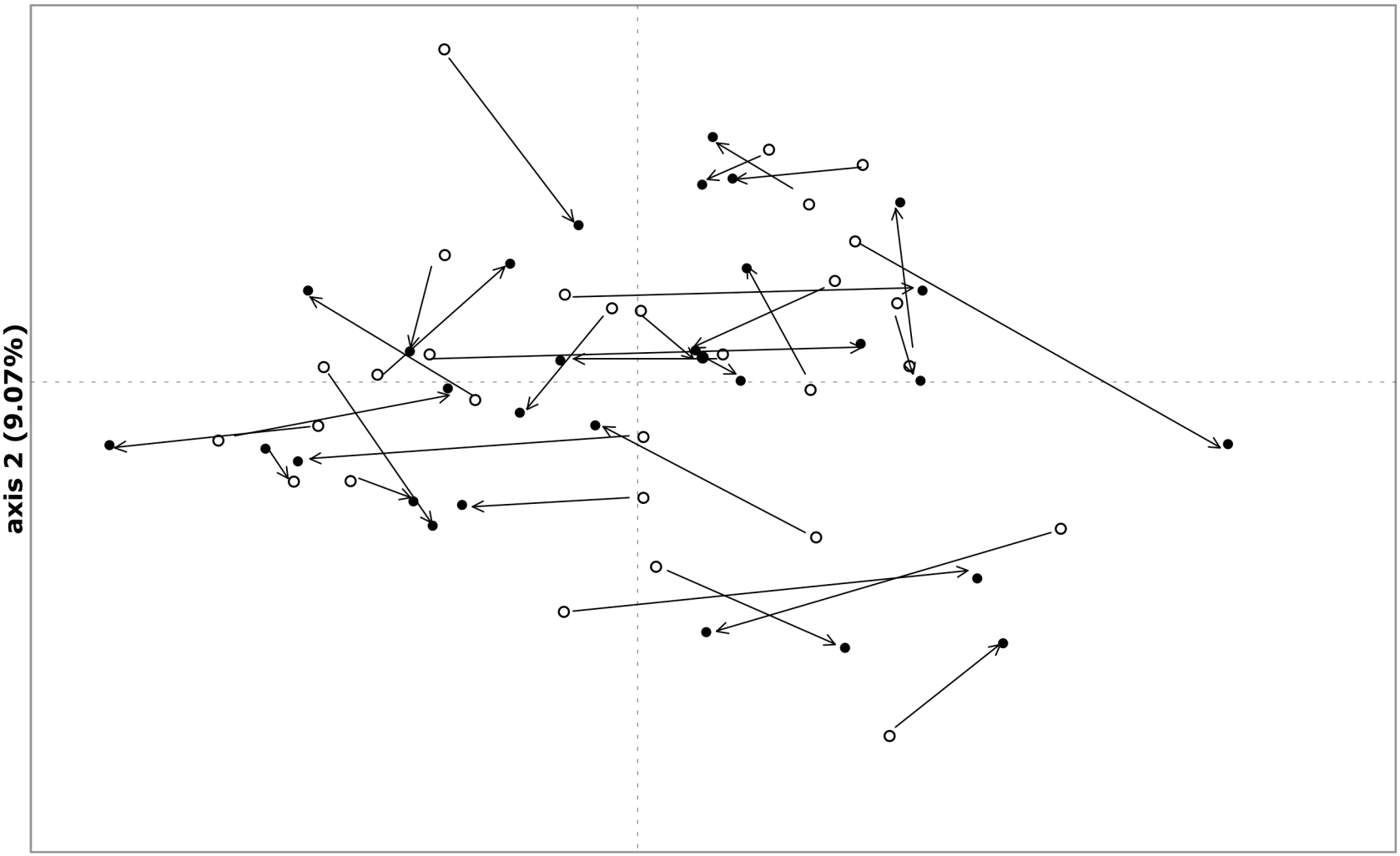


Fig 1.13. Multivariate ordination (PCoA, binary, Sorenson) of understory plots with and without green alder (*Alnus viridis* ssp. *crispa*(Ait.) Pursh) in a boreal Jackpine forest at the Sandilands. Closed symbols: alder plots. Open symbols: reference plots.

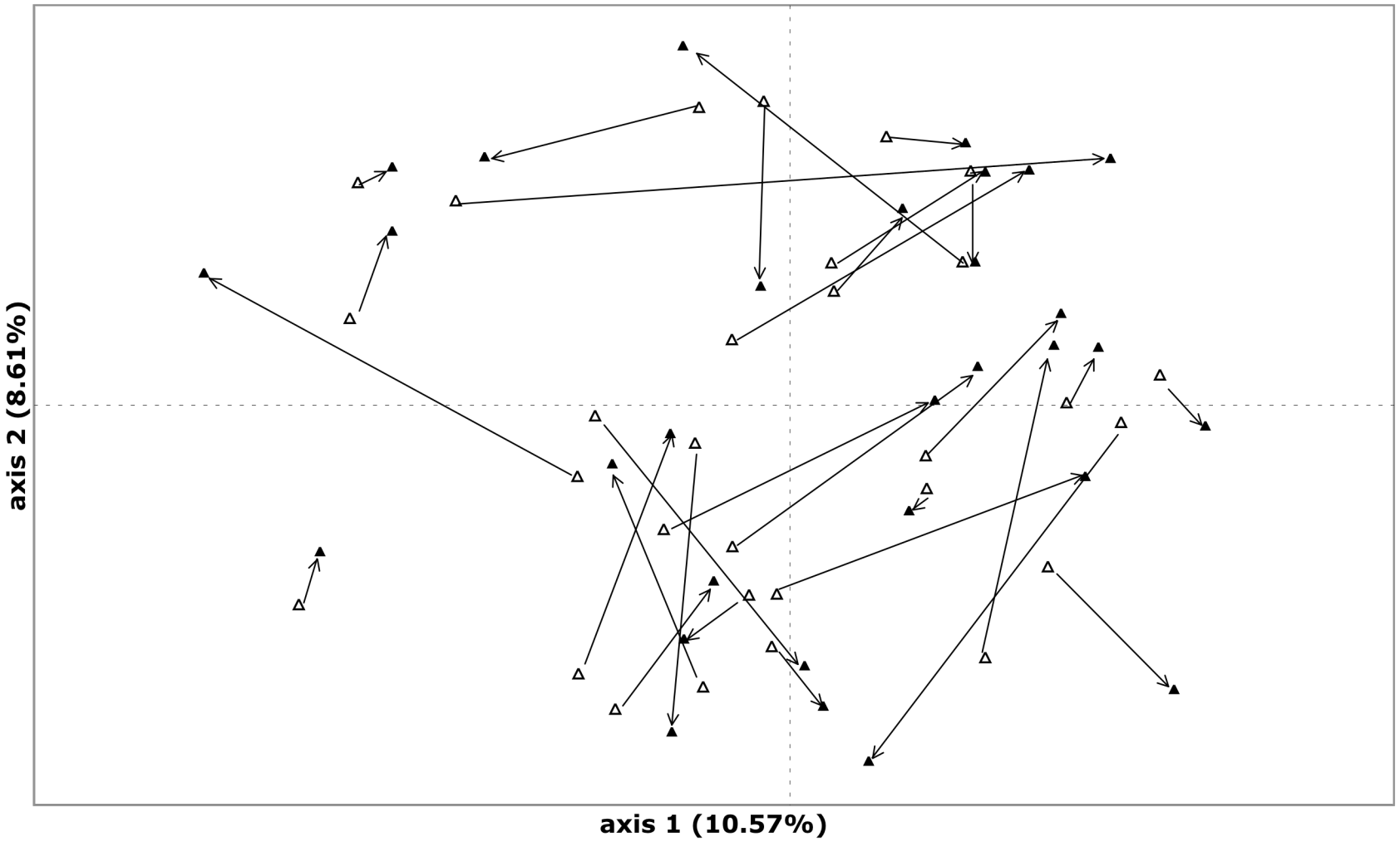


Fig 1.14. Multivariate ordination (PCoA, binary, Sorenson) of understory plots with and without green alder (*Alnus viridis* ssp. *cripsa* (Ait.) Pursh) in a boreal Jackpine forest at Star Lake. Closed symbols: alder plots. Open symbols: reference plots.

alder plots. Despite their proximity in multivariate space, these plots show little spatial correlation in the sampling area.

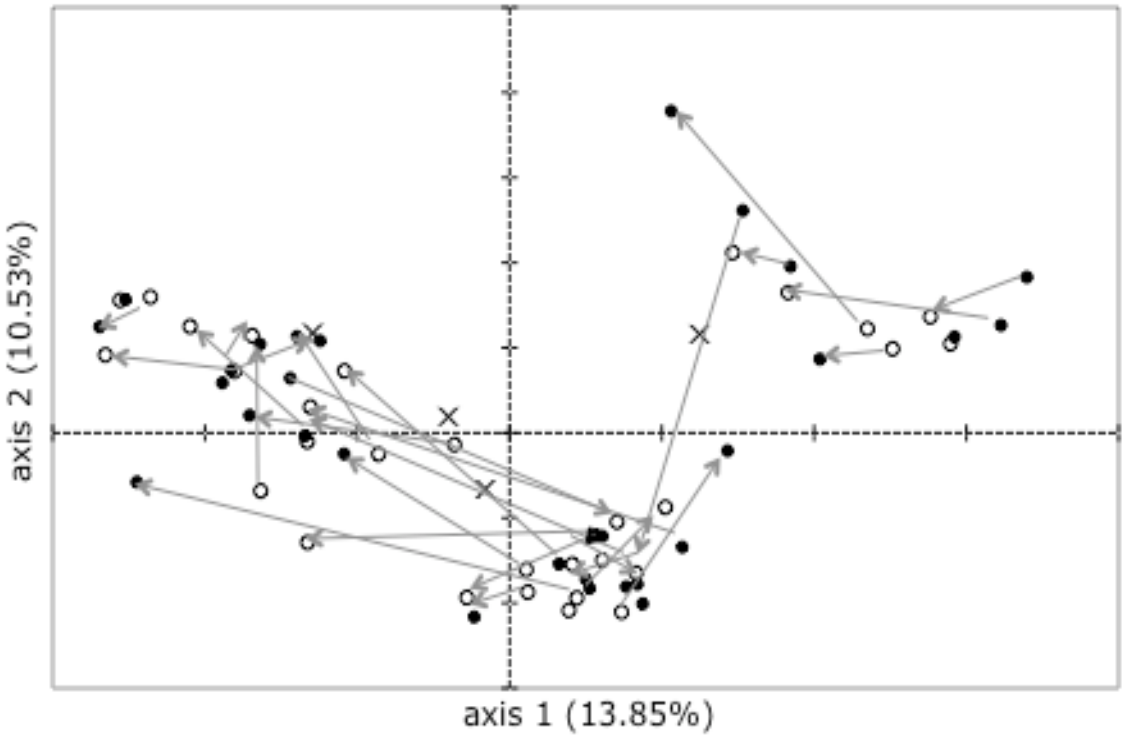
Ordination of Abundance Matrix

Plot Data

There was no overall separation of alder and reference plots visible in the multivariate ordinations for either the Sandilands (Fig 1.15) or Star Lake (Fig 1.16). There was also no consistent direction of magnitude of the reference-alder trajectories at either site. Removal of alder and hazel from the plot data did not change the ordination output.

The ordination of the understorey plots at the Sandilands produced a clumped point swarm that suggests three groups of plots (Fig 1.15a). The leftmost group hovers around y=0 on the negative side of the first axis, and containing almost all of the plots with negative scores on the first axis. The second group sits in a fairly tight clump in the second quadrant (+,-). The final group occupies the first quadrant (+,+) showing a half-moon shape. Three of the species at the Sandilands – *Pteridium aquilinum* (bracken fern), *Diervilla lonicera* (bush honeysuckle) and *Maianthemum canadense* (wild lily-of-the-valley) – were pulled away from the origin by the ordination (Fig 1.15b). Both *P. aquilinum* and *D. lonicera* showed strong correlations with both axes 1 and 2. The two species showed a high degree of separation on the first axis, but showed virtually no separation on the second axis. Deviation of *M. canadense* from the origin occurred primarily along the second axis, and was only about half the magnitude of the deviation

a)



b)

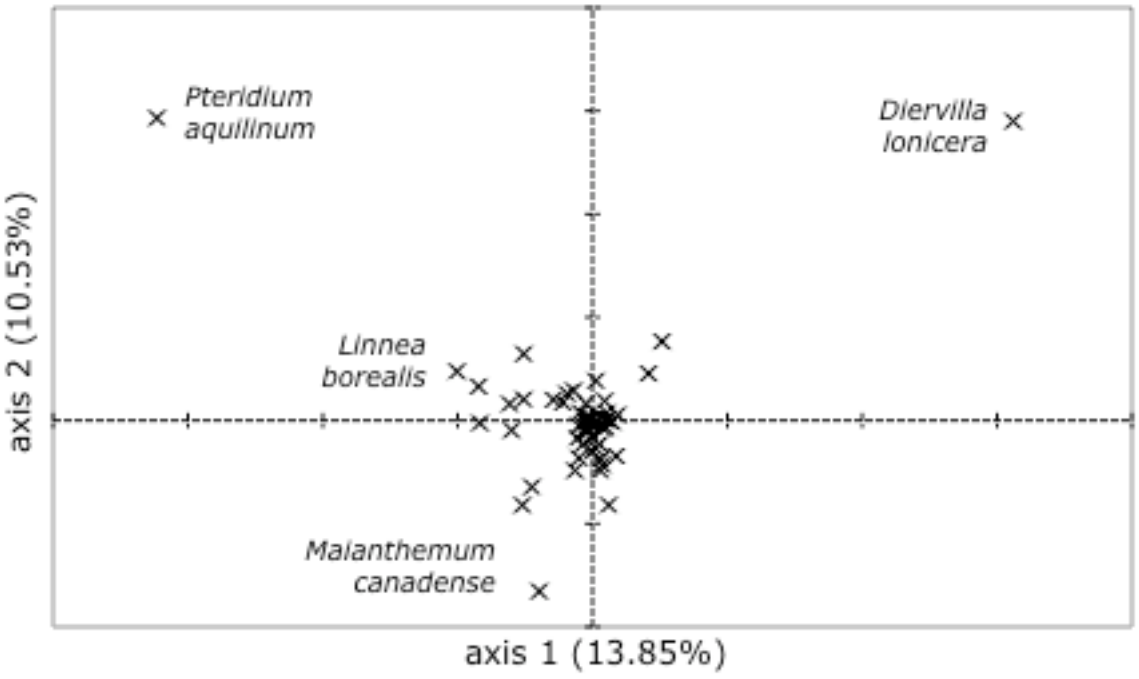


Fig 1.15. Multivariate ordination (PCA, covariance, log transformed, Euclidean) of understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in a boreal jack pine forest in the Sandilands Provincial Forest in Southeastern Manitoba. (a) plot scores and outermost species scores, (b) species scores. Closed circles: alder plots. Open circles: reference plots. X indicate species. Arrows indicate trajectories.

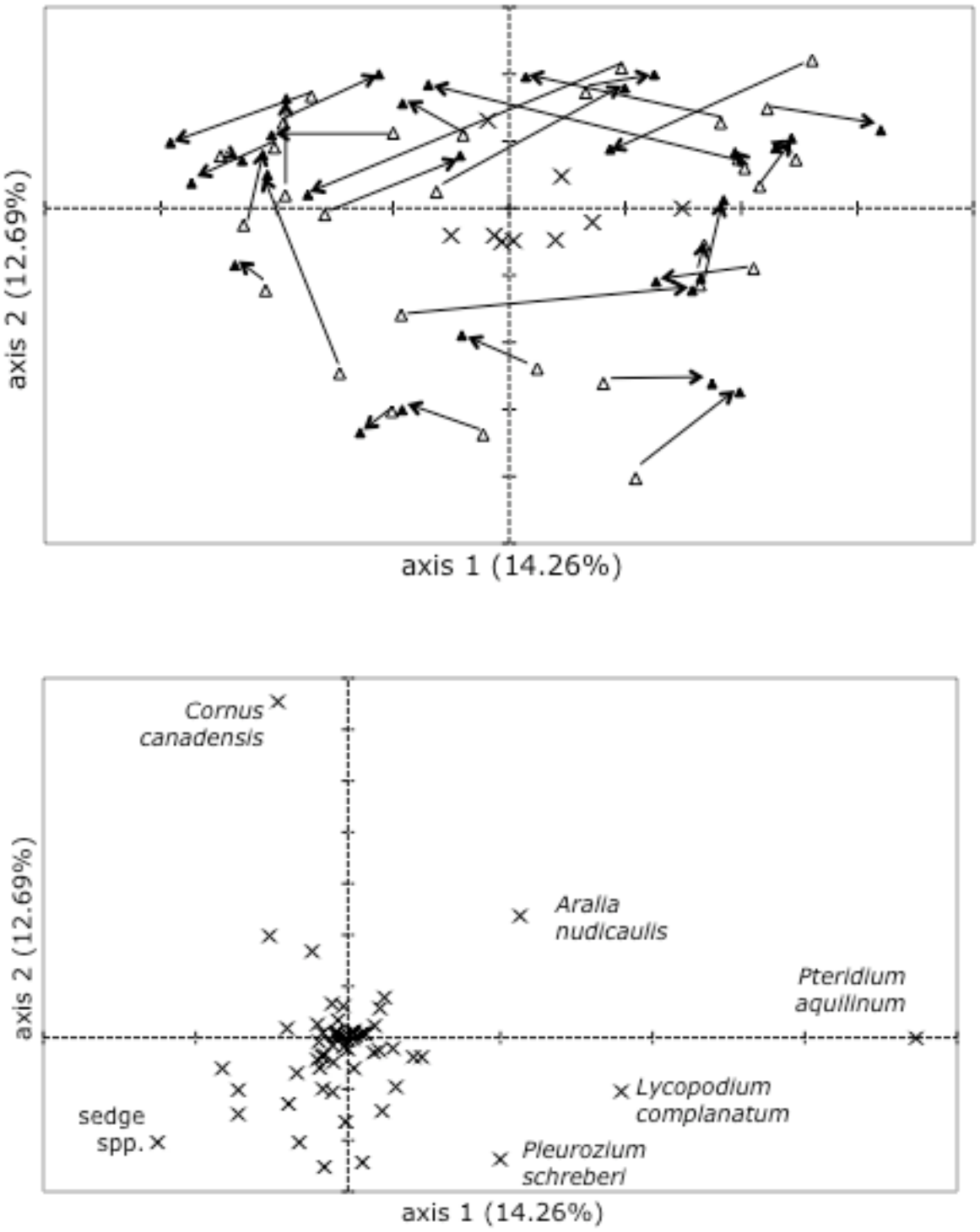


Fig 1.16. Multivariate ordination (PCA, covariance, log transformed, Euclidean) of understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in a boreal jack pine forest near Star Lake in Southeastern Manitoba. (a) plot scores and outermost species scores, (b) species scores. Closed triangles indicate alder plots. Open triangles indicate reference plots. X indicate species. Arrows indicate trajectories.

of *P. aquilinum* and *D. lonicera*. The two species that were used to define the plots (*Alnus viridis* ssp. *crispa* and *Corylus cornuta*) both scored very weakly on both axes (alder: -0.007, 0.003, beaked hazel: -0.026, -0.097).

The ordination of the understorey plots at Star Lake produced a fairly uniform point swarm, with no indication of natural groups (Fig 1.16a). More species showed a strong separation from the origin in the ordination of the understorey plots at Star Lake compared to the Sandilands. The first axis was most strongly correlated with *Pteridium aquilinum* and the second axis was most strongly correlated with *Cornus canadensis* (Fig 1.16b). Many other species also pulled away from the origin to an intermediate extent, including *Aralia nudicaulis* (wild sarsaparilla), *Lycopodium complanatum* (ground cedar), *Pleurozium schreberi* (big red stem feather moss) and the sedges. *Lycopodium complanatum* and *Pleurozium schreberi* trended in very similar directions. The sedge spp. and *Aralia nudicaulis* trended in opposite directions from each other.

Site Data

The ordination of the site data showed a consistent separation of alder and reference communities along the second axis (Fig 1.17a). There was a greater difference between alder and reference communities at Star Lake (d=0.892) than at the Sandilands (d=0.704). There was virtually no separation of alder and reference plots along the first axis.

There were three species that showed a strong separation from the origin – *Cornus canadensis*, *Corylus cornuta* and *Alnus viridis* ssp. *crispa* (Fig 1.17b). *Cornus canadensis* (bunchberry) was strongly associated with the first axis, with Star Lake at

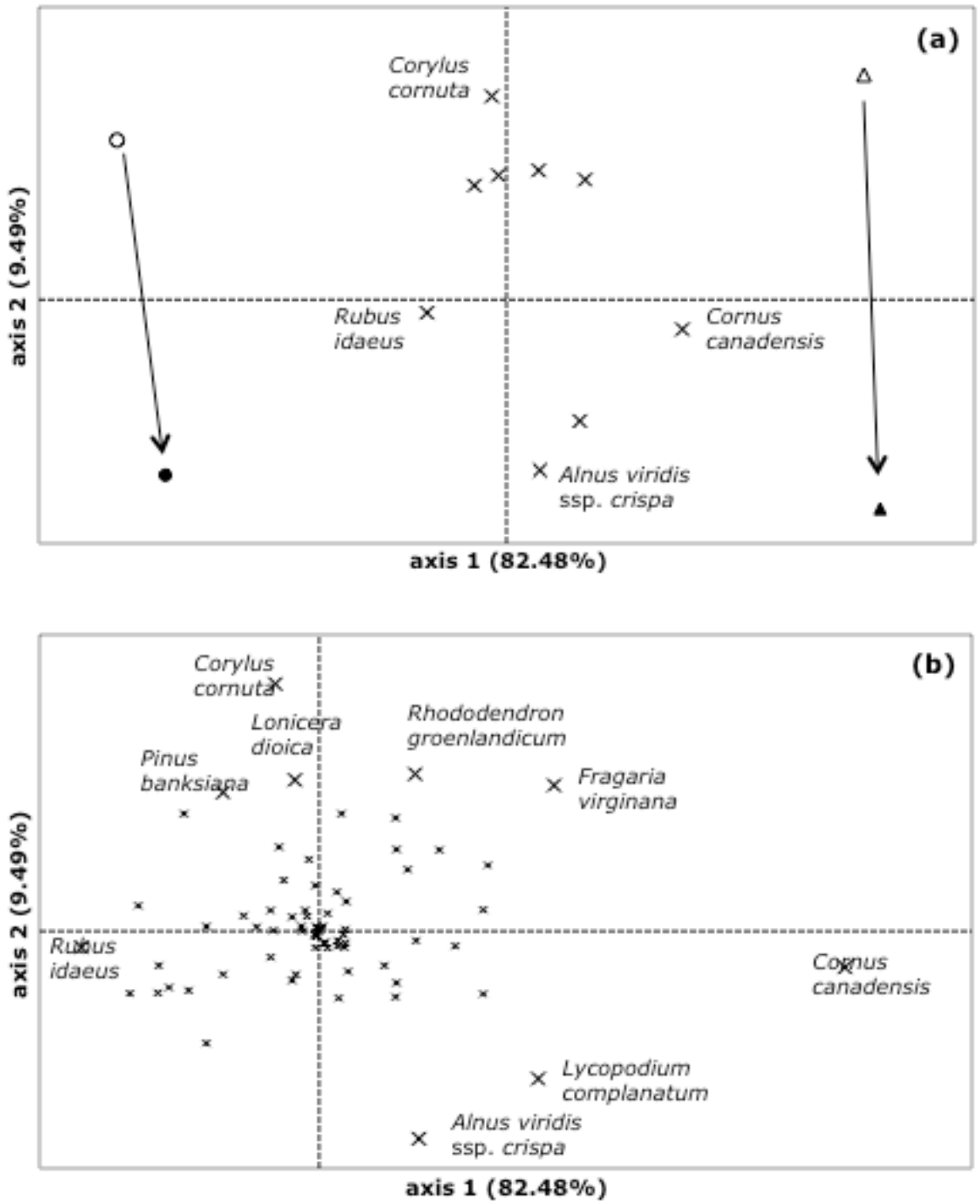


Fig 1.17. Multivariate ordination (PCA, covariance, log transformed, Euclidean) of understorey communities with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. (a) plot scores with outermost species scores, (b) species scores. Circles indicate the Sandilands. Triangles indicate Star Lake. Closed symbols indicate alder plots. Open symbols indicate reference plots. X indicate species. Arrows indicate trajectories.

the high end and the Sandilands at the low end. *Fragaria virginiana* and *Lycopodium complanatum* also trended in the same direction as *Cornus canadensis*, while *Rubus idaeus* trended in the opposite direction.

The species that trended most strongly with the second axis were *Corylus cornuta* and *Alnus viridis* ssp. *crispa*. Other species that trended with the second axis included *Rhododendron groenlandicum*, *Lonicera dioica*, *Fragaria virginiana* and *Pinus banksiana* in a positive direction, and *Lycopodium complanatum* in a negative direction. I removed both alder and hazel from the ordination to see if the trends still held (Fig 1.18). The removal of these two species from the ordination changed the spatial relationship between the treatments at Star Lake, shortening the trajectory considerably. The alder communities maintained their position on the positive end of the second axis, with the reference communities on the negative end, suggesting that there is some lasting difference in community composition and structure between the treatments apart from the presence or absence of alder.

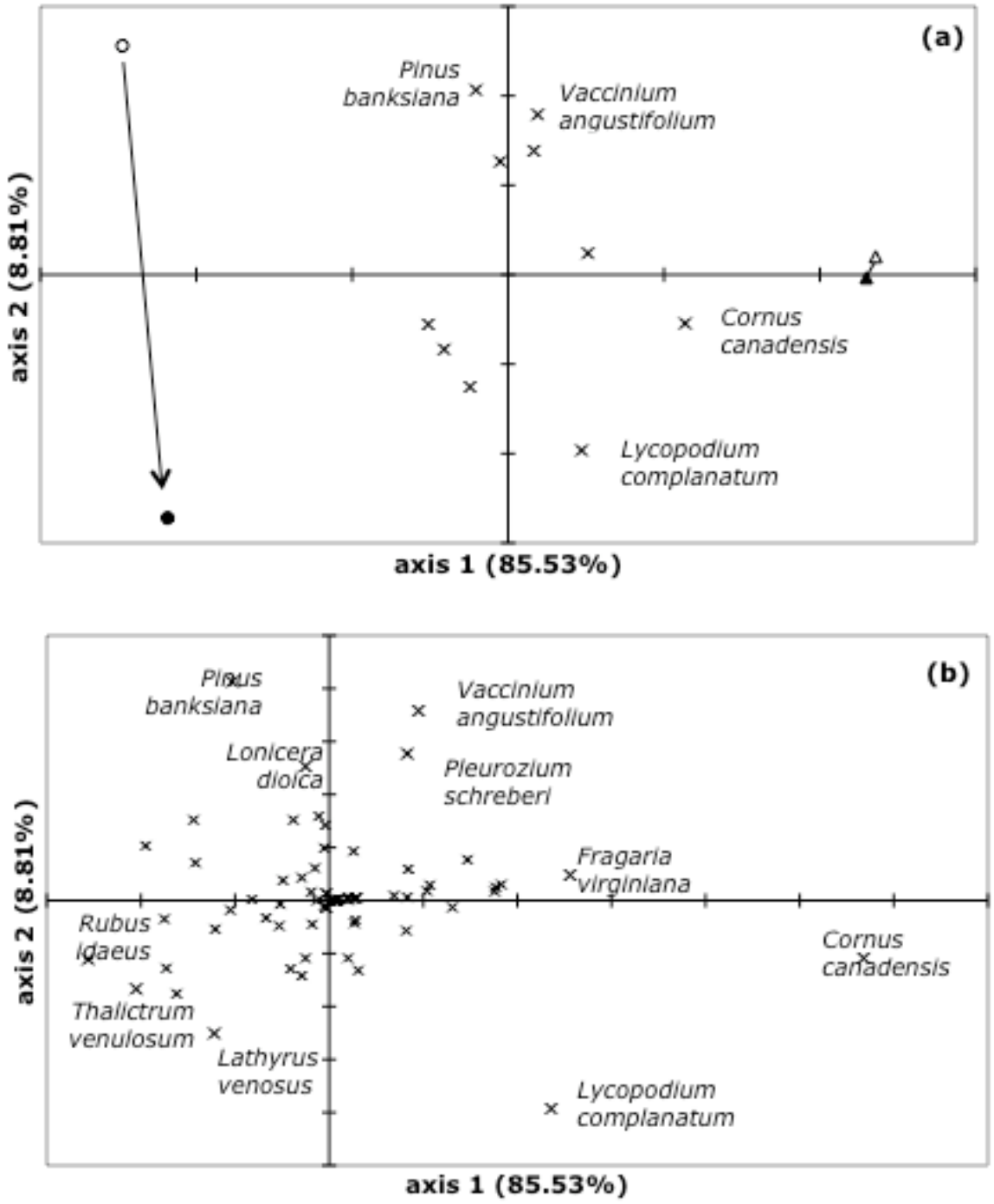


Fig 1.18. Multivariate ordination (PCA, covariance, log transformed, Euclidean) of understorey communities with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in Southeastern Manitoba. (a) plot scores with outermost species scores, (b) species scores. Circles indicate the Sandilands. Triangles indicate Star Lake. Closed symbols indicate alder plots. Open symbols indicate reference plots. X indicate species. Arrows indicate trajectories.

5. Discussion

5.1. Environmental Parameters

5.1.1. Abiotic Environment

If alders are in fact actively fixing and contributing N to their local area, then we would expect to find higher N in the alder plots, but I found no evidence to suggest that these alders were enhancing the local inorganic N pool. There was no statistically significant difference in [inorganic N] between the alder and the reference plots at either the Sandilands or Star Lake. I had expected that these alders would be fixing N because of the low soil N at these sites, but the benefits of fixation may have been outweighed by unsupportable energetic costs. The fixation pathway must be financed by energy gained from photosynthesis, and cannot proceed if the alder cannot obtain enough energy to fund the necessary reactions. Alder-fixation is highest is early successional stages and symbiotic N fixation is low in mature forests with a closed canopy (Rastetter et al. 2001). The forests selected for this study are certainly far past early succession, and the maturity of the canopy may confine the alders to a non-fixing state.

Without having physically checked the alders' nodules, I cannot discount the possibility that the alders are fixing N, but that my sampling failed to revealed it. This may have resulted from the commission of a type II error, which occurs when the variation in the data set is too large to be resolved by the sample size. The result is a low p(α) coupled with a low p(β), as was seen in my data. There was a high amount of variation in plot [inorganic N] at both of my sites, which would certainly have decreased the power of the T-tests. This variation is not unprecedented; there is a high degree of

variation in N under natural conditions and it exists both spatially and temporally, even on very small scales (Schimel and Bennett 2004). However even if the observed values could be resolved to a level of statistical significance, the enrichment of the IN pool isn't biologically significant. In a fertilization experiment in a boreal coniferous forest in northern Sweden, Nordin et al. (1998) used a level of 0.5 kg N/ha as their control treatment application, which they expected to cause no effect. This level is on par with the difference observed in my study, suggesting that the enrichment of the IN pool observed at my sites is negligible.

Despite the lack of enrichment of the IN pool, there may still be alder-enrichment that expresses itself as a change in N flux. It is possible to have an increase in the N flux within the system without a corresponding increase in the total amount of NH_x + NO_x if plants immediately take up fixed N as soon as it becomes available. Previous research has indicated that N flux may be a better predictor of the amount of N available to plants in some sites (Näsholm and Persson 2001, Schimel and Bennett 2004), and perhaps an investigation of N flux would have yielded different results in my study.

There may have also been a violation of the intrinsic assumptions of my experiment. I assumed that an alder-enrichment effect would occur in a localized manner around the individual alders. Wurtz (1995) discussed the results of Valentine (1990), who attempted to map N in an Alaskan site and correlate it with the distribution of *Alnus viridis* ssp. *crispa*. When he failed to find a relationship, Valentine speculated that it could be due to the heterogeneity of N in the area or due to redistribution of alder litter by wind. This could be the case in my sites as well. I assumed that the moderately dense shrub layer would restrict wind and confine alder litter underneath the parent

plant, but I did not attempt to quantify litter spread. A simple paint experiment could clarify this issue.

Soil pH was higher in the alder plots than in the reference plots at Star Lake. This is contrary to what would be expected if the alders are fixing nitrogen. N fixation activity is generally linked to a decrease in pH because of acidification by the nitrification process, which converts ammonia (NH_3) to nitrate (NO_3^-) (Hart et al. 1997). Wurtz (1995) found that alder soils had lower pH than non-alder soils in two Alaskan boreal forests. She found a decrease of 0.6 units under alder in a closed white spruce forest, and decrease of 0.5 units in an open paper birch stand. In northwest Alaska, Rhoades et al. (2001) found a decrease in pH under *Alnus viridis* ssp. *crispa* by 0.1-0.3 units ($CaCl_2$ methodology). Contrary to both of their findings, I found a 0.2 unit increase under *A. crispa* at Star Lake. The baseline pH was lower at Star Lake (~4.0) than at Rhoades' sites (~4.6 tundra, ~ 6.0 valley slope and ~7.4 floodplain), and perhaps nitrification is being restricted in my plots by low pH (De Boer and Kowalchuk 2001), but the pH at my sites was very comparable to the pH in Wurtz's study.

5.1.2. Biotic Environment

Despite my efforts to select plot pairs such that environmental differences were minimized, I still found some differences between the treatments at both sites. The most troubling disparity is the difference in canopy openness between the treatments at Star Lake. This is especially concerning because a difference in light levels would almost certainly affect the individuals in the understorey community. Although the difference in canopy openness between treatments may seem small - only 1.7% - the magnitude of

the difference is ~10% of the mean. The green alders were found in patches with higher canopy openness at Star Lake. This would make sense if the alders were fixing nitrogen, since light is needed to support the fixation pathway, but my measurement of inorganic soil N offers evidence to suggest that they were.

Although there was no difference in canopy openness between the treatments at the Sandilands, there were differences in other measures of the overstory. There were some interesting differences that approached significance, most notably larger jack pine CBH and higher tree density in the alder plots. Although these differences failed to reach a standard level of significance, they are supported by the fact that there was significantly greater total basal area in alder plots than in reference plots. The difference is almost entirely attributable to *Pinus banksiana*, which accounted for 83% of the total basal area in the alder plots. The observed increase in basal area and CBH are in accord with the observations of Vogel and Gower (1998) who found that *P. banksiana* growing in association with *A. crispa* had greater basal area in forests in Manitoba and Saskatchewan. The observations are also in accord with the observations at Binkley's (1983) infertile site in Mt. Benson, British Columbia, in which they found that *Pseudotsuga menziesii* had larger diameters when growing in association with *Alnus rubra*, a tree type alder.

5.2. Species-Level Response

There was no evidence to suggest that any single species was responding strongly to the presence of alders. Turkington et al. (2002) found a significant positive

response to artificial nitrogen fertilization (17.5 $gN/m^2/y$) in a boreal meadow by *Mertensia paniculata* (tall lungwort), *Epilobium angustifoilium* and *Achillea millefolium* and a negative response by *Linnaea borealis* (twin flower) and *Arctostaphylos uva-ursi* (bearberry). The three species that showed a positive response are all herbaceous and can be considered competitors, while the two species that showed a negative response (*L. borealis* and *Arctostaphylos uva-ursi*) are ericoid and can be considered stress tolerators (sensu Grime). These five species were all present in one or both of my study sites, but none of these species showed responses consistent with Turkington's fertilization experiment in the Yukon (Table 1.10). It should be noted however that Turkington's fertilizer treatment also included enrichment of phosphorus (5.0 $gP/m^2/y$) and potassium (2.5 $gK/m^2/y$), while my green alders are expected to enrich N only, creating a fundamental difference between the two experiments.

Table 1.10. Abundance (aerial % cover) of selected understorey species in plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests at the Sandilands and Star Lake in southeastern Manitoba. Species were included to compare to results obtained by Turkington et al. (2002), and expected response indicates the response to artificial fertilization in their study. A dash (-) indicates absent species. P-values from 2 tailed, paired t-tests.

		Sandilands			Star Lake		
Species	Expected	Alder	Ref	p	Alder	Ref	p
Achillea millefolium	increase	-	-	-	-	0.2	0.20
Epilobium angustifolium	increase	1.9	3.0	0.41	0.2	0.2	0.85
Mertensia paniculata	increase	0.8	0.3	0.36	0.7	0.8	0.94
Arctostaphylos uva-ursi	decrease	0.1	0.1	1.00	1.7	1.8	0.87
Linnaea borealis	decrease	2.4	1.9	0.30	4.6	3.2	0.32

Turkington's results were supported by Nams et al. (1993), who studied the response of woody and nonwoody species to artificial N fertilization in a boreal white spruce (*Picea glauca*) forest. They added ammonium nitrate in levels up to 12.5 kgN/ha

over the course of 2 years and found that cover by both *Achillea millefolium* and *Epilobium angustifolium* increased in response to N addition. Although the overall direction of response was similar in these two species, the nature of the response was different. While *E. angustifolium* increased stalk biomass in response to an increase in N, *Achillea millefolium* increased leaf size. These observations are consistent with Turkington's findings, but neither species showed a response to alders in mine.

Nams et al. (1993) found no response by *Arctostaphylos uva-ursi* to artificial fertilization, contrary to Turkington's study, in which this species decreased cover. The observation is however in agreement with my results, which showed no difference between alder and reference treatments.

Although nine of the understorey species in my study showed significant differences in cover between the treatments, no species showed significant trends at both sites. Two of the nine species were *Alnus viridis* ssp. *crispa* and *Corylus cornuta*, whose saplings were included in the understorey community. These differences are not meaningful since the plots were selected to have different levels of both alder and hazel. Of the remaining 7 species, over half (4 of 7 spp.) are known or suspected to use an alternate N-source, such as atmospheric N_2 (n=1) or organic N (n=3).

Species with alternative N-acquisition strategies have a competitive advantage under N-limited conditions and should be able to increase their growth relative to the other members of the community (Pennings et al. 2005). In areas where there is very little N available in the inorganic N pool, species that obtain N from alternate sources can overcome nutrient limitation by tapping into an abundant and less exploited N sink, such as atmospheric N_2 or organic nitrogen. As the other members of the community

compete for inorganic N, alternative N-users can use the N they obtain from alternate pools to create new tissues and increase their abundance relative to the other competitors. If the nutrient limited condition is ameliorated, these species lose their advantage and decline in abundance (Pennings et al. 2005). If the alders in my study are relieving N-limitation, species with the ability to harness an alternative N-source should lose their primary competitive advantage and decline in abundance. This would translate into lower cover by N-fixers and ON users in the alder plots than in the reference plots.

Three species that are known or strongly suspected to be organic N users had lower cover in alder plots - *Fragaria virginiana* (wild strawberry), *Pleurozium schreberi* (big red stem feather moss) and *Vaccinium angustifolium* (low sweet blueberry). Both *F. virginiana* and *P. scherberi* are known to use organic nitrogen (Krab et al. 2008, Reeve et al. 2008, Markham 2009). To date, there does not seem to be any research to indicate whether *V. angustifolium* uses ON, but many other members of the *Vaccinium* genus (*V. myrtillus*, *V*. *oxycoccus*, *V*. *vitis-idaea*) have been shown to use and even prefer organic nitrogen (Näsholm et al. 1998, Persson and Näsholm 2001, Persson et al. 2003). Two of these species (*V*. *oxycoccus* and *V*. *vitis-idaea*) co-occur with *V*. *angustifolium* in boggy areas in my study sites. It seems reasonable to assume that *V*. *angustifolium* has the ability to use organic nitrogen, especially given the close phylogenetic relationship with other ON users that share a similar geographic range and occupy similar environmental niches.

Species that use organic nitrogen are expected to have lower cover in alder plots, and all three ON-users were observed to conform to this prediction. *Fragaria*

virginiana was only observed at Star Lake where it was found to have lower cover in alder plots. *Pleurozium schreberi* had lower cover in the alder plots at the Sandilands, but did not show any significant difference between treatments at Star Lake. *Vaccinium angustifolium* also showed a decrease in cover in alder plots at the Sandilands, but showed the opposite trend at Star Lake, exhibiting a marginally significant increase in alder plots at Star Lake.

Other than alder, there were only two N-fixing species captured in my understorey plots – *Lathyrus venosus* - a member of the legume family – and *Pleurozium schreberi* – which is both an ON-user and an associative N-fixer (Markham 2009). Contrary to the expectation that N-fixers would decline in abundance in the presence of alders, *L. venosus* had significantly higher cover in the alder plots at the Sandilands (3.9% alder v. 1.8% reference, $p=0.04$). This positive correlation could indicate that *L. venosus* and alder are both taking advantage of conditions that are favorable for N-fixation and thus are distributed similarly. It seems unlikely that they are jointly exploiting favorable light patches since there was no difference in canopy openness between the treatments (17.3% alder v. 18.1% reference, $p=0.19$) at this site. *Pleurozium schreberi*, which can also use organic N, did conform to the expectation of lower cover in the alder plots at the Sandilands but did not at Star Lake. This moss likely isn't fixing an appreciable amount of N, as *Pleurozium schreberi* has been shown to fix N at a lower rate than other moss species at and near the Sandilands site (Markham 2009).

Almost all of the understorey species (7 of 8) that showed significant differences with respect to cover did not show differences in frequency. This suggests that the

species are responding by adjusting the size or number of individuals rather than adjusting their distribution. They are not being captured more or less often, they simply have higher or lower cover when they are encountered. The only exception to this observation was *Lycopodium complanatum* (ground cedar), which showed greater abundance (2.7% alder v 0.3% reference, p=0.10) and higher frequency (20.0% alder v 3.3% reference, p=0.06) in the alder plots, suggesting that this species was responding by adjusting both its cover and its distribution.

5.3. Community-Level Response

I found minimal evidence that the alders were exerting an effect on the structure and composition of their associated understorey community. The effects were primarily observed in the community indices including diversity.

Although previous researchers have observed responses by functional categories of species to fertilization (Turkington et al. 2002), there were no meaningful differences in my study. This discrepancy may be explained by the nature of my particular communities. The community at Star Lake had a high proportion (~45%) of ericaceous species, which sometimes show no response to fertilization (Nordin et al. 1998). Grasses commonly increase in response to fertilization, but the presence of grasses was low at both the Sandilands (~10%) and Star Lake (~7%) and no differences were observed.

The rank-cover curves showed slight differences between the alder and reference understorey communities. There was an upwards displacement of the alder

rank-cover curve at both sites, indicating that the top-ranked species in the alder communities exist at a slightly higher abundance than the top species in the reference plots. Increased availability of N in these areas would allow the top-ranked species to increase their abundance, but there was no evidence to suggest that N was more available in the alder plots.

The rank-frequency curves indicate a greater shift in the Star Lake communities than those at the Sandilands. There was a prominent upwards displacement of the alder rank-frequency curve at Star Lake, indicating a shift in the frequency of the mid-ranked species such that the mid-ranked species have higher frequency in the alder plots than in the reference plots. This straightening of the rank-frequency curves indicates that the alder plots are more homogeneous (i.e., even) than the reference plots at Star Lake, which was weakly supported by the assessment of the evenness indices (see Sec. 5.3.1).

The shift in the community structure at Star Lake was also seen in the Raunkaier J curves, which showed a great deal of discrepancy between the alder and reference communities. The 60-79.9% frequency category was the only category that did not show a difference between the treatments. The alder plots had far fewer species in the 0-19.9% category and more species in the 20-39.9% category, indicating a shift in the frequency of species in the lowest ranks to a slightly higher level. There was also a minor shift at the higher ranks, with fewer species in the 80-100% category in the alder plots.

Unlike Star Lake, where the effects of alders on the community were largely seen in the bottom and mid ranks, the effect at the Sandilands seemed to be largely confined

to the highest ranks. The alder J curve produced a deeper dip in the 60-79.9% category and stronger recovery in the 80-100% category. Displacement in the higher ranks was also seen in the rank-frequency curves for this site, which showed an erratic incongruity in the highest ranks. There was no displacement seen in the mid or bottom ranks in either the rank-frequency curves or in the J curves. This may indicate that at the Sandilands, N-enrichment by alders affects the frequency of individuals in the top ranks of the community (i.e., the dominant species) more than the mid and bottom ranks (i.e., the intermediate and rare species). There is no support for this statement in the observations of individual species. Only two species showed a significant difference in frequency between the alder and reference treatments – *Lycopodium complanatum* and *Spiraea alba* – both of which were part of the lower ranks.

5.3.1. Community Attributes and Indices

Despite the common observation of increased productivity (i.e., total cover) in other fertilization studies (Pennings et al. 2005), there were no differences in total cover between treatments at either site. This is not surprising considering that I found no fertilization effect, at least as far as total inorganic N is concerned. What was surprising was that despite the insignificant increase in inorganic N by alders, I did observe effects on the understorey that were in accord with the predictions of the FEH and DCH, most notably a decrease in species richness and an increase in evenness in the alder associated understorey communities.

Species richness was observed to be lower in the alder plots at Star Lake. Although the difference may seem small (Δs=1.5 spp/m^2), it represents a 10% loss of

species richness in the alder plots. The decline in species richness fits the prediction of both the FEH and phase II of the DCH, both of which assumed that as N increased, species richness would decrease in response to the exclusion of rare species.

All three evenness indices (E6, EQ and Evar) indicated that evenness was higher in the alder plots in the Sandilands. This observation is in agreement with the predictions of phase II of the Diversity Curve Hypothesis. This model predicted that as N increased, species that are inferior competitors will be excluded and superior competitors will strongly dominate.

There were no significant differences between the treatments for either the Shannon-Weiner (H) or the Gini-Simpson (D) diversity indices at either site. Power analysis indicated that the T-tests for both diversity indices had extremely low power (p_{β}=0.45-0.83), indicating a high probability of committing a type II error. It would be unwise to reject the null hypothesis (H_o: $diversity_{alder}$=$diversity_{reference}$ versus H_a: $diversity_{alder}$≠ $diversity_{reference}$) without increasing the sample size in order to determine whether a difference does in fact exist. Post-hoc power analysis indicated that the sample size should be doubled in order to adequately assess this relationship.

5.3.2. Assessment of the Proposed Models

The two hypotheses – the Fertilizer Effect Hypothesis and the Diversity Curve Hypothesis – each predicted different combinations of effects of N-enrichment by alder on the understorey community (Table 1.11). The Fertilizer Effects Hypothesis did not perform well; the observed differences do not agree with the predictions of the hypothesis. Although there was lower species richness in alder plots at Star Lake, which

Table 1.11. Expected effects on understorey attributes of N-enrichment by N-fixing alders as predicted by the Fertilizer Effect and Diversity Curve Hypotheses. Where “increase” indicates a value that is larger in the alder plots than the reference plots, “decrease” indicates a value that is smaller in the alder plots than in the reference plots, “no change” indicates a value that not expected to show either an increase or a decrease, “ns” indicates an observed value with no significant difference. Asterisks indicate significant differences (***α=0.01, **α=0.05, *α=0.10).

	Fertilizer Effect	Diversity Curve			Observed	
		Phase I	Transition	Phase II	Sandilands	Star Lake
Total cover	increase	increase	increase	increase	ns	ns
Diversity	decrease	increase	no change	decrease	ns	ns
Species richness	decrease	increase	no change	decrease	ns	**decrease*** **
Evenness	decrease	decrease	no change	increase	**increase***	ns

agrees with the FEH, there was higher evenness in the alder plots at the Sandilands, which disagrees with the predictions.

The data did conform to the Diversity Curve Model (DCM), albeit very weakly. The transition phase of the DCM predicts that there will be an increase in total cover but no change in species richness, evenness or diversity, while phase II predicts that the alder plots should have higher total cover and evenness as well as lower species richness and diversity. Both phases predict that when the level of N is below the inhibition point (i.e., $N_i<N_I$) there should be higher total cover in alder plots. This was not observed at either site, perhaps suggesting that the level of N at my sites has surpassed the inhibition point (i.e., $N_i \geq N_I$). There were no significant differencesobserved with respect to either diversity index (H or D), but the differences in species richness and evenness were in agreement with phase II of the DCM's predictions.

Overall, the model that performed best was the Diversity Curve Hypothesis. It was supported by the observations of lower plot species richness and higher evenness in the alder plots.

Further Development of the Diversity Curve Model

I found a minimal amount of evidence that weakly supported the Diversity Curve Model. Although the existing model did not adequately describe the observations, further development may be able to improve the accuracy of its predictions. As mentioned previously (sec 1.4.2), the Diversity Curve Model is a gross oversimplification of a natural system and other regulating factors would have to be considered in order to

increase the accuracy of the model. Including the effects of N on light availability would most likely be especially important in further development of the model.

The availability of N should affect productivity, increasing total cover as N increases, although I found no evidence of this in my sites. The increase in productivity should cause a corresponding decrease in light availability in the understorey. The effect of decreased light availability would manifest itself at the high end of the N spectrum, and would restrict community membership to those species with the ability to tolerate light limitation. Although this effect has not been directly included in this model, the effect of light limitation should reinforce the decline in species richness in phase II.

The weak relationship between [inorganic N] and the community attributes may indicate that [NH_x + NO_x] is not a good predictor variable for the effect of alders on the understorey community, and further development of this model should consider other environmental parameters, such as N-flux, in conjunction with or in lieu of [NH_x + NO_x].

5.3.3. Multivariate assessment

For the most part, there was very little information provided by the multivariate ordinations. There were some interesting observations, which I will discuss below, but they certainly do not constitute strong evidence of an effect of nutrient enrichment by alders on the understorey community.

Binary data

The ordination of the presence/absence data showed plots with converging trajectories, suggesting a homogenizing effect of alders on the membership of the

understorey community. The trajectories at Star Lake showed a greater deal of similarity with regards to trajectory direction than the trajectories for the Sandilands, suggesting a more consistent shift in the Star Lake communities in response to alder.

Abundance Data

Ordination of Site Data

I assessed the site data to determine which species were most strongly associated with the alder and reference communities. The ordination emphasized the difference between the two locations – Sandilands and Star Lake. This certainly isn't surprising considering the differences in relative cover by functional categories at the two sites, especially with regard to the cover by ericoid species and low woody species, and to a lesser extent by herbs and graminoids (Fig 1.17, Fig 1.18).

The second axis separated the alder and reference communities, but only accounts for 8.81-9.49% of the total variation, suggesting that any difference caused by alder accounts for no more than 8.81-9.49% of the variation in the community. Species with strong scores on this axis are most responsible for discriminating the treatments, i.e., these species are more strongly associated with one plot type than with another. Only one species - *Lycopodium complanatum* - showed a strong correlation with the alder side of the ordination. *Lycopodium complanatum* was more frequent and had higher cover in alder plots at the Sandilands. This species shows the most positive association with alder of any of the individual species in my communities. *Lathyrus venosus* also showed an association with the alder plots when alder and hazel were removed from the data set, and was twice as abundant in the alder plots at Star Lake.

The species that showed the strongest correlation with the reference side of the second axis were *Pinus banksiana* and *Lonicera dioica*, and to a lesser extent, *Vaccinium angustifolium*, *Pleurozium schreberi*, *Rhododendron groenlandicum* and *Fragaria virginiana*. These species are all very different with respect to growth form, including trees, vines, low growing ericoid species and mosses, as well as nutritional strategies, including species that are restricted to using inorganic N and those that can use ON and/or N_2. Three of these six species - *F. virginiana, P. schreberi* and *V. angustifolium* - showed a significant difference between treatments in the understorey. All three are ON users, and as discussed previously (sec 5.2), all had lower cover in the alder plots, in agreement with the dissociation between these species and alder in the ordination diagram.

Ordination of Plot Data

I also assessed the plot data to determine whether any species were responding to alder in a single site, but the analysis did not reveal any obvious trends at either the Sandilands or at Star Lake. There were no trends apparent in the plot trajectories with respect to either direction or magnitude, and the alder plots and reference plots were interspersed relatively evenly throughout the point swarms.

Only one species - *Pteridium aquilinum* - showed a strong deviation from the origin at both sites, suggesting that it is a particularly important component of the understorey community when separating plots in multivariate space. The separation is not related to treatments in any way, and most likely represents some other gradient present in the site – likely light. I did not find a correlation between the abundance of

bracken fern and canopy openness (R^2<0.19), but my experiment was not designed to address this relationship.

5.4. Assessment of the Sampling Effort

There are many possible reasons that the observed results were so weak. The negligible effects on the understorey community is likely attributable to the negligible increase in [NH_x + NO_x] at these sites. The lack of a statistically significant increase in [NH_x + NO_x] may be evidence that there is no N enrichment occurring at these sites, but I am hesitant to assume that the alders are not contributing fixed N to the soil pool, especially considering the possibility that the enrichment effect may manifest itself as an increase in N flux but not in the total amount of inorganic N (section 5.1.1).

Was the Sample Size Adequate?

One of the major concerns of any study is obtaining a sufficient sample size to adequately address the research question and rule out both type I (determining that there is a difference when there is none) and type II (determining that there is no difference when there is one) errors. The sample size was not determined *a priori*; it was determined in the field according to the size of the green alder population and the heterogeneity of environmental conditions at the two sites. I was able to find 30 suitable alder-hazel pairs at each site, i.e., 30 samples per plot type. This is comparable to the 32 samples per treatment used by Turkington et al. (2002) in a similar experiment, but many of my tests suggested that the sample size was inadequate to rule out type II

errors. Both the species richness curves and the jackknife estimates suggested that we missed many species. If the sampling effort missed a large proportion of species, then the community was not adequately described and further sampling is required to capture the species that were missed.

I used post-hoc power tests to determine whether the sample size was sufficient to accurately determine whether any difference exists between the treatments, and for the most part, the results indicated that it was not. There was very little power (p_{β}>0.10) to most of the tests on the community attributes and indices. The power analysis indicated that doubling the sample size in Star Lake could increase the power to accurately describe trends in evenness and diversity but the difference between the means is very small and while the results may become statistically significant they may not be biologically significant.

While collecting more samples could be beneficial, it may not be possible to do so. The sample size was limited to the population of green alders with suitable reference shrubs within a fairly homogeneous landscape. I captured the majority of the alders in the study area, and it seems overly optimistic to presume that another 30 sample pairs could be found within the boundaries of the present study site. It may be possible to expand the boundaries of the study sites to capture more individuals, but it would require sampling across a more heterogeneous landscape.

5.5. Conclusions

There was no evidence to suggest that the green alders studied are enriching their surroundings with N, and so it is not surprising that there was very little evidence to suggest that the alders are exerting an effect, even a weak one, on understorey community structure. The two species that showed the strongest responses to alder were *Lycopodium complanatum*, which showed a positive association with *A. crispa*, and *Fragaria virginiana*, which showed a negative association with *A. crispa*. There was significantly lower species richness (13.6 vs. 15.1 spp./m^2, p=0.01) in Star Lake, and higher evenness (E6: 0.687 vs. 0.646) in the Sandilands to a moderately significant degree (p=0.08). These observations support phase II of the Diversity Curve Model, which predicts decreased species richness and increased evenness in the presence of N-enrichment by alders.

5.6. Recommendations for Future Research

The power analysis of this data set showed that the power was fairly low in the assessment of many of the community indices. Increasing the sample size with further data collection at these and other comparable boreal jack pine sites could significantly improve confidence in these parameter estimates. It is also possible that there may be an N-enrichment that manifests itself in the N-flux without affecting the absolute amount of inorganic N in the soil pool, and N-flux could be a useful measurement in future studies.

Chapter 2

Alder Tissue in the Decomposition Environment

The inclusion of *Alnus viridis ssp. crispa* (Ait.) Pursh (green alder) tissue in litter mixes does not result in higher decomposition rates of associated litter in lab or field incubations.

Abstract

Previous litterbag studies have concluded that alder litter may increase the decomposition rates of associated litter species. In the past, species mixes have been very simplistic, restricted to pairwise combinations of litter species. This study has incorporated two separate methodologies. The first part studied the decomposition of natural litter assemblages under field conditions. Litterbags were filled with litter collected directly from the forest floor and incubated either under *Alnus viridis ssp. crispa* (Ait.) Pursh (green alder) or *Corylus cornuta* Marsh. (beaked hazel) in a boreal jack pine forest for one year. Birchwood chopsticks were incubated alongside the litterbags to provide a standard substrate for comparison to the litterbags. There was no significant difference in the decomposition rates of either the litterbags (p=0.62) or the chopsticks (p=0.23) between the alder and reference treatments. The second part of this study investigated the decomposition of artificially constructed litter mixes (up to 4 species) under controlled conditions in a dark growth chamber for 12 weeks. After 12 weeks of incubation, *A. crispa* tissue had lost significantly less (p=0.01) mass than *Rubus idaeus* L. var. *strigosus* (Michx.) Maxim. (red raspberry) and showed no significant difference (p=0.11) in mass loss from *Cornus canadensis* L. (bunchberry). There was no difference (p=0.08) in mass loss between mixes with alder (50.2%) than mixes without alder (54.7%). There was no evidence from either experiment to suggest that *A. crispa* increases the decomposition of associated litter species.

1. Introduction

1.1. Decomposition in the Boreal Forest

Decomposition is largely a biotic process, with microorganisms responsible for the majority of the breakdown of organic tissues. Cool temperatures inhibit the growth and metabolism of saprophytic microorganisms and consequently retard decay processes in the boreal forest (Bonan and Shugart 1989, Nams et al. 1993, Turkington et al. 2002). When decomposition is constrained, the compositional elements of the litter are not converted into inorganic forms, but remain bound in organic forms such as amino acids and proteins. Thus the nitrogen (N) in boreal systems is distributed such that the majority exists in organic forms, with very little in inorganic forms. Unfortunately organic N can only be metabolized by certain plant species (see Chapter 1), so despite the abundant N stored in the organic pool, it is largely inaccessible. Plant species that can only access inorganic N are limited to sharing the small pool of inorganic nitrogen. Thus, the slow turnover of nutrients from dead tissues is responsible for widespread nutrient deficiency throughout the boreal forest.

1.2. Litter Quality and Decomposition Rates

Biotic factors, including litter quality, also affect decomposition rates. The ability and efficiency of microbes to metabolize tissues is affected by the physical and chemical nature of their substrate. Previous research has pointed to various chemical constituents as important components of litter quality, including N, lignin, cellulose and secondary compounds (Facelli and Pickett 1991, Cornelissen 1996). Of the various leaf

quality parameters N:lignin is considered the most important litter quality factor governing decomposition rates by some researchers (Melillo et al. 1982, Taylor et al. 1989, Scott and Binkley 1997). The two components of this ratio have opposite effects on decomposition. The availability of N partially controls the growth of microorganisms and shows a positive correlation with the rate of decomposition. Lignin shows the opposite effect. Most organisms, with the exception of saprophytic species, do not metabolize lignin, due to its highly stable structure. Tissues with a greater proportion of lignin are more difficult to digest, and so lignin is negatively correlated with the rate of decomposition.

In natural systems, the litter layer generally contains both high and low quality substrates from different species, and the microorganisms acting on the various components interact with each other (Gartner and Cardon 2004). It has been postulated that the decomposition of low quality litter could be promoted through an association with high quality litter, which may provide a N-rich substrate for decomposer organisms (Taylor et al. 1989). The presumed mechanism behind this interaction is that the high-quality tissue provides a nutrient rich substrate for decomposer organisms, creating a more favorable decomposition environment around the low-quality litter. Thus the organisms breaking down the low-quality litter are able to benefit from the nutrients released from the decomposition of the deciduous litter. If the decay rates of recalcitrant species could be accelerated through the association with high quality litter, the rate at which nutrients become available for plant uptake should increase.

1.3. Alder Tissue in the Decomposition Substrate

Alders produce rapidly decomposing, high quality litter that may be able to increase the decomposition rates of associated litter species (Fyles and McGill 1987, Fyles and Fyles 1993, Clein and Schimel 1995, Pérez-Corona et al. 2006). These studies found that alder litter decomposes faster than the comparison species and that it can also accelerate the decomposition of the other species when included in the decomposition environment. Comparison tissues were often highly recalcitrant, such as *Populus tremuloides* (trembling aspen) leaves (Taylor et al. 1989) and *Pseudotsuga menziesii* (Douglas fir) needles (Prescott et al. 2000a, Prescott et al. 2000b).

Comparing alder to conifers seems somewhat trivial. We know that evergreen needles are much more resistant to decay than deciduous leaves. Deciduous species shed their leaf tissue on a yearly basis and so invest less energy into the protective features for their leaf tissue (Chabot and Hicks 1982). Conversely, evergreen species retain their leaf tissue for prolonged periods of time. These species must protect their tissues from herbivores and microorganisms and have developed various structures and phytochemicals to maintain and protect their tissues. One of the most important structural elements that protects evergreen tissues is a very thick waxy cuticle, which acts as an effective barrier to both water loss and to invasion by microorganisms (Facelli and Pickett 1991, Cornelissen 1996). Although deciduous species also produce a cuticle, it is much thinner. Additionally, evergreen species invest a large amount of energy into the production of secondary compounds that discourage herbivory and inhibit decomposition. Coniferous leaves contain a large amount of resins, which have antimicrobial properties and inhibit protein digestion (Bryant and Kuropat 1980). Conifer

needles also contain a high proportion of lignin, tannins and resins, which significantly decrease palatability to both large herbivores as well as microorganisms (Bryant and Kuropat 1980, Prescott et al. 2000b).

To date, there are very few studies comparing alder to other high-quality litter species. Although it is certainly useful to have investigated the effects of alder on low-quality tissues, it would also be useful to pair alder with high-quality litter species. *Alnus glutinosa* Gaert. (black alder) was found to have considerably slower decomposition than *Fraxinus angustifolia* (ash) in a riparian forest in the Mediterranean (Pérez-Corona et al. 2006).

The question of whether alder litter will interact with extremely recalcitrant litter seems much more trivial than the question of whether alder litter will interact with other highly decomposable litters. The question then focuses on why would we expect alder to show faster decomposition than other high quality litter species. When considering decomposition processes, the distinguishing characteristics of alder tissue are that it is (a) deciduous litter from (b) a shrub or tree that (c) potentially derives a portion of its component N from N fixed from the atmospheric N_2 pool by its endosymbiont – *Frankia* – a N-fixing actinomycete. None of these characteristics taken separately is unique to alder; there are many species that belong to one or two categories, but there are far fewer species, if any, with all three characteristics.

The first characteristic – the production of deciduous tissues - certainly seems to be a relevant factor when considering decomposition rates. It has been previously shown that deciduous tissues decompose at a faster rate than evergreen tissues (Prescott et al. 2000a, Prescott et al. 2000b). The second characteristic – a shrub or

tree growth form - will largely affect the amount of litter produced, with herbaceous species producing very little leaf mass, shrubs producing an intermediate amount of leaf mass, and tree species producing the most leaf mass. The last characteristic – the source of tissue N – may or may not exert an effect of decomposition rates. The main difference between N derived from the inorganic N pool versus from the atmospheric N_2 pool is the relative proportions of heavy N (N^{15}) and light N (N^{14}). Fixed N sometimes has a smaller proportion of heavy N (N^{15}) than inorganic N forms (Boddey et al. 2000), but it is unclear whether this should have any bearing on the decomposition rate. If decomposers prefer tissues with a low N^{15}:N^{14}, then alder tissue should have a greater decomposition rate.

1.4. Litterbag Studies

Since their first use in 1957 by Bocock and Gilbert, litterbag experiments have become the standard methodology in the study of litter decomposition (Prescott 2005). Litterbag studies conducted both in the field (Sharma and Ambasht 1987, Taylor et al. 1989, Trofymow et al. 1995, Prescott et al. 2000a, Prescott et al. 2000b, Pérez-Corona et al. 2006) and in the lab (Daubenmire and Prusso 1963) have used pure and mixed species litterbags to determine whether interactions between litter species affect decomposition rates. Their findings have been inconsistent (Taylor et al. 1989, Nilsson et al. 1999, Prescott et al. 2000a, Prescott et al. 2000b, Wardle et al. 2003, Prescott 2005). In a review paper by Hättenschwiler et al. (2005), they estimated that 50% of mixing effects were synergistic (i.e., total decomposition is greater than the sum of its

parts), 30% were additive (null) and 20% of the interactions were antagonistic (i.e. total decomposotion is less than the sum of its parts), suggesting that litter-mixing effects are largely idiosyncratic. The inconsistent results obtained among previous studies leave us with many unanswered questions about the interaction between high and low quality litters.

One of the most significant limitations of litterbag experiments is their extension to natural systems. In the majority of studies, species mixtures were kept very simple, generally restricted to pairwise species combinations. This type of design limits the applicability of the results, since a two species situation is unlikely to occur naturally (Wardle et al. 2003, Hättenschwiler et al. 2005). More recently some researchers have started to explore more complex species mixtures. Zhang et al. (2008) created artificial mixes by combining equal portions of litter from 10 species, and then incubated litterbags in 21 sites across Canada to compare 5 decomposition models. Hector et al. (2000) filled litterbags with mixtures of up to 11 species, with the relative contribution of each species reflecting its natural proportion in a living assemblage. Although their mixtures reflected artificially revegetated plots, their methodology can certainly be applied to natural communities. By doing so, we can compare the differences in decomposition of natural species assemblages in order to look at the decomposition dynamics for an entire plant community.

1.5. Research Focus

We know that alders can condition their soils and lessen nutrient deficiency by shunting N from atmospheric sources to the soil pool, and by adding high quality litter to the litter layer and affecting the decomposition process. But despite the research that has been conducted in the past, we are still left with the question of whether alder tissue increases the decomposition rates of other species, especially relative to other high-quality deciduous species. I addressed this question using two separate experiments. The first experiment was conducted in a growth chamber, using known quantities of different litter types. This experiment was designed to compare alder litter to three other species, as well as to compare litter mixes with alder to litter mixes without alder. The second experiment was conducted in the field, using litter collected directly from the forest floor as well as a standardized substrate (birchwood chopsticks). This study was designed to compare the decomposition rates of these substrates when incubated on alder-conditioned soils to soils that had not been conditioned by green alder.

The double design that I have employed in this study, which integrates both field and lab components, is intended to mitigate the drawbacks of both field and lab experiments. Field experiments have the benefit of capturing a more realistic view of the process as it would occur under natural conditions. The major drawback of field experiments is that it is impossible to account for the complex effects of dynamic environmental conditions in the analysis. Natural systems are completely uncontrolled and demonic intrusions cannot be fully accounted for. Laboratory experiments have the benefit of control; the experimenter can regulate and document all of the conditions in the system. Unfortunately these conditions do not accurately represent those that would

exist in nature and thus lab experiments may not adequately describe processes under natural conditions. By incorporating both field and lab components, I can truth the results of the controlled lab experiment with the results of the more realistic field experiment, and vice versa.

2. Methods

2.1. Decomposition of Complex, Natural Assemblages in the Field

In August 2007, litterbags and chopsticks were deployed in the field in conjunction with the sampling outlined in Chapter 1. Litterbags and chopsticks were indubater under either green alder (*Alnus viridis* ssp. *crispa*) or beaked hazel (*Corylus cornuta*) with 30 litterbags and 30 pairs of chopsticks in each treatment for a total of 60 litterbags and 60 pairs of chopsticks at each site. Litterbags were filled with fresh litter collected from beneath the alder and reference shrubs. The bags were constructed from black fiberglass window screening with a mesh size of 1.16 mm to form pockets measuring 10 cm x 15 cm. The mesh size was chosen to exclude large detritivores so as to more closely approximate the growth chamber experiment (see Sec. 2.2. Decomposition of Simple, Contrived Assemblages in the Lab). Litter was removed from an undisturbed 10 cm x 15 cm area from within the understorey plots at each green alder and beaked hazel from Chapter 1. Litter was collected down to the mineral soil layer in various states of fragmentation, from intact to small fragments. Large woody tissue that would not fit in the litterbags was removed, and the rest of the litter was placed into the litterbag. The litterbags were then sewn shut, weighed and incubated in the place that the litter had been collected. The litterbags were not buried, as they were intended to measure surface decomposition within the litter layer. Additional litter samples were collected concurrently, dried in a 60°C oven for 1 week and weighed to determine dry weight equivalents (DWE) for the incubated litter samples.

Birchwood chopsticks were incubated in pairs along either side of their corresponding litterbag. The inclusion of a standardized substrate allows for the

separation of the effects of incubation environment (i.e., alder or reference soils) and the decomposition substrate. The litterbags are affected by both the incubation environment and the decomposition substrate (i.e., the composition of the litter community in each litterbag), but the chopsticks are only affected by the incubation environment.

I intended to allow the litterbags and chopsticks to incubate for a full year before collecting them in August 2008. Two months before the scheduled collection date, all of the samples incubated at the Sandilands were lost to a forest fire. On May 24, 2008 a ground fire destroyed 33 km^2 of forest in the Sandilands, including the site used in this study. The fire was largely confined to the ground and devastated the understorey community. The litterbags and chopsticks were all affected and in some cases were completely consumed. The damage was far too severe to salvage any information from these samples and they had to be removed from the analysis.

Litterbags and chopsticks were collected from Star Lake in August 2008, approximately one year after incubation. Some of the samples could not be relocated and were presumably removed by animals. There was evidence of animal interference on many of the recovered litterbags, including shredding and mauling and, in one case, colonization by ants. Most of the animal damage was superficial and only the colonized litterbag was affected severely enough to warrant exclusion from analysis. After the litterbags were harvested and returned to the lab, the tissue remaining in the litterbags was removed and dried in a 60°C oven for 1 week. The dried litter was then weighed to determine mass loss and the annual surface decomposition rate.

2.2. Decomposition of Simple, Contrived Assemblages in the Lab

In September 2007, I collected fresh leaves from four boreal species to be incubated in the lab in single species (pure) and multiple species (mixed) litterbags. The pure bags are used to determine the decomposition rate for each individual species, which can then be used to predict the rates for the mixes, based on the abundance of each species in the litter mix (Gartner and Cardon 2004). If the predicted rates describe the rate for the mixed bags well, then the rate in the mixed bags are additive and there is no net effect of mixing litter species on decomposition rates. If there is an effect of litter mixing, the rate in the mixed bags should be non-additive. If the predicted rates underestimate the rate in the mixed bags, mixing had a synergistic effect of decomposition. If the predicted rates overestimate the rate in the mixed bags, mixing had an antagonistic effect on decomposition.

I used two recipes for the mixed litterbags – one with and one without *A. crispa*. If alder tissue has the ability to enhance the decomposition of associated litter species, then litterbag mixes that include *A. crispa* should decompose faster than those without alder. The design also incorporated species with different decomposition rates in order to elucidate any synergistic or antagonistic effects in the litter mixes, relative to the pure bags.

2.2.1. Species Selection

The species were selected on the basis of expected decomposition rate as well as their presence in the study communities in Chapter 1. I chose species from these communities to allow for a better comparison with the field experiment, which used litter

collected directly from the forest floor. The natural litter assemblages in the field-deployed litterbags would likely have contained contributions from these species. Two of the four species were expected to have high decomposition rates – *Alnus viridis* ssp. *crispa* (Ait.) Pursh (green alder) and *Rubus idaeus* L. var. *strigosus* (Michx.) Maxim. (red raspberry) - and two were expected to have low decomposition rates - *Cornus canadensis* L. (bunchberry) and *Pleurozium schreberi* (Brid.) Mitt. (big red stem feather moss).

Like many other alder species, *Alnus viridis* ssp. *crispa* has been shown to have a high decomposition rate (Fyles and McGill 1987). One of the objectives of the experiment was to determine whether alder showed a higher decomposition rate than other comparable deciduous tissue. I initially intended to use *Corylus cornuta* (beaked hazel) as the comparison species because of similar physiology and close taxonomic relationship with alder, as well as its use as the reference species in the study outlined in Chapter 1. Unfortunately *C. cornuta* senesces much earlier than *A. crispa* and at the time of collection (September 17, 2007) the hazel leaves were yellowing and beginning to senesce. Due to the low quality of the *C. cornuta* litter, *Rubus idaeus* was selected as the deciduous comparison species. Both *A. crispa* and *R. idaeus* are deciduous shrubs and are commonly found growing together in the alder plots at the study sites from Chapter 1 (80% frequency in alder plots at the Sandilands, 17% frequency in the alder plots at Star Lake). I expect *Rubus idaeus* to be highly decomposable like its blackberry relatives, *R. allegheniensis* and *R. argutus* (White et al. 1988). Although alders can incorporate fixed N in their tissues, the component N in raspberry tissue is derived solely from the soil inorganic-N pool. If alders have an especially high decomposition

rate above and beyond that of other deciduous shrubs, then tissue from *A. crispa* should show greater mass loss than tissue from *R. idaeus*. However if alder tissue is no different than tissue from other deciduous shrubs, the decomposition rate for *A. crispa* should be similar to or less than that of *R. idaeus*. I expect *Rubus idaeus* to be highly decomposable like its blackberry relatives, *R. allegheniensis* Porter and *R. argutus* Link (White et al. 1988).

I also included two recalcitrant species that represent two very different growth forms – wintergreen species and mosses – both of which are integral components of boreal forest communities. Natural litter assemblages from my forest communities would doubtlessly include contributions from both functional classes, and my mixtures were formulated to acknowledge this.

The representative wintergreen species is *Cornus canadensis* (bunchberry), a low growing (~10cm tall) shrub species from the dogwood family (Cornaceae). Although there do not appear to be any studies of the decomposition of this species to date, I expect *C. canadensis* leaves to be highly recalcitrant given the tough and leathery texture of the leaves, and their heavily sclerified vascular tissue.

The representative moss species is *Pleurozium schreberi* (big red stem feather moss) – a very common moss in boreal forest systems and the most common moss species in my study areas (see Chapter 1). This species is found to have a very slow decomposition rate (Fyles and McGill 1987, Hättenschwiler et al. 2006, Nilsson et al. 1999, Turetsky 2003).

2.2.2. Decomposition Period

I chose to limit the experiment to the first 12 weeks of decomposition because the most rapid mass loss occurs at the beginning of decomposition. Minderman (1968) recognized three phases of decomposition – a lag phase, followed by rapid mass loss and finally slow mass loss (Minderman 1968). The lag phase is generally short, but low temperatures can lengthen the duration of this phase. In areas with long, cold winters such as the boreal forest, the lag phase may last from fall until the spring, especially for species such as alders that drop their leaves after the first snowfall. The timing and duration of the rapid mass loss phase is influenced by multiple factors including environmental factors, such as moisture and light levels, as well as edaphic factors, including litter characteristics and fragmentation (Taylor 1998). During this phase the highly decomposable compounds are metabolized, leaving behind the recalcitrant tissues. The final phase is generally the longest phase, and is characterized by the gradual breakdown of recalcitrant compounds such as lignin and cellulose.

Coûteaux et al. (1995) only recognized two phases in their model of decomposition in Scots pine (*Pinus silvestris)* needle litter - an early phase (phase I) in which mass loss is high and a late phase (phase II) in which mass loss slows. Their model predicts that decomposition in the early phase will be increased by rate enhancing components (N, P, S) in the litter and will result in the loss of soluble components and non-lignified carbohydrates. Decomposition in the late phase is inhibited by rate-retarding components (lignin) and results in the loss of lignified carbohydrates and lignin. The retarding effect of lignin on decomposition is modified by climate, with less of an effect in harsh environments (e.g. Arctic) and more of an effect

in warm, moist environments. High concentrations of N also inhibit decomposition during the late phase. These phases were also observed in a study of the decomposition of *Alnus glutinosa* (L.) Gaertn (black alder)*, Populus x hybrida* (poplar hybrid)*, Fraxinus angustifolia* Vahl. (narrow leaved ash) in a Mediterranean riverine system (Pérez-Corona et al. 2006).

2.2.3. Experimental Protocols

Standard black plastic seed trays were each filled with 2.5 L of LECA (Light Expanded Clay Aggregate) beads. Litterbags were constructed from black fiberglass window screening with a mesh size of 1.16 mm. The litterbags were fastened together using hot glue to form pockets measuring 10 cm x 15 cm.

Fresh leaf litter was collected from the Sandilands in September 2007. Healthy, intact leaves were collected directly from the parent plant, bagged and stored in a refrigerator for 24 hours. A 10.0 g (fresh weight) subsample of each litter species was removed and dried in an 80°C oven for 7 days and used to determine the dry weight equivalents (DWE) of the incubated fresh litter. A total of 6.0 g of fresh litter was added to the bottom 10 cm of the litterbags according to the following treatment schedule (Table 2.1).

Table 2.1. Fresh weights (g) of each of 4 species added to litterbags in a decomposition experiment with 6 treatments (A: pure *Alnus viridis ssp. crispa*, P: pure *Pleurozium schreberi*, R: pure *Rubus idaeus*, C: pure *Cornus canadensis*, M: mix without alder, MA: mix with alder).

	Litterbag treatment					
Species	A	P	R	C	M	MA
Alnus viridis ssp. crispa	6.0	0.0	0.0	0.0	0.0	1.5
Pleurozium schreberi	0.0	6.0	0.0	0.0	2.0	1.5
Rubus idaeus	0.0	0.0	6.0	0.0	2.0	1.5
Cornus canadensis	0.0	0.0	0.0	6.0	2.0	1.5

The filled litterbags were arranged on top of the LECA, with a single tray housing all six litterbags for one replicate. Each treatment was replicated 5 times, for a total of 180 litterbags (6 bags per replicate x 5 replicates per treatment x 6 treatments). Distilled water (1 L) was poured over the filled litterbags and the trays were sealed with Glad Press N' Seal. Reservoir levels were checked and refilled as needed to maintain a constant depth. Trays were stacked on seed plug racks in a dark growth chamber at a constant temperature of 15°C for the duration of the experiment.

Litterbags were randomly selected for removal from each tray after 1, 2, 3, 5, 8 and 12 weeks. Harvested litter was transferred from the litterbag to a paper bag and dried in a 60°C oven for 7 days. Once dry, the leaves were weighed to the nearest 0.01 g. Due to unexpected complications, the moss had to be removed at all sampling periods (see The *Pleurozium* Problem). The remainder of the litter was weighed as a mix and where possible, as each separate species component. Unique physiological features of certain species facilitated the identification and separation of species in the decomposing mixes (see Discussion). Unfortunately by the 8th week, the litter was too highly decomposed to be separated with any reasonable semblance of accuracy and weights were only possible for the mixes as a group from this sampling period forward.

3. Analysis

3.1. Field Incubation

The experiment was designed to use paired, 2-sided T-tests to compare the decomposition rates in paired alder and reference plots. Unfortunately there were large losses of samples, presumably due to animal interference. At the time of incubation, 30 litterbags were incubated for each of the four treatments (Sandilands alder, Sandilands reference, Star Lake alder, Star Lake reference) for a total of 180 litterbags. All 60 of the litterbags at the Sandilands were lost in the forest fire, and by the harvest almost 20% of the samples at Star Lake had been lost as well (Table 2.2).

Table 2.2. Sample size (n) remaining after one year for decomposition experiment.

	Litterbags	Chopsticks
Alder	23	25
Reference	24	25
Intact pairs	20	22

Often only one sample from an alder-hazel sample pair was recovered – i.e., the alder sample was recovered but the reference sample could not be located, or vice versa – making the use of paired T-tests inappropriate for unmatched samples. I designed the experiment to facilitate paired tests because they have greater analytical power than unpaired tests, and it seemed unwise to completely abandon the paired design. Instead the analytical methodology was modified to test both the remaining intact pairs with paired, 2-sided T-tests in conjunction with unpaired, 2-sided T-tests on

the entire group of samples, including matched and unmatched samples. Both the data from the matched pairs and the unmatched samples will be presented in the analysis.

3.2. Growth Chamber Incubation

The individual mass remaining (%MR) values were averaged for each of the 5 remaining treatments. The pure bag treatments were compared to each other using fixed effects ANOVA. The observed %MR values from the pure bags were used to calculate predicted %MR for the mixed bags. The predicted values were determined by calculating the average %MR from the litter components in their pure bag treatments (Formulas 2.1 and 2.2). The predicted values were compared to the observed values using z-tests. I conducted a post-hoc power analysis using G*Power 3.0.10 (Faul et al. 2007).

Formula 2.1: predicted $\%MR_{(M)} = (\%MR_{\textit{Rubus}} + \%MR_{\textit{Corylus}})/2$

Formula 2.2: predicted $\%MR_{(MA)} = (\%MR_{\textit{Alnus}} + \%MR_{\textit{Rubus}} + \%MR_{\textit{Corylus}})/3$

4. Results

4.1. Field Incubation

There was comparable mass loss from the litterbags and the chopsticks in the two treatments (Fig 2.1). The birchwood chopsticks lost ~75% less mass than the litterbags, which contained a mixture of woody and leafy litter. There was no significant difference in mass loss between treatments for the chopsticks or the litterbags according to both the matched and the unmatched data, even after the exclusion of outliers. There was no correlation between the mass loss from chopsticks and litterbags at the plot level (Fig 2.2), nor was there a correlation between the level of soil nitrogen and decomposition in the plots (Fig 2.3).

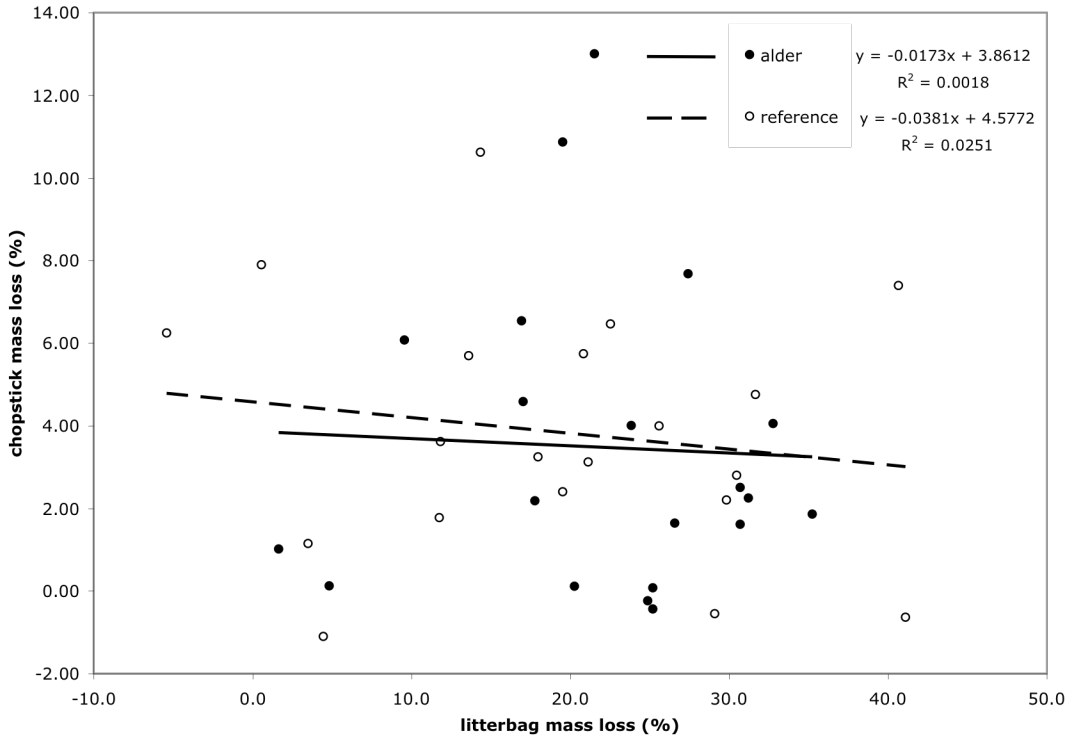


Fig 2.2. Correlation between mass loss (%) from litterbags containing natural species assemblages and from birchwood chopsticks after one year of decomposition when incubated in plots under green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) and non-fixing reference shrubs (beaked hazel: *Corylus cornuta* Marsh.) in a boreal jack pine forest near Star Lake in Southeastern Manitoba.

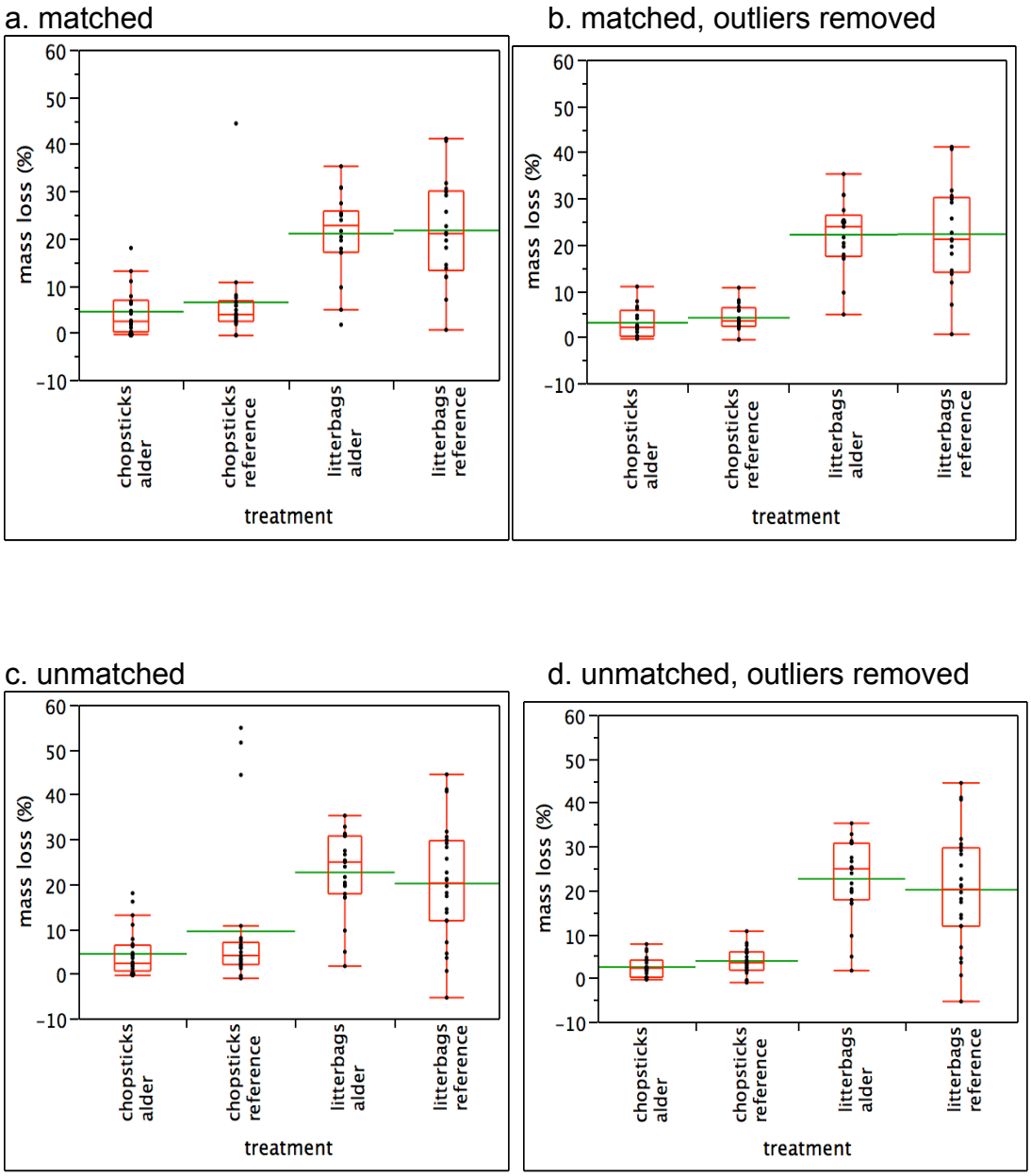


Fig 2.1. Mass loss (%) from litterbags containing natural species assemblages and from birchwood chopsticks after one year of decomposition when incubated under green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) and non-fixing reference shrubs (beaked hazel: *Corylus cornuta* Marsh.) in a boreal jack pine forest at Star Lake in Southeastern Manitoba. (a) and (c) include all data points, while (b) and (d) exclude points identified as outliers by JMP. Bars indicate treatment means. Whiskers indicate 95% confidence intervals, boxes indicate the median as well as the upper and lower quartiles.

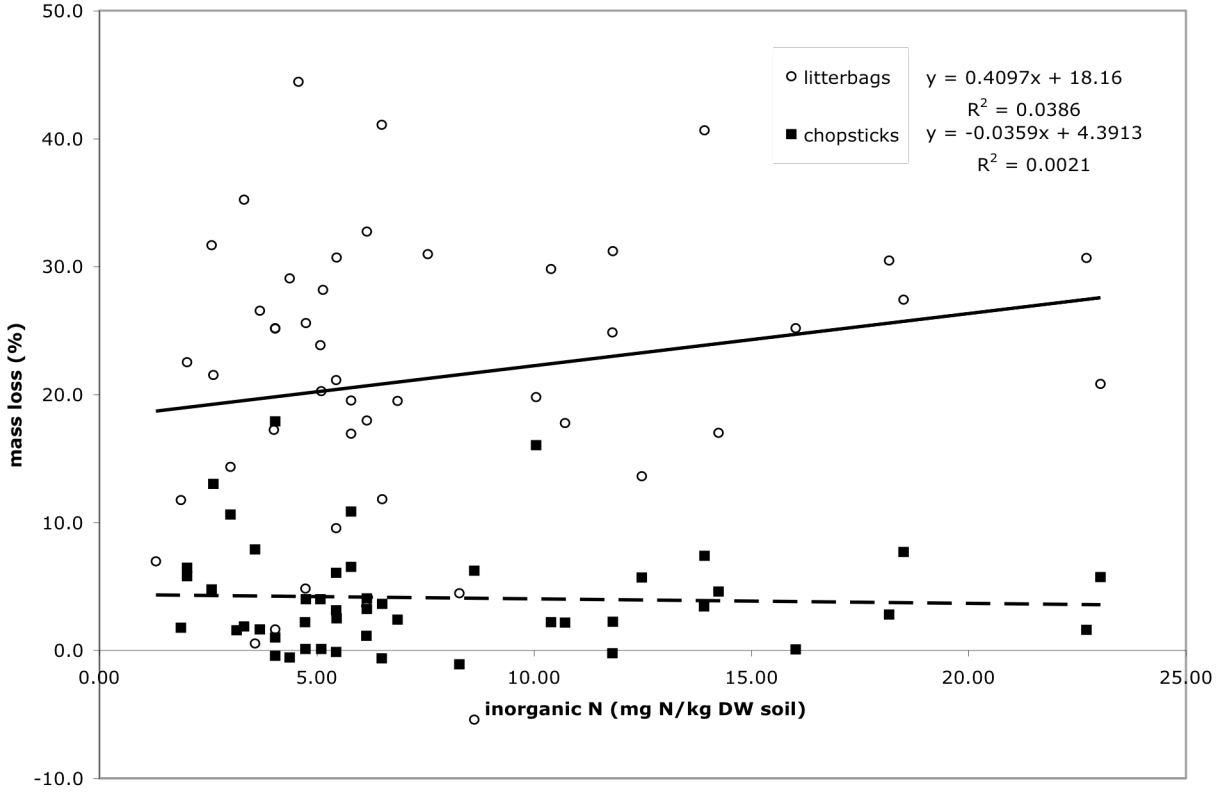


Fig 2.3. Mass loss (%) from litterbags containing natural species assemblages and from birchwood chopsticks after one year of decomposition when incubated under green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) and non-fixing reference shrubs (beaked hazel: *Corylus cornuta* Marsh.) in a boreal jack pine forest at Star Lake in Southeastern Manitoba as predicted by the level of inorganic N in the plots.

4.2. Growth Chamber Incubations

There were significant differences between treatments in the mass remaining in the lab-incubated litterbags at each of the sampling periods (Table 2.3). The pure treatment with the highest %MR at all sampling periods was the *Cornus canadensis* bags (C), and the treatment with the lowest %MR was *Rubus idaeus* (R). *Alnus viridis* ssp. *crispa* (A) showed an intermediate decomposition rate, less than *R. idaeus* and greater than *C. canadensis*.

Table 2.3. Mass remaining (%) in pure and mixed (with and without green alder: *Alnus viridis* ssp. *crispa* (Ait.) Pursh) species litterbags during the first 12 weeks of decomposition. Litterbags were incubated in a 15°C dark growth chamber and harvested randomly after 1, 2, 3, 5, 8 and 12 weeks. n = 5 for all treatments, except where superscript indicates a reduced number of samples. P-values compare treatment means via fixed effects ANOVA (S-plus). Bolded values indicate a significant difference at α=0.05.

	Weeks after incubation					
Species/mix	1	2	3	5	8	12
Alnus viridis ssp. crispa	96.0	83.0	78.6	71.5	59.5	52.4
Rubus idaeus	95.4	78.9	73.2	64.5	51.2	43.7[4]
Cornus canadensis	99.9	92.2	92.5	86.1	65.9	55.5
Mix without alder	86.7[1]	84.5	80.7	71.8	57.5	45.3
Rubus idaeus	-	-	70.7	63.9	-	-
Cornus canadensis	-	-	93.9	91.1	-	-
Mix with alder	91.8[1]	85.5	80.1	71.4[4]	60.1	49.8
Alnus viridis ssp. crispa	-	-	76.3	68.9[2]	-	-
Rubus idaeus	-	-	74.5	73.0[3]	-	-
Cornus canadensis	-	-	90.0	80.2[3]	55.0[1]	-
p-value	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

In the first week, *A. crispa* showed a comparable mass loss to both *R. idaeus* and *C. canadensis*, although *R. idaeus* lost significantly more mass than *C. canadensis* (4.6% R v 0.1% C, p=0.005 from pairwise T-test) (Fig 2.4). Between weeks 2 and 8, *R. idaeus* showed significantly faster decomposition than both *A. crispa* and *C. canadensis* (p<0.027 from pairwise T-test), and *A. crispa* showed significantly faster decomposition than *C. canadensis* (p<0.020 from pairwise T-test). By week 12, *R. idaeus* still showed the greatest rate of decomposition (p<0.009 from pairwise T-test), but the difference in mass loss between *A. crispa* and *C. canadensis* was not significantly different (p=0.215 from pairwise T-test).

There was only one sample for each mix at the first sampling period due to problems associated with the inclusion of *Pleurozium schreberi* in the mixes (see The *Pleurozium* Problem). The moss tissue had to be removed from the mixes, and mass

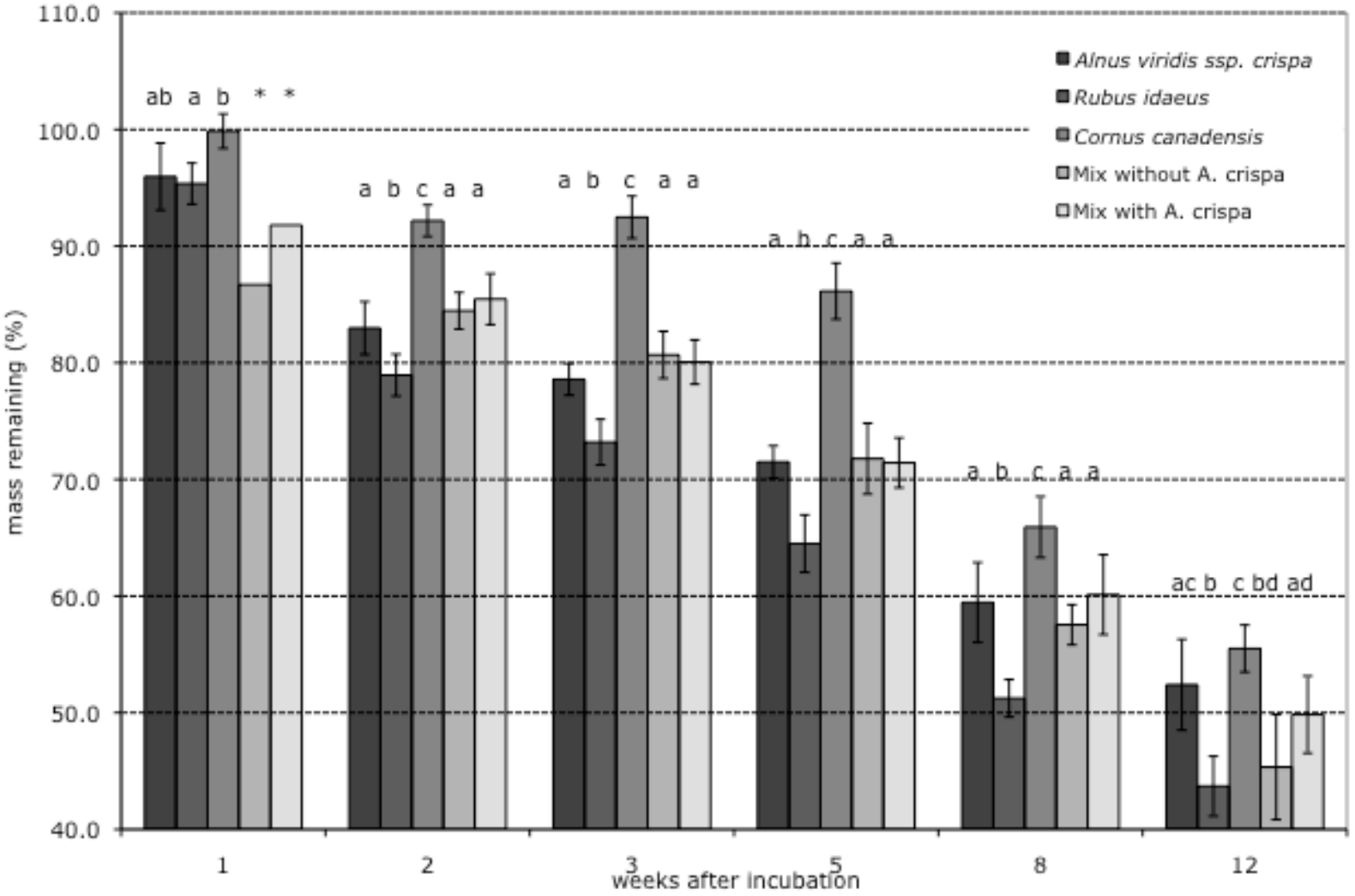


Fig 2.4. Mass remaining (%) in litterbags incubated in a 15C dark growth chamber during the first 12 weeks of decomposition. Letters indicate significant differences (a=0.05). Asterisks (*) indicate values derived from a single observation. Error bars indicate 95% confidence intervals.

loss was determined for the remaining litter components. Consequently the mix with alder had three species (*A. crispa*, *C. canadensis* and *R. idaeus*) and the mix without alder had only two species (*C. canadensis* and *R. idaeus*). Throughout the experiment, there was no significant difference between the mass remaining in the two mixes.

Values for individual species within the mixes were available for the full complement of species (n=3) at weeks 3 and 5. At week 5, the *A. crispa* tissue had decomposed more rapidly in the mixed bags than in the pure bags, but the relationship failed to reach a standard level of significance (78.6% MR pure v 76.3% MR mixed, p = 0.08). At the same sampling period (week 5), the *R. idaeus* tissue showed the highest mass loss in the mix without alder (63.9% MR), higher than both the pure bag (64.5% MR R, p=0.06) and the mix with alder (73.0% MR A, p=0.04). After 5 weeks, the *C. canadensis* tissue in the mixed litter treatments had ~6% less mass remaining in the mixed bags (M and MA) than in the pure bag, but the result was more significant for the M treatment (86.1% MR pure v 80.0% MR M, p=0.08) than for the MA treatment (86.1% MR pure v 80.2% MR MA, p=0.26). Although the mean MR for the M and MA treatments was very similar (80.0% MR MA v 80.2% MR M), there was a great deal of difference in the p-values (p=0.26 MA v p=0.08 M). This discrepancy may be due to the smaller number of samples in the MA treatment (n = 3) than in the M treatment (n = 5).

There were no significant differences between the predicted %MR values and the observed MR values for either of the mixed litterbag treatments (M, MA) at any of the harvest periods (Table 2.4).

Table 2.4. Mass remaining (%) in mixed (with and without green alder: *Alnus viridis* ssp. *crispa* (Ait.) Pursh) species litterbags during the first 12 weeks of decomposition. Litterbags were incubated in a 15°C dark growth chamber and harvested randomly after 1, 2, 3, 5, 8 and 12 weeks. Predicted values were determined by averaging the pure bag treatments. P-values determined using a z-test (ANOVA). A dash (-) indicates that the p-value could not be calculated due to insufficient sample size. n=5 for all treatments, except where superscript indicates number of samples.

	Weeks after incubation					
Species/mix	1	2	3	5	8	12
Mix without alder						
predicted	97.7	85.6	82.9	75.3	58.6	49.6
observed	86.7[1]	84.5	80.7	71.8	57.5	45.3
p-value	-	0.92	0.98	0.99	0.88	0.97
Mix with alder						
predicted	97.1	84.7	81.4	74.0	58.9	50.5
observed	91.8[1]	85.5	80.1	71.4[4]	60.1	49.8
p-value	-	0.25	0.92	0.98	0.24	0.66

5. Discussion

5.1. Field Incubations

Unfortunately due to the losses from the forest fire, only half of the intended data set is available from the field experiment. The data that was obtained does not suggest that the mixes incubated underneath and containing litter from *A. crispa* have higher decomposition rates than mixes that do not contain *A. crispa* litter and are incubated underneath a non N-fixing shrub species. This observation is in agreement with Perez-Corona et al. (2006), who found that alders had an intermediate decomposition rate when they incubated monospecific litterbags under black alder (*Alnus glutinosa*), poplar (*Populus x hybrida*) and ash (*Fraxinus angustifolia*).

There was an indication that further sampling may reveal that decomposition was slower underneath *A. crispa*. The chopsticks (unmatched group) lost half as much mass when incubated underneath *A. crispa* than when incubated under *Corylus cornuta* at Star Lake. Although this trend is not statistically significant, the failure of this trend to reach statistical significance may be an artifact of inadequate sampling. Post hoc power analysis showed that the power of the test was low (power=0.32), most likely due to the extremely high standard deviation for the reference treatment (15.7). This is one of the drawbacks to field experiments; heterogenous conditions produce high degrees of variation. There is much less variation in the growth chamber study, in part due to the carefully controlled environmental conditions.

There were environmental differences between the alder and hazel plots at Star Lake that may have influenced the decomposition rates. Decomposer organisms, like all life forms, require N for the production of amino acids and proteins. In theory, if there is

a greater availability of inorganic N under alders, it should provide nutrients for more decomposer organisms and thus favor decomposition in the *A. crispa* plots. I found no difference in N between treatments and the unmatched chopsticks, indicating that decomposition may actually be favored in the *C. cornuta* plots. The less favourable N conditions under *C. cornuta* may have been overridden by more favourable light conditions in these plots. The hazel plots had lower canopy openness and correspondingly receive less sunlight. The shadier conditions may constrain evaporative water loss, creating more favorable moisture conditions for saprobes, since decomposition is positively correlated to soil moisture up to its saturation point (Walse et al. 1998).

There were no significant differences (α=0.10) observed between the alder and hazel treatments in any of the parameters measured in the field experiment. This suggests that *A. crispa* litter does not increase the decomposition of recalcitrant litter more than litter from beaked hazel - another deciduous shrub species.

5.2. Growth Chamber Incubations

5.2.1. Quantitative Data

Green alder did not show the fastest decomposition rate of the three species – green alder (*Alnus viridus* ssp. *crispa*), bunchberry (*Cornus canadensis*) and red raspberry (*Rubus idaeus*). The mass loss from *A. crispa* was greater than *C. canadensis* at all but the last harvest (12 weeks) at which point the mass loss from the two treatments was indistinguishable. A plot of the decomposition constant (k) over the

course of the experiment shows that the decomposition rates of both species decreased, but the decline in k was greater for *C. canadensis* (Fig 2.5). The rates likely slowed after the soluble and easily metabolized components of the tissue were gone, leaving the more resistant components (White et al. 1988). Alder leaves can contain a high proportion of resistant components such as lignin, despite their generally high quality and high proportion of N. Taylor et al. (1989) found that *A. crispa* leaves from a 40-year-old trembling aspen stand in the Front Range of the Rocky Mountains west of Calgary had a higher proportion of lignin (17.4%) than *Populus tremuloides* (10.5%) leaves.

Fyles and Fyles (1993) found that leaves from *Alnus rubra* Bong. (red alder) decomposed faster than *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) needles and *Gaultheria shallon* Pursh (salal) leaves. Litter from *A. rubra*, *P. menziesii* and *G. shallon* were mixed in various combinations and incubated in a dark environment at 30°C. Mass loss was highest in the *A. rubra* which lost ~28% mass after 10 weeks and ~34% after 20 weeks. These values are much lower than the 48% mass loss from my *A. crispa* bags after 12 weeks. This is especially surprising considering that Fyles and Fyles (1993) used ground tissues. In general, fragmentation should increase decomposition by increasing the surface area that can be attacked by decomposer organisms (Gartner and Cardon 2004). However the greater mass loss from *A. crispa* in my experiment can be explained by differences in the experimental protocols between my experiment and that of Fyles and Fyles (1993). Firstly, their experiment used a different species and *A. crispa* may have a greater decomposition

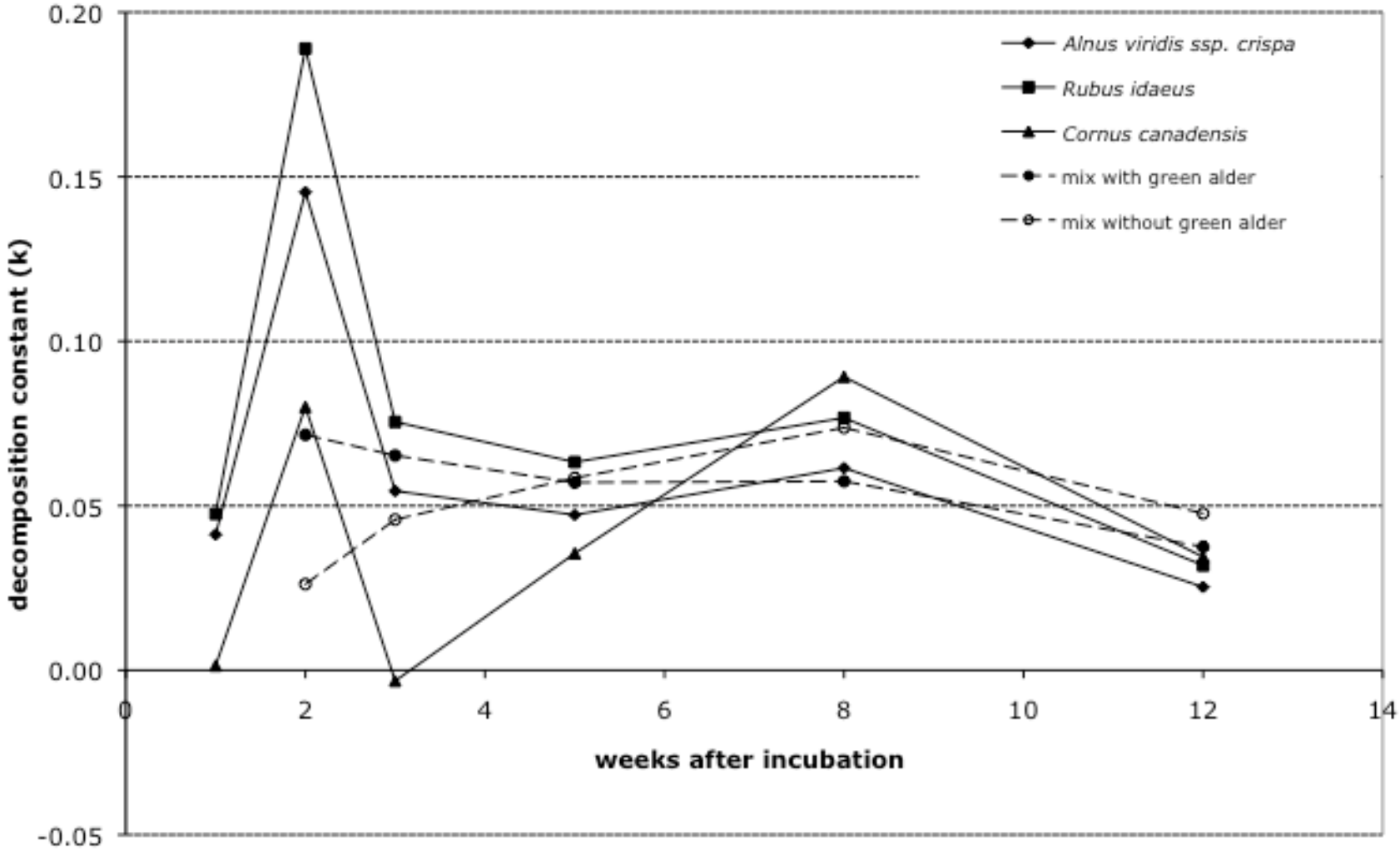


Fig 2.5. Decomposition constant (k) of pure and mixed species litternags incubated in a 15°C dark growth chamber during the first 12 weeks of decomposition. Mixes also contained tissue from *Pleurozium schreberi* (big red stem feather moss). This species was excluded in the measurements of mass remaining due to unforeseen complications. N_0 was determined from the previous harvest.

rate than *A. rubra*. A study designed to directly compare the decomposition rates of various species of alder could reveal whether *A. crispa* truly decomposes faster than *A. rubra* and other tree type alders. Also, despite the overall similarity, there were many differences between the experiments that make direct comparison unwise – including litter pretreatment (fresh and intact versus air-dried and ground), temperature (15°C versus 30°C) and litter mixes.

The presence of *A. crispa* in the mixed bags did not have a synergistic effect on the decomposition of associated litter. To the contrary, the presence of alder negatively affected decomposition of *R. idaeus*. At week 5, there was more raspberry mass remaining in the mix with alder than in the mix without alder and in the pure bag. There was also no statistically significant difference between the total mass remaining in the mix with alder and the mix without alder at any of the sampling periods ($p>0.16$), but the difference between the two treatments became more significant as decomposition proceeded and a difference between the treatments may have become apparent past the 12 week run time of this experiment, i.e., during the final slow mass loss stage.

When the decomposition rates from the pure bags were used to predict the decomposition rates in the mixed bags, the predicted values adequately described the observed values. There were no significant differences between the observed decomposition rates in the mixed bags from what would be expected if each species decomposes at the same rate as in its pure bag ($p>0.88$ mix without alder, $p>0.24$ mix with alder), showing that the decomposition rates in the mixed bags were additive. If *A. crispa* tissue had a synergistic effect, the observed mass loss in the mixed bags with *A. crispa* should be greater than the predicted mass loss. The lack of a difference between

the observed and predicted values suggests that *A. crispa* tissue did not affect the decomposition of associated litter in my litterbags.

The species that showed the fastest decomposition over the course of my experiment was *Rubus idaeus*. With the exception of the first week, *R. idaeus* consistently showed faster decomposition than *A. crispa* ($p \leq 0.01$). In fact, *R. idaeus* had the largest mass loss of any treatment at all but the last sampling period, at which point the mass remaining in the *R. idaeus* litterbags was not significantly different from the mix without alder (43.7% R v 45.3% M, p=0.56). These observations are in accord with the findings of White et al. (1988), who found that blackberry leaves (*R. allegheniensis* and *R. argutus*) had a higher decomposition rate than *Robinia pseudo-acacia* L. (Black locust), a N-fixing tree species.

As the most highly decomposable species in this experiment, *Rubus idaeus* may have a positive effect on the decomposition of associated litter. In week 5, *A. crispa* in the mixed bag (MA) had significantly less mass remaining than in its pure bag (68.9% MA v 71.5% A, p=0.08) and *C. canadensis* had less mass remaining in the mixed bag (M) than in its pure bag (80.0% M v 86.1 C, p=0.08).

5.2.2. Qualitative Observations

From the first harvest, certain physical changes to the litter suggested that decomposition rates were quite different among the species. At the first harvest (1 week), the *R. idaeus* tissue had undergone the largest amount of physical change. The leaves had turned bright red and yellow, and the small remaining patches of green had become much paler and took on a yellowish hue. The *R. idaeus* leaves lost all green

colouration by the second week. The adaxial (upper) surface turned completely black, in strong contrast to the white abaxial (lower) surface. The pronounced colour difference on the abaxial and adaxial surfaces made *R. idaeus* particularly easy to distinguish from the *A. crispa* leaves. In the third week, the pure *R. idaeus* litterbags showed the largest amount of fungal colonization and the majority of colonization in the mixed bags was associated with *R. idaeus* tissue.

The *A. crispa* leaves appeared to decompose more slowly than the *R. idaeus* in the early weeks of the study. They maintained their dark blue-green colour for almost 2 weeks before turning black. Although the *A. crispa* tissue seemed to decompose slowly at first, by the end of the 12 weeks the *A. crispa* litterbags appeared to have as much if not more fungal colonization than the *R. idaeus* litterbags. This may be evidence that the decay rate of *A. crispa* leaves would have increased in the later stages of decomposition, i.e., past the 12-week mark.

Cornus canadensis was the slowest species to decompose and remained physically unchanged the longest. It wasn't until the third week that *C. canadensis* started changing colours, and the leaves retained green tissue past the 8-week mark. *Cornus canadensis* was the last of the species to show visible colonization by fungi, with the pure litterbags showing the first outward signs of fungal colonization after 5 weeks of incubation – 3 weeks later than the tissues from *A. crispa* and *R. idaeus*.

5.3. Conclusions

There was no evidence to suggest that *A. cripsa* tissue increased the decomposition of associated litter. Litter mixes did not decompose faster when *A. cripsa* tissue was included in the mix or when associated with alder-conditioned soils. The alder tissue (*Alnus viridis* ssp. *crispa* leaves) decomposed faster than the wintergreen tissue (*Cornus canadensis* leaves), but did not decompose faster than the deciduous comparison tissue (*Rubus idaeus* leaves). The *A. crispa* tissue actually decomposed slower than the deciduous comparison tissue, showing that the *A. crispa* tissue does not decompose faster than tissue from similar species that do not obtain their component N from N-fixation.

5.4. Recommendations for Further Research

My original experimental design incorporated a maximum of four species in the litter mixes, but only provided information about three species after the removal of *Pleurozium schreberi*. Although it is an improvement on a pairwise design, it would be useful to extend the methodology to mixes with larger number of species. The experiment included two recalcitrant species (*Cornus canadensis* and *Pleurozium schreberi*), but only incorporated one highly decomposable tissue (*Rubus idaeus*) other than green alder (*Alnus viridis* ssp. *crispa*). Extending the mixes to include other high quality tissues would allow for comparison between the decomposition rates of alder and other species with high quality litter.

Beaked hazel (*Corylus cornuta*) would be an interesting comparison species in future mixes. It is very commonly found growing in the same areas as *A. crispa* and is taxonomically very similar. I had originally intended to include *C. cornuta* in the litter mixes in lieu of *R. idaeus*, but was unable to do so. *Corylus cornuta* senesces much earlier than *A. crispa* and at the time of litter collection the quality of the *C. cornuta* leaves had deteriorated to a point that it seemed unwise to include them in the study.

It would also be useful to run two time series concurrently. A more complete picture of the decomposition process could be obtained by matching a short-term set that investigates the rapid mass loss phase and a long-term set that investigates the slow mass loss phase.

The *Pleurozium* Problem - A commentary on the use of fresh litter for litterbag incubations

Abstract

Most litterbags studies have included some type of pre-treatment to the litter tissue, including drying and fragmentation, without concern for the effects these processes may have on the subsequent decomposition processes. The use of fresh litter would likely yield a view of decomposition that is more applicable to natural systems. A litterbag experiment was conducted using fresh litter from four boreal species – *Alnus viridis ssp. crispa* (Ait.) Pursh (green alder), *Rubus idaeus* L. var. *strigosus* (Michx.) Maxim. (red raspberry), *Cornus canadensis* L. (bunchberry) and *Pleurozium schreberi* (Brid.) Mitt. (big red stem feather moss). All of the species except *Pleurozium schreberi* proved to be suitable for fresh-tissue incubations. Samples of the leaves from each species were dried to determine dry weight equivalents (DWE) for the incubated fresh litter. The DWE calibration described the three angiosperm species very well, but was completely unsuitable for *Pleurozium schreberi*. The high degree of variation in the moisture content of the moss tissue prevented the DWE calibration from having any degree of accuracy. The moss tissue also did not die after transitioning to a dark growth chamber, and remained green and continued to create new shoots even after 12 weeks of incubation. Although the moss tissue was clearly not suitable for fresh litter incubations, the three angiosperm species behaved very well. I would recommend fresh litter as a viable alternative to air-dried litter in subsequent litterbag experiments, except in the case of bryophytes.

1. Introduction

Litterbag experiments have become very popular since their first use by Bocock and Gilbert in 1957 (Prescott 2005). At first glance the methodology may seem very straightforward - add litter to a mesh bag and measure mass loss after a predetermined amount of time. However a surprisingly large amount of variation exists in the methodology, especially with regards to the pretreatment of litter tissue.

Previous experiments seem to have incorporated litter treatments largely for convenience, without considering the effects on the subsequent decomposition processes or the ecological relevance. Most studies have included some form of drying, whether oven (Fyles and McGill 1987) or air-drying (Daubenmire and Prusso 1963, Melillo et al. 1982, Sharma and Ambasht 1987, Taylor et al. 1989, Fyles and Fyles 1993, Nilsson et al. 1999, Wardle et al. 2003, Pérez-Corona et al. 2006). Some studies have also imposed mechanical damage, such as fragmentation (Daubenmire and Prusso 1963) and grinding (Berg and Staaf 1981, Fyles and Fyles 1993).

Alterations to the chemical or physical structure of the leaf will undoubtedly affect decomposition. Taylor (1998) found that air-dried litter decomposed more slowly than fresh litter. He postulated that the loss of adsorbed water caused the complexation and precipitation of cell components, increasing their resistance to enzymatic attack (Taylor 1998 p. 411). Fyles and McGill (1987) found that the decomposition rate of pine needles doubled when they were cut into 1cm fragments before incubation. It follows that other litter treatments should also affect the rate of decomposition, whether positively or negatively.

If our goal is to understand decomposition as it occurs in nature, we must attempt to simulate the guiding factors regulating decomposition. In nature litter would senesce and reach the forest floor in a relatively fresh state. To make lab studies more closely approximate decomposition processes in nature, we should use fresh litter in decomposition experiments.

2. The Experiment and What Went Wrong

Litterbags were filled with fresh litter from four boreal forest species - *Alnus viridis ssp. crispa* (Ait.) Pursh (green alder), *Rubus idaeus* L. var. *strigosus* (Michx.) Maxim. (red raspberry), *Cornus canadensis* L. (bunchberry) and *Pleurozium schreberi* (Brid.) Mitt. (big red stem feather moss) – as described in the previous section (see Chapter 2, Section 2.2 for a detailed explanation of methodology).

Two problems arose over the course of this experiment, both of which stemmed from the inclusion of *Pleurozium schreberi*. The first problem arose when trying to calculate DWE values for incubated fresh tissue samples. It became apparent while filling the litterbags that although 6.0 g of *Alnus viridis* ssp. *crispa, Rubus idaeus* and *Cornus canadensis* leaves gave a consistent volume of tissue, the volume of 6.0 g of *Pleurozium schreberi* was highly variable. Although the moss had been kept in a sealed plastic bag for 24 hours to allow for an equilibriation of moisture, there were obviously wetter and drier patches of moss within the bag. Inconsistent moisture content of the litter causes the conversion of FW to DWE to give inaccurate estimates. Fresh weight

(FW) was underestimated for any samples that were drier than the DWE calibration sample, and overestimated for wetter samples.

Despite the initial observation that the volume of the moss tissue was inconsistent, I continued to run the experiment as designed in the hopes that this problem was not as severe as anticipated. Once the first batch of litterbags was harvested and analyzed, it was clear that there were definite problems with the DWE *for Pleurozium schreberi*. Some of the sample weights (DW) after 1 week of decomposition indicated that mass had almost doubled – an improbable result, if not impossible (Table 2.5). Results from subsequent sampling periods also showed chaotic results. The DWE were obviously inaccurate, and the *Pleurozium schreberi* tissue was removed from the analysis.

Table 2.5. Mass remaining (%) in pure *Pleurozium schreberi* litterbags incubated in a 15C dark growth chamber during the initial rapid mass loss phase of decomposition. Mass loss measurements were made 1, 2, 3 and 5 weeks after incubation.

	Weeks after incubation			
Replicate	1	2	3	5
1	118.0	70.0	135.8	56.3
2	99.7	94.0	90.4	100.7
3	170.5	139.4	140.2	153.2
4	78.3	78.1	107.4	166.1
5	130.9	155.4	123.0	110.8

The second problem arose due to the modular nature of mosses. The leaves from the angiosperms were removed from the parent plant before incubation. Since these species are unable to regenerate from a single detached fresh leaf, they could not maintain themselves in a fresh state after removal from the parent plant. In the case of

the moss however, the microphylls were not removed from the stems; I used the entire intact moss gametophyte for the incubations. Although moss gametophytes can regenerate easily, I had assumed that the moss would not survive the move to a dark growth chamber. Contrary to my expectations, the transition to the dark growth chamber had very little effect on the moss. From the first sampling period, it was noted that the moss remained bright green. Even after fungal colonization was observed in the second week, the moss retained its green colour. After 5 weeks in the dark growth chamber, new shoots emerged growing through the mesh of the litterbags. Surprisingly, the majority of the new growth was associated with the lower surface of the litterbags. The shoots continued to grow over the course of the experiment, showing new growth even after 12 weeks in total darkness. The largest of the emergent shoots reached almost 3cm in length by the end of the experiment. The shoots were very slender, less than 1.0mm in width, but had identifiable microphylls.

Fresh litter from the three angiosperms performed very well in the experiment. They produced very consistent estimates for dry weight equivalents, and all showed significant amounts of decay (~50% mass loss) after the 12 weeks of incubation.

3. Conclusions about Fresh Litter Incubations

This experiment suggests that leaf tissue from *Alnus viridis* ssp. *crispa*, *Cornus canadensis* and *Rubus idaeus* are all suitable for fresh litter incubations. I would expect that most other angiosperm species would behave similarly. Unfortunately *Pleurozium schreberi* showed itself to be entirely unsuitable for fresh litter incubations. The

inconsistent tissue moisture content would likely be a problem with other moss species as well, which renders them unsuitable for DWE calculations. Despite the problems associated with using dried litter for decomposition studies, it would seem to be the more suitable option for mosses. Drying could eliminate both of the problems observed in this experiment; it would eliminate the need for DWE calculations and may stop further growth of the moss gametophytes.

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Appendix

Table A1. GPS locations of plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests at the Sandilands and Star Lake in southeastern Manitoba. A dash (-) indicates a plot locations that is unavailable.

Sandilands alder		Sandilands reference		Star Lake alder		Star Lake reference	
plot	GPS location	plot	GPS location	plot	GPS location	plot	GPS location
03A	N49 24 06.2 W96 16 54.3	03R	-	33A	N49 44 45.3 W95 15 24.2	33R	N49 44 45.4 W95 15 24.5
04A	N49 24 02.0 W96 16 53.5	04R	N49 24 02.0 W96 16 53.5	34A	N49 44 45.2 W95 15 25.0	34R	N49 44 45.2 W95 15 25.0
05A	N49 24 06.2 W96 16 42.3	05R	N49 24 06.7 W96 16 42.2	35A	N49 44 43.8 W95 15 26.9	35R	N49 44 44.0 W95 15 26.9
06A	N49 24 04.2 W96 16 44.6	06R	N49 24 04.7 W96 16 44.6	36A	N49 44 41.9 W95 15 22.7	36R	N49 44 41.7 W95 15 22.7
07A	N49 24 05.1 W96 16 42.5	07R	N49 24 04.9 W96 16 43.0	37A	N49 44 43.8 W95 15 20.8	37R	N49 44 44.1 W95 15 20.5
08A	N49 24 05.6 W96 16 42.3	08R	N49 24 06.1 W96 16 41.3	38A	N49 44 43.1 W95 15 21.3	38R	N49 44 42.8 W95 15 21.7
09A	N49 24 06.9 W96 16 41.4	09R	N49 24 06.9 W96 16 40.4	39A	N49 44 41.8 W95 15 23.7	39R	N49 44 41.8 W95 15 23.2
10A	N49 24 01.1 W96 16 55.2	10R	N49 24 00.4 W96 16 55.9	40A	N49 44 41.4 W95 15 18.5	40R	N49 44 41.1 W95 15 18.6
11A	N49 24 00.9 W96 16 54.5	11R	N49 24 00.3 W96 16 54.4	41A	N49 44 37.3 W95 15 24.1	41R	N49 44 37.1 W95 15 24.2
12A	N49 24 00.1 W96 16 53.9	12R	N49 24 00.4 W96 16 53.6	42A	N49 44 37.0 W95 15 23.9	42R	N49 44 37.0 W95 15 24.1
13A	N49 24 00.9 W96 16 53.9	13R	N49 24 01.2 W96 16 54.3	43A	N49 44 36.4 W95 15 22.7	43R	N49 44 36.3 W95 15 22.8
14A	N49 24 01.0 W96 16 53.6	14R	N49 24 01.0 W96 16 54.1	44A	N49 44 36.0 W95 15 23.3	44R	N49 44 35.9 W95 15 23.1
15A	N49 24 05.4 W96 16 54.2	15R	N49 24 05.1 W96 16 54.7	45A	N49 44 38.1 W95 15 32.9	45R	N49 44 38.1 W95 15 33.1
16A	N49 24 05.6 W96 16 54.8	16R	-	46A	N49 44 38.3 W95 15 33.6	46R	N49 44 38.3 W95 15 33.6
17A	N49 24 01.7 W96 16 51.9	17R	N49 24 01.8 W96 16 52.0	47A	N49 44 38.3 W95 15 32.8	47R	N49 44 38.0 W95 15 33.0
18A	N49 24 01.5 W96 16 51.5	18R	N49 24 01.3 W96 16 52.0	48A	N49 44 38.7 W95 15 33.6	48R	N49 44 39.3 W95 15 33.5
19A	N49 24 04.3 W96 17 13.7	19R	N49 24 04.5 W96 17 13.4	49A	N49 44 46.4 W95 15 21.9	49R	N49 44 46.7 W95 15 22.5
20A	N49 24 05.3 W96 17 12.7	20R	-	50A	N49 44 46.3 W95 15 22.6	50R	N49 44 46.3 W95 15 23.1
21A	N49 24 05.1 W96 17 13.1	21R	-	51A	N49 44 46.7 W95 15 23.2	51R	N49 44 46.6 W95 15 22.9
22A	N49 24 08.2 W96 17 09.7	22R	N49 24 08.5 W96 17 09.4	52A	N49 44 46.9 W95 15 22.2	52R	N49 44 47.1 W95 15 22.4
23A	N49 24 00.5 W96 17 02.9	23R	N49 24 01.0 W96 17 03.0	53A	N49 44 47.3 W95 15 22.5	53R	N49 44 47.5 W95 15 22.7
24A	N49 24 00.1 W96 17 02.7	24R	-	54A	N49 44 47.9 W95 15 22.8	54R	N49 44 48.0 W95 15 22.5
25A	N49 23 59.7 W96 17 02.4	25R	N49 23 59.9 W96 17 02.8	55A	N49 44 47.6 W95 15 23.5	55R	N49 44 47.7 W95 15 23.7
26A	N49 24 01.6 W96 17 02.3	26R	N49 24 01.3 W96 17 01.8	56A	N49 44 47.6 W95 15 23.9	56R	N49 44 47.6 W95 15 24.2
27A	N49 24 10.3 W96 16 37.5	27R	N49 24 10.6 W96 16 37.0	57A	N49 44 48.1 W95 15 25.3	57R	N49 44 48.0 W95 15 25.0
28A	N49 24 09.2 W96 16 37.4	28R	N49 24 09.6 W96 16 37.6	58A	N49 44 48.4 W95 15 26.0	58R	N49 44 48.5 W95 15 26.1
29A	N49 24 07.9 W96 16 38.3	29R	N49 24 07.8 W96 16 37.8	59A	N49 44 49.3 W95 15 25.8	59R	N49 44 49.4 W95 15 25.8
30A	N49 24 07.7 W96 16 36.4	30R	N49 24 07.6 W96 16 36.3	60A	N49 44 49.9 W95 15 27.2	60R	N49 44 50.1 W95 15 26.3
31A	N49 24 07.5 W96 16 35.7	31R	N49 24 07.3 W96 16 35.9	61A	N49 44 49.9 W95 15 26.1	61R	N49 44 50.1 W95 15 26.0
32A	N49 23 58.8 W96 16 59.7	32R	N49 23 58.7 W96 16 59.3	62A	N49 44 50.5 W95 15 25.6	62R	N49 44 50.4 W95 15 25.5

Table A2. Species list of understorey plots with and without green alder (*Alnus viridis* ssp. *crispa* (Ait.) Pursh) in two boreal jack pine forests in the Sandilands and Star Lake in southeastern Manitoba.

Latin name with author citation	Common name
Mosses	
Brachythecium velutinum (Hedw.) Schimp.	Velvet feather moss
Dicranum spp.	Fork mosses
Eurhynchium pulchellum (Hedw.) Jenn.	Common beaked moss
Hylocomium splendens (Hedw.) Br. Eur.	Stair-step moss
Pleurozium schreberi (Brid.) Mitt.	Big red stem feathermoss
Polytrichum juniperum Hedw.	Juniper moss
Ptilium crista-castrensis (Hedw.) De Not.	Knight's plume
Unknown moss spp.	
Ferns and Allies	
Equisetum arvense L.	Field horsetail
Lycopodium clavatum L. var *megastachyon* Fern. & Bissell.	Running club-moss
Lycopodium complanatum L.	Ground cedar
Lycopodium obscurum L.	Ground pine
Pteridium aquilinum (L.) Kuhn var. *latiusculum* (Desv.) Underw.	Bracken fern
Herbaceous species	
Achillea millefolium L.	Common yarrow
Anemone quinquefolia L. var. *interior* Fern.	Wood anemone
Antennaria canadensis Greene	Broad leaved pussytoes
Aralia nudicaulis L.	Wild sarsaparilla
Arenaria lateriflora L.	Grove sandwort
Aster spp.	Aster
Clintonia borealis (Ait.) Raf.	Blue-bead lily
Epilobium angustifolium L.	Fireweed
Fragaria vesca L. var. *americana* Porter	Woodland strawberry
Fragaria virginiana Duchesne	Smooth wild strawberry
Galium septentrionale R. & S.	Northern bedstraw
Galium triflorum Michx.	Sweet-scented bedstraw
Heuchera richardsonii R. Br.	Richardson's alumroot
Lathyrus venosus Muhl. var. *intonsus* Butt. & St. John	Veined peavine
Lithospermum canescens (Michx.) Lehm	Hoary pucoon
Maianthemum canadense Desf.	Wild lily-of-the-valley
Melampyrum lineare Desr.	Cow-wheat
Mertensia paniculata (Ait.) G. Don.	Tall lungwort
Taraxacum officinale Weber	Common dandelion
Thalictrum venulosum Trel.	Veiny meadow rue
Trientalis borealis Raf.	Northern starflower
Viola rugulosa Greene	Canada violet
Viola spp.	Violet

Table A2 cont.

Latin name with author citation	Common name
Ericoid	
Arctostaphylos uva-ursi (L.) Spreng.	Common bearberry
Chimaphila umbellata (L.) Bart. var. *occidentalis* (Rydb.) Blake	Prince's pine
Gaultheria procumbens L.	Teaberry
Ledum groenlandicum Oeder	Labrador tea
Linnaea borealis L. var. *americana* (Forbes) Rehd.	Twinflower
Oxycoccus microcarpus Turcz.	Small cranberry
Potentilla tridentata Ait.	Three-toothed cinquefoil
Pyrola secunda L.	One-sided pyrola
Vaccinium angustifolium Ait.	Low sweet blueberry
Vaccinium myrtilloides Michx.	Velvet-leaf blueberry
Unknown woody species	
Low-growing woody species	
Cornus canadensis L.	Bunchberry
Diervilla lonicera Mill.	Bush honeysuckle
Juniperus communis L.	Common juniper
*Lonicera dioic*a L. var. *glaucescens* (Rydb.) Butters	Twining honeysuckle
Rosa acicularis Lindl.	Prickly rose
Rubus idaeus L. var. *strigosus* (Michx.) Maxim.	Wild red raspberry
Rubus pubescens Raf.	Dwarf raspberry
Spiraea alba Du Roi	Narrow-leaved meadowsweet
Symphoricarpos occidentalis Hook.	Wolfberry
Viburnum edule (Michx.) Raf.	Mooseberry
Viburnum rafinesquianum Schultes	Downy arrow-wood
Seedlings and saplings	
Alnus viridis ssp. *crispa* (Ait.) Pursh	Green alder
Abies balsamea (L.) Mill	Balsam-fir
Acer spicatum Lam.	Mountain maple
Amelanchier alnifolia Nutt.	Saskatoon
Betula papyrifera Marsh.	Paper birch
Corylus cornuta Marsh.	Beaked hazelnut
Pinus banksiana Lamb.	jack pine
Populus tremuloides Michx.	Trembling aspen
Prunus pensylvanica L. f.	Pin cherry
Salix scouleriana Barratt	Scouler's willow