# Climate-informed restoration of white pine: Impacts of seed source, planting site, and earthworms

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# Abstract

Habitat suitability for forest species is expected to shift with changes in climate, resulting in new and altered species assemblages. These alterations will be most notable near species boundaries, where feedbacks between disturbance, regeneration and recruitment are especially sensitive to temperature. How forest ecosystems respond will depend on the rate of climate change, dominant disturbance regime, the arrival of suitable propagules and the ability of those propagules to survive local climate, herbivory, invasive species and interspecific competition. Local adaptation of tree populations has been well documented in common garden experiments and may have a significant impact on the regeneration and recruitment in disturbed forests. In Northern Minnesota, USA, forest-climate models predict large-scale shifts in forest cover, and climate-driven changes in forest regeneration have already been documented at ecotone boundaries. Local populations of white pine (*Pinus strobus*) that are expected to increase may not be able to survive and reproduce successfully due to population bottlenecks, slow migration rates, habitat fragmentation, and intense herbivory. This is coupled with reduced reproduction of other temperate species, such as maples (*Acer spp.),* by herbivory and introduced detritivores. Given the rate of climate change, the migration and evolution of locally adapted populations and species are expected to lag behind optimal climate. My work documents restoration of white pine to assess the effects of seed source climate and site attributes, such as light environment and earthworm abundance, on survival, growth and phenology of planted seedlings. I also investigate the impacts and abundance of non-native earthworms in disturbed areas, as well as the surrounding intact forest to look for interactions between these invaders and disturbance. This data could be further used to improve models and inform management that will help sustain forest health and productivity.

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**Introduction**

Forest ecosystems cover almost 1/3 of the land area on earth and can act as major sinks or sources in the global carbon cycle. The forest industry in the U.S. alone accounts for $200 billion dollars in sales annually, and continued productivity in forest ecosystems is important for sustaining the economic benefits and ecosystem services they provide (US EPA 2015). Due to anthropogenic disturbance global forest cover is declining, especially at low latitudes where rates of deforestation for agriculture and development are relatively high. At mid and high latitudes, such as the Great Lakes region of North America, timber harvest and land use change are the main causes of forest loss (Dixon 1994, Hansen *et al* 2010). These intense disturbance regimes, coupled with changing climate, make the regeneration and recovery of forest ecosystems an important and relevant topic in modern population and conservation biology. We must address how feedbacks among disturbance, recruitment, and canopy cover are altered by climate change, anthropogenic disturbance, non-native species and the adaptation of local tree populations.

Although forest ecosystems have recovered from intense disturbance and changing climate in the past, the current barriers to establishment and recovery are unprecedented. Rapid rates of climate change, intense herbivory, habitat fragmentation, and introduced competitors all pose serious obstacles to continued forest regeneration and threaten to interrupt feedbacks in forest dynamics (Dale 2001, Cote 2004, Catford *et al* 2011). These barriers restrict gene flow and can limit the capacity of species to adapt to change or migrate across the landscape. The fitness and success of forest tree species is determined by the influx of propagules after disturbance, as well as the ability of those propagules to compete and survive in the local environment (Davis 1981). Once established, these individuals must maintain productivity as the local climate varies in order to compete. This process has been interrupted by anthropogenic disturbance and climate change, but the impact on reforestation efforts has not been quantified. Currently, our knowledge of the establishment and survival of species following forest disturbance is relatively unknown, and the way that this will be affected by anthropogenic environmental changes is even less well understood. We need accurate data on all life history stages in order to understand climate-driven changes in forest regeneration and productivity (Price 2001).

Adaptation to local climate and soil characteristics may improve the fitness of tree seedlings after disturbance. Phenotypic divergence of physiological and reproductive traits in tree

populations have been documented in common gardens over the ranges of many species; however, the way this phenotypic variation will influence regional scale regeneration and individual tree fitness in natural systems is unknown. Changes in forest species assemblage are predicted to accompany climate change, and the magnitude of these changes will depend on management techniques, carbon emissions, and the ability of species to compete for available resources (Pastor and Post 1988, Duveneck *et al* 2013). Forests that exist on ecotone boundaries, such as the transition from temperate to boreal forest, may be particularly susceptible to changing climate (Frelich *et al* 2012). Furthermore, introduced earthworms are currently altering soil and seed bed conditions at these ecotone boundaries. The mobilization or soil nutrients and reduction of organic matter caused by these invaders is novel in this once earthworm-free habitat, forcing local species to regenerate in previously encountered conditions (Frelich *et al* 2006). Phenotypic divergence and propagule pressure from well-adapted species and populations will be especially important for the maintenance of local populations and productivity in these ecosystems in a changing climate.

The following thesis examines the effects of seed source and site attributes on the survival and growth of planted white pine (*Pinus strobus*) seedlings in northern Minnesota, USA, and also considers the role of invasive earthworms and how they interact with disturbance. First, we must understand the feedbacks that determine the composition of forest ecosystems and how climate and migration of populations interact with these processes. Then we will explore how different seed sources and the presence of earthworms might alter these feedbacks and interactions.

**Dynamic Feedbacks in Forest Composition**

Disturbance is a natural and important process in forest ecosystems which alters the feedback between canopy cover and regeneration in the understory. These events open up space, change nutrient availability and can completely alter forest community structure. Disturbance occurs on different spatial and temporal scales depending on the forest and soil type as well as weather and climate. Fire is the dominant disturbance in many boreal forests and is important for determining the distribution of different cover types (Weber and Flannigan 1997). In temperate hardwood forests, the size of single tree fall and blowdown gaps was historically important in determining species regeneration (Runkel 1982). Gradual shifts in climate and the disturbances regimes that accompany them, such as glaciations and climate change, can also restructure forest communities on longer time scales.

Following the Wisconsin glaciation, forest communities in North America expanded from refugia and a gradual succession of species occurred over the next 14,000 years. Species ranges expanded across the landscape as the climate changed and habitat suitability shifted. The rate of species migration was dependent not only on the rate of climate change, but also on the availability of suitable substrate, the arrival of propagules, and the ability of those propagules to survive and compete with the current vegetation (Brubaker 1975, Davis 1981). For these reasons, the rate of migration was not uniform across species or within a species range. Spruce (*Picea spp.*) migrated north quickly and at a relatively constant rate across eastern North America while white pine (*Pinus strobus* L.) moved up the east coast from the Carolinas to New England before spreading west into the Great Lakes region (Fig. 1) (Davis 1981).

The adaptive potential and genetic structure of migrant populations may have also influenced the ability of these species to move across the landscape by increasing the fitness and competitive ability of propagules. These traits may have also changed during the migration process. Pollen samples from lake beds and peat formations in temperate and boreal forests that have been used to track species migration could also be used to track genetic structure of populations across space and time, if they contain viable genetic material. If so, this evidence might point to genetic changes that were important for the competitive release and expansion of species. Arrival of new genetic material or propagules from different areas of the species range and alterations of local disturbance could influence the timing of species range expansion and migration.

Although we know that species have migrated with climate in the past, human caused disturbance and fragmentation will also play a role in the way that species respond to rapid, modern changes in climate. In the following work, I examine the regeneration in forest ecosystems that have recently been disturbed by modern logging. These sites also contain artificial regeneration, which has the possibility of altering the trajectory of forest succession.

Forest succession is dictated by a feedback among disturbance, canopy cover, propagule pressure, regeneration, and recruitment of species. The current canopy, litter quality, and soil texture influence the nutrient and water availability at a site (Pastor and Post 1988). Competition for nutrient and water resources, population size, and herbivory can all influence the probability that a species arrives at a site and is able to establish or increase. The relative influence of these drivers is changing due to increasing temperature, altered disturbance regimes, and non-native competition (Frelich *et al* 2012). Specifically, non-native earthworms can affect regeneration in forest ecosystems directly, by consuming fine roots of seedlings, and indirectly, by altering soil nutrient availability and mycorrhizal fungi associations (Hale *et al* 2006, Alban and Berry 1994,

McLean *et al* 2006).  However, the ways in which earthworm invasion interacts with anthropogenic disturbance has not been identified and put into a management perspective.

I propose that the most important drivers of forest regeneration and recovery can be identified through experimentation in natural ecosystems, and that manipulating these feedbacks may be essential for maintaining the economic and ecological value of forest systems. For example, in our experiments we manipulate the influence of white-tailed deer herbivory by caging each individual and increase the probability of survival and eliminate this variable. I will explore how the feedbacks between seed source, local adaptation, and climate affect the fitness of propagules following disturbance. Another important and relatively recent addition to northern forest ecosystems that I will focus on is non-native earthworms, which alter seedbed conditions and the trajectory of forest regeneration and recruitment.

**Climate Change and Temperate-Boreal Forests**

My study takes place in Northeast Minnesota; a well-studied and heavily forested area where boreal spruce-fir forests reach their southern limits and are interspersed with northern hardwood stands and other temperate species. Forests dominated by red pine (*Pinus resinosa*) and white pine (*Pinus strobus*) are also common, although timber harvest has reduced them from 20% to just 5% of forest cover since European settlement (Friedman and Reich 2005). Under several climate scenarios, long-term models for this area predict major shifts in forest composition and the formation of new species assemblages, even with alternative management (Pastor and Post 1988, Iverson and Prasad 1998, Ravenscroft *et al* 2010, Duveneck *et al* 2014).These models predict that red maple (Acer rubrum) and northern hardwoods will increase productivity on sites that retain water and nutrients. Southerly species such as oak or drought resistant species such as jack pine will increasingly dominate the forest in areas with sandy soil and low availability of nitrogen and water (Pastor and Post 1988). This will be coupled with decreasing regeneration and productivity of boreal species such as quaking aspen (*Populus tremuloides*), balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*)(Fig. 2). Other species, such as red oak and white pine, are also predicted to increase. Low populations due to over harvesting have led to low levels of reproduction and heavy deer browse has minimized regeneration, recruitment, and adaptive potential of species that are predicted to increase. (Anderson *et al* 2003, White 2010, Duveneck *et al* 2013).

Predicted shifts in forest composition in Minnesota have been supported by empirical studies in naturally regenerating transitional forests. Boreal species regeneration has declined while seedling and sapling productivity of temperate species has increased in Minnesota's

transition forests at local and regional scales (Fisichelli *et al* 2014). However, the success of temperate seedlings and saplings was heavily reduced by browse pressure from white-tailed deer (*Odocoileus virginiana*), highlighting the importance of herbivory in the feedback between climate, propagule pressure, and regeneration (Fisichelli *et al* 2012). Forests farther from temperate seed sources may have a similar reduction in boreal species productivity coupled with reduced influx of well-adapted propagules and heavy browse pressure. Furthermore, although invasive earthworms prefer the leaf litter of temperate species they also feed on the fine roots of boreal seedlings and also reduce the success of some temperate seedlings, especially sugar maple (Frelich *et al* 2012). If boreal species do reproduce successfully, they will not be as productive in the future as the climate becomes less suitable. The reductions in boreal species regeneration and productivity as well as the inability of temperate species to invade these forests may leave gaps in regenerating forests, potentially leading to 'savannafication' of the current forested areas (Frelich *et al* 2009). Despite these challenges, forest ecosystems may persist through the migration and adaptation of local populations.

**Local Adaptation in Forest Trees**

Variable temperature, precipitation, and growing season length over a species range can lead to phenotypic divergence and local adaptation of tree populations. In a common garden study, saplings of *Eucalyptus marginata* from drier climates in Australia had smaller diameter but increased survival when compared to saplings from wetter climates (O'Brien *et al* 2007). Growth of North American tree species was greatest where minimum temperatures matched the seed source, and 8 of the 10 species studied actually declined in growth in warmer climates (Carter 1996). This has important implications for climate change: If well adapted populations are not able to migrate quickly enough to match the rate of change, warmer temperatures may actually decrease productivity in many North American forests.

High levels of divergence and phenotypic plasticity are apparent in several species of the genus *Pinus,* of which white pine is a member. Inland and coastal populations of a Chilean pine, *Pinus radiata*, differed in 8 morphological traits after 15 months of experimental growth in water stressed conditions. Coastal populations grew slowly but were more likely to survive (Espinoza *et al* 2014). Species with high levels of differentiation can maintain productivity over wide temperature ranges. In reciprocal transplant studies, growth and survival of *Pinus contorta* populations from western Canada varied with climate but locally adapted populations maintained 80% of maximum growth in a 6°C annual temperature range. (Rehfeldt *et al* 1999). Fitness of *Pinus sylvestris* from central Sweden is highest close to their home site, but they are also able to

survive and grow away from this site (Erikkson *et al* 1980). In a greenhouse, northern populations of this species set buds earlier, and highest variance for this trait was found at the center of the range (Samela 2014) (Fig. 3). This is an excellent example of adaptation to a thermal cline and shows evidence that at the center of the range, where the climate is the most variable, directional selection for bud set timing has been weaker. Bud set is an important characteristic for climate adaptation and seedling survival because it determines the timing of winter dormancy and the amount of root stores for initiating growth the next spring. Higher levels of plasticity, especially in phenological traits, will be advantageous in a variable and changing climate because they will allow individuals to adjust their growth response to match the climate. In common gardens, *Pinus strobus* seedlings from southeastern Ontario, Canada were taller and had later bud burst and bud set than those from the northwestern range of species (Li *et al* 1997). Taxa, such as the genus *Pinus,* which have well documented phenotypic divergence and make up significant portions of global forest cover should be used to test the importance of genetic differentiation in forest regeneration, recruitment, and productivity.

Divergence in these species may lead to variable fitness of individuals from different populations depending on climate and other environmental factors. This differentiation may be advantageous to regenerating species in a changing climate, if populations can migrate or adapt. On the other hand, invasive species, herbivory, and competition may override any advantages of phenotypic differentiation and increased gene flow.

**Seed Source, Regeneration, and Climate**

My work assesses the influence of local adaptation on regeneration by assessing white pine from three seed sources at 20 sites in northeastern Minnesota. Phenotypic divergence and local adaptation of specific seed sources could have an impact on the feedbacks between nutrient availability, canopy cover, and disturbance that control forest regeneration and establishment by allowing for seedlings to compete in the wider range of conditions that will accompany climate change. Introduction of diverse genetics might also increase the ability of restored populations to adapt to changing climate in the future (Harris 2006). The current climate in many areas does not match the historic climate that species have become adapted to. This mismatch between the current climate and the climate which selected for phenotypes and genotypes in the past might result in conditions that are not favorable for local propagules to compete and survive. Seeds and pollen from warmer climates with variable temperatures, precipitation and growing season length might have an advantage over local varieties, especially in species like white pine, which have gone through genetic bottlenecks due to over-harvest, herbivory, and disease (Anderson *et al*

2002, White 2012). The long life history and limited dispersal of pollen and seeds restricts the movement of tree species and their genetic material. This is coupled with intense habitat fragmentation that discourages migration and gene flow in most forest ecosystems.

Movement of seedlings between populations could have positive or negative effects on establishment and fitness of future propagules. Gene flow from central to peripheral populations in a species range has been shown to cause outbreeding depression and could reduce the fitness of introduced populations and subsequent generations produced by mixed seed sources (Lenormand 2002, O'brien *et al* 2007). However, this relies on the assumption that current selection regimes closely match those in the past and that migration of the species matches climate closely enough that peripheral populations are at or near the physical limits at which they can exist. Even now, species can lag behind their phenotypic optima and growth can be increased in slightly warmer or cooler climates (Rehfeldt 1999, Carter 1996). Rapidly changing climate, wide-spread disturbance, and habitat fragmentation make these assumptions hard to apply for long-lived species like forest trees.

Conversely, future genetic recombination of disparate populations may lead to hybrid vigor and novel phenotypes that will be advantageous in the future. Current rates of climate change may interrupt the feedback between climate and local adaptation, especially in long-lived species (Davis and Shaw 2001, Etterson and Shaw 2001). Phenotypes that currently exist in the canopy were selected for 50-100 years in the past, depending on species, and may not be fit in a new climate. Recombination may lead to unique phenotypes that are better able to compete in a warmer environment with exotic competitors and high levels of herbivory. The most advantageous and plastic of these new recombinations will give species a higher potential to adapt and will be selected for.

I will address the effects of seed source and local adaptation on forest regeneration by conducting an assisted migration (AM) experiment that measures growth and survival of multiple seed sources of white pine in recently disturbed logging cuts in a range of climates. This will put the results of common garden provenance trials into a management perspective and will help to identify any differentiation between seed sources in northern and central Minnesota. The most important seed sources will be those that survive well in the current climate and will potentially maintain that ability as climate continues to change and conditions vary.

I will also assess the impacts of non-native earthworms in both intact and disturbed forest ecosystems. It is already known that these detritivores cause major changes in soil biota and reductions in temperate tree reproduction. It is unknown, however, how these invaders interact with climate change and altered land use to help determine future forest composition. I will

explore the relationship between earthworm impacts, abundance, and forest disturbance in order to identify if and how these relationships interact. I will also use earthworm data in my analysis of seedling performance to see if earthworms impact the fitness of planted seedlings. In the future, these findings can be used to inform studies that explicitly link earthworms, disturbance and natural regeneration. Furthermore, experimental studies of germination and recruitment in earthworm invaded sites using multiple seed sources could bring climate and local adaptation into the context of soil structure and nutrient cycling.

The Lake Superior region in eastern North America is warming more quickly than many other areas due in part to feedbacks between rising regional temperatures, reduced ice cover, and increased heat absorption by the lake and northern Minnesota is predicted to increase in average temperature by 4.0°C by 2060 (Austin and Colman 2007, Galatowitsch *et al*, 2009). Northern Minnesota also contains diverse, transitional forest types that are predicted to shift with climate. These conditions may exacerbate the effects of climate on feedbacks between forest disturbance and regeneration. Survival and competition will be very sensitive to temperature and precipitation changes in this area and in similar environments. Seed source and local adaptation may play an important role in composition and succession of these forest ecosystems, as will interactions between regenerating seedlings, herbivores, and introduced detritivores (Frelich *et al* 2012). Studying restoration and regeneration in this area will help maintain the productivity and ecosystems services of regional forests.

**Conclusion**

Climate change is predicted to cause changes in the biotic community across all taxa (Parmesan 2006). Forest ecosystems are especially susceptible to change due to human caused disturbance, invasive species, and increased herbivory. The health and productivity of forest ecosystems is dependent on the successful regeneration and recruitment of individuals. We must begin to explore how these processes are responding to climate and other anthropogenic disturbances. Local adaptation, gene flow, and migration all play a role in the ability of a population to adapt. The rate of climate change high degree of habitat fragmentation, and non-native invaders present new and unparalleled barriers to adaptation and resilience in forest ecosystems.

In order to maintain the productivity and services that these ecosystems provide, we must understand the regeneration process and how feedbacks between propagule pressure, canopy cover, and disturbance are influenced by local adaptation. We must also take into account the impact of anthropogenic disturbance, habitat fragmentation, and the impact of non-native

earthworms. By implementing and monitoring assisted migration efforts, I will assess the impact of local adaptation and propagule pressure on forest dynamics. This reforestation effort utilizes multiple seed sources to show how genetic differentiation of local populations will impact regional forest regeneration and species fitness. Detailed survival and growth data for early life history stages will improve models that aim to predict future forest cover in areas that will be sensitive to changing climate. These efforts will also identify seed sources that compete well in the current climate and have potential for continued performance in the future. Genetic recombination of disparate populations of the same species may lead to an increase in the adaptive potential of tree species and may prove to be an important component for climate adaptation of both forest and human populations.
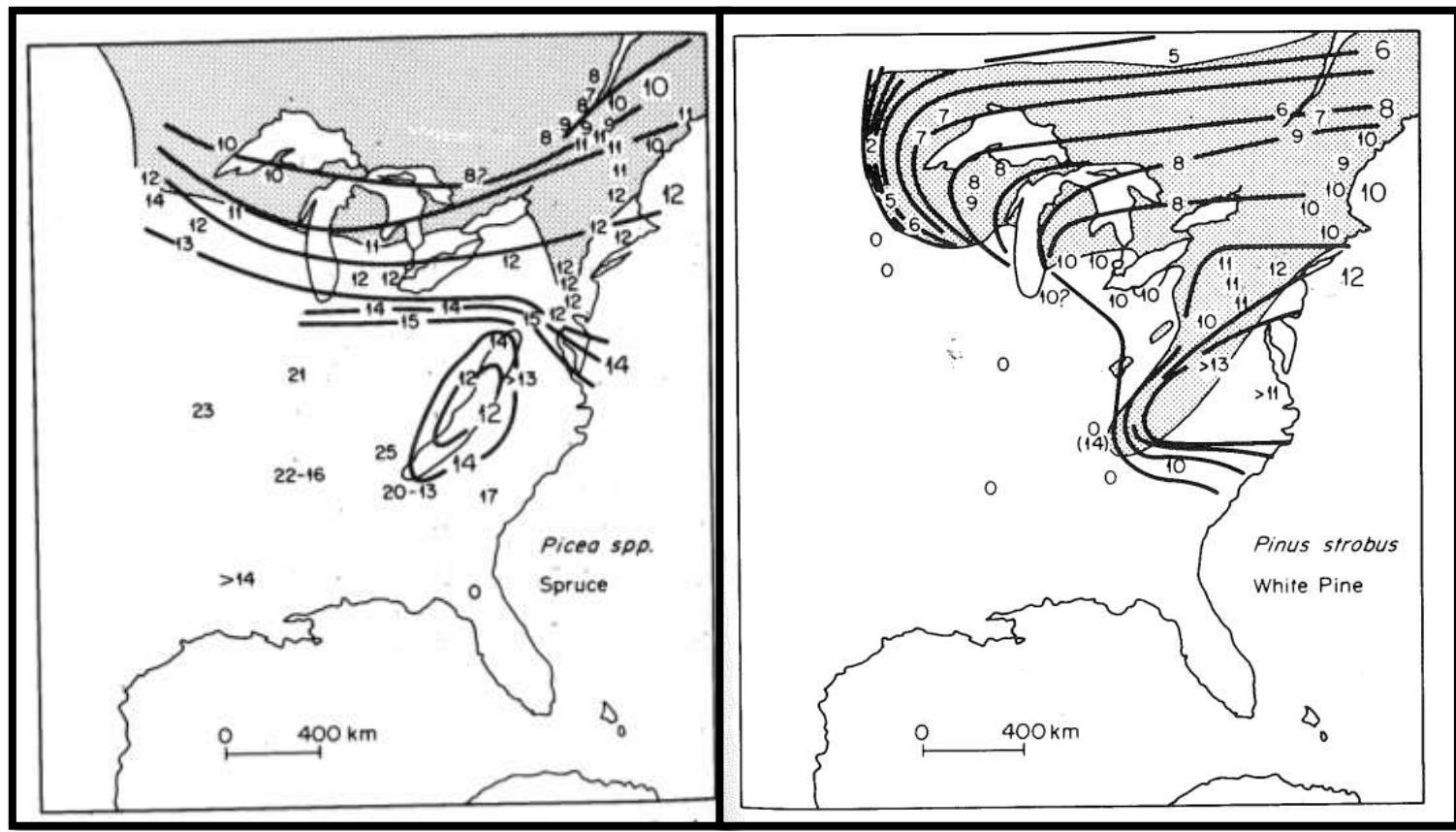
**Figure 1**. Migration rates of a) *Picea* spp.(Spruce) and b)*Pinus strobus* (Eastern White Pine) following the Wisconsin glaciation. Sediment cores containing pollen evidence from 62 locations were used to map species migration and expansion from glacial refugia. Lines indicate isoclines for arrival date of a taxa (thousands of years before present). From Davis 1981.
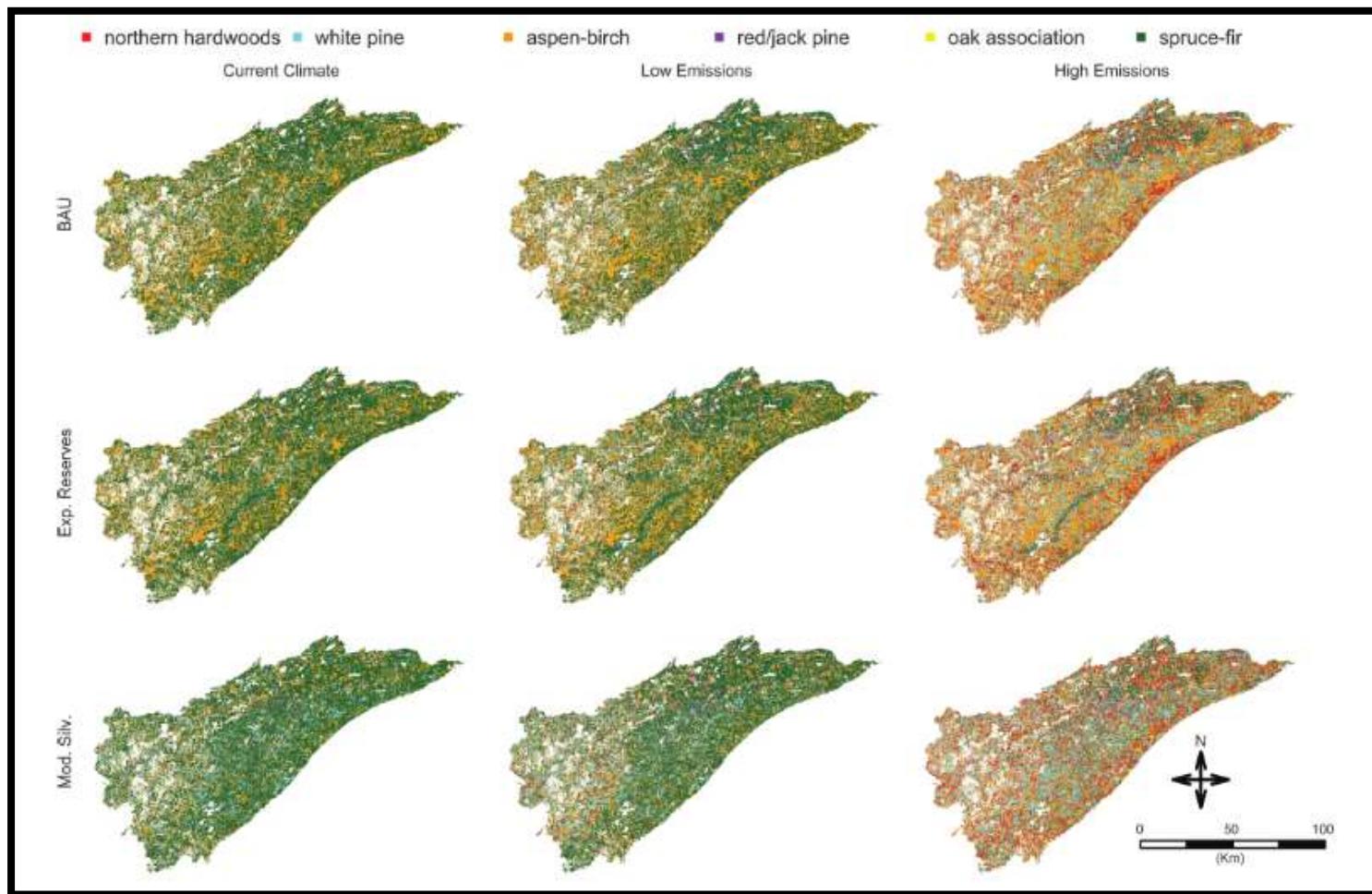
**Figure 2.** Predicted forest cover for northeastern Minnesota, USA under 9 different climate and management scenarios. BAU is Business-as-usual forestry methods, Exp. Reserves represents a 10% increase in the amount of unmanaged forests and Mod. Silv. is modified silviculture and includes altered harvest regimes and restoration of pine and oak species. From Duvenek 2014
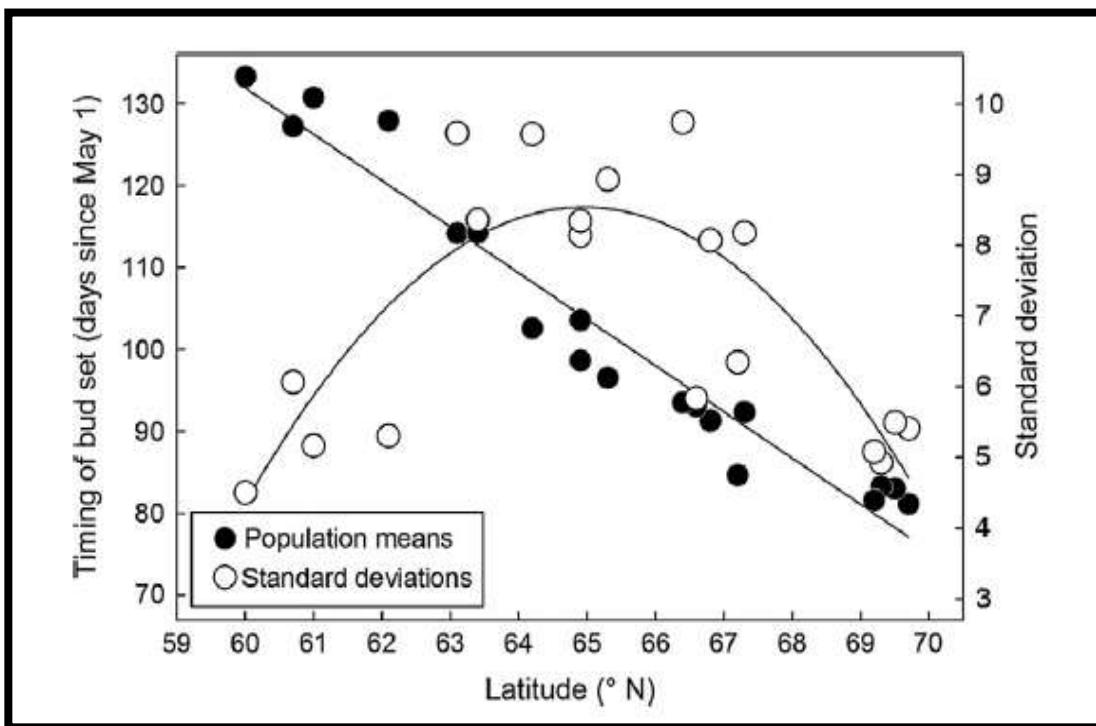
**Figure 3.** The mean and standard deviation of bud set date for 19 populations of *Pinus sylvestris* (Scots Pine) in common gardens in Finland. Latitude shows the population source. From Salmela 2014.

**Climate-informed restoration of white pine: Contrasting growth and survival of seed sources**

**Introduction**

*Climate Change and Forest Tree Migration*

Forest trees are long-lived plant species that often occupy large geographic ranges. In the northern hemisphere, tree species have undergone large range contractions and expansions with the advance and retreat of glaciers during the most recent ice age. In North America, the rate of range expansion from glacial refugia has been estimated using both fossil pollen and cpDNA evidence. According to fossil pollen, the rate of expansion for several species has been estimated at 100-1000m per year (Davis 1982). However, cpDNA evidence has revealed more northern refugia which results in more conservative estimates of 10-100m per year following glacial retreat (McLacklin 2005). Importantly, this migration also occurred on an unfragmented landscape that would potentially allow for more rapid movement.

Adaptive divergence of local populations is well documented over the range of many forest tree species (O'Brian *et al* 2007, Rehfeldt 1999, Rehfeldt 2002, Savolainen *et al.* 2007, Carter 1996). Variation in growth, bud phenology, and cold hardiness have been found along geographic clines in temperature, elevation, and precipitation for many species. Variation in these traits is especially important for the adaptation of temperate tree populations to local climate. Individuals that maximize growth have a competitive advantage over conspecifics. However, the timing of this growth response is also important to avoid frost damage in the spring and fall. Differences in growing season length over the range of the species leads to selection that balances the maximization of growth with timing of phenology that matches the growing season (Fig. 3) (Salmela 2014).

Adaptation to local climates may have helped species to respond to past changes in climate and can help species respond to current climate change as well, but only if well-adapted alleles are able to pass from one population to another or individuals from well-adapted populations are able to migrate along with the suitable climate (Davis and Shaw 2001, Etterson and Shaw 2001). In many species, this in unlikely to occur naturally given the fragmentation of many forest ecosystems and the reduction of populations size and genetic variation following over-harvest of valuable timber species. With climate change, the major cover types in many forests are predicted to change (Pastor and Post 1988, Iverson and Prasad 1998). The shift in

forest species assemblage will be most notable at ecotone boundaries, where species meet there abiotic limitations.

Due to the rapid rate of modern climate change, models predict that tree species migration may lag behind climate optima for individual species and populations within species (Jump and Penuelas 2005). Furthermore, adaptation in place is unlikely because selection acts slowly on tree species, even for very heritable traits (Savolainen *et al* 2004). In order to maintain the health and productivity of ecosystems in the face of climate change, assisted migration (AM) has been proposed to match species and populations to suitable climates that currently exist or are predicted to occur in the future. While opponents of AM deem this strategy inviable and warn of the risks of species invasion (Ricciardi and Simberloff 2009), this technique can be beneficial because it can bolster both the reproductive output and the genetic diversity in species that are predicted to remain stable or increase with climate change (Savolainen *et al.* 2004, Aitken *et al.* 2008). Assisted migration could also allow species to move along natural migration routes that have been fragmented by human disturbance (Vitt *et al.* 2009). Furthermore it is important to distinguish the AM being suggested for forestry purposes, which aims to move species within their range limits, with more controversial AM that suggests movement of species outside of their current distributions (Pedlar *et al.* 2012).

*Transitional Mixed Forests and Climate Change*

This study takes place in northeastern Minnesota, a forested and relatively unfragmented area where boreal spruce-fir forests reach their southern limits and are interspersed with temperate hardwood stands. Forest dominated by red pine (*Pinus resinosa*) and white pine (*Pinus strobus*) is also common, although it has been reduced from about 20% to less than 5% of total area since European settlement. Under several climate scenarios, long-term models for this area predict major shifts in forest composition and the formation of new species assemblages, even with alternative management (Pastor and Post 1988, Ravenscroft *et al* 2010, Duveneck *et al* 2014). This shift includes an increase in red maple (*Acer rubrum*) dominated communities coupled with a decrease in the regeneration of boreal species such as quaking aspen (*Populus tremuloides*), balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*). Other species, such as red oak and white pine, are also predicted to increase. However, low populations due to over harvesting and poor regeneration under heavy browse from white tailed deer (*Oidecoileus virginiana)* have hindered these species (Anderson *et al* 2002, White 2012). Predicted changes in forest composition in Minnesota have been supported by empirical studies. There is a significant decline in boreal species regeneration and an increase in temperate seedling and sapling

productivity in Minnesota's transition forests at a local and regional scale (Fisichelli *et al* 2014). However, heavy browse pressure diminishes increased growth response and productivity of temperate species (Fisichelli *et al* 2012).

In order to maintain the productivity and resilience of transitional forest ecosystems, a redistribution of genetic material across the landscape may be necessary (Savolainen *et al.* 2007, Joyce and Rehfeldt 2013). If temperate species are unable to migrate and increase at a sufficient rate, boreal species may continue to grow in northeast Minnesota but in less than optimal climates. Alternatively, a single temperate species, such as red maple (*Acer rubrum*), may become increasingly dominate on the landscape (Duveneck *et al.* 2014, Ravenscroft *et al.* 2010). In order to mitigate these effects, assisted migration of populations may be useful to help forest trees in Minnesota adapt to changing climate. New alleles may allow species to be more competitive in future climates and recombination of local and disparate genotypes may lead to novel phenotypes that are better adapted to uncertain future climate scenarios. By planting multiple seeds sources in the same environment, it can be determined how these populations differ in growth response to the same climate and seed sources can be matched to areas where they can compete in the present climate and maintain productivity in the future.

Common garden provenance trials have a long history in forest science, but the results of these trials have not been used to inform climate-based management. Currently, forest managers are aware of climate change impacts on forest ecosystems, but most have not begun to incorporate this knowledge into silviculture practice and prescription (Milad *et al.* 2013, Keenan 2015). Anecdotally, local varieties of many species are suggested for reforestation following harvest to attain maximum growth and productivity in forest trees. In 1974 the Minnnesota Department of Natural Resources developed a seed zone map that suggests using planting stock from the local area. However, many populations already lag behind there climate optima or are competitively excluded from optimal habitat by conspecifics (Carter 1999, Rehfeldt 2002). Furthermore, asymmetric gene flow from the center of the geographic range to the periphery has led to outbreeding depression in peripheral populations and prevented local optimization of phenotypes (Rehfeldt 2002). As the climate warms, populations that exist in cooler that optimal climates may increase in productivity and those in optimal or warmer than optimal conditions may suffer. Assisted migration within the species boundary is appropriate here given these mismatches in climate and local adaptation, along with the current rate of climate change.

*Current Study*

Here, three seed sources of white pine (*Pinus strobus)* from northern Minnesota (MN DNR seed zone 104), central Minnesota (MN DNR seed zone 105), and central Michigan are used to assess the effects of local adaptation on the phenology, survival, growth, and morphology of planted seedlings in recently logged forests in northeastern Minnesota. The impact of temperature, moisture, forest type, light availability, competing vegetation and non-native earthworms on the results of this climate change mitigation technique are also assessed by utilizing sites over the range of environments that exist in the study area. This experiment will help to put the results of common garden provenance trials into management perspective and will test the role that seed source and local adaptation play in regeneration in disturbed ecosystems.

It is predicted that the Michigan ecotype, from the warmest climate, will display the earlier bud burst, higher growth, larger diameter, a larger number of fascicles and smaller specific leaf area (SLA) ($cm^2 g^{-1}$) when compared to northern MN seedlings at the same sites. As a result of its extended growth period and early bud burst, survival will also be significantly lower in the Michigan ecotype (O'Brien *et al* 2007, Rehfeldt *et al* 1999). Minnesota seed zone 104, from the coldest climate, likely exists in environments that are colder than its optimum and so will be most productive at sites that are slightly warmer than its local climate, which is near the edge of the prairie-forest ecotone boundary in northwestern Minnesota (Rehfeldt *et al* 2002). It is also predicted that this seed source will have later bud burst, smaller growth increment, less of fascicles and larger SLA than both central MN and Michigan seedlings, because it comes from a very cold and dry environment near the western edge of the species range. Central MN seedlings will display earlier bud burst, greater height growth, and smaller SLA than northern MN because its local environment is warmer and moister than that of northern MN. Furthermore, survival for these two ecotypes will be equal due to the ability of seedlings to alter their growth response in order to maintain survival across a range of environments.

**Methods**

*Study Species*

`Eastern white pine (*Pinus strobus*) is a long- lived, temperate conifer that is found in a variety of habitats from well-drained sandy sites to small ridges in wetlands and poorly drained sites (Barnes and Wagner 2004). This species is distributed throughout the forested areas of Minnesota and extends north to Ontario and south into the Appalachian mountains of northern Georgia. Eastern white pine is wind pollinated and is reproductively mature at about 30 years of

age. Seedlings germinate and compete well after fire and can live up to 500 years, with an average life span of about 200 years (Wendel and Smith 1990).

Eastern white pine was extremely important in pre-settlement forests of Minnesota but was reduced from over 20% to less than 5% of total forest cover by extensive logging at the end of the 19th century (Abrams 2001, Larson 2007). Regeneration and establishment have been hindered by increased herbivory from white tailed deer and the suppression of fire from the landscape. The diverse habitats that white pine is found in suggests that plantings should survive well on a variety of sites that might increase or decrease in productivity in future climates (Pastor and Post 1988). White pine is well-suited for assisted migration because its populations have been reduced due to over harvesting (Larson 2007), regeneration has been hindered by herbivory (Abrams 2001, Anderson *et al*. 2003), and this species is predicted to increase with climate change in our study area (White 2012).

*Study Sites and Planting*

In order to assess the effect of seed source on phenology, survival, growth, and morphology of planted white pine seedlings, two sampling blocks were established at each of 20 logged sites located in four forest types in northeast Minnesota (Fig. 4, Table 1). These sites were chosen because they represent the most abundant and frequently managed forest types in northeastern Minnesota. Each site was logged 1-3 years prior to the start of the study using modern methods. In the fall of 2014, the planted area was thinned using a brush saw treatment to reduce the populations of woody competitors. Hardwood sites (Minnesota Forest type MHn35, MHn45) were dominated by sugar maple or red oak, with lesser amounts of yellow birch, basswood and red maple (MN-DNR 2003). Boreal Mixed forest (Minnesota forest type FDn43) were dominated by quaking aspen, white birch, and balsam fir, and contained lesser amounts of white spruce, white pine, and red maple. Mesic pine (Minnesota forest type FDn43) sites were dominated by quaking aspen, paper birch, red pine, or white spruce with variable amounts of white cedar, white pine and balsam fir (MN-DNR 2003). Dry-Mesic Pine sites (Minnesota forest type FDn33) were dominated by red and white pine with balsam fir and white birch often in the understory (MN-DNR 2003).

In spring of 2014, fourteen sites in all four forest types on Superior National Forest and MN-DNR land were planted with containerized seedlings from two MN seed zones: 104 (Bemidji, MN) and 105 (Hill City, MN) (Fig. 4, Table 1). Minnesota 104 seedlings will be

referred to as "northern MN" and MN 105 seedlings will be referred to as "central MN" for the remainder of the manuscript.

Due to planting restrictions on federal and state property and limitations in planting stock, the remaining six sites in hardwood and boreal mixed forest, on Lake and St. Louis County forest land, were planted with bare-root seedlings from MI (near Kalkaska and Baldwin) and bare-root seedlings from MN seed zone 104 (Bemidji, MN)(Fig. 4, Table 1). These two sets of planting sites are treated as separate experiments due to non-overlap in the sites and planting stock. Data and analyses for these experiments are presented separately throughout the manuscript. Northern Minnesota (104) seedlings were from the coolest and driest climate, near the edge of the prairie-forest ecotone, central Minnesota (105) seedlings were from a moderate climate, and Michigan seedlings were from a much warmer and wetter climate in the east-central portion of Michigan's Lower Peninsula (Table 2). 20 individuals of each seed zone were planted in two planting blocks at each site for a total of about 1600 individuals (Table 1).

*Seedling Measurements*

Survival, growth, morphology, and phenology of each seedling was measured in the 2015 growing season. Starting in late April, bud burst phenology was monitored using visual checks at each site once per week for a total of 8 weeks. An ordinal scale was used to score the progression of phenology for each seedling (Desprez-Loustau and Dupuis 1994) (Fig. 5). During the first phenology check, we also measured the diameter of each seedling to the nearest 0.1mm at 2.5cm from the forest floor using a digital caliper. In September and October 2015, the incremental growth of each individual was measured to the nearest 0.5 cm. 2014 and 2015 growth were inferred by measuring the distance between bud scars on each seedling. In addition, the number of fascicles on the dominant leading stem of each individual was recorded, its location was marked with GPS, and a single needle was collected from the uppermost fascicle to be used for SLA ($cm^2$ $g^{-1}$) measures. Finally, diameter measurements were repeated as described above.

To calculate SLA, each needle was dried for 48 hrs at 75ºC. After drying, needles were massed to the nearest 0.1 mg and scanned using an HP ScanJet 4C/T scanner designed for use with WinRHIZO. Surface area ($cm^2$) was calculated using ImageJ software and the analyze particle command. SLA ($cm^2$ $g^{-1}$) was calculated by dividing the area ($cm^2$) of each needle by its dry mass (g).

*Climate*

Climate at each site was determined from the closest weather station using the Minnesota Climatology Center data summaries. Average annual temperature, average minimum January temperature, average summer temperature (Jun-Aug), growing degree days $>5^{\circ}$C, and total annual precipitation were included in our data summary (Table 1). A moisture index that is a ratio of total precipitation to GDD $>5^{\circ}$C was also calculated (Rehfeldt *et al.* 2002).

*Light*

Light availability was quantified as both percent open canopy and as a ratio of red:far red light. Canopy openness was assessed at each individual, 1m from ground level using hemispherical photos shot at dawn and dusk on a Canon Rebel T5i digital camera with a Lenbaby $185^{\circ}$ fish eye lens. Photos were converted to binary using Image J software and the binary conversion tool. At the same time, red:far red ratios were measured at the height of each seedling using Skye Instruments light sensor model SKR 100 with sensor 660/730.

*Earthworms*

Earthworm abundance was measured using a quantitative rapid assessment method. The rapid assessment protocol uses the abundance of earthworm castings and the condition of the forest floor to give relative impacts of non-native earthworms (Loss *et al.* 2013). This method uses an ordinal scale with 5 categories; 1 being uninvaded and 5 being heavily invaded. We completed 3 rapid assessments inside each planting block; one along the central vegetation transect and one on each transect East and West of the center.

The slope, aspect, and microtopography was documented in each block and soil surveys were completed to identify soil texture and horizon structure.

*Vegetation*

Species level surveys of all regenerating vegetation were completed in each block in the June and July of 2014 and 2015. At each block, a North-South transect was established at the center of the block and 1m radius plots were surveyed every 5m along this transect. Each herbaceous and graminoid species was recorded and its height and abundance was classified using a modified Braun-Blonquet floristic system. Woody stems (tree seedlings and shrubs) were counted in 3 height classes: 0-50 cm, 50-100 cm, and 100+ cm. We used the same abundance classes for woody stems as for herbaceous vegetation. We repeated this procedure every 10m east and west of the block center, until the extent of the planted area was reached. We aimed to sample at least 5% of the planted area with our vegetation surveys.

*Data Analysis*

Principle components analysis was used to summarize soil variables for each block and axis scores from the top two components were used as covariates in the overall data analysis. Percent sand, silt, and clay, organic layer thickness, A-horizon thickness, B-horizon thickness, E-horizon thickness, and pH were included in the PCA of soil for each block in this analysis.

Generalized linear mixed effects regression (*glmer*) in R package lme4 (R Core Team, 2014) was used to estimate survival probabilities based on seed source and site attributes. R package lme4 was also used to perform mixed effects ANCOVAs to assess the effect of seed source, forest type, and site attributes on continuous response variables (R Core Team 2013). These included growth increment in 2014 and 2015 (cm), diameter growth in 2015 (mm), SLA (cm$^2$ g$^-$1) and number of fascicles.  Each of these variables was transformed using an appropriate transformation, if necessary, to better fit model assumptions including normality and equal variance. Repeated measures ANOVA was utilized on spring phenology measurements to assess differences among seed sources, forest types and sites. We used likelihood ratio tests and AIC scores to compare models with different site and climate variables. A generalized full model for the analysis takes the form:

Response = (site:block)+(forest_type:site)+site MI*seed source MI+canopylight+RFR+ soilPCA1+soilPCA2+avgvegdensity+woodystemdensity+worm_assessment_score+$H_i$

Due to the uneven planting design and site restrictions, data from sites planted with northern and central containerized seedlings was analyzed separately from those sites containing bare-root Michigan and northern MN seedlings. Partial residual plots were utilized to show individual main effects as well as significant covariates from mixed effects regression models. These plots show the full model residual plus the partial regression coefficient for the predictor of interest plotted against the original predictor, in order to show the partial effects of each predictor (Fig 9, 10).

**Results**

Overall, adaptive restoration had mixed results depending on the seed sources used as well as the moisture level and temperature at the planting site. For survival, the best models were those that included seed source location and forest type. For the remaining variables, seed source and site moisture index (MI) were included in the best models. Local seedlings tended to perform best in survival and growth responses, while morphological variables did not vary between seed

sources. Light quality (R:FR) and the height of the seedling at the time of planting ($H_i$) were highly significant across the models of survival, growth, morphology, and phenology.

*Soil PCA*

The first two axes from the principle components analysis of soil data explained 53.17% of the variance in the data. The first 3 axes explained 68.41% of the variance. The first axis was most positively associated with percent sand and most negatively associated with percent clay (Fig. 6). The second axis was positively associated with soil pH and b horizon thickness and negatively associated with e horizon thickness (Fig. 6). We used the first two PCA axis scores in the following analyses as covariates to explain soil.

*Survival*

The survival probability of northern Minnesota seedlings was slightly higher than that of central Minnesota seedlings, while bare-root Michigan seedlings were much more likely to suffer mortality than northern MN seedlings. On average, MN seedlings were 9% more likely to survive ($p$=0.047), but this relationship did not vary across forest types ($p$=0.37, Fig. 7a, Table 3). For every one unit increase in red: far red (R: FR) light ratio survival for these seedlings increased by 11-13%, on average ($p$<0.0001) and, surprisingly, seedlings with a lower initial planting height ($H_i$) were 4-6% more likely to survive ($p$<0.0001, Table 3). Similiarly, the seed source had a significant influence on the survival of bare-root Michigan and northern MN seedlings, but the effect was larger at these sites. On average, Michigan seedlings, from a warmer and wetter environment, had 46% lower survival across all forest types ($p$<0.0001, Fig. 7b). Once again, increasing R:FR light by one unit caused a 12-50% increase in survival ($p$=0.003) and seedlings with smaller $H_i$ were 3-5% more likely to survive for every one cm decrease in initial height ($p$<0.0001, Table 3).

*Growth*

Height growth (cm) varied among planting site and seed source climate, but diameter increment (mm) varied only among seed source climate. Height growth of containerized Minnesota seedlings was greater at sites with higher MI but this relationship differed depending on the MI at the seed source (Table 4, Fig. 8a,b). Moisture index (MI) was also important at sites planted with bare-root seedlings. Bare-root Michigan seedlings, from a higher MI, grew more vigorously than their northern MN counterparts initially, but this response did not continue after the first year (Table 4, Fig. 8c).

In 2014, the first growing season following transplant, northern and central MN seedlings showed increasing growth at sites with higher MI, but the local, northern seedlings responded more strongly to this increase in planting site MI ($p=0.011$, Table 4, Fig. 8a). This trend did not continue in 2015, when trees from a higher MI in central MN actually showed a decline in growth with increasing planting site MI, while local northern MN seedlings had a fairly even growth response across sites ($p=0.002$, Fig. 8b). In 2014, growth increased by 4.45 cm for every one unit increase in R:FR light ratio ($p<0.0001$) and declined by 0.61 cm for every 1 cm increase in $H_i$ ($p<0.0001$, Table 4). These covariates were also important in 2015 but had smaller impacts on the growth rate of the planted seedlings (Table 4, Fig. 9a,b). In 2015, growth increased by 0.88 cm for every one unit increase in R:FR light ratio ($p<0.0001$, Fig. 9a) and declined by 0.05 cm for every 1 cm increase in $H_i$ ($p<0.0001$, Fig. 9b). Diameter of these seedlings also decreased by 1.62 mm with increasing seed source MI ($p=0.032$, Table 4) and increased by 0.37mm with increasing light quality ($p<0.001$, Table 4).

Similarly, the height growth response of bare-root seedlings increased with seed source MI at all sites in 2014 ($p<0.0001$, Fig. 8c), but in 2015 there was no difference in growth response among seed sources or sites with differing climates (Table 4). In 2014, growth increased by 85.62 cm for every one unit increase in R:FR light ratio ($p=0.041$) and declined by 0.54 cm for every one cm increase in $H_i$ ($p<0.0001$, Table 4). These same covariates were also important in 2015, but had different effects on the growth rate of the planted seedlings (Table 4, Fig. 9 c,d). Growth in 2015 increased by a small amount, 5.28 cm, for every one unit increase in R:FR light ($p=0.003$, Fig. 9c). Unlike 2014, $H_i$ had a positive impact on 2015 growth for bare-root Michigan and northern MN seedlings. In the second year following transplant, seedlings grew 0.11 cm more on average for every 1 cm increase in $H_i$ at sites planted with bare-root Michigan and northern MN seedlings($p=0.001$, Fig. 9d).

*Morphology*

Like survival and height growth, fascicle number differed significantly based on the climate at the seed source and planting sites. There was a significant interaction effect between seed source MI and planting site MI on the mean 2015 fascicle number for central and northern MN seedlings ($p=0.001$, Table 4). Similar to growth responses, seedlings actually declined in fascicle number at cool, moist sites (high MI), and this response was stronger in the central MN seedlings which are from a warmer and wetter environment (Fig. 8b). Fascicle number increased with R:FR light ratio at these sites and, on average, plants had 11.2 more fascicles for every one unit increase in R:FR ($p<0.0001$). Seedlings planted smaller (lower $H_i$) had more fascicles as

well. For every one cm increase in $H_i$, the number of fascicles declined by 0.43 fascicles, on average ($p$=0.002). At sites planted with Michigan and northern MN seedlings, increasing light quality (R:FR) by one unit increased average fascicle number by 15.1 fascicles ($p$<0.0001, Table 4). Fascicle number also had a significant negative relationship with soil PC2, and fascicle number decreased by 1.74 fascicles for every one unit increase in soil PC2 score ($p$<0.001, Table 4).

Seedling SLA was not significantly influenced by MI for either set of planting sites, but R:FR light ratio had a significant effect on the SLA of planted seedlings, further stressing the importance of light environment and planting stock for seedling performance (Table 4, Fig. 10 a,b). At sites planted with northern and central MN seedlings SLA decreased by 264.01 cm$^2$ g$^{-1}$ for every one unit increase in R:FR light ratio, on average ($p$<0.0001, Fig. 10a). For Michigan and northern MN seedlings, SLA decreased by 289.15 cm$^2$ g$^{-1}$ for every one unit increase in R:FR ratio ($p$<0.0001, Fig. 10b). Smaller seedlings also tended to have lower SLA at these sites and for every one centimeter increase in $H_i$ the SLA declined by 7.93 cm$^2$ g$^{-1}$ ($p$=0.016, Fig 10c).

*Phenology*

The timing of onset and development of new growth differed significantly with seed source and planting sites climate for all seedlings, but this difference was most pronounced between northern MN and Michigan seedlings (Fig. 11). Seedlings from the two MN seed zones responded differently to planting site climate and had a significant interaction between seed source and planting site MI over time ($p$= 0.029, Table 5). At the first check, central Minnesota seedlings, from a warmer climate, had phenology scores that were on average 0.08 stages lower than those from northern Minnesota, but only at the driest planting sites with low moisture indexes. This difference diminished over time and the seedlings were equal in their scores at the later phenology checks. The relationship between mean phenology stage and average woody stem density also varied significantly over time, with small effects at the first check only ($p$<0.0001, Table 5). Red: far red light ($p$= 0.004) and percent vegetation cover ($p$= 0.003) also impacted mean phenology stage but these relationships did not vary over the time of the phenology checks (Table 5). Seedlings in better light environments (high R:FR) tended to develop more slowly and were between 0.06 and 0.27 stages lower in their average phenology score. Seedlings in densely vegetated plots also developed more slowly over time, but this effect was weaker, with average phenology scores between 0.01 and 0.16 stages behind for every one unit increase in understory vegetation cover.

For Michigan and northern MN seedlings, seed source MI ($p=0.003$) and planting site MI ($p=0.006$) were both significant and these relationships both varied over time (Table 5). Michigan seedlings (higher MI) initiated growth, on average, at the same time as the northern MN seedlings, and the two seed sources were equal at the first check. Michigan seedlings developed more slowly into the later stages that northern MN seedlings, and average phenology scores were 0.33 lower at the fourth check and 0.07 lower at the 8th and final check (Fig. 11). Moisture index at the planting site had a significant effect only at the first check, when increases in site MI led to lower phenology scores, on average. The driest sites (MI=0.27) had an average phenology score of 1.47 and the moistest sites (MI=0.30) had an average score of 0.96. In addition to MI, mean phenology score also varied significantly with Axis 2 from our soil PCA ($p<0.001$) and earthworm assessment score ($p<0.0001$, Table 5). Soil PCA 2 scores were positively correlated with phenology scores at the first check only, and for every one unit increase in PCA 2 score, phenology scores increased by 0.17, on average.  Higher earthworm assessment scores also led to delayed onset of growth, and at the first check, phenology scores decrease by 0.23 units for every one unit increase in earthworm assessment score. Woody stem density had a significant negative impact (-0.14) on the mean phenology score of northern MN and Michigan seedlings and this relationship did not vary with time ($p<0.0001$, Table 5).

**Discussion**

Assisted migration of white pine seedlings within their native range had mixed results depending on the distance that populations were moved, the climate at the planting site, and the availability of photosynthetically active light.

Light quality was the best site covariate in several of our models for both sets of planting sites. Increasing Red: Far-red light ratio led to increased survival, growth, and fascicle number as well as decreasing Specific Leaf Area (SLA $cm^2 g^{-1}$)(Fig. 9, 10). Better light environments also led to slower development of new growth, which also may allow for larger growth increments, as seen in the Michigan seedlings measured here (Table 5). While not surprising, these results stress the importance of light quality and microenvironment within planting sites for seedling establishment. Restoration efforts following disturbance should be implemented on microsites with low levels of woody species establishment and managers should actively reduce the vegetation in restoration sites following planting, if necessary. At sites that were previously dominated by trembling aspen (*Populus tremuloides*), these efforts can be crucial due to the dense asexual reproductive response of this species following harvest (Brinkman and Roe 1975). Trembling aspen is expected to decline in productivity in the US with climate change. In order to

mitigate the loss of productivity at these sites, multiple species restoration efforts should be employed (Ravenscroft *et al.* 2010, Duveneck *et al.* 2014). It may also be crucial to reduce competition and ensure adequate light availability for planted seedlings. These findings highlight the need to considering site history and condition when prescribing management and estimating its long-term costs. Equally important is selecting the most appropriate planting stock for restoration.

In order to aid in this selection process, we tested three populations of white pine planted at the same sites. The importance of planting site and seed source moisture index (MI) in our overall models most likely occurred because this metric takes into consideration both the growing season temperature sum (GDD>5°C), which has been shown to be related to growth potential in this species (Joyce and Rehfeldt 2013), and water availability. Seedlings from northern MN survived slightly better that central MN seedlings at all sites and also grew larger at cool, moist sites in the first two years following transplant. At warmer, drier sites, with lower moisture indices, northern and central MN seedlings grew equally (Fig. 8 a,b). A lack of difference in survival was predicted for these two seed sources because similar temperate tree species have been shown to be plastic in their growth response and maintain survival when moved to slightly cooler and drier environments, however this was not the case, perhaps due to very cool conditions at these sites following transplant (Carter 1996, Rehfeldt *et al.* 1999, Rehfeldt *et al*. 2002). The unequal survival and growth of MN seedlings suggests local adaptation of the northern MN seed source (MN seed zone 104) to sites with cooler climate and higher MI (Fig. 7a, 8a,b). At these sites, northern MN seedlings maintained or improved performance, while central MN seedlings did not.

This differentiation did not reflect itself strongly in phenology scores, which were equal for these two seed sources at most sites and for most of the checks. In addition, more southern seedlings from central MN may perform well both now and in potentially warmer future climates, despite slightly lower survival at the early stages following transplant (Fig. 7a). If the average temperature at these sites does get warmer in the future, central MN seedlings may increase in growth more rapidly than the northern seedlings (Fig. 8b). At cool sites, this will lead to equal growth of both seed sources and at warm sites the central seed zone may surpass the maximum growth of local, northern seedlings which appear to be adapted to cool climates (Rehfeldt *et al* 1999, Rehfeldt *et al* 2002). The slightly lower survival of central MN seedlings may not mean this seed source will be less fit over time at these sites, especially if warmer temperatures allow these remaining seedlings to be more productive in the future.

In contrast, northern MN and Michigan bare-root seedlings had large differences in growth as well as survival. Michigan seedlings, from the warmest and wettest environment, were much less likely to survive than the local northern MN seedlings (Fig 7b). However, in 2014, the Michigan seedlings also showed a much greater growth response than the local northern MN seedlings (Fig. 8c). Taken together, this suggests that Michigan seedlings are locally adapted to their home site and were not able to adjust their growth response in order to match the cooler and drier environment in northeastern MN. Other species show similar patterns and individuals adapted to wetter environments have larger growth but lower survival when planted in common gardens (O'Brian *et al* 2007).

The importance of soil characteristics, such as earthworm invasion level, pH, and B-horizon thickness (Soil PC2), for the onset of new growth could have long-term consequences for the restoration plantings, as well (Fig. 6, Table 4,5). Later bud burst at earthworm invaded sites may lead to lower productivity or a larger chance of fall frost damage, and could result from drier surface soil due to reduced organic layer thickness (Laube *et al* 2014). Slower shoot development of Michigan seedlings may also contribute to the exaggerated growth response and increased mortality (Fig. 11). This response suggests that Michigan seedlings required a higher temperature sum, humidity level and perhaps a longer photoperiod for the development of new growth (Laube *et al.* 2014). This is further evidenced by the lack of trend in 2015 growth for these two seed sources. Although slower development and larger growth is advantageous in the warm and moist conditions of Lower Michigan, this response was not optimal in northeast MN. The larger growth of the Michigan seedlings in 2014 appears to be unsustainable, leading to increased mortality and equal growth in 2015. Michigan seedlings may have been moved too far from their source climate envelope and short range movement of populations is likely a more productive method for assisting species migration.

Additionally, the two years that this study took place had particularly cool and wet growing seasons. In 2014 the average temperature was 0.5-1.2 °C below the thirty year average and the precipitation total was 46-112 mm above average. 2015 was not as extreme and temperatures were equal to the thirty year normal while the precipitation levels were 15-47 mm above the thirty year normal. The response of different seed sources will be dependent on year to year variation in weather at these sites. This is reflected in the differences in growth trends for northern and central MN seedlings in 2014 and 2015. In the cool and wet season of 2014, local seedlings increased in growth with increasing MI more quickly than central MN seedlings (Fig. 8a). However, in the warmer and drier 2015 growing season, the central MN seedlings grew

larger that northern MN seedlings at the warm and dry sites, but quickly declined in cooler temperatures (Fig. 8b). Alternatively, growth responses may have differed between years because these seedlings were recent transplants and 2015 was the first full year for these seedlings in the restoration sites. Nonetheless, seed sources differed significantly in growth response over the range of climates tested here in both years measured.

In the future, comparing the growth response in average years for temperature and precipitation to more extreme years will give insight into the plasticity of these populations, as well as the impact of local weather on the competitive dynamics of white pine seedlings from different populations. If the years following transplant are cool and wet then the local seedlings may grow larger and establish themselves in the canopy, especially at the coolest sites tested. However, if those years are warm, more southern seedlings may outcompete local propagules. The outcomes of this assisted migration effort may well depend on the variation in weather and climate, and therefore growth response, across the region.  Future monitoring of these plots and similar experimental plantings should continue to parse out the effects of temperature and moisture on tree fitness, productivity and establishment for different populations of the same species. Further research that pairs population genomics with common garden experiments such as the one done here will also identify divergence in these populations that leads to phenotypic differentiation across the species range. Understanding local adaptation and climate response across the entire species range will allow managers to select planting stock that will be well adapted to the climates predicted for the future.

**Conclusions**

The results of this assisted migration restoration experiment suggest that short range movement of populations along natural dispersal and migration pathways is the best way to balance survival and local adaptations in growth and phenology. Northern and central MN seedlings both survived well at a variety of sites with differing climate and light availability. Meanwhile, local adaptation was evident from the larger growth of northern MN seedlings at cool sites and slightly higher overall survival. Bare-root Michigan seedlings also showed signs of adaptation to their warm and moist home environment, and grew significantly more than bare-root northern MN seedlings, but with diminished survival. This data serves as a basis for a long-term experiment in population ecology and forest tree assisted migration. Moving forward, researchers should aim to study annual growth patterns within and between populations across a range of climates to understand which are most sensitive to climate or might be the most likely to adapt.

While land managers know the potential threats of climate to forest health, most have not taken any real action to mitigate these potential effects (Milad *et al.* 2013, Keenan 2015), despite the fact that trees living today will most likely be alive in 2100, which is as far as most climate models predict. Due to the long life history of forest species we must act in the present to assist forests in adapting to climate change (Spittlehouse and Stewart 2003). The larger effort that this work studies experimentally is also an example of how climate change science can be applied by forest managers to help mitigate the predicted loss of productivity in forest ecosystems. With uncertainty looming in the future, using multiple seed sources and species during forest restoration will increase the phenotypic and functional diversity of the seedlings present. Diverse seedlings may reduce the predicted decline of forests due to drought and heat stress (Allen *et al.* 2010) as well as fire, insects and pathogens, which will all alter their behavior and impacts with climate change (Dale *et al.* 2001). Short-distance, in-range assisted migration can be seen as a bet hedging strategy for ensuring that the fittest seed sources are present in recovering forest ecosystems following natural or anthropogenic disturbance. Although intraspecific hybridization is 20 years away for these white pine seedlings (at minimum), mixing of these populations could lead to novel phenotypes and increase the ability of this species to adapt in a changing climate. Forest scientists should continue to work with land managers to document and share the results of both experimental field trials and silviculture prescriptions as well as laboratory and greenhouse studies.
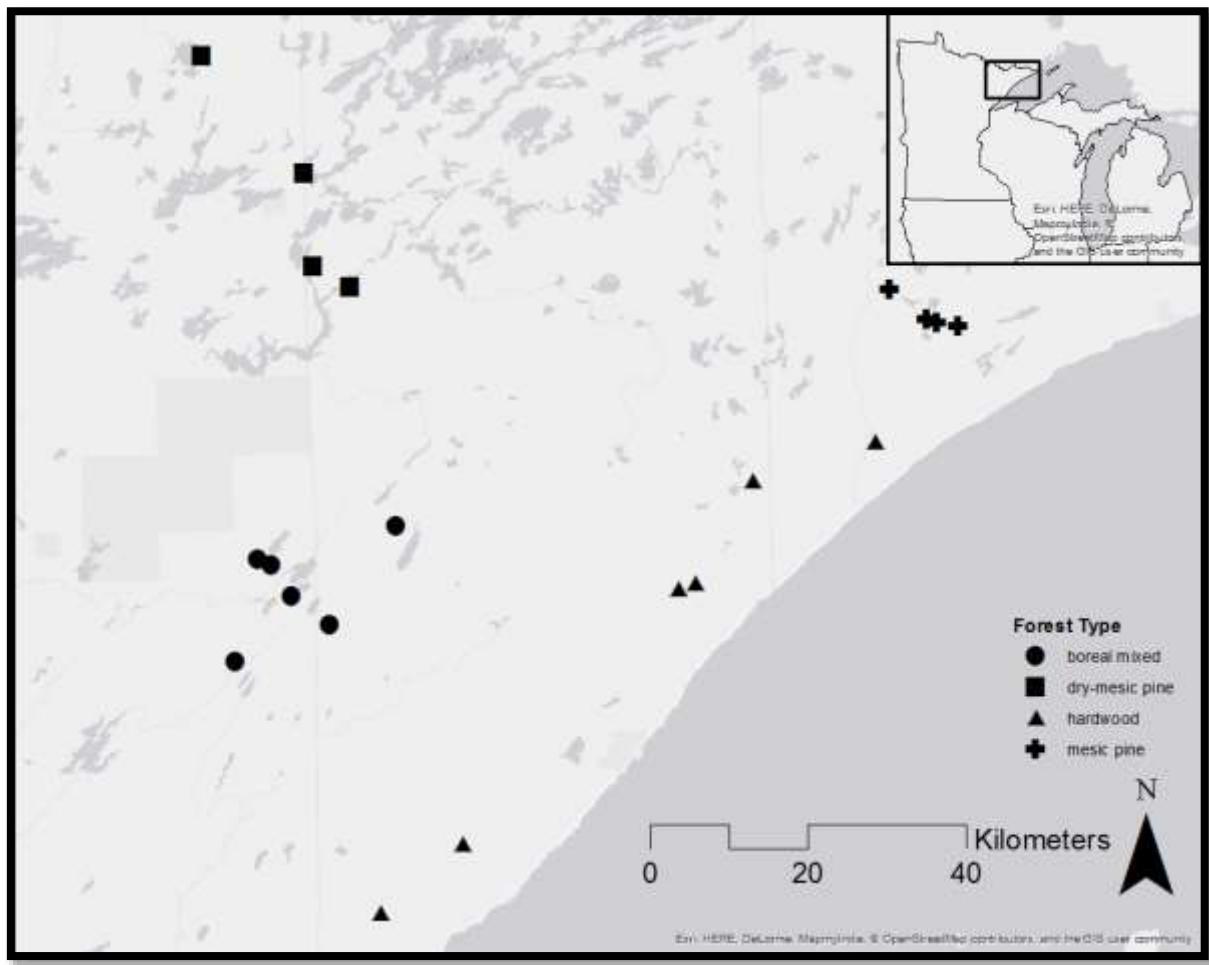
**Figure 4.** Map of reasearch sites showing the forest type for each site. The inset map shows the location of the study area in the upper Midwest, USA.
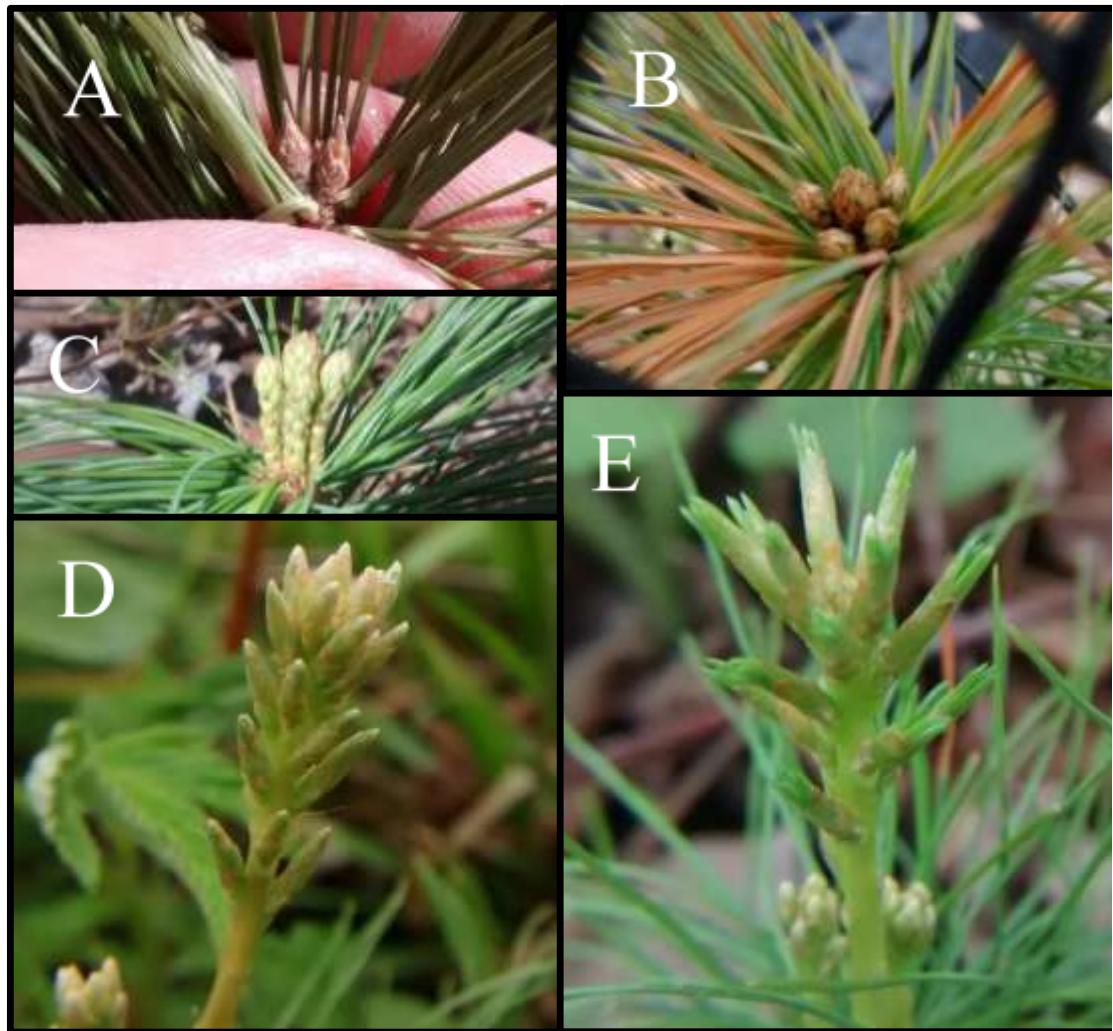
**Figure 5**. Phenology stages used for spring phenology checks. A) Stage 0 – Bud Dormant, No new growth visible. Bud very tightly bound. B) Stage 1 – Bud Swollen, new growth visible between neighboring scales. Bud still tightly bound. C) Stage 2 – Bud Broken, Separation is visible between neighboring scales. Bud not tightly bound. D) Stage 3 – Brachyblasts emerge from shoot (base of each fascicle). Shoot clearly elongated and visible. Discernable needles have not emerged. E) Stage 4 – Immature needles emerge from brachyblasts.
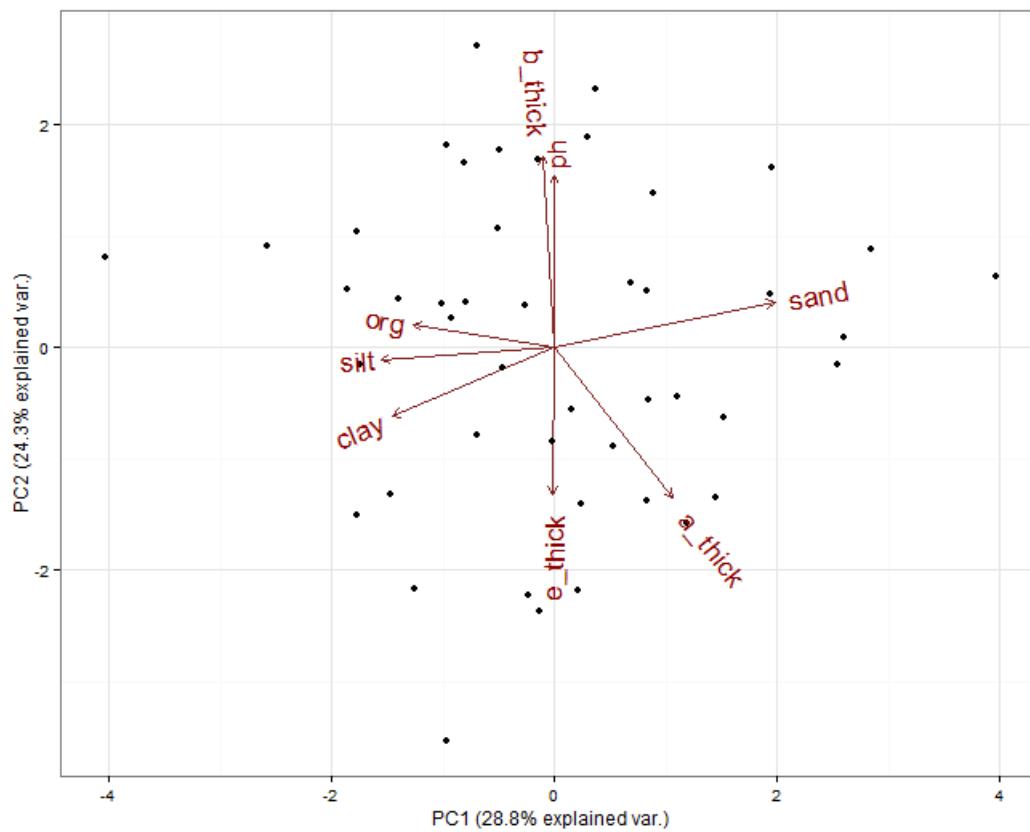
**Figure 6.** Scores and significantly associated from the principle components analysis (PCA) of soil variables. Soil PCA scores from these two components were used in the analysis to summarize soil characteristics. Arrows show the direction and strength of the relationship between predictor variables and PC scores.
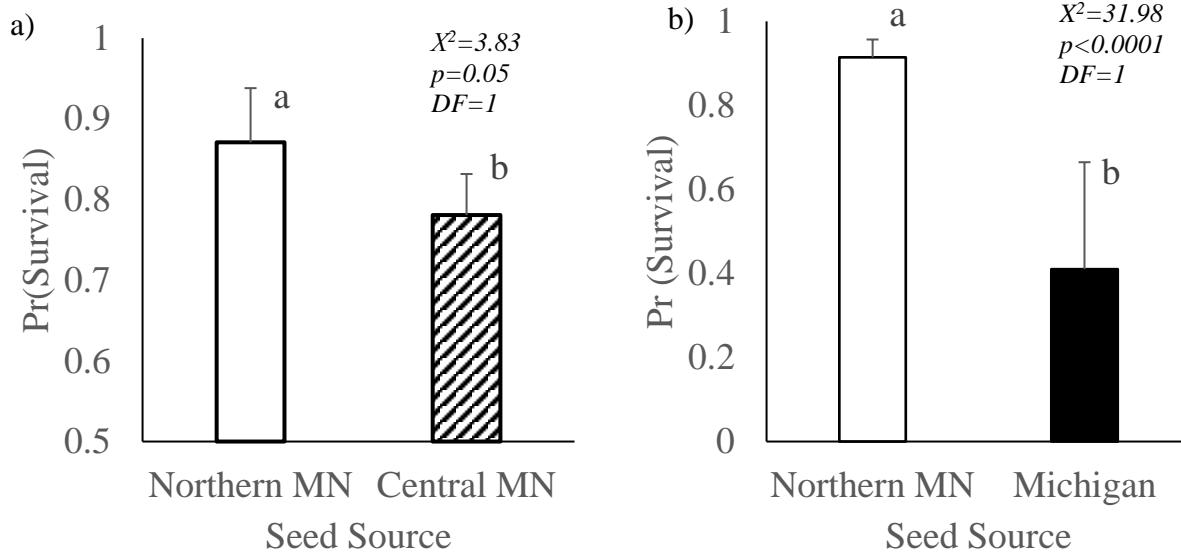
**Figure 7**. Survival probabilities for a) northern and central MN and b) northern MN and Michigan white pine seedlings two years following transplant. Each bar represents the mean survival probability of each seed source in 28 planting blocks at 14 sites in northeast MN. Error bars show a 95% confidence interval around each mean. Bars labeled with the same letter are statistically equal at the $\alpha$=0.05 level.
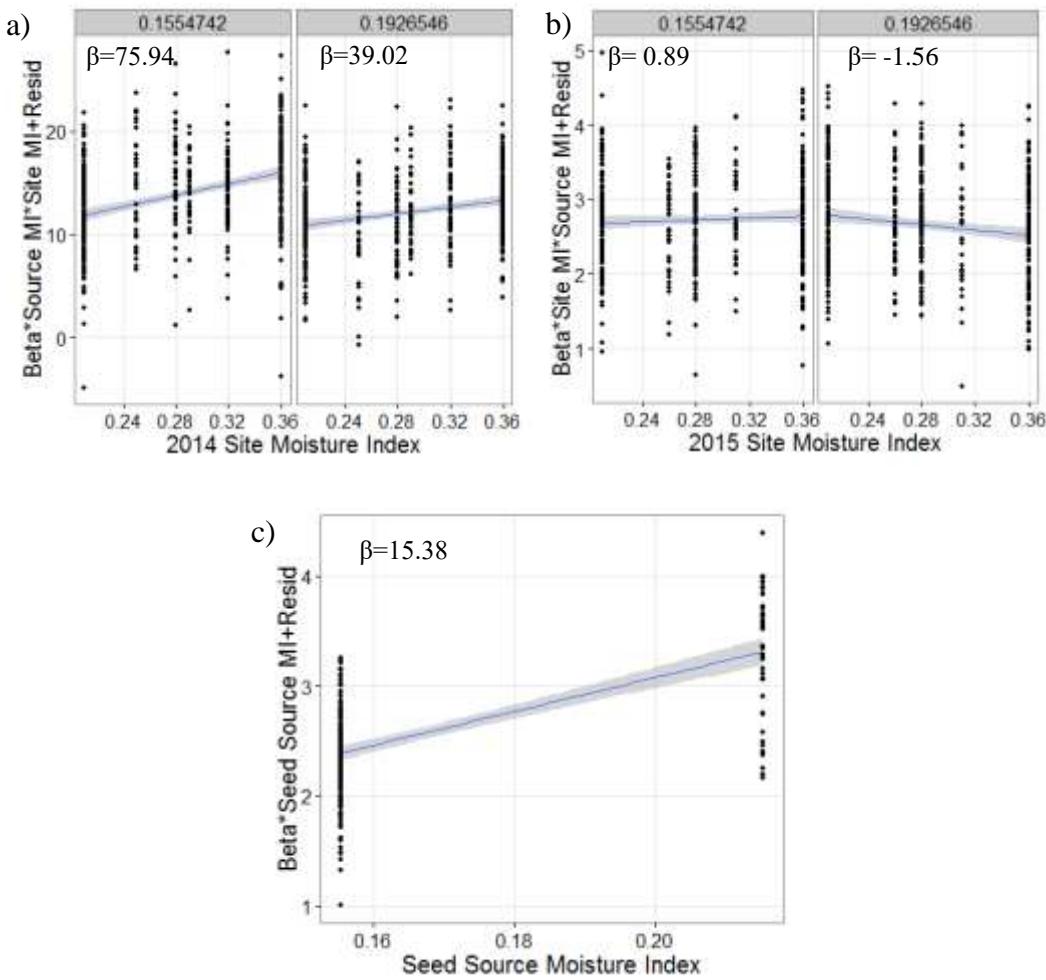
**Figure 8.** Partial regression plots for moisture index (MI) at the seed source (individual panels) and MI at the planting site (along the y axis) on a) 2014 and b) 2015 growth rates of northern and central MN seedlings planted at 14 sites. In The left panel in each figure represents the growth response of northern MN (MN 104, MI=0.155) seedlings and the right panel represents the response of central MN (MN 105, MI=0.193) seedlings. The bottom graph (c) shows the relationship between 2014 growth and seed source moisture index for northern MN and Michigan seedlings planted at six sites. Trend lines represent the least squares regression of the data with a 95% confidence interval shown around each prediction.
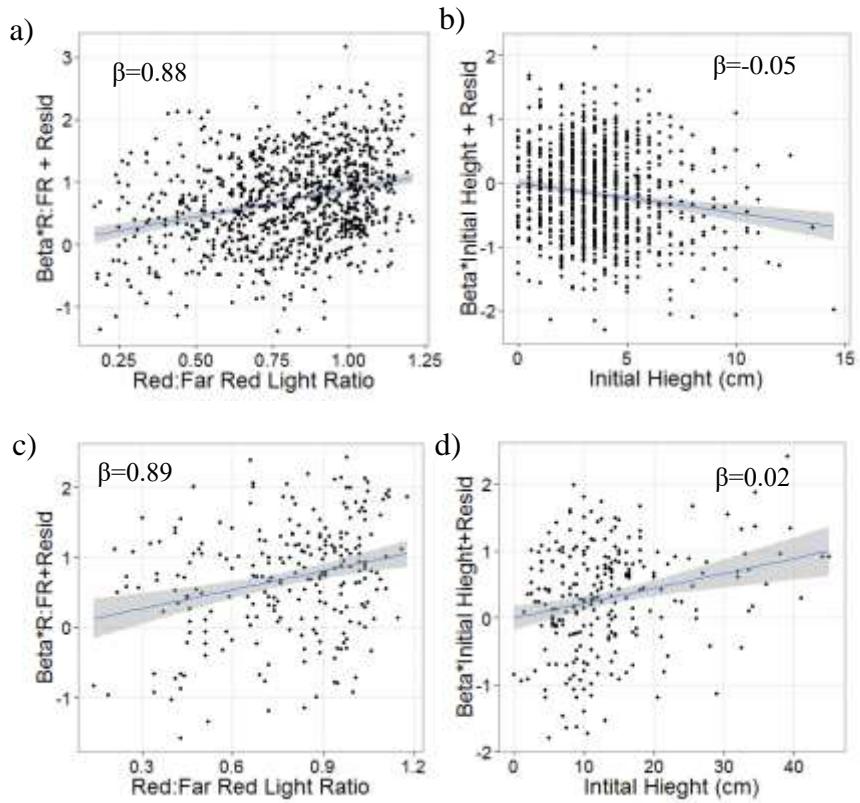
**Figure 9.** Partial regression plots for significant covariates, Red: Far Red light ratio and Initial Height, on 2015 growth rates of northern and central MN white pine seedlings planted at 14 sites (a,b) and bare-root northern MN and Michigan seedlings planted at 6 sites (c,d). Trend lines represent the least squares regression of the data with a 95% confidence interval shown around each mean. These graphs are representative of the effect of these covariates across models and years for each set of planting sites.
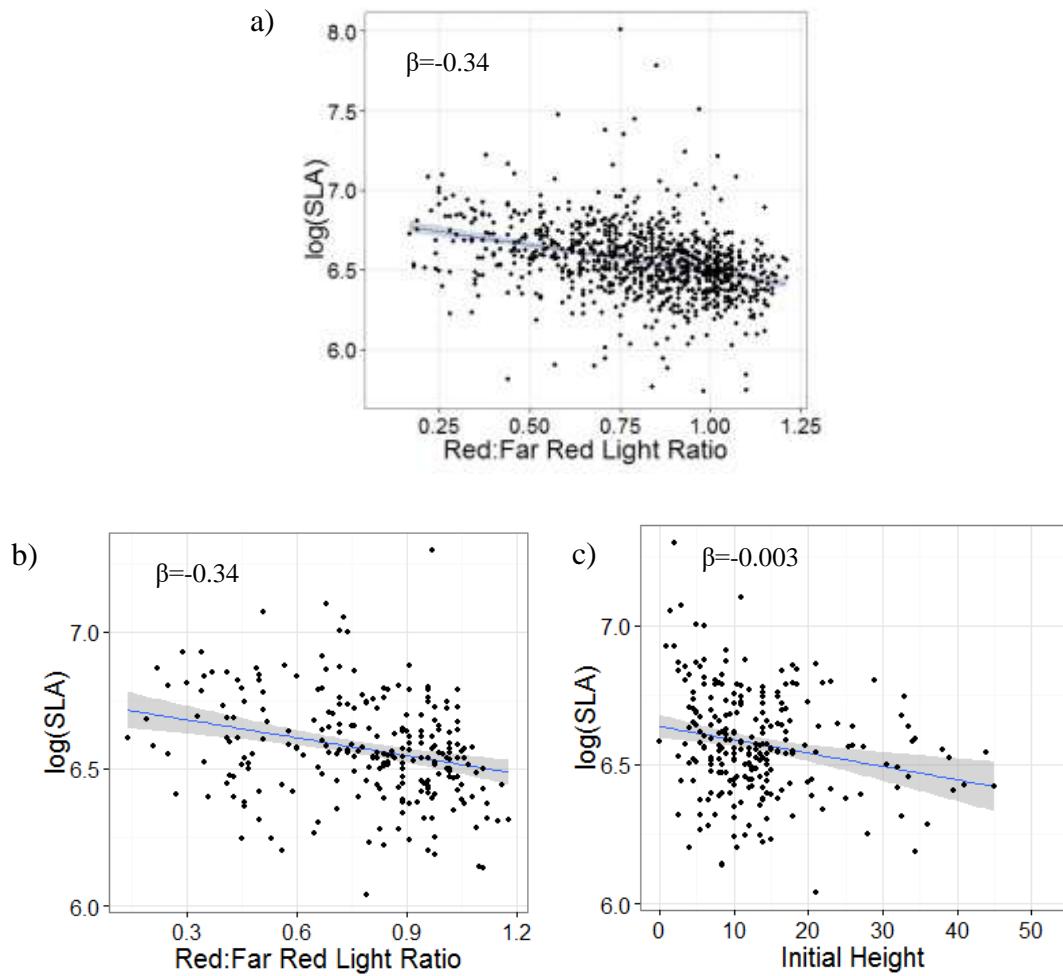
**Figure 10.** Effect of Red: Far Red light ratio and Initial Height on log (2015 SLA (cm² g⁻¹ )) of northern and central MN white pine seedlings planted at 14 sites (a) and northern MN and Michigan seedlings planted at 6 sites (b,c). Each data point represents an individual pine seedling. Trend lines represent the least squares regression of the data with a 95% confidence interval shown around each mean.
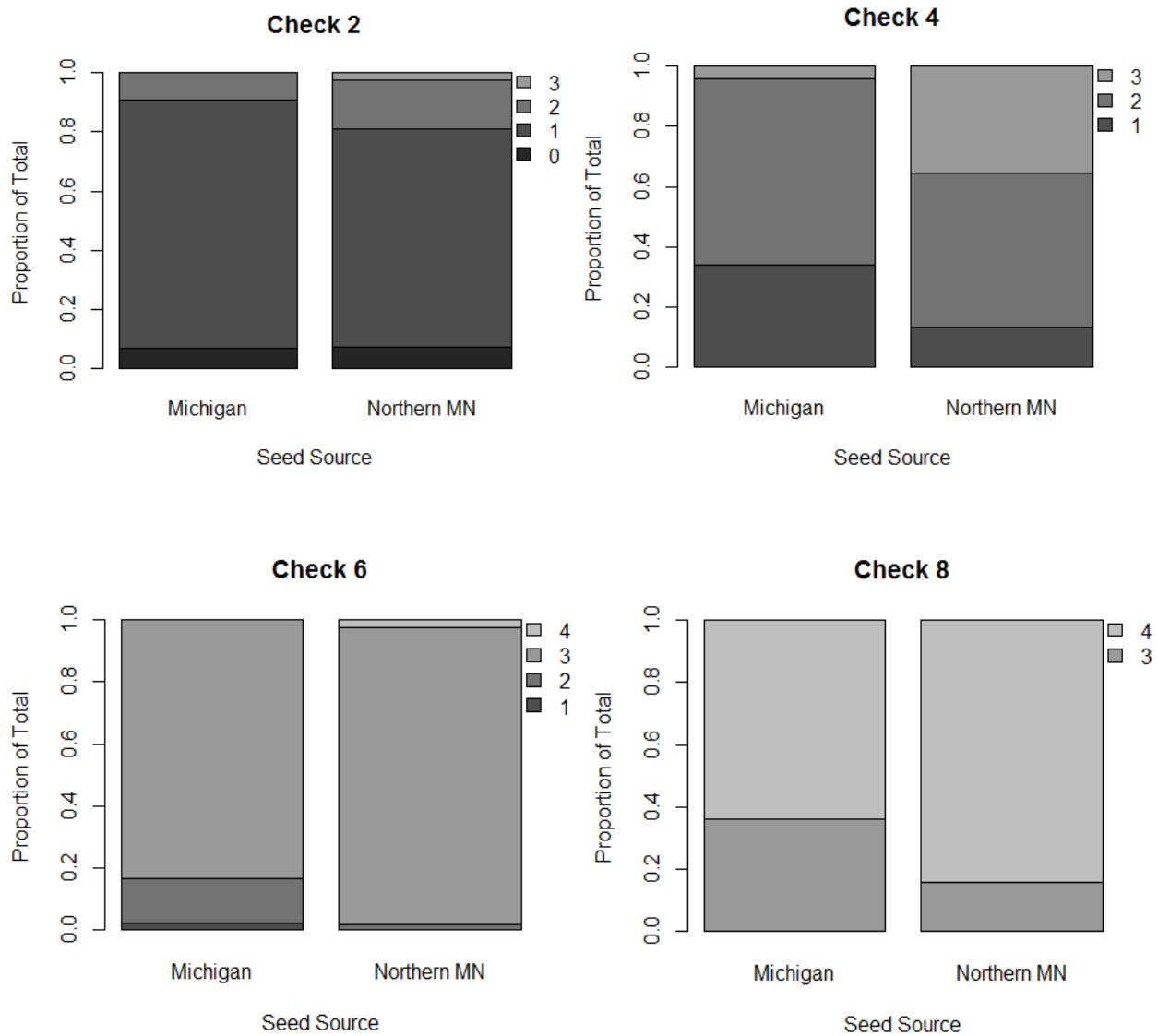
**Figure 11.** Proportions plots for phenology scores at 6 sites planted with bare-root Michigan and northern MN seedlings. Each bar represents the proportion of individuals assigned to each phenology stage during full spring phenology checks. Checks 4, 6, and 8 are significantly different (p<0.001). Lighter gray indicates layter stages, as indicated by the legend associated with each figure.

**Table 1.** Site attributes and seedlings totals. Climate variables are the average from the 2014 and 2015 growing seasons. MN-DNR is the Minnesota Department of Natural resources. SNF sites are under the ownership if the USDA Superior National Forest. Moisture index is a ratio of precipitation to GDD (Growing Degree Days). MN 104 seedlings are from MN-DNR seed zone 104 near Bemidji in north central MN. MN 105 seedlings are from MN-DNR seed zone 105 near Hill City in central MN. MI seedlings are from the lower peninsula of Michigan, USA near Kalkaska and Baldwin, MI in the Manistee National Forest. MN104 and 105 seedlings were planted with soil and MN 104 (br) and MI (br) seedlings were planted with bare roots (br).

| Site | Owner | Forest Type | GDD >5°C | Annual Precipitation (mm) | Moisture Index | MN104 | MN105 | MI (br) | MN104 (br) | Seedling Total |
|------|-------|-------------|----------|---------------------------|----------------|-------|-------|---------|-----------|----------------|
| Big Lake | MN-DNR | boreal mixed | 2501.5 | 745 | 0.30 | 40 | 40 | 0 | 0 | 80 |
| No More Stealing | SNF | boreal mixed | 2806.5 | 761 | 0.27 | 40 | 40 | 0 | 0 | 80 |
| Remodeled | St. Louis County | boreal mixed | 2570.5 | 675 | 0.26 | 0 | 0 | 40 | 40 | 80 |
| Ridgepole | SNF | boreal mixed | 2806.5 | 761 | 0.27 | 40 | 40 | 0 | 0 | 80 |
| Round Beaver | SNF | boreal mixed | 2885 | 861 | 0.30 | 40 | 40 | 0 | 0 | 80 |
| Smackdown | St. Louis County | boreal mixed | 2501.5 | 745 | 0.30 | 0 | 0 | 40 | 40 | 80 |
| Burgo | SNF | dry-mesic pine | 3074.5 | 650 | 0.21 | 40 | 40 | 0 | 0 | 80 |
| Echo Trail Big Lake | SNF | dry-mesic pine | 2885 | 861 | 0.30 | 40 | 40 | 0 | 0 | 80 |
| Kawishiwi | SNF | dry-mesic pine | 2239.5 | 799 | 0.36 | 40 | 40 | 0 | 0 | 80 |
| White Iron | SNF | dry-mesic pine | 3074.5 | 650 | 0.21 | 40 | 40 | 0 | 0 | 80 |
| DNR Sawbill | MN-DNR | hardwood | 3074.5 | 650 | 0.21 | 40 | 40 | 0 | 0 | 80 |
| Gopher Gaps | Lake County | hardwood | 2885 | 861 | 0.30 | 0 | 0 | 40 | 40 | 80 |
| Hare Lake | MN-DNR | hardwood | 3074.5 | 650 | 0.21 | 40 | 40 | 0 | 0 | 80 |
| Old Chevy Road | Lake County | hardwood | 2962.5 | 869 | 0.29 | 0 | 0 | 40 | 40 | 80 |
| Reeves Road | Lake County | hardwood | 2806.5 | 761 | 0.27 | 0 | 0 | 40 | 40 | 80 |
| VDM | Lake County | hardwood | 2885 | 861 | 0.30 | 0 | 0 | 40 | 40 | 80 |
| Clara Caribou | SNF | mesic pine | 2239.5 | 799 | 0.36 | 40 | 40 | 0 | 0 | 80 |
| Clara Pit | SNF | mesic pine | 2239.5 | 799 | 0.36 | 40 | 40 | 0 | 0 | 80 |
| Clara Steep | SNF | mesic pine | 2239.5 | 799 | 0.36 | 40 | 40 | 0 | 0 | 80 |
| NEPU4 | SNF | mesic pine | 2962.5 | 869 | 0.29 | 40 | 40 | 0 | 0 | 80 |
| Total | | | | | | 560 | 560 | 240 | 240 | 1600 |

**Table 2.** Climate data for seed sources. Annual average temperature, January Minimum, and Annual precipitation are 100 year averages (1915-2015). GDD>°5C is the average for growing seasons from 1985-2015. Moisture index is the ratio of precipitation to GDD.

| Seed Source | Annual Average Temperature (°C) | Average January Minimum Temperature (°C) | GDD>5°C | Annual Precipitation (mm) | Moisture Index |
|---|---|---|---|---|---|
| Northern Minnesota (MN 104) | 3.57 | -35.76 | 3279 | 510 | 0.15 |
| Central Minnesota (MN 105) | 4.56 | -35.39 | 3413 | 657 | 0.19 |
| Michigan | 6.28 | -27.97 | 3560 | 766 | 0.21 |

**Table 3.** Analysis of deviance for generalized linear models of seedlings survival. The model on the left represents data from 14 sites in four forest sites planted with northern and central MN seedlings. Model on the right represents data from 6 sites in two forest types planted with northern MN and Michigan seedlings. F values marked with a single asterisk (*) are significant at the α=0.05 level. . F values marked with a double asterisk (**) are significant at the α=0.01 level. . F values marked with a triple asterisk (***) are significant at the α=0.001 level.

|  | *Northern and Central MN* | | *Northern MN and Michigan* | |
|---|---|---|---|---|
|  | DF | Chi Squared | DF | Chi Squared |
| **Forest Type** | 3 | 3.14 | 1 | 0.28 |
| **Seed Source** | 1 | 3.83* | 1 | 31.98*** |
| **Red to Far-red Ratio** | 1 | 21.87*** | 1 | 8.50** |
| **Initial Hieght** | 1 | 15.08*** | 1 | 40.34*** |

**Table 4.** Results of mixed effects linear models for survival and mixed effects ANCOVA for growth and morphology data of white pine seedlings at 20 sites in NE Minnesota. The models in the top row represent data from 14 sites in four forest sites planted with northern and central MN seedlings. The models in the bottom row represent data from 6 sites in two forest types planted with northern MN and Michigan seedlings. F values marked with a single asterisk (*) are significant at the α=0.05 level. . F values marked with a double asterisk (**) are significant at the α=0.01 level. . F values marked with a triple asterisk (***) are significant at the α=0.001 level.

| | Northern and Central MN | | | Northern MN and Michigan | | |
|---|---|---|---|---|---|---|
| *2014 Height Growth* | Beta | DF | F | Beta | DF | F |
| **Site MI** | 75.94 | 1, 566 | 11.52** | 8.74 | 1, 3 | 2.04 |
| **Source MI** | 39.20 | 1, 929 | 1.19 | 15.38 | 1, 232 | 116.7*** |
| **Site MI x Source MI** | -311.54 | 1, 928 | 6.4** | | *NS* | |
| **R:FR light ratio** | 4.46 | 1, 940 | 38.08*** | 0.36 | 1, 167 | 4.24** |
| **Soil PC2** | | *NS* | | | *NS* | |
| **H$_i$** | -0.61 | 1, 935 | 73.4*** | -0.04 | 1, 232 | 67.1*** |
| *Sqrt(2015 Growth)* | | | | | | |
| **Site MI** | 10.70 | 1, 199 | 6.94** | | *NS* | |
| **Source MI** | 16.42 | 1, 930 | 7.63** | | *NS* | |
| **Site MI x Source MI** | -64.82 | 1, 929 | 9.87** | | *NS* | |
| **R:FR light ratio** | 0.88 | 1, 942 | 54.58*** | 0.89 | 1, 183 | 9.03** |
| **Soil PC2** | | *NS* | | | *NS* | |
| **H$_i$** | -0.05 | 1, 917 | 16.1*** | 0.02 | 1, 242 | 11.0** |
| *2015 Diameter* | | | | | | |
| **Site MI** | 1.11 | 1, 12 | 2.64 | | *NS* | |
| **Source MI** | -1.62 | 1, 960 | 6.18* | | *NS* | |
| **Site MI x Source MI** | | *NS* | | | *NS* | |
| **R:FR light ratio** | 0.37 | 1, 892 | 34.32*** | 0.55 | 1, 215 | 19.63*** |
| **Soil PC2** | | *NS* | | | *NS* | |
| **H$_i$** | | *NS* | | 0.005 | 1, 236 | 3.64* |
| *Sqrt(Fascicle #)* | | | | | | |
| **Site MI** | 139.88 | 1, 694 | 9.31** | | *NS* | |
| **Source MI** | 222.33 | 1, 894 | 9.41** | | *NS* | |
| **Site MI x Source MI** | -828.92 | 1, 893 | 10.62** | | *NS* | |
| **R:FR light ratio** | 11.23 | 1, 863 | 62.04*** | 1.77 | 1, 185 | 32.43*** |
| **Soil PC2** | | *NS* | | -0.27 | 1, 25 | 12.79** |
| **H$_i$** | -0.43 | 1, 871 | 9.21** | | *NS* | |
| *log(SLA)* | | | | | | |
| **Site MI** | | *NS* | | | *NS* | |
| **Source MI** | | *NS* | | | *NS* | |
| **Site MI x Source MI** | | *NS* | | | *NS* | |
| **R:FR light ratio** | -0.34 | 1, 950 | 122.15*** | -0.34 | 1, 216 | 32.50*** |
| **Soil PC2** | | *NS* | | | *NS* | |
| **H$_i$** | | *NS* | | -0.003 | 1, 238 | 5.90** |

**Table 5.** Results of mixed effects repeated measures ANOVA for phenology data of white pine seedlings at 20 sites in NE Minnesota. The models in the left hand column represent data from 14 sites in four forest sites planted with northern and central MN seedlings. The models in the right hand column represent data from 6 sites in two forest types planted with northern MN and Michigan seedlings. F values marked with a single asterisk (*) are significant at the α=0.05 level. . F values marked with a double asterisk (**) are significant at the α=0.01 level. . F values marked with a triple asterisk (***) are significant at the α=0.001 level.

| | Northern and central MN | | | Michigan and northern MN | | |
|---|---|---|---|---|---|---|
| | Num DF | Den DF | F | Num DF | Den DF | F |
| Source MI | 1 | 824 | 1.52 | 1 | 214 | 19.67*** |
| Site MI | 1 | 824 | 3.47 | 1 | 214 | 2.55 |
| Source MI x Site MI | 1 | 824 | 3.09 | 1 | 214 | 0.82 |
| % Open Canopy | 1 | 824 | 2.53 | 1 | 214 | 0.24 |
| Red: Far Red Light Ratio | 1 | 824 | 8.50** | 1 | 214 | 0.18 |
| Soil PC 1 | 1 | 824 | 5.76* | 1 | 214 | 0.11 |
| Soil PC 2 | 1 | 824 | 0.18 | 1 | 214 | 23.31*** |
| Avg. Woody Stem Density | 1 | 824 | 2.34 | 1 | 214 | 15.76*** |
| % Vegetation Cover | 1 | 824 | 9.05** | 1 | 214 | 3.13 |
| Worm Assessment | 1 | 824 | 14.57*** | 1 | 214 | 1.22 |
| Intitial Height | 1 | 824 | 0.45 | 1 | 214 | 0.16 |
| Time | 3 | 822 | 9.97*** | 3 | 212 | 2.37 |
| Time x Source MI | 3 | 822 | 1.57 | 3 | 212 | 4.25** |
| Time x Site MI | 3 | 822 | 2.09 | 3 | 212 | 4.23** |
| Time x Source MI x Site MI | 3 | 822 | 3.02* | 3 | 212 | 1.00 |
| Time x % Open Canopy | 3 | 822 | 1.18 | 3 | 212 | 0.12 |
| Time x Red: Far Red Light Ratio | 3 | 822 | 1.68 | 3 | 212 | 2.05 |
| Time x Soil PC 1 | 3 | 822 | 1.79 | 3 | 212 | 1.71 |
| Time x Soil PC 2 | 3 | 822 | 0.03 | 3 | 212 | 6.73*** |
| Time x Avg. Woody Stem Density | 3 | 822 | 7.35*** | 3 | 212 | 1.28 |
| Time x % Vegetation Cover | 3 | 822 | 2.42 | 3 | 212 | 0.66 |
| Time x Worm Assessment | 3 | 822 | 1.79 | 3 | 212 | 7.76*** |
| Time x Intitial Height | 3 | 822 | 0.52 | 3 | 212 | 1.41 |

# Interactions between invasion and disturbance: Evidence from earthworms in transitional temperate-boreal forests

## Introduction

### *Earthworm Impacts*

Biological invasions are considered by many to be a major contributor to ecological change across global systems (Mack *et al* 2000, Crooks 2002). As humans have broken down barriers to long-distance dispersal of organisms, many have been able to colonize and establish themselves in new habitats far beyond their native range and inherent dispersal limitations. The capacity of these species to establish is dependent on the frequency of introduction (propagule size and number), local and regional land-use, evolutionary potential of the invasive population, and the resilience of that system to invasion (i.e. competitors, resource limitation) (Lockwood *et al* 2005, Foxcroft *et al* 2010, Whitney and Gabler 2008, Kennedy *et al* 2002). Invasive species cause change in these systems not only when they are able to outcompete native species, but also when they accompany shifts in climate and native species communities or colonize previously unoccupied habitat space (Frelich *et al* 2012). This alteration in community structure and material flow in a system can facilitate the establishment of other invasive species, sometimes known as "invasional meltdown", or an alternate assemblage of native species (O'Dowd *et al* 2003, Richardson and Pysek 2006, Frelich *et al* 2012).

In North America, non-native European earthworms have been introduced to variable extents throughout the temperate and mixed forests east of the Great Plains region (Holdsworth *et al* 2007a). Due to recent glaciation, no native earthworms or comparable decomposers were present in these forests prior to introduction (Frelich *et al* 2006). This lack of large detritivores in the forest floor led to large build ups of organic matter at the soil surface in many broad-leaved forests including those dominated by maple (*Acer* spp.), oak (*Quercus* spp.), aspen (*Populus* spp.) and birch (*Betula* spp.). When earthworms are introduced they feed on the organic matter in the forest floor, breaking down leaf litter and speeding the rate of decomposition. The extent of damage to the forest floor depends mostly on the functional groups of earthworms present because these groups occupy different areas of the forest floor and soil (Hale *et al* 2005a, Hale *et al* 2005b). Epigeic species live solely in the organic layer of forests are often the first to invade. These small worms feed on fresh leaf litter, humus, and fungi at the soil surface and do not enter the mineral soil or have permanent burrows. Endogeic species migrate between the humus layer and the mineral soil, mixing these layers. Anecic species are the last functional group. They tend to build permanent burrows that can be identified by the presence of middens at the opening of

the burrow. These species come to the surface to feed on leaf material, pulling the organic matter from the soil surface as deep as 40 cm into the mineral soil and are often the last group to invade a site.

Combined, these earthworms cause dramatic changes in soil characteristics, plant communities and invertebrate assemblages. In a Minnesota aspen forest, an increase in earthworm abundance over 14 years caused nearly complete mixture of the organic layer and the upper layer of the mineral soil, forming a nutrient rich A-horizon at the mineral soil surface. This was coupled with the movement of N and C stores from the soil surface to the upper layers of the mineral soil (Alban and Berry 1994). Organic layer thickness and N and P availability also decreased with increasing earthworm biomass in previously earthworm-free northern hardwood forests in Minnesota (Hale *et al* 2005b)  In New York maple forests, earthworms caused a 28% decrease in soil C but did not influence N movement at a previously uninvaded site. In contrast, earthworms caused no net loss of C and accelerated leaching of $NO_3^-$ from the soil at a previously cultivated site. Together, these results suggest that the impacts of earthworms are not dependent solely on the species present, but also the land use history of the site (Bohlen *et al* 2004).  Earthworm invasion not only directly impacts soil structure and quality, but also has direct and indirect effects on the local biota.

Earthworms impact organisms of diverse size and function. Bacterial biomass decreased in the organic layer and increased at the surface of the soil following earthworm invasion. Invaded sites had lower abundance and density of fungal species in the soil and mychorizzal associations between plants and fungi were diminished (McLean *et al* 2006). Earthworms also increase bulk density of soil and alter seed bed conditions for vascular plants (Frelich *et al* 2006). Many forest herbs decline in the presence of earthworms and many invaded forest sites are dominated by a single understory species, *Carex pennsylvanica* (Hale *et al* 2006, Holdsworth *et al* 2007b). This reduction in abundance and diversity of many life forms may make earthworm invaded sites more prone to future invasions and less resilient to environmental change.

*Interactions with Climate and Disturbance*

Climate change and other disturbances to ecosystems interact with biotic invasion by altering the suitability of habitat (i.e. deer replacing moose in temperate-boreal transitions zones as warming occurs) and availability of resources (Frelich *et al* 2012). Climate change may facilitate the expansion of current invasive species and the establishment of new invaders (Didham *et al* 2005, Hellman *et al* 2008, Walther *et al* 2009). In new climates, the impact of invasive species may also increase while control measures become less effective (Hellman *et al* 2008), however the migration of species and land use are not frequently used in models of species

expansion (Thuiller *et al* 2008). In forest ecosystems, disturbances such as fire and wind-throw are expected to increase in frequency and intensity with climate change (Flannigan *et al* 2000, Dale *et al* 2001). Disturbed ecosystems are more prone to invasion because they are in transition and native species are also attempting to colonize and establish in the newly available space (Dukes and Mooney 1999, Didham *et al* 2005). Humans disturb forest ecosystems for agriculture and forest products, often bringing along with them non-native species of plants and animals. This increases the propagule pressure of non-natives and the number of potential invaders.

In northeastern Minnesota, USA, where earthworm invasion is well documented, forest ecosystems are predicted to shift in composition over the coming century with climate change. An increase in mesic hardwood species, like red maple (*Acer rubrum*) will be accompanied by a decline in boreal species like quaking aspen (*Populus tremuliodes*), balsam fir (*Abies balsamea*), and white spruce (*Picea glauca*) (Pastor and Post 1988, Iverson and Prasad 1998, Ravenscroft *et al* 2012). Other species, such as white pine (*Pinus strobus*) and red oak (*Quercus rubra*) are also predicted to increase (Duveneck *et al* 2014). However, low populations due to over harvest and excessive white-tailed deer browse may inhibit the regeneration and population expansion of these species; allowing boreal species to persist on the landscape (Frelich *et al* 2012). Furthermore, in order for predicted shifts to occur on the appropriate time scale, well-adapted species must now begin to colonize disturbed areas (Ravenscroft *et al* 2012). This requires not only a source population, but also favorable conditions on the forest floor where seeds can germinate and compete for water and nutrients. The direct impacts of earthworms on the seed bed soil structure, and fine roots could potentially play a role in determining the future composition of Minnesota forests. Therefore, we must understand if and how climate change and anthropogenic disturbance interact with earthworm invasion.

*Current Study*

Here, the impacts and abundance of non-native earthworms are examined both inside of recently logged sites and in the nearby intact forest in northeastern Minnesota, USA. Earthworm impact and abundance should be highest in the undisturbed forest for three reasons: 1) The canopy filters out UV radiation that is harmful to earthworms, 2) increased risk of desiccation occurs where the canopy is not present to reduce evaporation rates, and 3) the input of deciduous tree litter, a preferred food source, is highest under the canopy of the undisturbed forest. Increased UV exposure and lower litter input in the logged areas may lead to a reduced earthworm population and lower amounts of damage to the organic layer over time. Moreover, increased desiccation of the forest floor may lead to slower rates of decomposition as water begins to limit bacterial activity, further reducing the impacts of the earthworms

**Methods**

*Site Locations*

In order to assess the impact and abundance of non-native earthworms in both disturbed and intact forest, both a qualitative rapid assessment technique as well as qualitative extraction of earthworms was carried out at 20 recently logged study sites in northeastern Minnesota, USA. These sites were located in the four most common and heavily managed forest types located within the study area (Fig. 4, Table 6). Hardwood sites (Minnesota Forest type MHn35, MHn45) were dominated by sugar maple or red oak, with lesser amounts of yellow birch, basswood and red maple (MN-DNR 2014). Boreal Mixed forest (Minnesota forest type FDn43) were dominated by quaking aspen, white birch, and balsam fir, and with lesser amounts of white spruce, white pine, and red maple. Mesic pine (Minnesota forest type FDn43) sites were dominated by quaking aspen, paper birch, red pine, or white spruce with variable amounts of white cedar, white pine and balsam fir (MN-DNR 2014). Dry-Mesic Pine sites (Minnesota forest type FDn33) were dominated by red and white pine with balsam fir and white birch often in the understory (MN-DNR 2014). Cuts varied in size from ~0.1 hectares to over 50 hectares and logging technique varied by forest type and management goals and prescription.

*Rapid Assessment Technique*

In the summer of 2014, the rapid assessment technique was used to rank the relative abundance of earthworms present within each cut area and the surrounding intact forest. At each site, two study blocks, which were established by our partners at The Nature Conservancy, were sampled and three rapid assessments were performed at random points along established sampling transects in each block. The first was performed along a N-S transect through the plot center and the subsequent assessments were made at random distances along N-S transects 10m to the east and west of the block center. We also performed paired plots in the intact forest surrounding the cut. We made our best effort to place the intact forest plots within areas that resembled the assemblage of the cut area prior to disturbance. Intact forest plots were located in three cardinal directions from the block center (N, E, and W) and were located 15m from the forest edge in each direction. When appropriate intact forest was not available in three cardinal directions, we sampled in alternative directions that were more suitable or used intact forest patches inside of the cut to represent the intact forest.

We followed the assessment protocol in Loss *et al*, and used the structure of the organic layer, presence of fine roots, and abundance of earthworm castings and middens to determine the relative abundance of earthworms at each chosen location (2013). This protocol uses an ordinal scale with 5 categories; 1 being uninvaded and 5 being heavily invaded (Table 7). This method is

extended from hardwood forests, where it was developed, into three other common forest types in northeastern Minnesota, as well as disturbed areas in each of these forest types.

*Mustard Water Earthworm Extraction*

In June of 2015, earthworms were extracted from a subset of sites (2 in each forest type) using the mustard water extraction method developed by the Natural Resources Research Institute (NRRI 2015) (Fig. 12). At each sampling block in each site, earthworms were extracted from three, 30 cm x 30 cm sampling frames along the same vegetation transects from the previous year. A random number table was used to determine a random distance (m) from the north end of each transect to place the sampling frame. To begin, the loose organic material was removed and carefully inspected. Any earthworms found were collected. 4L of water mixed with 50g of yellow mustard powder was then poured into each frame (NRRI 2015). This mixture irritates the mucus membranes on the earthworms, forcing them to the surface of the soil. The mixture was carefully shaken and poured so that the mixture was even and the water did not flow outside of the sampling frame. After 20 minutes, all worms were removed from the upper 2.5 cm of the soil and preserved in 91% ethanol for identification in the lab. This procedure was repeated with paired plots in the surrounding intact forest, using the same technique described above.

*Earthworm Identification*

Extracted earthworms were identified and placed in an ecological functional group in the lab. Using a dissecting scope, worms were identified to species whenever possible, however the juvenile status of many worms made this difficult. We used a key to the introduced worms of North American that was developed by the NRRI to identify worm species when possible. Many juveniles fell into species complexes due to the fact that they did not display the sexual markings used to identify adult individuals. However, the specimens were placed into species complexes that are typically of the same functional group. The ecological role of each functional group captures the worm's impact as well as species, so the precision of our identification was appropriate for answering our questions.

*Data Analysis*

Blocked two-way ANOVA's were used to look for effects of forest type and sampling location (Disturbed v intact forest) on the mean rapid assessment score, earthworm abundance, and earthworm diversity across blocks and sites. Block and site were considered random effects in the analysis, while forest type and sampling location were considered fixed effects. We used $\alpha=0.05$ for all statistical tests in our analysis.

**Results**

The two methods that we used to assess the impacts and presence of non-native earthworms showed contrasting results. The rapid assessment method, which relies on indirect assessment of earthworm impacts, revealed that recently logged sites had higher levels of earthworm impacts than nearby intact forests. When earthworms were extracted from these same sites, there was no difference in the abundance or diversity of earthworms depending on extraction location, except in hardwood forests where the abundance was higher in the nearby intact forest than in the recently logged areas.

*Rapid Assessment*

According to the rapid assessment method, the impact of non-native earthworms was higher in recently disturbed areas than in the nearby intact forest. At sites within the recently logged areas the average assessment score was 2.66, while the intact forest had an average assessment score of 2.42 ($p<0.0001$, Table 8, Fig. 13). Between forest types, differences in assessment score were also significant, with hardwood forests having the highest average assessment scores (3.40) and dry-mesic pine sites having the lowest average assessment score (1.95) ($p=0.0002$, Table 8, Fig. 14). There was no significant interaction between assessment location and forest type for the rapid assessment approach ($p=0.28$).

*Earthworm Extraction*

The earthworm extraction using mustard water shared some similarities with the results of the rapid assessment method, with a few important exceptions. On average, the number of species observed was highest at hardwood sites ($p=0.042$, Table 8, Fig. 15). In the hardwood forest, where worm number and species abundance was highest, sampling plots in the intact forest yielded 2.01 more worms per plot, on average, than the sampling plots in the recently disturbed logged area ($p=0.04$, Table 8, Fig. 16). There was no difference in worm individual or species abundance between intact and disturbed areas for any of the other forest types that were sampled (Table 8, Fig. 16). Furthermore, worm diversity did not show any differences between forest types and sampling locations.

**Discussion**

The contrasting results of the rapid assessment and the quantitative extraction used in this study suggest an interaction between earthworm invasion and disturbance, which may lead to changes in the regeneration and recovery of these disturbed forest systems (Dale *et al* 2001, Frelich *et al* 2012). In the face of climate change, this interaction must be considered by both managers hoping to maintain productivity in forests and modelers who try to forecast the distribution and productivity of forest ecosystems into the future (Frelich and Reich 2009, Frelich

and Reich 2010). This study suggests that rapid assessment of disturbed areas may be misleading in certain forest types and that the effects of logging tend to mimic those of earthworm invasion, leading to unreliable rapid assessments.

It was predicted that both earthworm impacts and abundance would be higher in undisturbed areas for three main reasons: 1) decreased UV exposure, 2) decreased risk of desiccation, and 3) greater amounts of deciduous litter input. However, our rapid assessments indicated worm impacts were highest in the disturbed area in hardwood forests and there was no difference in earthworm impacts between sampling locations in the other forest types sampled. Hardwood forests also had the highest earthworm assessment scores of all four forest types (Fig. 14). This is likely because of the high amounts of deciduous leaf litter in these forests and the warmer temperatures that exist at these sites, which facilitate larger populations of earthworms. Furthermore, the boreal and pine forests that we sampled often lack a well-developed forest floor and are less suitable for invasion by non-native earthworms, although sites in all forest types supported small earthworm populations (MN-DNR 2003). In these forest types, the extraction of earthworms agreed with the rapid assessment method but in the hardwood forests the results of the extraction and rapid assessment did not (Table 8).

In hardwood forests, where earthworms were apparently causing more impacts in the disturbed areas, extraction revealed earthworm abundance was actually significantly *lower* in the disturbed area, as predicted (Fig. 13, 14, 16). The disagreement between these two sampling methods calls for an explanation that accounts for both the observed decreases in organic layer depth and fine root density in the disturbed area as well as the low abundance of macrodetritivores like earthworms. One explanation for this result is the potential for harvesting practices to mimic the impacts of earthworm invasion. Direct effects of physical disturbance of the upper layers of the soil during harvest could account for these observations, however most logging in these forests in conducted in winter when the soil is frozen. Indirectly, disturbance also could mimic the impacts of earthworms by altering understory plant and fungal communities that are essential to the maintenance of the forest floor. Alternatively, the impacts of logging and earthworm invasion may interact with one another. Removal of the forest canopy and changes in ground cover could exacerbate the loss of organic matter and fine roots from the O horizon at many of the hardwood sites. Abundant castings from previous and current earthworm activity, combined with the depleted forest floor would lead to rapid assessment scores that are not representative of the actual number of earthworms present at these sites.

In the face of climate change, the future composition and productivity of northern forest ecosystems is in question (Pastor and Post 1988, Davis and Shaw 2001, Duveneck *et al* 2014,

Ravenscroft *et al* 2012). Non-native invaders and the interaction of these invaders with land use changes, such as human disturbance and deforestation may alter the trajectory of forest succession of both local and landscape scales.

At the local level, naturally occurring regeneration in many forest stands is dictated, at least in part, by the seed bed conditions and the type of substrate available for germination. The loss of organic matter and the increase in the amount of bare mineral soil in hardwood forest ecosystem could have dramatic impacts on the species that are able to reproduce in newly created gaps. For example maple (*Acer spp.*) species, which germinate and compete well on intact forest floor, may be at a disadvantage, especially if they are not able to germinate prior to disturbance due to the presence of earthworms (Godman and Tubbs 1973). On the other hand, white birch (*Betula papyrifera*), white pine (*Pinus strobus*) and oaks (*Quercus spp.*), which germinate well on bare mineral soil and need higher levels of light than maple, may have an advantage in the conditions created by earthworms and forest harvest (Webb 1988, Sander 1979). Leaching of soil nutrients also increases with earthworm presence and lower levels of available nutrients may also favor species like white pine which can compete well, even in very nutrient poor conditions like peat bogs (Mader 1985). Alternatively, the direct impacts of earthworms may be a disadvantage to regenerating forests, regardless of the abiotic conditions that are present. Earthworms often eat fine roots in the upper layers of the soil which can stress and kill young tree seedlings. Furthermore, in order for species to take advantage of the conditions created by earthworms and disturbance a seed source for these species (i.e. a nearby population) is required. If propagules of well adapted species are not present or are unable to survive, suboptimal individuals may proliferate and lead to decreased forest productivity.

On a broader scale, the persistence of boreal regeneration in disturbed, managed, or invaded areas, due to earthworm invasion and deer browse, may slow the future advance of temperate species that would otherwise take advantage of warmer conditions at these sites. However, the migration of these temperate species is also hindered by landscape fragmentation and the rapid rate of current climate change. With several obstacles in forest regeneration, the artificial regeneration and assisted migration of certain populations within their native range may be beneficial in disturbed areas that are heavily invaded by earthworms or under constant browse pressure from deer and other herbivores. Planting multiple populations and species in close proximity to one another can be viewed as "bet hedging", where only the most vigorous individuals will succeed. Planting seedlings may also help to replace the depleted understory following earthworm invasion and timber harvest. Furthermore, certain seed sources may be

better able to compete in the novel conditions created by earthworms and could be beneficial in maintaining the resilience of these forests to further invasion. In any case, the monitoring of these and other disturbed areas should continue in order to observe how earthworm invasion and disturbance interact and how this may be altering the regeneration dynamics of transitional forests in northeast MN and  the northeastern United States as a whole.

**Figure 12.** (A) Earthworm sample plot cleared of debris, leaf litter and organic layer. (B) Pouring mustard solution for earth worm collection. (C) Collecting worms (D) Preserving earthworm specimen for later identification.
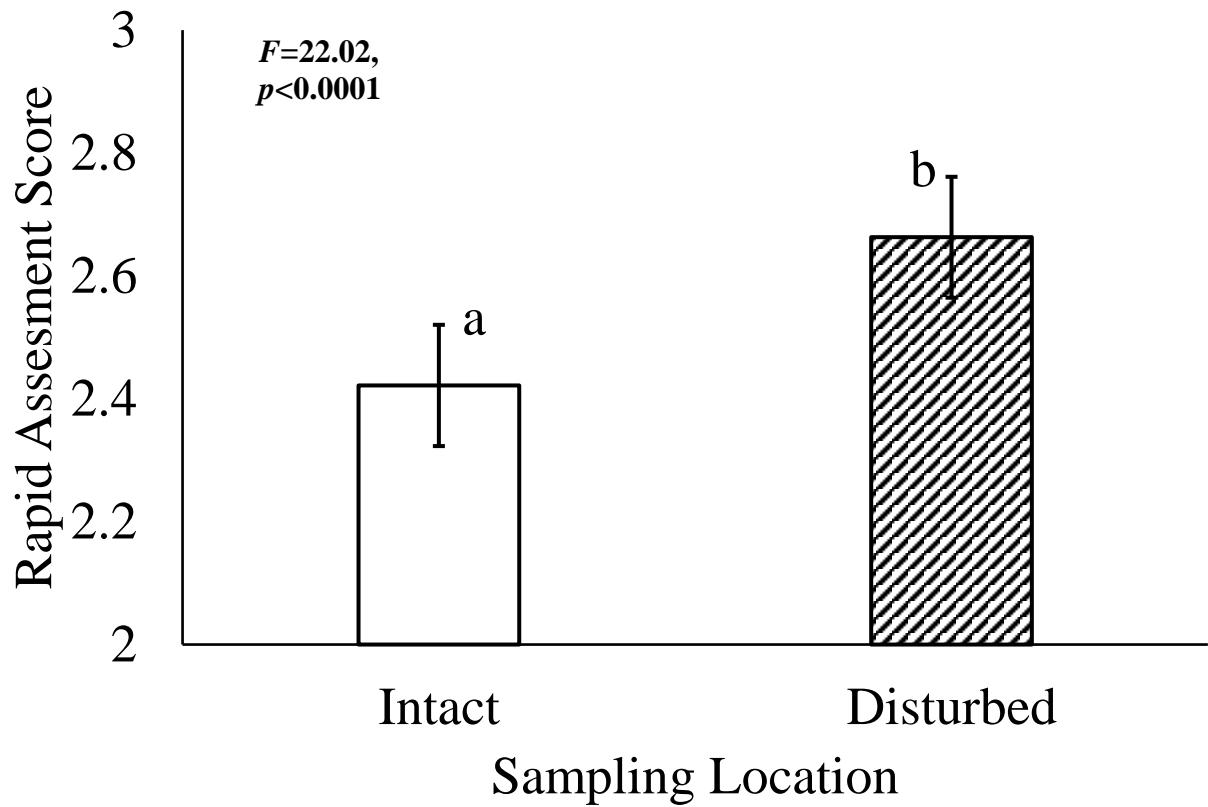
**Figure 13.** Mean rapid assessment score at 40 sampling blocks in 4 forest types in northern Minnesota. Intact plots were located in undisturbed forests and disturbed plots were located in recently logged areas of the same forest. Each bar represents the average earthworm impact score from 120 rapid assessments in each sampling location. Error bars show a 95% confidence interval around each mean. Bars marked with differing letters are significantly different at the $\alpha = 0.05$ level.
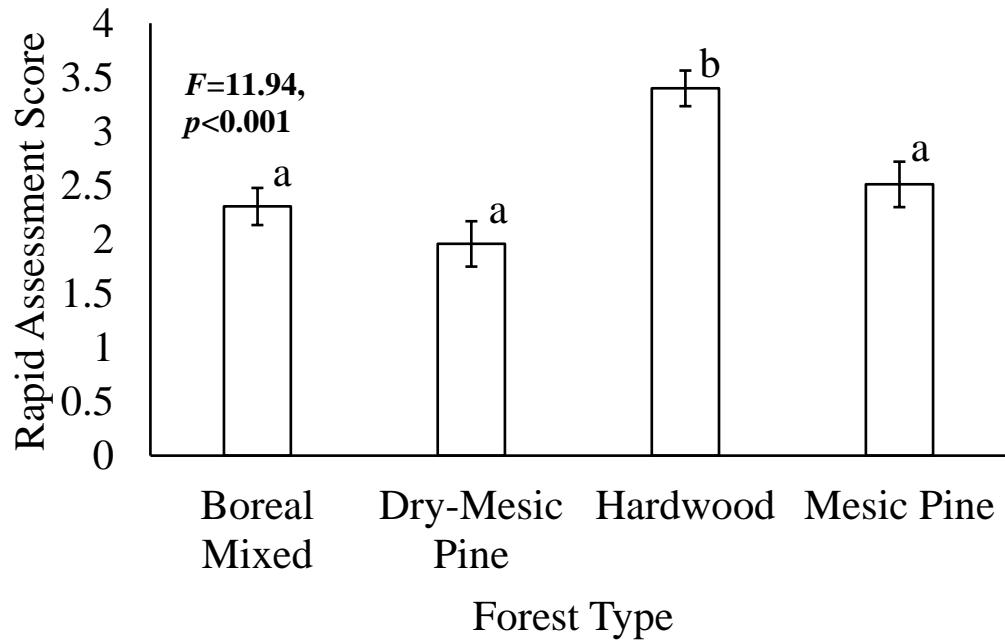
**Figure 14.** Mean rapid assessment score at 40 sampling blocks in 4 forest types in northern Minnesota.. Each bar represents the average earthworm impact score from 30 rapid assessments in both intact and disturbed forests for each forest type. Error bars show a 95% confidence interval around each mean. Bars marked with differing letters are significantly different at the α=0.05 level.
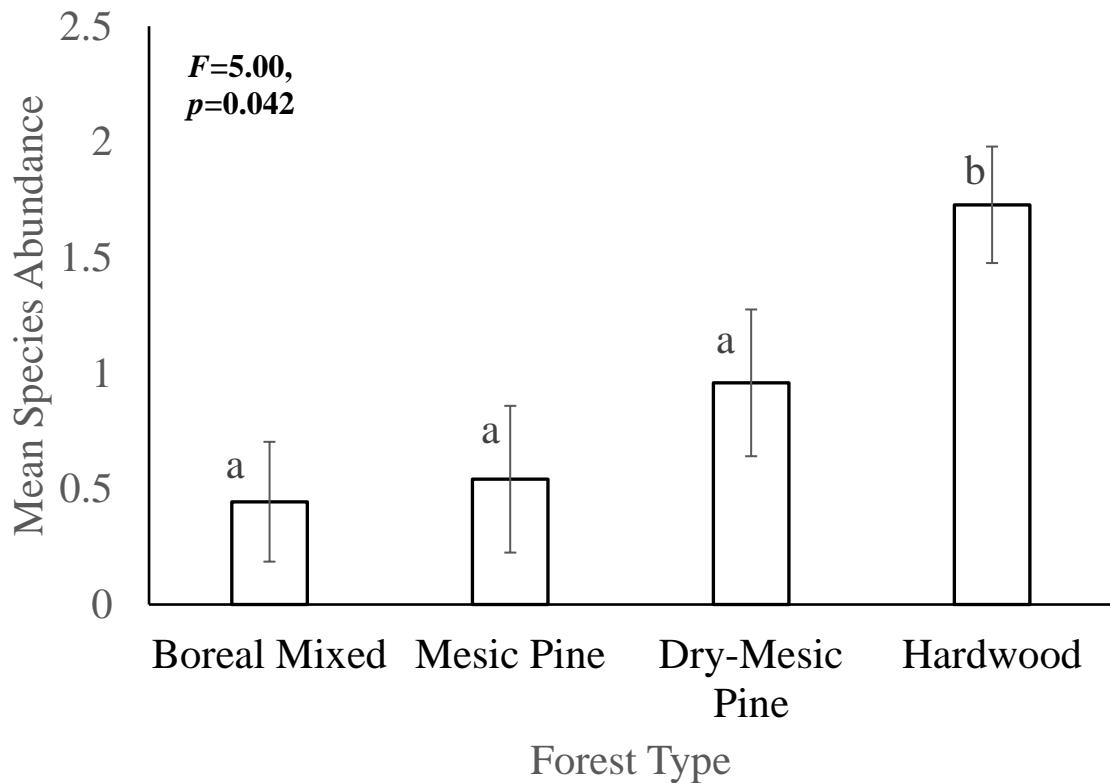
**Figure** 1**5.** Average number of earthworm species in each of 4 forests types in northern Minnesota, USA. Each bar is the mean number of species found in twenty-four 30cm x 30cm sampling plots in both intact and disturbed areas in each forest type. Error bars show a 95% confidence interval around each mean. Bars marked with differing letters are significantly different at the α=0.05 level.
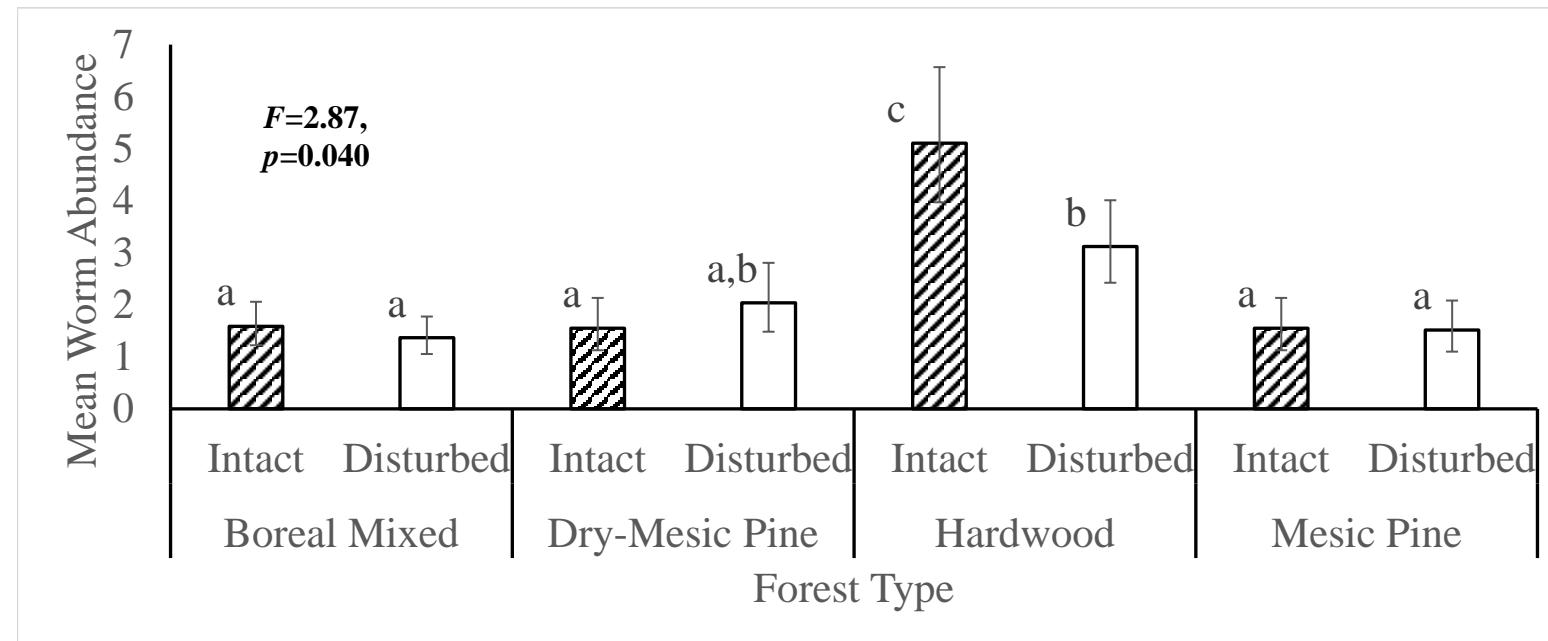
**Figure 16.** Average number of earthworms extracted from intact and disturbed areas of 4 forests types in northern Minnesota, USA. Intact plots were located in undisturbed forests and disturbed plots were located in recently logged areas of the same forest. Each bar is the mean number of worms found in twelve 30cm x 30cm sampling plots in each sampling location. Error bars show a 95% confidence interval around each mean. Bars marked with differing letters are significantly different at the $\alpha$=0.05 level.

**Table 6.** Site names, ownership, and forest type. MN-DNR sites are owned and managed by the Minnesota Department of Natural Resources. SNF sites are under ownership of the Superior National Forest, United States Department of Agriculture. St. Louis and Lake county sites are owned and managed by the respective counties in northeastern Minnesota, USA. Sites Marked with an "X" in the extraction column represent the subset of sites that were sampled with both rapid assessments and quantitative extraction of earthworm specimens.

| Site | Owner | Forest Type | Extraction |
|---|---|---|---|
| Big Lake | MN-DNR | boreal mixed | - |
| No More Stealing | SNF | boreal mixed | - |
| Remodeled | St. Louis County | boreal mixed | - |
| Ridgepole | SNF | boreal mixed | X |
| Round Beaver | SNF | boreal mixed | X |
| Smackdown | St. Louis County | boreal mixed | - |
| Burgo | SNF | dry-mesic pine | X |
| Echo Trail Big Lake | SNF | dry-mesic pine | - |
| Kawishiwi | SNF | dry-mesic pine | X |
| White Iron | SNF | dry-mesic pine | - |
| DNR Sawbill | MN-DNR | hardwood | - |
| Gopher Gaps | Lake County | hardwood | X |
| Hare Lake | MN-DNR | hardwood | X |
| Old Chevy Road | Lake County | hardwood | - |
| Reeves Road | Lake County | hardwood | X |
| VDM | Lake County | hardwood | - |
| Clara Caribou | SNF | mesic pine | X |
| Clara Pit | SNF | mesic pine | - |
| Clara Steep | SNF | mesic pine | - |
| NEPU4 | SNF | mesic pine | X |
| Total | | | |

**Table 7.** Ordinal scale used for rapid assessment of earthworm abundance and impacts.

| Stage | Characteristics |
|-------|-----------------|
| 1 | Litter from previous year intact ($O_i$) with layer forest floor ($O_e$ and $O_a$), Fragmented leaves present, Fine roots abundant in forest floor, No earthworms, castings, or middens present |
| 2 | Forest floor intact in patches ($O_i$, $O_a$, and $O_e$), Fine roots present, Some small earthworms and few castings present |
| 3 | Litter from previous autumn intact with some small leaf fragments but forest floor does not contain humus (no $O_a$), Fine roots absent from soil surface, Castings are apparent (≤50% of soil surface) |
| 4 | Some leaf litter from previous autumn present with mineral soil below, Fine roots absent, Castings are abundant (>50% of soil surface), Middens absent or present (≤5 middens in 5m radius of sampling point) |
| 5 | Some leaf fragments from previous autumn present with mineral soil below, Fine roots absent, Castings are abundant (>50% of soil surface), Middens abundant within 5m of sampling point (<5 middens in 5m radius of sampling point) |

**Table 8.** Results of mixed effects ANOVAs for rapid assessment scores, worm abundance, and worm diversity. "*" – significant at the α=0.05 level. "**" – significant at the α=0.01 level. "***" – significant at the α=0.001 level.

| Factor Variables | Rapid Assessment | | Worm Abundance | | Species Abundance | | Worm Diversity | |
|---|---|---|---|---|---|---|---|---|
| | DF | F | DF | F | DF | F | DF | F |
| **Forest Type** | 3, 15 | 11.94*** | 3, 6 | 3.59 | 3, 6 | 5.00** | 3, 6 | 1.78 |
| **Sampling Location** | 1, 232 | 22.02*** | 1, 94 | 1.02 | 1, 96 | 0.41 | 1, 60 | 0 |
| **Forest Type x Sampling Location** | 3, 232 | 1.29 | 3, 94 | 2.87* | 3,96 | 2.07 | 3, 59 | 0.93 |

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