Soil Respiration During Partial Canopy Senescence in a Northern Mixed Deciduous Forest

Thesis

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By

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Abstract

The mixed deciduous forests of the upper Midwest, USA are approaching an ecological threshold in which early successional dominant aspen and birch trees are reaching maturity and beginning to senesce. At the University of Michigan Biological Station in northern Michigan, we are combining long-term carbon (C) cycling measurements with a large-scale experimental manipulation to forecast how net ecosystem production will change in response to ongoing succession, disturbance, and climate variation. Our goal is to elucidate biophysical mechanisms that will constrain C storage in future forests. In the spring of 2008, we began the Forest Accelerated Succession ExperimenT (FASET), in which all aspen and birch (~35% canopy LAI) within 39 ha of an 85 yr old forest were stem girdled to accelerate mortality. The adjacent, untreated forest serves as a control. I hypothesized that, 1) aspen-birch senescence would decrease C allocation to root and microbial pools, resulting in increased root mortality and reduced root respiration, 2) that the treatment effect on soil respiration ($R_s$) would become more severe as time since girdling increased, and 3) the magnitude of the treatment effect would be proportional to the percent basal area of girdled aspen and birch. Sites with high percent basal area of girdled aspen and birch would yield lower $R_s$,
while sites with low percent basal area of girdled aspen and birch and control sites would have higher $R_s$.

We measured $R_s$ continuously using arrays of automated soil respiration chambers in the treatment and control sites. We also quantified above and below-canopy radiation, precipitation, air and soil temperature, and soil moisture. At the ecosystem scale, we calculated gross primary production from measurements of net CO$_2$ exchange between forest and atmosphere using eddy-covariance methods. Soil respiration was significantly reduced in the treatment relative to the control site in the first and second years after girdling, supporting my first hypothesis. However, the treatment effect occurred faster than anticipated, because root C stores were expected to maintain $R_s$ despite girdling in the first treatment year. I modeled the automated chamber $R_s$ data using a piece-wise linear regression and found that the magnitude of the treatment effect was significantly greater in 2009 compared to 2008, supporting my second hypothesis. Results from the basal area gradient study showed no significant difference between treatment and control site $R_s$. However, percent basal area of aspen and birch was a significant explanatory parameter in the model, revealing that as percent basal area of aspen and birch increased in the control site, so did $R_s$. The treatment site showed no relationship between $R_s$ and basal area, suggesting the treatment is beginning to deteriorate normal forest belowground functioning. My results provide an estimate of $R_s$ after a species specific disturbance and can be
extrapolated to other such disturbances such as those caused by stem girdling insects or diseases. My $R_s$ estimates can also be applied to calculations of net ecosystem productivity, because $R_s$ is the main component of ecosystem respiration.
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List of Abbreviations and Nomenclature

C=Carbon

$\text{CO}_2$=Carbon Dioxide

$[\text{CO}_2]$= Carbon Dioxide Concentration

DOY=Day of Year

HWA= Hemlock Wooly Adelgid

IRGA= Infrared Gas Analyzer

LAI= Leaf Area Index

LI-6400-09= LiCor 6400 with the soil respiration cuvette

N= Nitrogen

NEE= Net Ecosystem Exchange

NPP= Net Primary Productivity

$\text{O}_2$= Oxygen

PAR= Photosynthetically Active Radiation

$R_a$= Autotrophic Respiration

$R_e$= Ecosystem Respiration

$R_h$= Heterotrophic Respiration

$R_s$= Soil Respiration

SWC= Soil Water content (%vol. water/vol. soil)

$T_a$=Air Temperature ($^\circ$C)
\( T_s = \text{Soil Temperature (°C)} \)

\( Q_{10} = \text{Temperature Sensitivity of Soil Respiration} \)

QA/QC= Quality Assurance and Quality Control
Introduction

Background

The magnitude of the global soil carbon (C) pool is such that even a small increase in soil respiration ($R_s$) rates could result in a net reduction in C storage and an increase in the atmospheric carbon dioxide (CO$_2$) concentration. In the mixed deciduous forests of Michigan, USA, soils are the largest component of ecosystem respiration ($R_e$), comprising approximately 71% of the total flux of CO$_2$ (Curtis et al., 2005). Forest soils are a strong C source, making them an important component to quantify when studying the effect of disturbance on forests and CO$_2$ emission (Adair et al., 2009; Davis et al., 2009; Wang et al., 2009). The degree to which a forest stores C as biomass, or the C sink strength, is of greater interest as atmospheric CO$_2$ concentrations rise, contributing to changing global climates (IPCC, 2007). These climatic changes have increased the scientific community's efforts to quantify $R_s$, and better understand the factors controlling it.

Carbon Storage and Forests

Forest carbon storage potential varies as a function of numerous factors, including forest age (Gough et al., 2007). Many young forests are C sources, releasing more C as CO$_2$ into the atmosphere than is stored as plant biomass in a single growing season (Pregitzer and Euskirchen, 2004). In northern lower Michigan, the forests act as C sources in the spring and become a net C sink as photosynthetic rates increase (Gough et al., 2008a). Carbon storage rates for red pine and aspen trees were evaluated over a chronosequence in the mixed forests in
northern Minnesota, USA (Bradford and Kastendick, 2010). The authors found that cumulative C storage in tree biomass increased as the tree aged, but the rate at which total ecosystem C was sequestered decreased logarithmically to zero as the pines reached 100 years old (Bradford and Kastendick, 2010). However, the 140 year old aspen trees in this ecosystem still sequestered C at a rate above zero Mg C ha\(^{-1}\) yr\(^{-1}\), which supports the assertions of Luyssaert et al. (2008) that old growth forests can serve as C sinks (Bradford and Kastendick, 2010). This stands in contrast to early work by Odum (1969) where climax communities were described as being C neutral, where the ratio of energy fixed was equal to the energy required to maintain the biomass.

The C storage potential of different forest types is not equal. The sink strength of a particular forest is dependent not only on the forest age and composition, but also on harvest patterns in managed forests. The maple-alder, Douglas fir, and fir-spruce-mountain hemlock forests of the Pacific northwest were found to have the potential to store more C if their harvest was delayed five years (Foley et al., 2009). In contrast, many forests in both the northeast, and southwest United States did not increase C storage when harvesting was delayed (Foley et al., 2009). In the Pacific Northwest, USA Hudiburg et al. (2009) found that the C storage potential of forests could increase 46% if tree harvests were delayed 30-50 years, or if the total acreage harvested was reduced.

**Disturbance Effects on Soil Respiration**

Disturbances including fire, wind throw, ice storms, and insect outbreaks can have a large impact on forest C dynamics. These destructive forces redistribute large quantities of plant biomass. Trees which store large quantities of C are burnt, uprooted, delimbed, or severely damaged. As the C is redistributed from living biomass to coarse woody debris on the soil
surface the soil microbial community gains a C source. Some of the tree biomass is labile and available for rapid decomposition, while other parts of it are recalcitrant, requiring more time to be decomposed (Concilio et al., 2006; Gough et al., 2007). Forests disturbances thus vary in their effect on $R$, and the C storage potential of the forest.

Before European settlement, forests in the Eastern United States were fire maintained by Native Americans to enhance the production of mast species and fruit trees (Abrams and Nowacki, 2008). High frequency, low intensity fires maintained fruit bearing trees in park-like forests with large canopy gaps and productive understories (Abrams and Nowacki, 2008). Today fire in the forests of Eastern USA is infrequent, resulting in closed canopy forests, which hold a higher fuel load. Thus, when fires occur, they are often expansive and uncontrollable.

The altered present-day fire regime in the United States has led researchers to investigate the effect of fires on stand type, and forest composition to learn about forest responses to fire and the impact on C dymanics. A study conducted in the Cascade mountains of Oregon, USA evaluated the effect of fire intensity on C dynamics (Meigs et al., 2009). Meigs et al. (2009) found that severe fires lowered net primary production (NPP) 40% compared to sites with low-severity fires. However, no difference in $R$, was found between the burn severities, suggesting that $R$, in this ecosystem is resistant to disturbances (Meigs et al., 2009). While these findings may be valid over the short term (4-5 years), fire may result in long-term changes in forest soils and C storage potential. Gough et al. (2007) studied a forest burn chronosequence in northern lower Michigan, USA. In this study forest sites were clear cut and burned one time over the past 68 years (Gough et al., 2007). Forest stands which were burned 50 years ago had
60% larger \( R_s \) values than recently burned stands (Gough et al., 2007). This indicates that fire disturbance history plays an important role in \( R_s \) and the C balance of this ecosystem.

Forest thinning, the removal of dense patches of small diameter trees, serves as a proxy for small scale high frequency fires. Thinning is used to prevent wildfires and to study ecosystem responses to disturbance. In the southwestern United States studies on forest thinning, controlled burns, and their combination have been used learn how to increase forest C storage. A study conducted in ponderosa pine forests in Arizona, USA used thinning to reduce the tree density by 67% and basal area, calculated as the total cross sectional area of a trees at breast height (1.5 m), by 39% (Sullivan et al., 2008). This treatment led to a reduction in \( R_s \) in June and August one year after the trees were thinned. Sullivan et al. (2008) interpreted the decrease in \( R_s \) as due to root mortality which decreased autotrophic respiration (\( R_a \)). Autotrophic respiration is defined by Hogberg et al. (2009) as respiration by “live roots, their mycorrhizal fungal symbionts and other closely associated microorganisms dependent on the flux of recent photosynthate.” Autotrophic and heterotrophic respiration together comprise \( R_s \). The decrease in \( R_s \) occurred in spite of warmer soil temperatures, which normally increases \( R_s \) (Sullivan et al., 2008). Also, soil moisture did not increase at the treatment site, so heterotrophic respiration (\( R_h \)), respiration due to the decomposition of compounds such as litter and soil organic matter by microbial decomposers, did not increase because decomposition was limited by moisture (Hogberg et al., 2009; Sullivan et al., 2008). The thinning treatment therefore effectively reduced the release of new, labile photosynthates from the tree roots. Sullivan et al. (2008) concluded that forest thinning is a good management technique to minimize C loss from the dry forests of Arizona, because \( R_s \) did not increase in the first summer. Unfortunately, their study
did not follow the long term effect on $R_s$. It may be assumed that in later years the thinned above ground biomass could become available for decomposition, increasing soil organic matter availability, and thus increasing $R_s$. Concillio et al. (2006) in a similar ponderosa forest utilized burning, thinning, and burning+ thinning treatments. These authors found that three years after treatment, the more intensely burned sites had higher $R_s$ than less intensely burned sites and control sites. These findings suggest that the soil respiratory benefits of thinning that Sullivan et al. (2008) described may not be feasible over the long term in this forest.

Large scale wind disturbances also have substantial impacts on the C balance of forests. In the Russian taiga, a large wind throw removed trees from approximately 400,000 m$^2$. Knohl et al. (2002) studied the impact of this wind throw on C dynamics using eddy covariance measurements, which were subsequently compared to a control forest stand. The site became a major CO$_2$ source, releasing approximately 800 g CO$_2$m$^{-2}$yr$^{-1}$ (Knohl et al., 2002). Similarly, in 2005, a wind storm in Sweden removed approximately 66 million m$^3$ of stems. Lindroth et al. (2009) estimated the net CO$_2$ flux from the forest to range between 897 to 1259 gCm$^{-2}$yr$^{-1}$. In 1999 a northern Minnesota, USA boreal forest experienced a similar scale wind throw disturbance (Rich et al., 2007). Carbon dioxide flux measurements were not taken at this site, but given the extensive nature of the disturbance, C loss estimates similar to those in the Swedish and Russian studies would seem reasonable. Soil respiration in the three studies mentioned was not reported, but I predict it will remain high as tree biomass is slowly decomposed, resulting in sites which are long term C sources.
Impacts of Tree Girdling on $R_s$

Several studies have shown that recently produced photosynthate, sugars produced via photosynthesis, are a key driver of $R_s$ (Frey et al., 2006; Hogberg et al., 2001). Different forests show distinct $R_s$ and $R_h$ responses to forest disturbances. For example, Hogberg et al. (2001) performed a girdling study on Scots pine (*Pinus sylvestris* L.) in northern Sweden to study $R_s$. Girdling removes the bark and the underlying phloem in a strip around a tree (Figure 1), preventing the translocation of photosynthate, from the leaves to the roots (Bhupinderpal et al., 2003; Hogberg et al., 2001). Hogberg et al. (2001) used two girdling treatments, early season and late season girdles. The authors found $R_s$ in early season girdled sites decreased 27% compared to the control within the first five days of the study. In the late season girdle treatment, $R_s$ declined by 37% in five days, and in 14 days $R_s$ was 56% lower than the control. Other studies which have girdled deciduous tree species confirm that photosynthate drives $R_s$ however a slower, less drastic soil respiratory response was reported when compared to Hogberg et al. (2001). For example, Frey et al. (2006) girdled sweet chestnut (*Castanea sativa* Mill.) in a Swiss forest. As the roots of the girdled trees became C limited a 22% decrease in $R_s$ was noted over the 37 days of the experiment (Frey et al., 2006). The authors attributed this difference to lower utilization of fine root starch by deciduous and coniferous trees. For example, the deciduous chestnut trees in Frey et al. (2006) depleted 90% of the fine root starch within 37 days after girdling, while the Scots pines in Hogberg et al. (2001) depleted ~80% of the fine root starch over 102 days after girdling (Hogberg et al., 2001).
Figure 1. A stem girdled bigtooth aspen with the author’s hand for scale.

Species-specific responses to girdling can be highly variable. Chen et al. (2010) studied two species of two year old plantation trees to assess species specific responses to girdling. The authors found that *Acacia crassicarpa*, a nitrogen (N$_2$) fixer, responded to girdling with a 27% decrease in $R_s$, while *Eucalyptus urophylla*, which is prone to resprouting, showed $R_s$ declines of only 14% (Chen et al., 2010). The authors pointed to the C:N ratio of the roots as a possible cause for this difference in $R_s$. The roots of the N$_2$-fixing species had a lower root C:N ratio and were preferred by microbial decomposers resulting in increased microbial respiration and CO$_2$ production (Chen et al., 2010). The disparity in $R_s$ between the two plantation species was also proposed to result from resprouting. The new shoots photosynthesized, providing the roots with a renewed and labile C source. This C source slowed root mortality rates, which reduced the amount of root biomass available for decomposition, thereby reducing CO$_2$ production from soil respiration (Chen et al., 2010). However, a study conducted in eastern Tennessee, USA on the
tulip poplar (*Lirodendron tulipifera* L.) by Edwards and Ross-Todd (1979) produced unexpected responses. They monitored $R_s$ in *L. tulipifera* soils using open-system moving chambers and an infrared gas analyzer (IRGA) to quantify the CO$_2$ flux from the forest floor. The authors found no significant difference in $R_s$ between girdled and control sites over the two years of experimentation. The trees continued to grow and maintain leaves for two years after girdling. The authors cite root starch stores as the likely cause of the maintained $R_s$. Clearly this species responded to girdling differently than trees in the previously mentioned studies.

Phloem girdling insects can create large scale forest disturbances, so they are being studied to determine their impact on $R_s$ and C dynamics in forests. The forests of the United States are currently home to more than 360 non-native insects, 30% of which have become invasive pests (Moser et al., 2009). Several of the more recent, aggressive invaders are phloem girdlers including the hemlock woolly adelgid (HWA), pine bark beetles, and the emerald ash borer (Morehouse et al., 2008; Nuckolls et al., 2009; Poland and McCullough, 2006; Robertson et al., 2009). Nuckolls et al. (2009) studied the effect of phloem girdling HWA in comparison to manual girdling. The authors found that manual girdling and HWA infestation resulted in similar declines in $R_s$, demonstrating that the impact of HWA infestation is similar to manual girdling in this system (Nuckolls et al., 2009). The imminent death of infested hemlocks suggests forest productivity will decrease in the future as the dominant trees are lost from forests of southern Appalachia. The pine bark beetle, another phloem girdling insect was studied for one year in southwestern Arizona, USA by Morehouse et al. (2008). The authors found that $R_s$ in this system was unchanged despite the presence of the beetle and extensive tree mortality (Morehouse et al., 2008). The authors infer that the treatment and control sites had similar $R_s$ during this
period because stored root C was available for respiration. Also, CO$_2$ production may have increased as dead roots were decomposed. Lastly, as tree biomass was lost soil temperature and moisture increased, leading to conditions more favorable for $R_s$, which balanced other declines in $R_s$ (Morehouse et al., 2008).

**Environmental Controls and Soil CO$_2$ Fluxes**

Air temperature and solar radiation provide important controls over $R_s$. Soil respiration increases as soil temperatures rise, because microbial community abundance and activity both increase as soils warm (Bouma et al., 1997; Elberling, 2003; Schindlbacher et al., 2009). Increased solar radiation increases photosynthetic rates resulting in increase C availability to roots, thereby increasing $R_s$ (Gough et al., 2008a). Another study on solar radiation was conducted in the arid Patagonia region of Argentina. The authors found that photodegradation increased above-ground litter decomposition by 30% when compared to the control (no radiation exposure) (Austin and Vivanco, 2006). This finding represents a potentially large C loss from above-ground plant material. Campbell and Law (2005) found that in the forests of western Oregon the soil and climatic patterns result in greater variation in $R_s$ than the disturbance history. The climate of the Earth has been changing over recent decades and many studies report global increases in air temperature ($T_a$) and solar radiation (IPCC, 2007; Nemani et al., 2003; Wild et al., 2005). Curtis et al. (2005) found $R_s$ in a northern hardwood forest was well explained by $T_a$, which is driven by $T_o$.

Other abiotic factors shown to influence soil CO$_2$ production include soil moisture, nutrient availability, and soil type (Bond-Lamberty et al., 2004; Huxman et al., 2004; Johnson et al., 2000; Kane et al., 2005; Melillo et al., 2002; Yi et al., 2007). Soil respiration rates are sensitive
to both the magnitude and frequency of rain events which influences soil moisture (Elberling, 2003; Huxman et al., 2004; Jenerette et al., 2008). Large rain events soak deeper into the soil stimulating both the roots ($R_a$) and the microbial community ($R_b$), while small rain events that wet the top portion of the soil stimulate only the microbial community ($R_b$) that lives in the top organic matter rich soil layer (Huxman et al., 2004). It has been proposed that moist soils generally have higher $R_s$ than dry soils, because more labile forms of organic matter become solubilized, which in turn stimulates the microbial community (i.e. $R_b$) (Akinremi et al., 1999; Melillo et al., 2002). The main exception to this is saturated soils, where the microbial community becomes oxygen ($O_2$) limited, and hence $R_s$ decreases. At Harvard Forest, Massachusetts, USA researchers found that soils with very low and high water contents have reduced $R_s$. Maximal $R_s$ occurred at 60% soil water holding capacity for this site (Bowden et al., 1998). Curtis et al. (2005) found that $R_s$ was explained adequately by soil moisture content in their northern USA hardwood forest in the spring and fall, but was of variable importance in the summer.

Soil composition also affects $R_s$. Kane et al. (2005) found that labile light fraction soil organic matter was cycled more rapidly than dense fraction soil organic carbon as temperature and stand productivity increased resulting in greater $R_s$. The composition and presence of the organic matter in different soil horizons also has strong control over $R_s$. Buchmann (2000) found that removal of organic soil layers decreased $R_s$. Limiting nutrient availability also has a strong influence on $R_s$. In southwestern Australia, P as superphosphate and N as urea were applied in nutrient addition experiments to monitor their effect on microbial respiration (O’Connell and Mendham, 2004). The authors found a 19% increase in $R_s$ with the addition of P, while N
additions in this system did not affect $R_s$ over the five years of study. In another $R_s$ study, P limited forests in Africa were treated with nutrient additions. Gnankambary et al. (2008) found that adding glucose (C) and phosphate (P) together increased respiration rates much more than glucose (C) and urea (N) additions. In the N limited high arctic, a study was done to determine if the soils were N or C limited along a successional gradient. The authors found co-limitation of C and N, which when added to the soil, resulted in increased $R_s$ (Yoshitake et al., 2007).

Methods for Measuring Soil CO$_2$ Effluxes

Soil CO$_2$ effluxes are extremely sensitive to the methods employed to quantify the flux, so the methods used should be carefully reviewed to understand the potential biases associated with each system (Luo and Zhou, 2006). Soil respiration is often measured using either open or closed system chambers. With open system chambers air of a known CO$_2$ concentration ([CO$_2$]) is blown across the soil surface. As air leaves the chamber the [CO$_2$] is determined using an IRGA. The change in [CO$_2$] is used to calculate the flux of CO$_2$ from the soil using equation 1 below

$$CO_2 = \frac{V(C_{t1}-C_{t2})}{A(t1-t2)}$$

Eqn 1.

where $V$ is the chamber volume, $C_t$ is the [CO$_2$] inside the chamber at time $t$, and $A$ is the area of the chamber (Bouma et al., 1997; Widen and Lindroth, 2003). One limitation of the open system design is interference with the boundary layer above the soil surface as the chambers are sampled. That is, as the boundary layer is mixed by the movement of the air samples through the chamber the [CO$_2$] gradient between the soil and the chamber headspace is altered (Widen and Lindroth, 2003). Well-mixed boundary layers reduce the concentration gradient such that
more CO$_2$ is released from the soil and the calculated fluxes are overestimated (Widen and Lindroth, 2003). A second problem with open systems is caused by the flow of air through the chamber. If the flow of air through the chamber is not balanced as it is drawn to the IRGA, underpressurization or overpressurization can occur. In the former circumstance CO$_2$ is pulled from the soil, while overpressurization retards the mass flow of CO$_2$ from the soil. Both of these lead to inaccurate $R_s$ estimates (Davidson et al., 2002).

With closed system chambers, air is circulated from the chamber to an IRGA, and then returned to the chamber. This allows CO$_2$ to accumulate within the chamber, and the increase in [CO$_2$] is recorded. However, high CO$_2$ concentrations in the chamber can slow the diffusion of CO$_2$ from the soil, resulting in lower calculated fluxes (Davidson et al., 2002; LI-COR, 1998; Widen and Lindroth, 2003). The LI-COR LI-6400 and LI-6400-09 soil CO$_2$ flux chamber (Li-Cor, Lincoln, NE, USA) (hereafter referred to together as the LI-6400-09) is a portable closed system chamber which uses an IRGA to measure changes in chamber [CO$_2$]. This system allows the site specific ambient [CO$_2$] to be set on the instrument. This feature is important, because $R_s$ is most accurately quantified when the chamber conditions are similar to ambient conditions. Under ambient conditions, the soil [CO$_2$] gradient is preserved and the diffusion of CO$_2$ can be more precisely recorded. The LI-6400-09 further reduces errors associated with altered chamber [CO$_2$] gradients by scrubbing the [CO$_2$] in the chamber to just below the set ambient conditions. The IRGA measures and records the increase in chamber [CO$_2$]. Soil CO$_2$ flux is calculated from these measurements with equation 2.

$$
F_c = \frac{kPV}{S(T + 273)} \left( \frac{\partial C}{\partial t} + \frac{C}{1000 - W} \frac{\partial W}{\partial t} \right)
$$

Eqn. 2
Where \( k = 1.2028 \), \( P \) is the atmospheric pressure in kPa, \( V \) is the volume of the chamber in \( \text{cm}^3 \), \( S \) is the surface area covered by the chamber in \( \text{cm}^2 \), \( T \) is the chamber temperature in °C, \( C \) is the concentration of \( \text{CO}_2 \), \( t \) is time, and \( W \) is the water concentration of the chamber air in mmol water mol\(^{-1}\) air. Many closed systems do not utilize a \( \text{CO}_2 \) scrubbing feature, because it is unfeasible with large chambers or with automated chambers that run continuously. One way to minimize problems associated with altered soil \([\text{CO}_2]\) gradients is to close chambers for shorter periods of time and allow \( \text{CO}_2 \) to accumulate for more limited time intervals, thus reducing the negative impact of high chamber \([\text{CO}_2]\) on diffusional fluxes from the soil. For example, Davidson et al. (2002) recommended closing chambers for a maximum of five minutes.

Similar to open systems, pressure differentials between the chamber and the surrounding air and unevenly distributed chamber \( \text{CO}_2 \) concentrations are also problems associated with closed systems. Closing a chamber lid can change the internal chamber pressure, often slowing the diffusion of \( \text{CO}_2 \) from the soil leading to artificially low \( R_s \) calculation (Davidson et al., 2002; LI-COR, 1998; Ryan and Law, 2005). These pressure differences can be reduced by equalizing the pressure using a long thin piece of tubing that penetrates the side of the chamber (Jassal et al., 2005). Diffusion of \( \text{CO}_2 \) through the tube during the experiment is minimal, and the resulting flux measurements are more accurate because internal chamber pressure differences are minimized (Jassal et al., 2005). The concentration of \( \text{CO}_2 \) within a chamber can also be unevenly distributed while a chamber is closed. This results in \([\text{CO}_2]\) measurements that depend on where samples are taken within the chamber. To homogenize the \([\text{CO}_2]\) within a chamber, some closed systems utilize small mixing fans. However, the fans can disturb the boundary layer which might alter flux rates as described above (Jassal et al.,
Le Dantec et al. (1999) found that if within chamber wind speeds were maintained below 0.4 ms$^{-1}$, then potential negative effects on $R_s$ were avoided.

**Automated and Manual Chambers**

Open and closed system chambers can be further categorized according to whether they employ automated or manual operation. Automated chambers have been used extensively in a wide variety of ecosystems including barley fields, lab settings, arctic heath lands, Douglas fir forests, and tropical forests (Akinremi et al., 1999; Bouma et al., 1997; Elberling, 2003; Jassal et al., 2005; Mo et al., 2007; Schindlbacher et al., 2009). Automated chambers are often set up in arrays of several chambers which can be replicated at multiple sites. Automated chambers utilize a single centralized IRGA where air samples from the chambers are analyzed. The most useful feature of automated chambers is that they continuously collect data with a high temporal resolution (2 second [CO2] measurements). Using automated systems $R_s$ can be quantified through the night and during inclement weather, which would be impossible using a manual system (see below). However, there are also a number of disadvantages associated with automated systems. First, they are expensive to build, set up and maintain. Automated chamber arrays must be carefully deployed in a centralized location near a power source to run pumps and an IRGA. Automated chamber arrays typically cover a small area (~2000 m$^2$ maximum), so the spatial variation that the chambers encompass is rather small. When in operation, automated chambers require frequent maintenance, including the removal of plants or fungi growing in chambers. Automated chambers also need to be checked every two to three days to ensure that the chambers lids are creating a tight seal each time they close. If not, the chamber lids must be repositioned and stabilized. Automated chambers are also exposed to the
elements and forest organisms. For example, ants and other insects must be dislodged from tubing, and fans need metal shrouds to protect them from precipitation. Second, automated chambers require electronic programming and controls to run the chambers on a regular schedule and to record data in a logical, useful way. After the data are collected, a remaining challenge is processing the large datasets produced for each chamber at each site. Often the development of an additional computer program is required to aid in data processing and flux calculations.

Manually operated chambers provide many benefits and only a few disadvantages compared to automated chamber systems. First, manual chambers, such as the LI-6400-09, can be used to cover large spatial scale variations because the unit is mobile. Second, the LI-6400-09 automatically analyzes the \([\text{CO}_2]\) data to yield \(R_s\) directly, so results are obtained in nearly real time. Some manual chambers allow the ambient \([\text{CO}_2]\) to be set and utilize scrubbing features, which reduce disruptions to the natural soil \([\text{CO}_2]\) gradient. These features improve the accuracy of the calculated \(R_s\). Other manual chambers are open to ambient conditions which reduces pressure differential problems (Ryan and Law, 2005). One of the drawbacks of manual systems is that they cannot capture small temporal scale changes in fluxes to the same degree as automated systems. In addition, manual chambers can also be expensive, ranging around $30,000 for the LI-6400-09, however this is less expensive than automated chamber arrays. Finally, manual systems such as the LI-6400-09 weigh 13.7 kg, which is cumbersome when recording \(R_s\) in rough terrain, or over a wide region.
**Managing CO₂ Data Sets**

One of the challenges with automated chamber systems is the large data sets that are produced, which require sophisticated computer programs to yield \( R_s \) estimates. Automated chambers may record the [CO₂] as often as every two seconds and the large data sets produced by such high sampling rates require a program which is capable of handling a variety of data processing issues. For example, gaps in the data need to be recognized, so that the flux of CO₂ is calculated from full accumulations of CO₂. Second, the recording interval needs to be the same for all sites, or the program must be flexible to work with different sampling regimes. Third, the program must recognize where the recorded [CO₂] data switches between days, hours and chambers. This allows each day, hour, and chamber combination to be individually fit with a specific non-linear curve and the important coefficients output (see methods for more details). Lastly, after the fluxes are calculated, a system must be used to determine which data points are “good” and which are outliers. For example, if the chambers malfunctioned and produced inaccurate data, the system should be able to provide statistical guidelines about what data should be removed.

**Objectives and Hypotheses**

The overall objective of this project was to quantify \( R_s \) before and after a forest stand-level disturbance due to stem girdling. This involved the installation of an array of automated chambers at treatment and control forest stands, monitoring the chambers several times a week during the spring through fall of 2008 and 2009, and [CO₂] data processing using a customized MATLAB program (Appendix A) to determine hourly \( R_s \). I also sampled \( R_s \) over a
greater spatial area using manual chamber measurements with the LI-6400-09 to provide additional information about the extent of the treatment effect.

My hypotheses were:

1). The treatment site will have reduced $R_s$ compared to the control site because girdling prevents the movement of photosynthate to the roots, thereby reducing $R_a$.

2). The treatment site will have increasingly lower $R_s$ values over time as the treatment effect becomes more severe as time since girdling increases.

3). The treatment effect magnitude will be proportional to the percent basal area of girdled aspen and birch. Sites with high percent basal area of girdled aspen and birch will yield lower $R_s$, while sites with low percent basal area of girdled aspen and birch and control sites will have higher $R_s$.

4). The treatment site $R_s$ will show a greater sensitivity to changes in abiotic drivers such as soil temperature, soil moisture, and net radiation than the control forest. This will be shown later in an analysis completed with collaborators Matteo Detto and Gil Bohrer.
Methods

Site Description

Our study site at the University of Michigan Biological Station in northern Michigan (45°35.5' N 84°43' W) has a history of disturbance. In the early 20th century, old growth forests that covered the region were clear cut and burned repeatedly, first to clear slash (tree limbs etc.) from the logging process, and later as wildfires (Gough et al., 2007). This reset the successional clock of the region, and today approximately 35% of the basal area of the forest is dominated by early successional, relatively even aged *Populus grandidentata* Michx. (bigtooth aspen), *Populus tremuloides* Michx. (trembling aspen), and *Betula papyrifera* Marsh. (paper birch). Other trees that comprise the canopy include *Fagus grandifolia* Ehrh. (American beech), *Acer saccharum* Marsh. (sugar maple), *Acer rubrum* L. (red maple), *Pinus strobus* L. (white pine) and *Quercus rubra* (red oak). The early successional aspen and birch species are currently beginning to senesce and will continue to do so over the next 50 years. During that time, aspen and birch will cease to be the dominant forest trees as they are replaced by later successional species such as the maple, oak, and pine species mentioned above.
Figure 2. (Left) The University of Michigan Biological Station is located near Pellston, MI in the northern lower peninsula of Michigan. (Right) The girdled (treatment) area is located in the stippled region of the map. The control plot is located west of the treatment site. The dots found on the transects radiating from the central meteorological towers were sites where additional measurements were taken (including $R_s$).

On April 20-May 3, 2008 we stem girdled the early successional aspen and birch trees in a 33 ha plot, totaling approximately 6,400 trees (Figure 2 stippled area). Three forest stands of two ha each were also girdled to serve as replicates. A control forest with similar site and soil characteristics is located nearby (Figure 2). Radiating transects stretch from the central 1.1 ha plots in the treatment and control forests. At the 1.1 ha plots we recorded tree diversity, tree diameter at breast height, $R_s$, leaf area index (LAI), stand age, root turnover, gaseous $N_2$ and NO$_x$ effluxes, and other variables (Curtis et al., 2005; Giblin et al., 1994; Gough et al., 2007; Gough et al., 2008b; McCalley and Sparks, 2008). At the center of each study site is a meteorological tower that records measurements of wind speed, and CO$_2$ and water concentrations. These
variables are used to quantify the fluxes of CO$_2$ and water in this system, yielding net ecosystem exchange (NEE) and latent heat exchange. Net ecosystem exchange is the net amount of CO$_2$ produced by respiration and assimilated by photosynthesis in the forest, while latent heat exchange is the movement of water between the forest and atmosphere. In addition to meteorological data, air temperature ($T_a$), soil temperature ($T_s$), precipitation, and photosynthetically active radiation (PAR) were recorded every half hour at each plot. Within the center of each 1.1 ha plots are eight sets of PVC collars where measurements of $R_s$ are taken using a portable LI-6400-09 IRGA and soil respiration cuvette. These collars are dispersed in a roughly circular pattern near the center of each site (Figure 3).
Figure 3. Automated chamber and LI-6400-09 $R_s$ were recorded in the central 1.1 ha plot of the treatment (A) and control (B) sites. Circles indicate automated chambers, while small squares show the location of two LI-6400-09 PVC collars. Notice that the automated chamber array is in a circular pattern to cover the greatest spatial variation, while still remaining close to the IRGA, pumps, and the power source.
**Girdling Treatment**

Aspen and birch trees in the main treatment and replicate stands were girdled between April 20 and May 3, 2008 prior to bud break. At the treatment and the replicate stands a 25x25 m grid was set up with conduit pipe (1.5 cm diameter, 1.3 m height), so that all aspen and birch trees could be systematically located and girdled. Two professional sawyers were hired to saw two parallel circumferential cuts approximately 5-7 cm apart on each aspen and birch tree (Figure 1). The cuts went through the bark and phloem, but were shallow enough that the xylem was left intact. A vertical cut was made to connect the two parallel cuts. This created a gap where a pry bar could be inserted. Two to three researchers followed behind the sawyers with pry bars and hammers to remove the bark and phloem on each tree.

**Field Measurements**

*Automated Chambers*

At the treatment and control sites eight closed system automated chambers were set up on the forest floor (Figure 3). Each chamber consisted of an aluminum frame with four long pointed legs which allowed it to be secured into the ground (Figure 4). The frame was further stabilized with rebar. A pneumatic actuator was built onto the frame where the lid rotated from the open to closed position. The lid, which was also mounted on the frame, closed as pressure was applied to the actuator. The lid was made up of two pieces of metal. The first portion of the lid had large circular punches taken out to minimize weight (Figure 4 left). This piece formed the very top of the lid when the chamber closed. The second piece was the portion of the lid that came into contact with the chamber and consisted of solid aluminum which had adhesive backed foam applied to it. The foam acted as a gasket facilitating a tight seal between the lid
and the chamber. The two pieces of the lid were connected with four metal rods with springs that maintained a distance of approximately 5 cm between the two pieces. The rods and springs allowed the second piece to flex so that a tight seal could form on the chamber (Figure 4). The chamber itself was a clear plexiglass cylinder (radius 14.75 cm). Within each chamber was a manifold which returned sampled air to the bottom of the chamber. Near the top of the chamber was polyethylene coated aluminum tubing which sampled the chamber headspace and transported the sample to the IRGA for analysis. Lastly, a 2.54 x 2.54 cm fan was located 6.5 cm from the top of the chamber to homogenize the chamber air as samples were taken. The fan was placed in a steel shroud to protect it from rain (Figure 4).

Figure 4. (Left) Automated chambers closed when pressure was applied to the pneumatic actuator which created a seal between the lid and the chamber. The aluminum frame was stabilized with re-rods to prevent movement which could reduce the chamber seal. (Right) The closed system utilized a fan to lightly mix the air within the chamber headspace while the sample was being taken. Samples were collected from the black tube near the top center of the chamber, and analyzed air was returned via the perforated manifold at the base of the chamber.
The automated chambers required maintenance before data could be collected in the summer of 2008. First, the plexiglass chambers were adjusted so that each chamber contained a uniform volume of air. This required cutting the soil with a knife, so that the chambers could be inserted into the soil until exactly 20 cm remained aboveground. This yielded a standardized soil surface area of 0.068 m², and a headspace volume of 0.0144 m³. Small (2.54 x 2.54 cm), low voltage mixing fans with stainless steel shrouds were installed near the top of each chamber. The fans gently mixed the air column in the chamber while CO₂ measurements were recorded. A small hole was drilled in the plexiglass, so the wires for the fan could exit the chamber. These fans were connected to a power source and the chamber datalogger so the fans turned on as each respective chamber was being sampled. Next, the four long spiked legs on each aluminum frame were forced into the soil, and adjusted so the lid formed a tight seal with the chamber. The frames slowly loosened in the soil because they were jostled each time the lid popped open as the pressure was released. Therefore, beginning in summer of 2009, rerod was fastened to the chambers with stainless steel screw clamps. This reduced problems associated with shifting frames.

The chambers were controlled by a data logger (Model # CR23X, Campbell Scientific Inc., Logan, UT, USA) which directed pressure (~80-100 psi) from an air compressor to a manifold. From the manifold, eight solenoid values could be activated one at a time to direct pressure to the pneumatic actuator on each chamber. The actuator closed the chamber lid when pressure arrived. Chamber air was pulled from the top of the chambers, through polyethylene coated aluminum tubing via a second pump. The data logger, manifolds, and solenoid values which ran the programs and directed the samples at the treatment and control sites were stored in large
plastic chests which protected the contents from the elements. The tubing from each chamber entered the chest and went to a second manifold with eight solenoid valves. These valves opened and closed following the program on the data logger. From the manifold, a single line of tubing ran to the IRGA (Model LI-6252, Li-Cor, Lincoln, NE, USA) which recorded the [CO₂] every two or ten seconds (control and treatment sites respectively, this was later adjusted to two seconds for both sites). The [CO₂] of each chamber was recorded for 1.5 minutes before the lid closed. This flushed the previous sample and yielded an ambient CO₂ reading. Next, the chamber lid closed for 5.5 minutes. During this time, the buildup of CO₂ within the chamber headspace was recorded. After the air was analyzed, it was pushed by a second pump through the manifold and solenoid valves to the perforated manifold in the base of the chamber being sampled. Each chamber was sampled for seven minutes each hour, so the eight chambers in each array were sampled once every hour.

Automated chamber data were processed using a program I developed in Matlab (The Mathworks, version 7.6.0 R2008a) (Appendix A). For each chamber, the [CO₂] was recorded every two seconds for seven minutes each hour. The program fit a non-linear curve (Equation 3) to those data points which yielded coefficients from which Rₛ was calculated.

\[ C_c(t) = C_s - (C_s - C_o) * e^{-(k*t)} \]

**Eqn. 3.**

When \( C_c \) is the concentration of CO₂ in ppm at time \( t \), \( C_s \) is the soil [CO₂] (ppm), \( C_o \) is the [CO₂] (ppm) at time \( t=0 \), and

\[ k = \frac{S + g_s}{ρV} \]

**Eqn. 4**
where \( S \) is the chamber surface area (0.068 m\(^2\)), \( g_s \) is the soil conductance to CO\(_2\) (mol/mol), \( \rho \) is the molar density of air (41.6 mol/m\(^3\)), and \( V \) is the chamber volume (0.0144 m\(^3\)). To solve for \( C_s \) and \( k \) an estimate of \( C_0 \) is needed. To obtain \( C_0 \) a minimizing function was employed to find the lowest 20 second moving average within the first 70 data points (Appendix A). Using \( C_0 \), \( t \), and \( C_c \) data, a non-linear least squares fit with least absolute residuals returned estimates of \( C_s \) and \( k \).

Figure 5 shows examples of raw CO\(_2\) data, while Figure 6 illustrates the fitted curve for one hour of data from which coefficients were calculated. \( C_0 \), \( C_s \), and \( k \) values for each seven minute period for each site, year, day, hour, and chamber combination were used to calculate the diffusional flux of soil CO\(_2\) (\( D_c \)):

\[
D_c = (C_s - C_0) * g_s
\]

**Eqn. 5**

and since \( g_s = k * \rho V / S \) from equation 4, therefore:

\[
D_c = (C_s - C_0) * \left[ \left( \frac{\rho V}{S} \right) * k \right]
\]

**Eqn. 6**

Excel (Microsoft Office 2007) was used to remove \( D_c \) values where the chambers were suspected of have malfunctioning as follows. First, estimates that used a \( C_0 \) value less than 380 ppm, the current ambient air \([\text{CO}_2]\), were removed. Second, estimates where the \( R^2 \) value for the fitted line was below 0.9 were removed. The .9 level was determined to be high enough to assure close fits and accurate flux calculations, while low enough that the dataset remained robust. Third, negative \( D_c \) values and values above 15 \( \mu \text{mol/m}^2/\text{sec} \) were removed as outliers. Fluxes above 15 \( \mu \text{mol/m}^2/\text{sec} \) were determined to be outliers because they made up on average less than 1% of the data, and these data did not fall within the range of values reasonable for
our site. The data remaining after these operations were graphed using Sigma Plot (Systat Software, Inc. Sigma Plot 10.0). Soil respiration as defined in the present study is considered equivalent to $D_c$ because pressure gradients and hence advective fluxes of CO$_2$ are assume to be insignificant in our experimental approach (Appendix B).
Figure 5. DOY 220: initial graphing of raw data from the control site for chambers 1-4. The increase in [\(\text{CO}_2\)] was recorded during each seven-minute period while the chambers were closed. The four chambers pictured here show very different effluxes, some messier than others.
Figure 6. Raw [CO₂] data (blue line) were graphed for 330 seconds while CO₂ accumulated in the closed automated chambers. Each accumulation curve was fit individually with a non-linear curve (black line) in MATLAB (see methods). Coefficients were computed from the curve and exported to yield $R_s$ (soil respiration).
LI-6400-09 Measurements

A portable LI-6400-09 soil respiration chamber was also used to quantify \( R_s \) in the treatment and control forests. Soil respiration measurements were taken within the 1.1 ha central plots at eight (control) or six (treatment) locations on two, 10.16 cm radius PVC collars. The PVC collars were installed approximately 5 cm into the soil prior to the summer of 2008. This insertion depth left approximately 1 cm of collar above the soil surface for the soil chamber cuvette to rest on. Collars reduced soil disturbance which can occur if the chamber is forced into the soil (Norman et al., 1997). These eight sites were visited approximately once each week during the summers of 2008 and 2009. The data from each site were averaged for each day, yielding an average daily \( R_s \) rate. The within site variation of \( R_s \) values was assessed by calculating the average coefficient of variation for each site and year.

To quantify \( R_s \) using the LI-6400-09 the protocols described in the operation manual were followed (LI-COR, 2004). First, the ambient [CO\(_2\)] at the forest floor was determined and entered into the soil efflux program on the LI-6400. Next, the cuvette was set on the PVC collar. The LI-6400 reduced the [CO\(_2\)] in the cuvette to approximately 10 ppm below ambient (typically 370 ppm), then the internal IRGA analyzed and recorded the accumulation of CO\(_2\) until it was 10 ppm above the set ambient. This method reduced flux bias due to altered diffusional gradients by monitoring \( R_s \) in conditions near to ambient (LI-COR, 1998). As the air was sampled, it was returned to the chamber via a perforated manifold which gently mixed the chamber air. The data collected were fit with a non-linear regression to determine the slope of the increase in [CO\(_2\)], and from this regression the flux of CO\(_2\) from the soil was calculated (for more details see (LI-COR, 2004)). In addition to \( R_s \), soil temperature at 7.5 cm was recorded using a type E thermocouple which connected to the LI-6400. Soil moisture was recorded at 12 cm below the
soil surface using a Hydrosense soil moisture probe (CD620, Campbell Scientific Inc., Logan, UT, USA).

To quantify differences in $R_s$ at sites with varying aspen and birch distributions, the percent basal area of aspen or birch was determined for each site along the transects radiating from the central 1.1 ha treatment and control stands (Figure 2). I predicted that as total basal area of girdled species increased, $R_s$ would decrease. To test this prediction, during the summer of 2009 five PVC collars were inserted at 11 sites in the control and treatment stands. These sites ranged from ~1-84% aspen and birch basal area (Table 1). Weekly measurements of $R_s$ were performed at each site in the treatment and control forests with the LI-6400-09. Soil temperature and moisture were recorded as described above.

<table>
<thead>
<tr>
<th>Control</th>
<th></th>
<th>Treatment</th>
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<tr>
<td>Plot</td>
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<td>Total basal area (m²)</td>
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</tr>
<tr>
<td>A1</td>
<td>84</td>
<td>256</td>
</tr>
</tbody>
</table>

**Table 1.** Sites with similar percent basal area aspen and birch were found in the treatment and control forests. The total basal area of trees at each site is also provided. Soil respiration measurements were recorded at these sites on a weekly basis through the summer of 2009.
Statistical Methods

Automated Chambers

To analyze the 2008 and 2009 automated chamber data I worked with Dr. Christopher Holloman and Mr. Cong Liu of The Ohio State University Statistical Consulting Service. I developed a piecewise linear regression model with an autocorrelated (AR1) error term in SAS (SAS Inc. ver. 9.2). This model had five main components. The first component was the daily pattern in $R_s$. The data for each 24 hour period were first fit using a sine wave, then a cosine wave, each with a 24, 12, and 8 hour frequency. The modeled waves for each hour were added together in linear combination, resulting in a final wave that described the undulating daily pattern of $R_s$ (Appendix C). The second component utilized two seasonal indicators which defined the season to be early spring (DOY 110-139), summer (DOY 140-280), or late fall (DOY 281-312). The daily pattern (component 1) was fit separately for each season, accommodating different $R_s$ values early and late in the season.

The third component assigned change points for each year that identified where sudden increases or decreases in $R_s$ occurred (Table 2). These change points permitted the regression slope to change in a piecewise manner. Five change points were used in 2008 and 2009. The fourth component was year, either 2008 or 2009, because we expected different patterns in $R_s$ for each year. The fifth component was treatment effect, either treatment (girdled) or control, because we expected different $R_s$ responses from at the two sites. These five components and their interactions were used to develop variables which were tested for significance (Appendix D). The raw data from 2008 and 2009 served as the response variable $Y$ against all the variables which allowed for analysis of the model. The predicted values for the models were graphed and the difference between the modeled data in 2008 and 2009 were analyzed to determine if there was a treatment effect.
Table 2. Change points were set for days of year where the slope of the data changed rapidly in the 2008 and 2009 datasets. The first two change points in 2008 were not used, because treatment site data were not available early in the season.

Analysis of Manual Chamber Data

LI-6400-09 $R_s$ data were collected in the 1.1 ha plots and along the aspen and birch basal area gradient in the treatment and control stands. The 1.1 ha plots in the control and treatment sites were sampled during the summers of 2008 and 2009. These data were analyzed using an ANOVA test to determine if there was a significant difference between the sites or the measurement methods. The aspen and birch basal area gradient was only monitored in 2009. These data were analyzed using an ANOVA to test for a treatment effect. A second data set was developed to compare LI-6400-09 $R_s$ measurements to the average automated chamber $R_s$ for that day. These data sets were incorporated for each site, sampling date, and year. The data were also analyzed with an ANOVA test.
Results

Environmental Data

Air Temperature

Winter (DOY 0-110 and 313-366) air temperatures ($T_a$) in 2008 were typical of northern lower Michigan, with a daily average $T_a$ of -3.9°C (Figure 7 top). The minimum winter $T_a$ was -15.8 °C on DOY 41, while the highest $T_a$ was 16.4°C on DOY 107. Spring, designated as DOY 111-139, recorded average $T_a$ of 9.8°C. During summer (DOY 140-280) of 2008, $T_a$ averaged 16.2°C. The maximum temperature during this period was 22.9°C on DOY 159. The fall season, designated as DOY 281 to 312, had average $T_a$ of 7.9°C.

Air temperatures in 2009 followed a pattern similar to 2008 (Figure 7 bottom). The average winter 2009 $T_a$ was -3.4°C, with a maximum of 11.9°C occurring on DOY 107. The minimum winter $T_a$ in 2009 of -20.6 was recorded on DOY 35. Spring 2009 had average $T_a$ of 9.2°C. Summer 2009 was cooler on average than 2008, with a mean temperature of 15.6 °C. The high $T_a$ for the season was 25.1°C which was noted on DOY 175. The low $T_a$ of 5.7 °C was recorded on DOY 274. Fall 2009 was slightly cooler than 2008, with an average $T_a$ of 5.1°C.
Figure 7. Average daily air temperature in 2008 (top) and 2009 (bottom) calculated from half-hourly temperature measurements 1 m above the forest floor. Dashed lines indicate the seasonal demarcations where winter is day of year (DOY) 0-110 and 313-366, spring is DOY 111-139, summer is DOY 140-280, and fall is DOY 281-312.
Soil Temperature

All soil temperatures were measured at 7.5 cm depth. Winter soil temperature ($T_s$) was relatively constant in 2008, averaging 1.7°C and 2.0°C for the treatment and control sites respectively (Figure 8 top). The minimum winter $T_s$ were 0.4°C on DOY 92 and 0.5°C on DOY 98 at the treatment and control sites respectively. The soil began to warm in the spring 2008 on approximately DOY 110, with mean $T_s$ of 8.8°C and 8.5°C at the treatment and control forests. The high temperature was 11.1°C and 10.1°C on DOY 137 and 138 respectively for the treatment and control sites. Summer had similar average $T_s$ values at the treatment and control forests, with $T_s$ of 15.0°C and 14.7°C, respectively. Maximum summer $T_s$ values of 19.1°C and 18.8°C were recorded on DOY 236 for the treatment and control sites respectively. Fall 2008 $T_s$ averaged 9.2°C at both sites. Maximum $T_s$ values at the treatment and control sites were 13.6°C and 13.2°C on DOY 287, respectively.

Soil temperature in 2009 closely followed 2008 patterns (Figure 8 bottom). Winter soil temperature was similar to 2008, with average $T_s$ values of 1.7°C and 2.4°C at the treatment and control sites, respectively. Minimum winter $T_s$ values were 0.03°C on DOY 37 and 0.9°C on DOY 100 at the treatment and control sites respectively. In early spring mean $T_s$ values of 8.0°C and 8.1°C were recorded at the treatment and control forests, respectively. The maximum temperature recorded was 10.6°C on DOY 128 at both the treatment and control sites. Summer values averaged 14.1°C and 13.8°C at the treatment and control forests, respectively. Maximum $T_s$ in 2009 was similar to 2008, however, maximum $T_s$ occurred almost a week earlier in 2009 than in 2008. The maximum temperatures of 19.2°C and 19.0°C were recorded on DOY 229 for the treatment and control sites respectively. Fall 2008 was characterized by average $T_s$ of 7.5°C
and 7.7°C for the treatment and control forests, respectively. The maximum $T_s$ at the treatment and control sites was 10.1°C and 10.2°C, respectively on DOY 281.
Figure 8. Average daily soil temperature (°C) at 7.5 cm depth was recorded at the control and treatment sites in 2008 (top) and 2009 (bottom) using type T thermocouples. Measurements were logged every 30 minutes. Dashed lines indicate the seasonal divisions which were used. Winter is day of year (DOY) 0-110 and 313-366, spring is DOY 111-139, summer is DOY 140-280, and fall is DOY 281-312.
Soil Water Content

Soil water content (SWC) in 2008 showed soil drying during the summer and early fall, with punctuated rainfall events which increased the SWC briefly (Figure 9 top). Winter 2008 recorded average SWC of 12.5% and 12.2% volume of water per volume of soil for the treatment and control sites respectively. Between DOY 110 and 139, spring rains increased average SWC to 13.1% and 12.2% in the treatment and control stands, respectively. The maximum spring SWC of 16.8% and 16.4% occurred on DOY 117 for both the treatment and control sites, respectively. The minimum spring SWC of 11.6% and 10.0% occurred on DOY 115 at treatment and control forests, respectively. During summer 2008 the average SWC was 7.8% at both sites. The maximum summer SWC of 16.5% and 17.1% occurred on DOY 165, while the minimum SWC of 2.6% and 3.4% occurred on DOY 233 for the treatment and control sites, respectively. Mean fall SWC was 7.8% at both the treatment and control forests. The maximum recorded fall SWC was 11.9% and 12.8% on DOY 312 and 300 at the treatment and control sites respectively. In the fall the soil was driest on DOY 281, when SWC of 4.9% and 4.8% were recorded at the treatment and control sites, respectively.

Soil water content in 2009 was consistently higher at the treatment site than the control site (Figure 9 bottom). In winter 2009, SWC averaged 11.9% and 10.7% volume water per volume of soil for the treatment and control sites, respectively. In spring 2009, SWC values increased to average 11.7% and 10.1% for the treatment and control forests, respectively. The maximum spring SWC occurred on DOY 111 for both the treatment and control sites, respectively, with values of 13.6% and 12.0%, respectively. The minimum spring SWC of 10.0% and 8.7% occurred on DOY 126 at the treatment and control forests respectively. During the
summer months the average SWC was 9.6% and 6.4% at the treatment and control forest respectively. The maximum summer SWC of 16.5% and 12.0% occurred on DOY 148 and 160 for the treatment and control sites, respectively. This 4.5% divergence in SWC was the largest recorded difference between the two sites in 2009 or 2008. The minimum summer SWC of 4.6% and 4.0% occurred on DOY 202 for the treatment and control sites respectively. Mean fall SWC of 13.4% was recorded at the treatment site. The control site datalogger malfunctioned, so data for the remaining fall and winter were not collected. The maximum SWC at the treatment site was 16.2% on DOY 295, while the minimum of 11.4% was recorded on DOY 293.
Figure 9. Daily average soil moisture at control and treatment sites was recorded every 30 minutes at two locations at each site in 2008 (top) and 2009 (bottom). Representative error bars represent standard deviation of variation between the two sensors. The control site data logger ceased functioning on DOY 274 in 2009. Dashed lines indicate the seasonal divisions which were used. Winter is day of year (DOY) 0-110 and 313-366, spring is DOY 111-139, summer is DOY 140-280, and fall is DOY 281-312.
**Automated Chamber CO₂ Fluxes**

Representative automated chamber data are plotted in Figure 5 to illustrate the 7 minute accumulation of CO₂ recorded for each hour of the day, for each individual chamber. The four graphs in Figure 5 show chambers 1-4 on DOY 220, 2008 at the control site. Chamber 2 (top right) showed larger fluxes of CO₂ as evidenced by longer lines which indicate greater CO₂ accumulation within the chamber over each 7 minute measurement period. Chamber three (bottom left) recorded smaller Rs values, as evidenced by shorter lines (i.e. less CO₂ accumulation over 7 minutes). Chamber three may have malfunctioned between hours 15 and 16 where the accumulation of CO₂ in the chamber did not occur (Figure 5). The data for each 7 minute period were graphed and fit using the non-linear fit described previously. Figure 6 shows CO₂ accumulation data for chamber one at 10 am graphed with the fitted line. The coefficients C₀, Cₜ, and k were calculated in Matlab (see Methods), and used to calculate Rs.

The automated fluxes were calculated for each 7 minute collection period for each hour and site in 2008 and 2009. The fluxes were used to calculate the daily average Rs for each chamber. In 2008, the control site generally showed larger daily Rs values than the treatment site (Figure 10, top). The 2008 data set began on DOY 110 at the control site, while the treatment site chambers did not begin recording until DOY 176. Soil respiration at the treatment site was stable between DOY 110 and 139 (spring) averaging 1.6 µmol/m²/sec. A maximum Rs of 2.2 µmol/m²/sec was recorded on DOY 135, while a minimum Rs of 1.0 µmol/m²/sec was recorded on DOY 120. The summer of 2008 Rs at the control site averaged 4.4 µmol/m²/sec, while the treatment site averaged 3.5 µmol/m²/sec between DOY 176 and 280. Maximum effluxes of 5.7 µmol/m²/sec and 7.8 µmol/m²/sec were recorded on DOY 230 and 241 for the
treatment and control sites respectively. The minimum $R_s$ values were 1.6 $\mu$mol/m$^2$/sec and 1.2 $\mu$mol/m$^2$/sec on DOY 277 and DOY 142 for treatment and control sites, respectively. During fall 2008, data were recorded between DOY 281 and 311 (treatment site) or 312 (control site). The average $R_s$ values were 1.9 $\mu$mol/m$^2$/sec and 3.2 $\mu$mol/m$^2$/sec for the treatment and control sites, respectively. The maximum fall $R_s$ values of 3.1 $\mu$mol/m$^2$/sec and 4.3 $\mu$mol/m$^2$/sec were recorded on DOY 287 at the treatment and the control stands, respectively. Minimum fall daily $R_s$ of 1.1 $\mu$mol/m$^2$/sec and 2.2 $\mu$mol/m$^2$/sec occurred on DOY 303 and 307 at the treatment and control sites, respectively.

Spring 2009 $R_s$ was similar at the treatment and the control sites (Figure 10 bottom). Average $R_s$ of 1.5 $\mu$mol/m$^2$/sec and 2.0 $\mu$mol/m$^2$/sec were recorded at the treatment and control forests respectively. Maximum spring $R_s$ was 2.2 $\mu$mol/m$^2$/sec and 2.9 $\mu$mol/m$^2$/sec at the treatment and controls sites, respectively. These maxima occurred on DOY 128 and 127 for the treatment and control sites, respectively. Minimum spring $R_s$ values of 0.6 $\mu$mol/m$^2$/sec and 1.1 $\mu$mol/m$^2$/sec were recorded on DOY 112 at the treatment and control sites, respectively.

Summer 2009, the average $R_s$ values were 3.5 $\mu$mol/m$^2$/sec and 4.9 $\mu$mol/m$^2$/sec for the treatment and control sites, respectively. The site differences in $R_s$ in the summer of 2009 were larger than in 2008. During the summer 2009, maximum effluxes of 6.6 $\mu$mol/m$^2$/sec and 8.2 $\mu$mol/m$^2$/sec were recorded on DOY 229 at the treatment and control sites, respectively. Minimum summer 2009 $R_s$ values of 1.7 $\mu$mol/m$^2$/sec and 2.2 $\mu$mol/m$^2$/sec occurred on DOY 151 at the treatment and control stands, respectively. Average fall 2009 $R_s$ values were 1.5 $\mu$mol/m$^2$/sec and 2.4 $\mu$mol/m$^2$/sec for the treatment and control forests, respectively. The maximum $R_s$ values recorded were 2.0 $\mu$mol/m$^2$/sec and 3.1 $\mu$mol/m$^2$/sec on DOY 282 and 294.
for the treatment and control sites, respectively. At the onset of winter $R_s$ values for the sites became more similar, ending with minimum $R_s$ values of 1.7 µmol/m$^2$/sec and 2.0 µmol/m$^2$/sec for the treatment and control sites, respectively on DOY 290.
Figure 10. Average daily $R_s$ (soil respiration) in 2008 (Top) and 2009 (Bottom) recorded by automated soil respiration chambers at treatment and control sites. The error bars are standard error bars (n= 8), and the large error in mid-summer (2008) is due to small sample sizes for those dates. Dashed lines indicate the seasonal divisions which were used. Winter is day of year (DOY) 0-110 and 313-366, spring is DOY 111-139, summer is DOY 140-280, and fall is DOY 281-312.
Soil respiration measurements from the treatment and control sites were modeled to conduct a statistical analysis of the girding effect in 2008 and 2009 (Figure 11). T-tests showed there were significant differences between the modeled treatment and control site $R_s$ in both 2008 and 2009 ($\alpha=0.05$, $p\leq 0.022$ and $p\leq 0.0022$, 2008 and 2009 respectively) (Table 3). To determine if the girdling effect was greater in 2009, the magnitude of the difference in effluxes between the treatment and control sites for each year was analyzed as:

$$magn\text{itude of the respiratory difference} = (\text{control } '09 - \text{treatment} '09) - (\text{control } '08 - \text{treatment} '08)$$

Eqn. 5

A t-test indicated that the magnitude of the girdling effect became significantly greater in 2009 between DOY 219-252 ($p\leq 0.034$, $\alpha=0.05$) (Figure 12 and Table 3). The residuals for the modeled data were analyzed and found to be normal, with a few high outliers (Appendix E). The residuals versus year and date showed no overall trends (Appendix F).

<table>
<thead>
<tr>
<th>T-Test</th>
<th>df</th>
<th>t-value</th>
<th>$\alpha$</th>
<th>p-value (*indicates significance)</th>
<th>DOY range</th>
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<tr>
<td>Control and treatment site differences in 2009</td>
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<td>0.05</td>
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<tr>
<td>Magnitude of the change in efflux between sites and years</td>
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<td>2.18</td>
<td>0.05</td>
<td>0.034 *</td>
<td>219-251</td>
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</table>

Table 3. Automated data and LI-6400-09 data were analyzed to determine significant differences between treatments and measurement methods. T-tests showed significantly different automated chamber $R_s$ at the treatment and control sites for 2008 and 2009. A third t-test showed the magnitude of the difference between the treatment and control sites became significantly larger between DOY 219 and 252 in 2009. An asterisk (*) indicates a statically significant difference was detected between the two samples.
Figure 11. Predicted values for soil respiration ($R_s$) at the treatment and control sites in 2008 (top) and 2009 (bottom) were developed in SAS using a piecewise linear regression model. Change points (illustrated by arrows (Table 2)) were set in the model to accommodate changes in the slope of the original data sets. Dashed lines indicate the seasonal divisions which were used. Winter is day of year (DOY) 0-110 and 313-366, spring is DOY 111-139, summer is DOY 140-280, and fall is DOY 281-312.
Figure 12. Automated chamber soil respiration ($R_s$) data were modeled (Figure 11) for the treatment and control sites in 2008 and 2009. Then, the magnitude of the difference in predicted $R_s$ for the control and treatment sites between years was calculated and graphed above. The vertical lines indicate that between DOY 219-252 the respiration rates at the treatment and control site became significantly more different in 2009 than they were in 2008 ($p<0.05$). The dashed line indicates the end of the period we specify as summer on day of year 280, and the start of fall.
Soil respiration was monitored with the LI-6400-09 between DOY 172-218 or 288 (2008 or 2009) within the 1.1 ha plots at the base of the flux towers in the control and treatment sites (Figure 13). The average coefficient of variation for within site variation of the daily average $R_s$ values was 26%. In 2008, $R_s$ was monitored with the LI-6400-09 more frequently, but for a shorter portion of the summer. The control and treatment sites in 2008 had similar $R_s$ values, which averaged 5.5 µmol/m$^2$/sec and 5.8 µmol/m$^2$/sec, respectively (Figure 13 top). In 2009, $R_s$ was monitored with the LI-6400-09 longer into the summer, but less frequent measurements were taken (Figure 13 bottom). Soil respiration in 2009 averaged 3.8 µmol/m$^2$/sec at both sites.

An ANOVA test of LI-6400-09 measurements from the two sites revealed that 2009 had significantly lower mean $R_s$ values than 2008 ($p<0.001$). Day of year was also a significant parameter in the model, with $p=0.03$. Interestingly, the treatment and control sites were not significant parameters in the model, so based on these statistics we can conclude there was no site differences in $R_s$ values in 2008 and 2009 ($p=0.6$) (Table 4).

The LI-6400-09 1.1 ha $R_s$ results contrast with the automated chamber data. Soil respiration rates for automated chambers were determined for each day when LI-6400-09 measurements were taken (Figure 14). In 2008, the control site had an average $R_s$ of 5.6 µmol/m$^2$/sec, while the mean $R_s$ for the treatment site was 4.3 µmol/m$^2$/sec (Figure 14 top). This site difference in automated chamber $R_s$ was seen again in 2009. The treatment and control sites recorded $R_s$ values of 4.5 µmol/m$^2$/sec and 3.4 µmol/m$^2$/sec respectively (Figure 14 bottom). An ANOVA test for the automated data showed significantly lower $R_s$ in 2008 than in 2009 ($p=0.001$) and that DOY was a significant parameter ($p=0.045$) (Table 4). The ANOVA also
revealed that over the two years of data the control site had significantly greater $R$, than the treatment site ($p<0.001$). The interaction term of year and day was also a significant parameter ($p=0.02$) (Table 4).
Figure 13. LI-6400-09 soil respiration ($R_s$) measurements were taken at the treatment and control sites in 2008 (top) and 2009 (bottom) in the 1.1 ha plot. The average $R_s$ for each day was calculated and graphed with the standard error (max n=6). No significant difference in $R_s$ was found between the treatment and control sites (2008 $p=.45$, 2009 $p=.95$).
Figure 14. Automated chamber soil respiration ($R_s$) measurements at the treatment and control sites in 2008 (top) and 2009 (bottom) in the 1.1 ha plot. These data are the daily average $R_s$ for days when LI-6400-09 measurements were taken. The standard error (n=8) is shown for each point. A significant difference in $R_s$ was found in both 2008 and 2009 between the treatment and control sites (2008 $p=0.007$, 2009 $p=0.049$).
Automated chamber and LI-6400-09 measurements taken in the 1.1 ha plot in 2008 were compared to determine if the two measurement methods agreed. In 2008, data were available through DOY 218 (Figure 15). The calculated $R_s$ values for the control sites were similar and averaged 5.5 µmol/m$^2$/sec and 5.6 µmol/m$^2$/sec using the LI-6400-09 and automated chambers respectively (Figure 15 top). In contrast, the treatment site in 2008 showed a large difference between the LI-6400-09 and the automated chamber methods (Figure 15 bottom). The average 2008 $R_s$ values for the LI-6400-09 was 5.8 µmol/m$^2$/sec, while the automated chambers averaged $R_s$ values of 4.3 µmol/m$^2$/sec. The ANOVA test demonstrated that site was a significant parameter, with the control site having greater mean $R_s$ than the treatment site ($p=0.035$) (Table 4). Day of year and the measurement method (LI-6400-09 or automated chamber) were also significant parameters. As DOY increased so did $R_s$, while the automated chambers recorded lower $R_s$ than the LI-6400-09.

In 2009 the control site LI-6400-09 $R_s$ averaged 3.8 µmol/m$^2$/sec, while the automated chambers averaged 4.5 µmol/m$^2$/sec (Figure 16 top). At the treatment site in 2009 the LI-6400-09 had an average $R_s$ of 3.8 µmol/m$^2$/sec, while automated chambers averaged 3.4 µmol/m$^2$/sec (Figure 16 bottom). An ANOVA test of $R_s$ values in 2009 yielded very different results than in 2008. No significant difference was found between the treatment and control stands ($p=0.13$) (Table 4). Also, the DOY and measurement method (LI-6400-09 or automated chambers) were not significantly different from each other ($p>0.05$) (Table 4).

Overall, when we compared the data for the treatment and control sites in 2008 and 2009 taken with the LI-6400-09 or the automated chambers we find that site was a significant parameter with higher $R_s$ values occurring at the control site ($p<0.001$) (Table 4). Year and DOY
were also significant parameters, with 2009 having lower $R_s$ than 2008 ($p=0.001$), and increasing $R_s$ as DOY increased ($p=0.003$). No difference was found between the two measurement methods when the results from both sites and years were taken together. Several interaction parameters were also significant including site and measurement method ($p<0.001$), DOY and year ($p=0.003$), and year and efflux indicator ($p=0.039$) (Table 4).
Table 4. ANOVA tests were conducted on $R_s$ data collected at the control and treatment sites in 2008 and 2009 using the LI-6400-09 and automated chambers. In the table above year is 2008 or 2009, site is treatment or control, and measurement method is LI-6400-09 or automated chamber. The interactions between the parameters were also analyzed. Parameters were considered significant if $p$ was less than $\alpha=0.05$. Significant parameters were indicated with a check (✔), while non-significant parameters were indicated with an empty set (ø).
Figure 15. The automated chamber and LI-6400-09 measurements of soil respiration ($R_s$) were compared in 2008 at the control (top) and treatment (bottom) sites. No significant difference was found between the methods at the control site ($p=0.84$). However, a significant difference was found at the treatment sites between the two methods ($p<0.001$). Error bars are standard error bars were $n=8$ for the automated chambers, and $n_{max}=6$ for LI-6400-09.
Figure 16. The automated chamber and LI-6400-09 soil respiration ($R_s$) measurements were compared in 2009 at the control (top) and treatment (bottom) sites. No significant difference was found between the measurement methods at the control or treatment sites ($p=0.22$ and 0.39 respectively). Standard error bars are shown for the LI-6400-09 (max $n=6$) and automated chamber ($n=8$) measurements.
**Percent Basal Area Aspen and Birch Gradient**

The basal area gradient monitored during the summer of 2009 did not show obvious trends due to the girdling treatment (Figure 17). The control site recorded the largest $R_s$ values at approximately 51% basal area aspen and birch. Soil respiration declined at the control site as the percentage aspen and birch increased to 65%. The largest efflux recorded at the treatment site was $7.9 \, \mu\text{mol/m}^2/\text{sec}$ and was recorded at 45% basal area aspen and birch on DOY 223. However, high $R_s$ measurements were also recorded at 0% aspen and birch sites throughout the season (Figure 17 top). The treatment site location with 66% basal area aspen and birch recorded showed much lower $R_s$ ($3-6 \, \mu\text{mol/m}^2/\text{sec}$) over the 35 days of study, compared to the control site where $R_s$ ranged from 4.5-8.5 $\mu\text{mol/m}^2/\text{sec}$.

An ANOVA test was used to analyze the data. No significant difference in $R_s$ was found between the girdled and control sites ($p= .19$) (Table 5). However, DOY was a significant indicator. As the DOY increased so did the average $R_s$ at both sites ($p <0.01$). The percent basal area aspen and birch was a significant term in the ANOVA analysis ($p=0.019$). A regression analysis was used to compare the treatment and control site $R_s$ over the basal area gradient. As the control site percent basal area of aspen and birch increased so did $R_s$ ($R^2=.19$) (Figure 18). However, at the treatment site there was no relationship between percent basal area aspen and birch and $R_s$ ($R^2=.006$) (Figure 18).
<table>
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<th>p-value</th>
<th>Significance</th>
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<td></td>
<td>Treatment</td>
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<td>0.19</td>
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Table 5. The results of the ANOVA indicate that there were no significant differences between treatment and control site $R_s$ over the percent basal area aspen and birch gradient. DOY and percent basal area were significant indicators of $R_s$ ($\alpha=0.05$).
Figure 17. Treatment (top) and control (bottom) site soil respiration ($R_s$) rates were recorded with the LI-6400-09 along a percent basal area aspen and birch gradient between day of year (DOY) 188-223 in 2009. A site with 80% basal area aspen and birch was available at the control site, but not at the treatment site. Error bars show the standard deviation of the mean (n=5).
Figure 18. Soil respiration ($R_s$) was monitored along the basal area aspen and birch gradient in 2009 at the treatment and control sites. At the control site basal area aspen and birch was a positive predictor of $R_s$ ($R^2 = .19$), while at the treatment site there was no relationship between basal area and $R_s$ ($R^2 = .006$).
Discussion

Treatment Effects on Soil Respiration

Soil respiration in the treatment site was significantly lower than in the control site in 2008, with the treatment effects becoming larger in 2009 (Figure 10). These results supported hypothesis 1 that the girdling treatment would result in lower $R_s$. Additionally, automated chamber $R_s$ was modeled to analyze the effect of the girdling treatment, and the modeled data for each year demonstrated that the treatment significantly lowered $R_s$, with the effect becoming greater in 2009, especially in mid to late summer (Figures 11 and 12). The magnitude of the treatment effect also increased in 2009, supporting hypothesis 2, that the effect of girdling would become more severe with time (Figure 10 bottom).

Attributing the difference in $R_s$ in 2008 to the girdling treatment alone may, however, be problematic. Despite girdling in the spring of 2008 we predicted that the large reserves of carbohydrates in the roots and trunk below the girdle would maintain living roots and normal belowground tree functioning (Andersen et al., 2005; Chen et al., 2010; Gough et al., 2009). Hence, unchanged $R_s$ values were expected for the first year after girdling. Edwards and Ross-Todd (1979) girdled all trees taller than 40 cm in the tulip poplar dominated forests of Tennessee, USA. Open system automated chambers found that the treatment sites maintained pre-girdling $R_s$ values and normal tree function for two years following the treatment. In more recent studies, stored root C was identified as an important component that allowed girdled
trees to maintain their $R_o$ (Andersen et al., 2005; Ryan and Law, 2005). Schier (1978) examined the effect of girdling young greenhouse grown aspen trees, and found that girdled aspen had more rapid and extensive root decay than control trees. However, in the present study it seemed reasonable to assume that mature aspen, such as those in this study with large root C stores (Gough et al., 2009) would respond more slowly to girdling than greenhouse saplings. Furthermore, we did not observe changes in girdled tree fluorescence (an indicator of photochemical efficiency) until summer 2009. We found a trend of declining fluorescence as time since girdling increased, which is indicative of declining photosynthetic capacity (Hardiman pers. comm.). However, photosynthetic rates were not found to be significantly different between the control and treatment stands.

Treatment versus control site differences in $R_o$ during the summer of 2008, the pretreatment year, were not easily explained. The treatment and control forests had similar site characteristics including soil type, vegetation, slope, disturbance history, etc., which led us to predict similar $R_o$ at both sites. Perhaps one or more additional site variables such as soil organic matter content, N mineralization rate, root activity, mycorrhizal infection rate, or site fertility were not assessed, resulted in the difference in $R_o$.

Seasonality of Soil Respiration

Throughout 2008 and 2009 there was an interesting pattern of similar $R_o$ in treatment and control sites in the spring, divergence of $R_o$ in the summer, followed by convergence in $R_o$ values in the fall (Figure 10). In spring 2009, $R_o$ was within 0.5 μmol/m$^2$/sec at the two sites and began to diverge on DOY 180 (Figure 10 bottom). Bhupinderpal et al.(2003) also observed a similar pattern in $R_o$ in their girdled Scots pine forest. By mid September $R_o$ values at the
ungirdled treatments converged with the low $R_s$ values observed at the girdled sites. However, by following June the treatment site $R_s$ values were double those at the control site (Bhupinderpal et al., 2003).

In the present study, differences in soil moisture were results likely associated with the girdling treatment. Girdling itself does not alter the soil moisture because the roots continue to function after girdling (Hogberg et al., 2001). However, since girdling culminates in tree death, at some point in time root water uptake will decline as root function is lost. In 2008, the treatment and control sites had very similar SWC (Figure 7 top). However, in the early spring of 2009 (~DOY 115) SWC at the two sites began to diverge with the treatment site having higher SWC than the control site. The magnitude of this difference increased over summer 2009. By the end of the summer 2009 the treatment site had SWC nearly double that of the control site (Figure 7). Increased SWC at the treatment site could be due to aspen and birch root death which resulted in decreased root water uptake.

Decreased soil water uptake could have several cascading effects on $R_s$. First, soil moisture would increase, and a number of studies have shown that moist soils typically have higher $R_s$ than dry soils (Jin et al., 2008; Mo et al., 2007; Sullivan et al., 2008). At the treatment site, we predicted that wetter soils would increase $R_s$ by the microbial community, and that $R_o$ would increase as roots of non-girdled trees and understory species took advantage of extra water (Bouma et al., 1997; Jin et al., 2008). However, $R_s$ at the treatment site was lower than in the control sites. Thus, it appears that the decline in $R_s$ due to girdling (lower $R_o$), or pre-existing site differences was greater than any potential increases in $R_s$ due to increased soil moisture.
*Data Gap Assessment*

In 2008, approximately 17.0% and 15.9% of the CO$_2$ fluxes were removed at the control and treatment sites respectively due to quality assurance and quality control (QA/QC) procedures described in the methods (Figure 19). In 2009, the control site had the highest percentage of $R_s$ measurements removed at 29.1%, while the treatment site in 2009 had 15.7% of the $R_s$ measurements removed. On average over both sites and years approximately 19.5% of the data were removed.

*Figure 19.* The percentage of $R_s$ values removed for quality assurance and quality control (QA/QC) purpose for the treatment and control sites in 2008 and 2009. No error bars are shown because these are raw percentages out of the total number of fluxes recorded.
The first season of data collection (2008) was predicted to have more data removed for QA/QC because I was less familiar with chamber maintenance, chambers were not checked as often, and troubleshooting took longer. However, more data were removed in 2009 than in 2008 (Figure 19). The control site in 2009 had the most data removed, but the reason why is not clear. The tubing, IRGAs, curve fitting, and QA/QC methods were the same in 2008 and 2009. The only change in 2009 was improved chamber maintenance which lasted for a half hour 1-2 times a week.

Comparison of LI-6400-09 and Automated Chamber Soil Respiration Measurements

Soil respiration as measured using the automated chambers and the LI-6400-09 system were compared to investigate if the two measurement methods yielded similar findings about the magnitude and timing of the treatment effect. Generally, the two measurement methods showed similar patterns in $R_s$ as both sites responded to similar changes in environmental conditions (Figures 15 and 16). However, different conclusions where reached when either automated data, or only LI-6400-09 data alone were used to evaluate the treatment effect. An ANOVA of LI-6400-09 $R_s$ from 2008 and 2009 showed no significant site differences ($p>0.05$, Table 4 and Figure 13), while, automated chamber $R_s$ data from the same period reported a significant difference in $R_s$ between the treatment and control sites (Table 4 and Figure 14). These contrasting conclusions about the girdling effect were probably due to $R_s$ values determined using the automated chambers at the treatment site which were consistently lower than $R_s$ values for the control site.

In 2008, $R_s$ for the control site using both measurement methods and for the treatment site using the LI-6400-09 alone averaged 5.6 µmol/m²/sec., while the treatment site automated
chamber $R_s$ averaged 4.3 $\mu$mol/m$^2$/sec (Figure 15). Potential reasons for the low automated chamber $R_s$ values at the treatment site are not readily obvious. However, this trend did not continue in 2009, and no difference was found between sites or measurement methods (Table 4, Figure 16). The LI-6400-09 was adequately calibrated, the program parameters were correct, and the instrument was performing well, so these differences were not caused by measurement problems. The difference in $R_s$ also cannot be attributed to abiotic factors such as soil moisture, $T_a$, or $T_s$, all of these parameters were similar at the treatment and control sites in 2008 (Figures 7, 8, and 9).

In general, the automated data set would be predicted to be more reliable because it contains continuous measurements of $R_s$. The LI-6400-09 measurements could have been taken on an anomalous day or days, which may have skewed the results relative to the automated chambers. In addition, the LI-6400-09 measurements were only taken for a few minutes at each collar approximately once a week, so the dataset is less robust and has lower temporal coverage. Unfortunately, these potential explanations do not lead to unambiguous conclusions about the accuracy of the measurement methods, or the extent of the treatment effect.

**Soil Respiration Along an Aspen and Birch Basal Area Gradient**

The effect of girdling on $R_s$ was also assessed by comparing $R_s$ along a gradient of aspen and birch basal area in the treatment and control sites in 2009. It was hypothesized that treatment sites with high percent basal area of girdled aspen and birch would exhibit lower $R_s$ than at either treatment sites with low percent basal area of girdled aspen and birch, or at control sites. However, the $R_s$ data collected with the LI-6400-09 between DOY 188-223, 2009 did not directly support hypothesis 3 (Figure 17 top, Table 4). No overall significant difference in
were found between the treatment and control sites (p=0.19). The lack of site differences (treatment effect) in Rs over the basal area gradient contradicted conclusions developed from automated chamber data (Figures 10, 14). The basal area gradient Rs data revealed that at both sites DOY was a significant predictor of Rs. This relationship makes sense because Ts increased over the summer, creating conditions suitable for increased Rs. Interestingly, Rs at the control site increased as the percent basal area of aspen and birch increased, while the treatment site revealed no such relationship (Figure 18). This partially supports hypothesis 3 where I predicted a decline in Rs in sites with high percent basal area of girdled aspen and birch compared to control sites. This change in the positive association between percent basal area of aspen and birch and Rs may be an early sign of a treatment effect. The uncoupling of basal area and Rs at the treatment site may be an initial sign of deteriorating tree function due to girdling. Fast growing early successional trees may have greater Rs than slower growing late successional species (Curtis et al., 2005). The decline in Rs after girdling at sites with high percent basal area of aspen and birch may indicate that the trees are beginning to decline and this was noted in Rs. Greater uncoupling of other forest processes is predicted as girdled trees die. A negative relationship between percent basal area aspen and birch and Rs in the treatment site is predicted for 2010 because of greater root mortality than in control sites of similar basal area aspen and birch. Thus, the girdled sites are predicted to exhibit a decline in Rs resulting in lower Rs in future years.

The wide range in Rs over the basal area gradient between sites in the treatment and control stands may be due to site variability. In general, these forests grow in similar soils, but other site characteristics such as tree productivity, soil organic matter content, LAI, N
mineralization, etc. may have varied in subtle ways. Such variability may have minimized differences in observed $R_s$ between the control and treatment stands. The variation in the total basal area between all the sites was moderate, having a coefficient of variation of 24% (Table 1). Thus, total basal area differences cannot explain the $R_s$ results. We must therefore also consider the limited locations within sites where measurements were taken. The five collars at each site were located within approximately 1.5 m$^2$. As a result, the true variation at each site or the site response to girdling may not have been captured.

**A Delayed $R_s$ Response to Girdling?**

Assuming the 2008 site differences were due to pre-existing conditions, there exists no evidence from the study for a rapid decrease in $R_s$ after girdling as was seen in studies by Hogberg et al. (2001), Andersen et al. (2005), Frey et al. (2006), and Johnsen et al. (2007). Johnsen et al. (2007) utilized phloem chilling as a mechanism to physiologically girdle pine trees. The authors reported a decrease in $R_s$ after three days of chilling. Frey et al. (2006) observed a significant decrease in $R_s$ within 9-20 days after girdling sweet chestnut trees in Switzerland. In contrast, sites with girdled tulip poplars and chestnut trees in Tennessee, USA showed no decrease in $R_s$ during two years of monitoring (Edwards and Ross-Todd, 1979). Hogberg et al. (2001) reported a decrease in respiration rates within a few days of girdling Scots pine. However, that study took place in a plantation where 100% of LAI was girdled. This contrasts with the present study, where only approximately 35% of the LAI was girdled. Thus, we would expect a smaller decrease in $R_s$ compared to Hogberg et al. (2001). The decline in $R_s$ might be further reduced as changes in soil N and water occur over time. Initial studies at our site have shown increased soil N availability, and N losses as girdled tree root function declines (Hardiman...
pers. comm., Nave pers. comm.). Non-girdled trees, understory species, and the microbial community may be able to take advantage of extra soil water and N availability, resulting in root proliferation and microbial community growth, leading to increased $R_s$ (Hodge et al., 1999; Jin et al., 2008; van Vuuren et al., 2003). These increases in $R_s$ may initially be offset by the decreases in $R_s$ due to girdling.

Species-specific responses to girdling may also be important. Chen et al. (2010) found two young plantation species had very different $R_s$ values after girdling. A N$_2$-fixing tree (Acacia crassicarpa) showed a greater decline in $R_s$ than the root sprouting tree (Eucalyptus urophylla). Andersen et al. (2005) found a rapid decrease in $R_s$ under beech trees after girdling, but not under girdled spruce trees. The authors argued that this difference may have occurred because the spruce trees had greater root C reserves than did the beech trees. This interpretation could explain the delayed $R_s$ response to girdling at our treatment site. The mature aspen and birch trees at our site have considerable root C stores, which could be exploited to maintain root and microbial respiration (Gough et al., 2009). Similarly, Frey et al. (2006) found a 90% decline in sweet chestnut fine root starch content after girdling. However, the roots continued to function for 37 days (the end of the experiment), despite the reduction in stored C. If the roots at our site continue to function despite the girdling treatment, then a reduction in $R_s$ would not necessarily be observed.

**Conclusions**

Curtis et al. (2005) estimated that 71% of ecosystem respiration at our study site was due to $R_s$. Their study was conducted at our control site from 1999-2003, with $R_s$ measurements made using a LI-6400-09. These point measurements were extrapolated as described in Curtis et
al. (2005) to daily and yearly $R_s$. Average daily $R_s$ from Curtis et al. (2005) and from the automated chamber data in the present study were graphed for comparison (Figure 20). Average daily $R_s$ values in 1999-2003 were very similar to average daily $R_s$ in 2008-2009 at the control site using the two different measurement approaches. Average treatment site daily $R_s$ from 2008-2009, however, was much lower than the five year average of daily $R_s$ in the control site (Curtis et al., 2005). These prior $R_s$ data suggest that mean $R_s$ as measured in 2008-2009 at the control site were not anomalous. Because no $R_s$ data exist for the treatment site between 1999 and 2003, we can not definitively differentiate whether the treatment site consistently has lower $R_s$ or whether this effect is a result of girdling.

![Figure 20](image_url). Average daily soil respiration at the control site, 1999-2003 from Curtis et al. (2005) (black), average daily $R_s$ 2008-2009 at the treatment (green) and control (red) sites. Prior data were collected using a LI-6400-09, while recent data were collected using automated chambers.
Findings from the present study demonstrate that the treatment site had lower $R_s$ than the control site in 2008 and 2009. It may therefore be concluded that these differences in $R_s$ are due primarily to site differences, particularly 2008, and secondarily due to girdling, as manifested in 2009. Despite initial ambiguity between site differences and treatment effects, this study provides important information about the soil respiratory response to a species-specific tree mortality event. Average yearly $R_s$ loss of C of 429 gC/m$^2$ for the treatment site may be estimated between DOY 176-300, compared to the control site $R_s$ of 602 gC/m$^2$, or 29% less (Figure 21).

**Figure 21.** Cumulative soil respiratory carbon (C) loss calculated from the summed daily average soil respiration ($R_s$) between DOY 176 and 300 for each site and year.
These estimates of C changes in $R$, may be applied to a tree species-specific disturbance event such as the emerald ash borer, or hemlock wooly adelgid insect pest outbreak. If an aspen and birch specific pest or disease removed those tree species from forests in northern lower Michigan, we could estimate the loss of soil CO$_2$ over for the first two years. These estimates could be extrapolated to forests of similar composition in the upper Great Lakes. These C loss estimates could also be used in conjunction with estimates of C storage and might prove useful as C trading and C offsets gain commercial prominence.
References


Appendix A:
Matlab Code Used To Process Raw Carbon Dioxide Concentration Data
Matlab Files Used to Process Automated CO\textsubscript{2} Data in Order of Use

Note: All files must be opened in Matlab for the program to run

SEACFILE.m

clear all
close all
tic

skip=20; \% this skip will be changed to 20 when the 2 second data is encountered. =4 with 10sec data This removes the first 40 seconds of data because the CO2 readings are erratic as chambers transition from 1 to the next.
nmovavg=10; \% with the old (2008) data need to put 2 so that it takes in 20 seconds of data, with 2 second data

data_path = 'C:\Users\Jennifer\Documents\Jen''s Documents\The Ohio State University\SEAC data\Ameriflux raw data\SEAC';
infile = [csvread([data_path '09280.sec'],0,0);... csvread([data_path '09281.sec'],0,0);... csvread([data_path '09282.sec'],0,0);... csvread([data_path '09283.sec'],0,0);... csvread([data_path '09284.sec'],0,0);... csvread([data_path '09285.sec'],0,0);... csvread([data_path '09286.sec'],0,0);... csvread([data_path '09287.sec'],0,0);... csvread([data_path '09288.sec'],0,0)];

This is for early 2008 data
Year = infile(:,2);
DOY = infile(:,3);
hour_min= infile(:,4);
Chamber= infile(:,5); \% Chamber=5 for older data that doesn't include a seconds column
Lid= infile(:,6); \% Lid=6 for older data
CO2 = infile(:,10);
clear infile

Year = infile(:,2);
DOY = infile(:,3);
hour_min= infile(:,4);
Chamber= infile(:,5); \% Chamber=5 for older data that doesn't include a seconds column
Lid= infile(:,6); \% Lid=6 for older data
CO2 = infile(:,10); \% CO2=9

Year = infile(:,2);
DOY = infile(:,3);
hour_min= infile(:,4);
Chamber= infile(:,5); \% This is the set up for the 2009 data
Lid= infile(:,6);
Chamber= infile(:,7);
CO2 = infile(:,10);
clear infile

skip=20; %faset
nmovavg=10; %the old ('08) data at Faset is also 2 second data, so
20 and 10 stay for both '08 and '09 data

data_path = 'C:\Users\Jennifer\Documents\Jen''s Documents\The Ohio
State University\SEAC data\Faset raw data\F_SEAC\';
% % % % %
[infile] = readJenFiles(data_path, ending);

infile = [csvread([data_path '09280.FAC'],0,0);
csvread([data_path '09281.FAC'],0,0);
csvread([data_path '09282.FAC'],0,0);
csvread([data_path '09283.FAC'],0,0);
csvread([data_path '09284.FAC'],0,0);
csvread([data_path '09285.FAC'],0,0);
csvread([data_path '09286.FAC'],0,0)];

% % % % % % % % % % % % % % % % % % % % % % % % % %
%column headers for old 09 data
DOY = infile(:,2);
hour_min = infile(:,3);
Chamber = infile(:,5);
CO2 = infile(:,6);
clear infile

maxlength=length(DOY); %maxlength tell how long the files are
b=1;
a=1;
while a<=maxlength; %maxlength needs to be equal to the number of
values in the files that are being
concatinated.
b=find(hour_min==hour_min(a) & DOY==DOY(a));
hour_min(b)=(hour_min(b)+(0:length(b)-1')/length(b)); %this
adds the extra piece to the hour_min values so
there's not 30 11:56's...etc
a=a+length(b);
end
UTS = [(Chamber.*10^10) + (DOY.*10^7) + (hour_min*10^2)];

dayrange=unique(DOY); % this shows which days are included in the files
that I'm concatenating.

[ccl cc21 nw] = findOBSwindows(8, Chamber);
results = zeros(nw,8);
ci=1;
for c=1:8
    for w=1:length(cc2l(Jassal et al.)) %c, w, ccl, cc2l, hour_min, CO2, skip, plotYN, dofit, nmovavg
        [choppedCurve startwin endwin co20 nodata] = grabwindow(c, w, ccl, cc2l, hour_min, CO2, skip, 0, 0, nmovavg);
        if nodata==0
            [fcs, gof2] = JenFitRobust(choppedCurve, co20);
            results(ci,:)=[c w DOY(startwin) hour_min(startwin-skip)
                           fcs.co fcs.cs fcs.k gof2.adjrsquare];
        end
        ci=ci+1;
    end
end
save([data_path 'results09_280-311.txt'], 'results', '-ASCII');
toc

findOBSwindows.m

function [ccl cc2l nw] = findOBSwindows(nchambers, ChamberVector)
%functions take in inputs nchambers and ChamberVector (8 and Chamber as
given in SEACFILE) and output ccl cc2l and nw
nw=0; %nw is the number of windows
for c=1:nchambers %looping over chambers (between 1 and 8)
    ccl{c}=find(ChamberVector==c);
    cc2l{c}=[find(({ccl{c}(2:end)-ccl{c}(1:end-1))>1); length(ccl{c})];
    %where the difference is greater than 1, we know that we're between
    windows
    nw=nw+length(cc2l{c});
end
end

grabwindow.m

function [windowdata startwin endwin co20 nodata] = grabwindow(c, w, ccl, cc2l, hour_min, CO2, skip, plotYN, dofit, nmovavg)
%c is chamber number
%w is window number
nodata=0;
cc=ccl(c);
cc2=cc2l{c};
if nargin <10 %nargin is the number of arguments that have been input,
    if it's less than nine, then I don't make it do the
    fitting.
        nmovavg=10;
    end
    if w==1 %for the first window
        startwin = cc(1)+skip; %skip equals 20, to remove the first 40
        seconds of each window...can be changed in
        SEACFILE
    }else
endwin = cc(cc2(1));
if endwin-startwin<5*skip
    nodata=1;
    co20=0;
    windowdata=0;
    return
end
[co20 skip2]=getco20(CO2(startwin:endwin),nmovavg);
startwin= startwin+skip2-1;
sec =
    floor(hour_min(startwin:endwin)/100)*3600+60*mod(hour_min(startwin:endwin),100);  %this gets us the seconds for each time the
    windowdata=[hour_min(startwin:endwin) sec CO2(startwin:endwin)];  %this puts the hour_min and CO2 for the first start to end window
else
    startwin = cc(cc2(w-1)+1)+skip;
    endwin = cc(cc2(w));
    if endwin-startwin<5*skip
        nodata=1;
        co20=0;
        windowdata=0;
        return
    end
    [co20 skip2]=getco20(CO2(startwin:endwin),nmovavg);
    startwin= startwin+skip2-1;
    sec =
        floor(hour_min(startwin:endwin)/100)*3600+60*mod(hour_min(startwin:endwin),100);  %yields seconds
    windowdata=[hour_min(startwin:endwin) sec CO2(startwin:endwin)];
end

windowdata(:,2)=windowdata(:,2)-windowdata(1,2);
if nargin <9  %nargin is the number of arguments that have been input,
    if it's less than nine, then I don't make it do the fitting.
        dofit=0;
end

if dofit ==1  % if dofit is equal to 1, then it does the equation to
determine cs and k and it does the goodness of fit.
    [fcs,gof2] = JenFitRobust(windowdata,co20);
    if plotYN==1
        figure (2)
        plot(windowdata(:,2),fcs(windowdata(:,2)),'-b',windowdata(:,2),windowdata(:,3),'-r')
xlabel('Time [sec]')% line above: plots the secfit for the
period of time specified by the window with parameters in
blue, then seconds and CO2 in red.
ylabel('CO_2 [ppm]')
legend('Curve fit','Data')
title([num2str(floor(windowdata(1,1)/100)) ':'
num2str(floor(mod(windowdata(1,1),100))) ', C=' num2str(c)])
end
else
if plotYN==1
figure (1)
plot(windowdata(:,2), windowdata(:,3))
xlabel('Time [sec]')
ylabel('CO_2 [ppm]')
title([num2str(floor(windowdata(1,1)/100)) ':'
num2str(floor(mod(windowdata(1,1),100))) ', C=' num2str(c)])%title
end
end end

c020.m

function [co20, Ico20] = getco20(co2,nmovavg)
endavg=min(70,length(co2)); %finds the minimum value within the first
70 observations of CO2 data

cavg=smooth(co2(1:endavg),nmovavg); %smooth is a function for a moving
average that is within the first 70 observations, based on 10 (with
2 sec data, or change to 2 with 10 sec data) observation
means
co20=min(cavg); %want to find the minimum CO2 within the moving
average. This will serve as Co in the equation
Ico20=find(cavg==co20,1); %create an index of where Co2 is at the
minimum within the moving average CO2
end

JenFitRobust.m

function [fcs,gof2] = JenFitRobust(choppedCurve,co20) %Input the curve
that has the first 100 sec removed, and it's windowdata and CO2 from
grabwindow.m

s=fitoptions('Method','NonlinearLeastSquares', 'Robust',
'LAR','Lower',[360 0],'Upper',[6000 1],'StartPoint',[600
0],'MaxFunEvals', 1000, 'MaxIter', 1000, 'Display', 'Off');
f=fittype('cs-(cs-co)*exp(-k*x)', 'problem', 'co', 'options',s);
[fcs,gof2]=fit(choppedCurve(:,2), choppedCurve(:,3), f, 'problem',
co20); end
used the nonlinear least squares fit available in matlab, lower bounds for CO2 is 360, and for k is 0, upper bounds are 6000, or 1 for CO2 or k respectively. The maximum number of iterations and function evaluations are each 1000.

seacfit.m

function [seac]=seacfit(x,co,cs,k) \texttt{\%this is the equation used to calculate the variables needed to calculate the flux.}

seac = cs-(cs-co)*exp(-k*x);
end
Appendix B:
Note about Advective Flow and Air Water Content for Flux Calculations
High humidity can slow the diffusion of CO$_2$ within automated chambers, resulting in inaccurate calculations of $R_s$. At our site the dilution effect due to water was assumed to be small relative to $dC/dt$ (change in [CO$_2$] over time), so it was ignored in the calculations of $R_s$. There is no advective flow of CO$_2$ at our site, so the advective flow term was also not included in my calculations of $R_s$ (LI-COR, 1998).
Appendix C:
Predicted Daily Fluxes for Spring, Summer and Fall
Predicted effluxes for each hour of the day were created for three seasons, early spring, mid year, and late fall based on the linear combination of the modeled sine and cosine waves with 8, 12 and 24 hour frequencies.
Appendix D:
Variables Used for Model Development
Many variables were used in the models. The table below shows their estimated values, the standard deviation of the estimate, the degrees of freedom, the t value and lastly the p-value.

<p>| Effect                  | trt         | Estimate | Standard Error | Degrees of Freedom | t value | Pr &gt; |t| |
|-------------------------|-------------|----------|----------------|--------------------|---------|------|---|
| Intercept               |             | -1.0107  | 0.5229         | 26                 | -1.93   | 0.0642 |
| hour_s24                |             | -0.1944  | 0.02491        | 1.60E+04           | -7.81   | &lt;.0001 |
| hour_c24                |             | 0.3279   | 0.02489        | 1.60E+04           | 13.17   | &lt;.0001 |
| hour_s12                |             | -0.07642 | 0.01858        | 1.60E+04           | -4.11   | &lt;.0001 |
| hour_c12                |             | -0.1401  | 0.0186         | 1.60E+04           | -7.54   | &lt;.0001 |
| hour_s8                 |             | 0.05851  | 0.01428        | 1.60E+04           | 4.1     | &lt;.0001 |
| hour_c8                 |             | 0.002277 | 0.01425        | 1.60E+04           | 0.16    | 0.8731 |
| hour_s24<em>ind_earlysp    |             | 0.04418  | 0.04851        | 1.60E+04           | 0.91    | 0.3624 |
| hour_c24</em>ind_earlysp    |             | -0.03158 | 0.04803        | 1.60E+04           | -0.66   | 0.5109 |
| hour_s12<em>ind_earlysp    |             | 0.02523  | 0.03649        | 1.60E+04           | 0.69    | 0.4894 |
| hour_c12</em>ind_earlysp    |             | 0.1078   | 0.03619        | 1.60E+04           | 2.98    | 0.0029 |
| hour_s8<em>ind_earlysp     |             | -0.1008  | 0.02801        | 1.60E+04           | -3.6    | 0.0003 |
| hour_c8</em>ind_earlysp     |             | -0.09735 | 0.02783        | 1.60E+04           | -3.5    | 0.0005 |
| hour_s24<em>ind_latefal    |             | 0.1956   | 0.05393        | 1.60E+04           | 3.63    | 0.0003 |
| hour_c24</em>ind_latefal    |             | -0.2874  | 0.05344        | 1.60E+04           | -5.38   | &lt;.0001 |
| hour_s12<em>ind_latefal    |             | 0.04927  | 0.04032        | 1.60E+04           | 1.22    | 0.2218 |
| hour_c12</em>ind_latefal    |             | 0.1067   | 0.03997        | 1.60E+04           | 2.67    | 0.0076 |
| hour_s8<em>ind_latefall    |             | -0.04854 | 0.0309         | 1.60E+04           | -1.57   | 0.1163 |
| hour_c8</em>ind_latefall    |             | 0.02701  | 0.03076        | 1.60E+04           | 0.88    | 0.38   |
| doy2008                 |             | 0.0197   | 0.00391        | 1.60E+04           | 5.04    | &lt;.0001 |
| c_08_1                  |             | 0.2395   | 0.01884        | 1.60E+04           | 12.71   | &lt;.0001 |
| c_08_2                  |             | -0.242   | 0.02079        | 1.60E+04           | -11.64  | &lt;.0001 |
| c_08_3                  |             | 0.1624   | 0.02274        | 1.60E+04           | 7.14    | &lt;.0001 |
| c_08_4                  |             | -0.2791  | 0.02487        | 1.60E+04           | -11.23  | &lt;.0001 |
| c_08_5                  |             | 0.1146   | 0.01281        | 1.60E+04           | 8.94    | &lt;.0001 |
| c_08_6                  |             | -0.1057  | 0.01405        | 1.60E+04           | -7.52   | &lt;.0001 |
| c_08_7                  |             | 0.1037   | 0.01446        | 1.60E+04           | 7.17    | &lt;.0001 |
| doy2009                 |             | 0.02412  | 0.003922       | 1.60E+04           | 6.15    | &lt;.0001 |
| c_09_1                  |             | 0.08694  | 0.01005        | 1.60E+04           | 8.65    | &lt;.0001 |
| c_09_2                  |             | -0.1381  | 0.01376        | 1.60E+04           | -10.04  | &lt;.0001 |
| c_09_3                  |             | 0.5666   | 0.05355        | 1.60E+04           | 10.58   | &lt;.0001 |
| c_09_4                  |             | -0.5452  | 0.05119        | 1.60E+04           | -10.65  | &lt;.0001 |
| c_09_5                  |             | -0.06794 | 0.007083       | 1.60E+04           | -9.59   | &lt;.0001 |
| trt girdle              |             | -0.1843  | 0.8364         | 26                 | -0.22   | 0.8274 |
| trt nogird              |             | 0        | 0              | .                  | .       | .     |   |</p>
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Appendix E:
Histogram of Model Predicted Values
Below is a Histogram of the results from the automated chambers for model development. The histogram indicates rather evenly distributed values, with some high outliers, from this we deem the model to be appropriate.
Appendix F:
Residuals versus Year and Day
Residual values were evaluated against each day for each year for the models that were developed in SAS. There are a few high residuals, but no pattern is visible.