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Diurnal Variation in CO2 Efflux by Pine Seedlings and Root-Associated Mycorrhizal Fungi

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DIURNAL VARIATION IN CO_2 EFFLUX BY PINE SEEDLINGS AND ROOT-ASSOCIATED MYCORRHIZAL FUNGI

by
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A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

This project was part of a larger experiment conducted by Amber Horning and Jason Hoeksema in the Department of Biology at the University of Mississippi, which was set up to investigate variation (over time, and with different light levels) in resource exchange between pine seedlings and two different species of ectomycorrhizal (EM) fungi. The experiment was conducted in a growth chamber, with mycorrhizal pine seedlings growing in plexiglass "mycocosms." I worked with two EM fungal species (*Rhizopogon roseolus* and *Pisolithus arhizus*, hereafter "*Rhizopogon*" and "*Pisolithus*"), which were collected locally near Oxford, Mississippi under loblolly pine. I selected a subset of replicates from the main experiment, three each for each combination of fungal species and light level (high and low). These twelve mycocosms were examined at 5 time points over a 24-hour period (0100, 0500, 0900, 1400, and 2000) in November 2017 and February 2018. I found that carbon efflux rates varied throughout the 24-hour period and increased during the night in the November measurements. I also found that the amount of light exposed to the mycocosm altered the respiration rates, with the low-light treatment primarily having higher carbon dioxide efflux than the high-light treatment, particularly in the November measurements. I also found that carbon dioxide efflux rates of the plant-side of the mycocosm differed based on which mycorrhizal fungi was associated with the *P. taeda*. I concluded that it is not ideal to extrapolate soil respiration rates taken at one time point throughout an entire 24 hours.

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Introduction

In order to understand global carbon cycles, we need to know the key biotic sources of respired carbon dioxide (CO_2), and the factors that drive their variation (Ostle et al. 2009). Soil microbes are one key source, but the factors controlling variation in their respiration rates are still relatively poorly understood (Schlesinger et al. 2000). One point of uncertainty is the degree to which soil microbial respiration rates change over a twenty-four-hour period, known as *diel fluctuations*, and especially what factors control those fluctuations (Vargas et al. 2008). Many organisms increase their metabolic activities in response to increases in temperature, which typically fluctuates during a twenty-four-hour day, so it seems likely that diel fluctuations in soil respiration may be driven by temperature. In natural systems, the sun is often the driver of diel fluctuations in temperature and light. Diel fluctuations in metabolic processes often seem to be driven by temperature but can be difficult to decouple from other causal mechanisms, such as internal clocks and circadian rhythms (Huang et al 2011, Lacour et al 2012). Here, I studied diel fluctuations in soil respiration in a symbiosis between pine seedling roots and their symbiotic ectomycorrhizal fungi, holding temperature constant to allow quantification of diel fluctuations not driven by temperature.

Prior research has shown a possible correlation between temperature and soil respiration rates. Rueher and Knohl (2009) found that soil respiration was associated with both temperature and water saturation, with a delay occurring between the increase in the external factor and the increase in respiration. Tang et al. (2005) found a seemingly linear relationship between soil respiration and temperature and claimed that soil respiration could also be highly reliant on autotrophic respiration. Some studies revealed that fluctuations in the temperature of the soil could have effects on the soil respiration rates, whereas others found that increasing ambient air

temperature led to increases in soil respiration without a correlated increase in soil temp (Rueher and Knohl 2009, Heinmeyer 2009, Iain et al. 2009, Xia et al 2009).

In terrestrial ecosystems, temperature, ambient light levels, soil moisture, and plant photosynthesis rates can all be factors that affect diel fluctuations in soil respiration. In particular, the photosynthetic products of autotrophs associated with root-symbiotic microbes have also been linked to variations in the soil respiration rate. This makes mechanistic sense because photosynthesis provides the source of carbohydrates for plant roots and their symbiotic fungi and bacteria. Therefore, when there is an increase in the amount of carbohydrates available for allocation, the total potential respiration rate should increase. Several studies have found that there could be a time delay between the peak times for photosynthesis rates of the autotrophs in an ecosystem, and the peak times of soil respiration, measured through CO_2 release (Tang et al. 2005, Drake et al. 2008). Very little is known, however, about the relationship between the time of day and soil respiration rate while controlling for temperature, which is one of the reasons I had for conducting the experiments described in this thesis.

Diel fluctuations in metabolic processes are widespread, common, and frequently studied in various ecosystems. Gonzalez-Pinzon et al. (2015) discovered changes in the rate of respiration and in levels of enzyme activity in the microbiota of a headwater stream on a diel timescale over several measurement periods. Hendrix and Huber (1986) found many that processes and nutrient concentrations in cotton plants varied on a diel scale as well, including variations in enzyme activity that seem to rely on the presence or absence of light. Cyanobacteria have many of their internal mechanisms (such as gene expression) under the control of an internal clock and exhibit "circadian rhythms" (ca. 24 hour rhythms), with proteins responsible for keeping track of this time keeping mechanism that can continue to keep a 24-hour biochemical cycle going with just purified protein components and ATP (Cohan, 2006). Diel fluctuations in fungi have been studied

before, but not much research has been done into Ectomycorrhizal fungi, which is the focus of this study (Salichos, 2010).

Although we are beginning to understand how plant photosynthesis may drive soil respiration, a key part of that mechanism could be root symbiotic fungi known as mycorrhizal fungi. These fungi are associated with plant roots, through direct penetration of the root cells themselves and/or formation of a network of hyphae very closely surrounding the root network (Allen, 1996; Smith, 2017). One major type of mycorrhizal fungi, the ectomycorrhizal (EM) fungi, typically form an external sheath and a "Hartig net" that penetrates between the cortex cells of the root and are associated with many of the Northern Hemisphere's most dominant trees, such as oaks and pines. Plants and mycorrhizal fungi usually have a mutualistic relationship, with the plant providing the fungi with sugars and the fungi providing the plant with mineral nutrients from soil (Schöll et al. 1998, Hodge et al., 2001). EM fungi are also vital for the survival of many tree species, including pines, and therefore are very widespread in their ecosystems, and this relationship has been highly documented (Smith, 2017). Since host plants provide carbohydrates to mycorrhizal fungi, it can be surmised factors that influence photosynthetic rate can therefore lead to changes in soil respiration through the changes in respiratory activity of the mycorrhizal fungi upon receipt of carbohydrates from their host plants.

Understanding the circumstances that lead to differences in fungal respiration rates is important, since fungi contribute a major, but often overlooked, portion to total soil respiration (Buchman, 2000). Total soil respiration rates are also important for understanding the total carbon output of forests and other similar ecosystems, which means that fungi would play a large role in our modelling of global carbon cycles. In order to accurately incorporate fungi into global carbon cycles, we need to understand fungal respiration rates, how they change over time, and if they

would change over as short a time span as a single day. Understanding the diel fluctuations in the fungal respiration would help us to more accurately model and predict carbon cycling.

I used growth chamber mycocosms (Rygiewicz et al. 1988) with loblolly pine (*Pinus taeda*) seedlings and two different EM fungi (*Pisolithus* and *Rhizopogon*) to test whether soil respiration (associated with the EM fungi on their own or in combination with the roots) changed over the course of twenty-four hours, with temperature held constant. By keeping light levels on a strict schedule, and keeping the temperature constant, I wanted to see if any diel variations in soil respiration would occur without the influence of temperature. I also aimed to determine if there is a difference in diel CO_2 efflux between high- and low-light level treatments. I hypothesize that mycorrhizal fungal respiration is higher in the seedlings exposed to high amounts of light, because they will have a higher photosynthetic potential, equating to more carbon being transferred to the soil.

Methods

Overview. This project was part of a larger experiment conducted by Amber Horning and Jason Hoeksema in the Department of Biology at the University of Mississippi, which was set up to investigate variation (over time, and with different light levels) in resource exchange between pine seedlings and two different species of EM fungi. The experiment was conducted in a growth chamber with mycorrhizal pine seedlings growing in plexiglass "mycocosms." I worked with two EM fungal species (*Rhizopogon roseolus* and *Pisolithus arhizus*, hereafter "*Rhizopogon*" and "*Pisolithus*"), which were collected locally near Oxford, Mississippi under loblolly pine. I selected a subset of replicates from the main experiment, three for each combination of fungal species and light level (high and low). These twelve mycocosms were examined at 5 time points over a 24-hour period (0100, 0500, 0900, 1400, and 2000) in November 2017 and February 2018.

P. taeda L., loblolly pine, is a coniferous tree species native to the southeastern United States. EM mutualisms are particularly important in facilitating pine establishment in acidic, nutrient poor soils, which were utilized in this experiment (Brundrett 2009). *P. taeda* seeds were obtained from open-pollinated families that were originally selected for *Leptographium* disease resistance (Picullel et al. 2018, Singh et al. 2014). Pine seeds were sterilized in a 3% H_2O_2 solution for 24 hrs and then rinsed with running water for 2 min. To promote germination, seeds were cold stratified by keeping by keeping them at 4 ºC for 40 days in moist conditions and agitated daily to deter mold growth. To further prevent contamination after stratification, seeds were soaked in 10% bleach for 5 min, 70% alcohol for 1 min, and 10% bleach again for 1 min, followed by a sterile water rinse for an additional minute. Seeds were germinated in a Conviron Model ATC40 environmental chamber in groups of four on 10-inch water agar plates tilted at 70º in order to orient the direction of shoot and root growth. Foil was used to cover the lower half of plates in order to further encourage downward root growth. Seeds were germinated on a 13-hour

photoperiod (400 μmol/m^2/sec) with a consistent temperature of 18 ºC until seedlings were 2 to 3 inches in length, approximately 3 to 4 weeks.

Ectomycorrhizal inoculation of seedlings

Pine seedlings were dip-inoculated from spore slurries of fungal sporocarps from two target fungi (*Pisolithus* and *Rhizopogon*) collected from under *P. taeda* trees in Oxford, MS in 2016 and 2014 respectively. *Pisolithus* and *Rhizopogon* are common in pine forests, important for seeding establishment, and are early and thorough colonizers of pines, making them ideal for this seedling study. Identities of all fungal isolates used for inoculation, and of ectomycorrhizal root tips from harvested seedlings, were confirmed through Sanger DNA sequencing and comparison of sequences with public databases (as in Hoeksema et al. 2018, Rasmussen et al. 2018, Craig et al. 2016, and Rua et al. 2015).

For the spore slurry, sporocarps were blended with DI water, and spore concentrations were adjusted to ~10^7 spores/mL. Pine seedling root systems were slurry dipped and planted in cones filled with the same sterile soil substrate used in the timed experiment (described below) and inoculated for five months, exposed to full light (400 μmol/m^2/sec) on a 13-hour photoperiod in an environmental chamber (Conviron ATC40) at a constant temperature of 25 ºC. Seedlings were watered to saturation on a weekly basis.

Mycocosm assembly

Mycocosms, modified by M. Booth from the original design of Rygiewicz et al. (1988), were constructed of two clear polycarbonate plates (23 cm tall by 38 cm wide) separated on the sides and bottom by three sections of PVC 2.5-cm thick, adhered with wing nut bolts and general-purpose silicone sealant. The volume of the mycocosm was separated into halves by a PVC spacers (2.5 cm thick) routed to 90% openness, filled with a mix of fine and course sand substrate

and covered on both sides with a nylon mesh (44μm) to allow for the passage of fungal hyphae but block *Pinus* root growth between sections. After sufficient growth of mycorrhizal fungi through the mesh barrier, this creates a "tree-side", in which pine roots and mycorrhizal fungi are growing, and a "fungus-only" side, in which only mycorrhizal fungi are growing. Each mycocosm was sterilized in a 10% bleach solution for 30 min, rinsed with DI water and stored in a room protected with a HEPA air filter to reduce the likelihood of contaminants from non-target fungi before being filled with a growth substrate. The substrate was composed of a 1:20 soil:sand mixture, where the sand was a 1:1 mixture of commercial play sand to natural sand sourced from northern Mississippi, and the soil was a loamy field soil collected from beneath *P. taeda* trees in Oxford, MS. The substrate was sieved to 1 mm to remove coarse particles and autoclaved twice at 121 ^{o}C for 1 hr, with a 24-hr waiting period between sterilizations. Each half of all mycocosms was filled with approximately 800 mL of the substrate, then covered with 50 μm thick black plastic bag material to reduce algal growth and entrance of airborne fungal spores. One liter of fresh homogenized field soil was suspended in 6 liters of DI water and filtered to 5 μm to create a microbial wash. To each half of the mycocosms, 10 mL of microbial filtrate was added before planting.

Experiment setup

The full experiment was a 3 x 2 x 3 factorial design: two ectomycorrhizal fungal species and controls, crossed with two light levels, crossed with three harvest times. Each combination of fungal species, light level, and harvest time was replicated six (*Rhizopogon*) or eight (*Pisolithus*) times, for a total of 84 experimental mycocosms. For my experiment, I used 3 replicates of high-light and low-light for each of the two fungi, *Rhizopogon* and *Pisolithus*. The two light levels tested were high sunlight (400 μmol/m^{-2}/s^{-1}), to simulate an early successional environment, and low sunlight to simulate a late successional, closed-canopy environment (135 μmol/m^{-2}/s^{-1}) on a 13 hr light cycle., with the lights turning on at 7:00 AM and turning off at 8:00PM. For my

experiment, only one environmental chamber was utilized (Conviron Models ATC40), which had two shelves of growing space, one of which was set at the low-light level and one at the high-light level.

Seedling planting

After a 5-month inoculation period in cones, 10 mL of background soil microbe slurry and 10 mL of a 50% diluted MMN solution without carbon source were added to each cone. This was done four weeks before trees were planted in mycocosms to encourage fungal growth. Mycorrhizal colonization of root tips was verified on each seedling by visual inspection before planting.

Respiration measurements

Efflux of CO_2 from soil was estimated separately on the tree side and the fungus-only side of each chamber at each time point using a modified version of the protocol described by Meachum and Hoeksema (in prep) using a LiCor 6400XT infrared gas analyzer (IRGA) mounted to a custom polycarbonate box (Figure M1). LiCor soil CO_2 Flux System software was used with a "closed" method, wherein a 'Δ' value is selected and the target concentration of CO_2 is set at ambient. The CO_2 is scrubbed out of the chamber with soda lime until the measurements equal target – Δ, at which point the chamber concentration of CO_2 is allowed to rise. The software then begins to measure concentration of CO_2 in the chamber over time until target + Δ is reached, and the instantaneous flux rate of CO_2 is estimated as the slope of the function of the concentration of CO_2 over time where it intersects ambient concentration of CO_2. In this experiment, this process was repeated three times (separated by approximately six minutes) per side of each mycocosm, to obtain replicate estimates. For the tree side of the mycocosm, a mylar sheet was tied around the shoot of the pine seedlings during the readings to reduce the impact of a photosynthesizing plant inside the chamber. Ambient CO_2 levels were reassessed at the beginning of each set of three

measurements and Δ set at 5 ppm. Measurements were taken at the following five time points: 9:00 AM, 2:00 PM, 8:00 PM, 1:00 AM, and 5:00 AM over a 24-hr period in order to test for diurnal fluctuations in CO_2 respiration. Measurements of the *Rhizopogon* and *Pisolithus* mycocosms were separated by 24 hours.

The 24-hour cycle of measurements was first conducted between the first and second harvest of the main experiment (12-15 weeks after start of experiment) and then again between the second and third harvest (26-27 weeks after start of experiment). For the first bout of measurements, the high-light mycocosms were measured in early October 2017, for time points 0900, 1400, and 2000. All low-light measurements were taken in early November 2017, as well as high-light measurements for times 1 and 5. This disjunction of measurements was due to a malfunction in the LiCor machine, which was replaced and no longer a problem for the rest of the experiment.

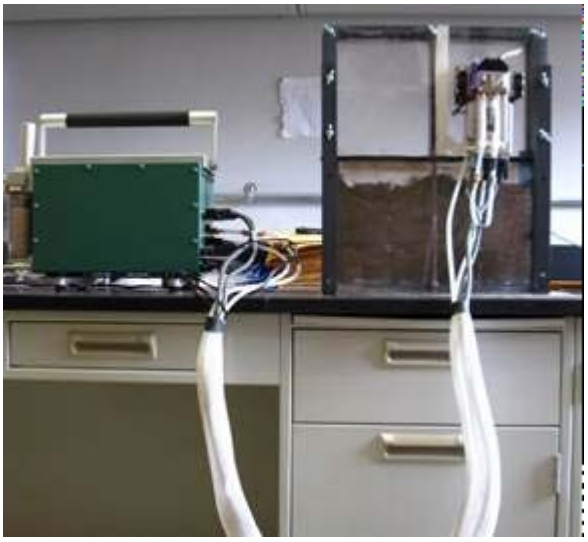

Figure 1 – Photo exhibiting custom polycarbonate box gas chamber. The chamber is separated into two sections to seal the top of experimental mycocosms. One half of the chamber is used for CO_2 measurements and the other is sealed off entirely with closed cell foam (CCF) at base. The sensor head is attached to the center of the chamber wall and sealed with CCF. CCF is mounted

to the base of the chamber to create a seal between the gas chamber and the soil surface of mycocosms, and the chamber is filled with curved pieces of CCF in order to promote constant movement of CO_2 during measurements. The Mylar Bag is wrapped around the shoot of the pine seedlings during the readings to reduce the impact of a photosynthesizing plant inside the chamber.

Data analysis

All analyses were conducted using R version 3.5.2. To test for effects of time, light and fungal species on instant efflux rates I used linear mixed effects models using the *lmerTest* package, with time (9:00 AM, 2:00 PM, 8:00 PM, 1:00 AM 5:00 AM), light level (high and low) and EM fungal species (Rhizopogon and Pisolithus), and their interactions as fixed effects, and tree genotype and individual mycocosm as random effects to account for differences in tree genotype and individual mycosoms for repeated sampling, respectively. In the case of significant effects of time sampled or light treatment, means were separated using Tukey HSD adjustment of pairwise P-values using the *lsmeans* package.

Results

On the fungus-only side in November 2017, CO_2 efflux for *Pisolithus* growing in low-light was similar to that in high-light but gradually diverged throughout the night, and respiration in low-light became significantly higher by 5 am (Fig. 2). CO_2 efflux for *Rhizopogon* growing in low-light was higher than *Rhizopogon* growing in high-light, and was significantly higher at 1 am, until the high-light CO_2 efflux spiked at 5 am to become significantly higher than the low-light *Rhizopogon* (Fig. 3). These species-specific responses of CO_2 efflux to light levels over time resulted in a significant three-way interaction between time, light, and fungal species ($F_{4,141.6}$= 6.18, P=0.0013, Figures 2 & 3).

On the fungus-only side in February 2018, CO_2 efflux for *Pisolithus* growing in low-light was higher than for *Pisolithus* growing in high-light for the daytime hours and was significantly higher at 2:00 PM. After this point, the efflux under high-light was higher than under low-light and was significantly higher at 1:00AM. These species-specific responses of CO_2 efflux to light levels over time again lead to a three-way interaction between time, light, and fungal species ($F_{4,150.1}$ = 3.97, P=0.0043, Figures 4 & 5).

On the tree-only side in November 2017, between fungal species, CO_2 efflux was typically lower for *Pisolithus* compared to *Rhizopogon*, except for 1:00 AM when it was significantly higher (Figure 6), whereas *Rhizopogon* had significantly higher CO_2 efflux at 2:00 PM and 5:00 AM (Figure 6). Regardless of fungal species, CO_2 efflux was typically higher for low-light but was only significantly higher at 8:00 PM; mycocosms kept in high-light only had higher CO_2 efflux rates at 9:00 AM (Figure 7). For CO_2 efflux on the tree side in November 2017, there was a significant two-way interaction between time and fungal species ($F=_{4,148.1}$=8.535, P<0.0001), and a significant two-way interaction between time and light ($F=_{4,148.1}$=9.57, P<0.0001).

On the tree-only side in February 2018, between fungal species, the CO_2 efflux rates were very similar, but efflux in *Pisolithus* mycocosms was significantly higher at 9:00 AM and 5:00 AM, and in *Rhizopogon* mycocosms it was significantly higher at 2:00 PM (Figure 8). Between light levels, the CO_2 efflux were also very similar; however, the mycocosms in high-light had significantly higher efflux rates at 1:00 AM compared to the low-light mycocosms, regardless of fungal species (Figure 9). For CO_2 efflux on the tree side in February 2018, there was a significant two-way interaction between time and fungal species ($F=_{4,150.5}=6.132$, $P<0.001$), and a significant two-way interaction between time and light ($F=_{4,150.5}=5.517$, $P<0.001$).

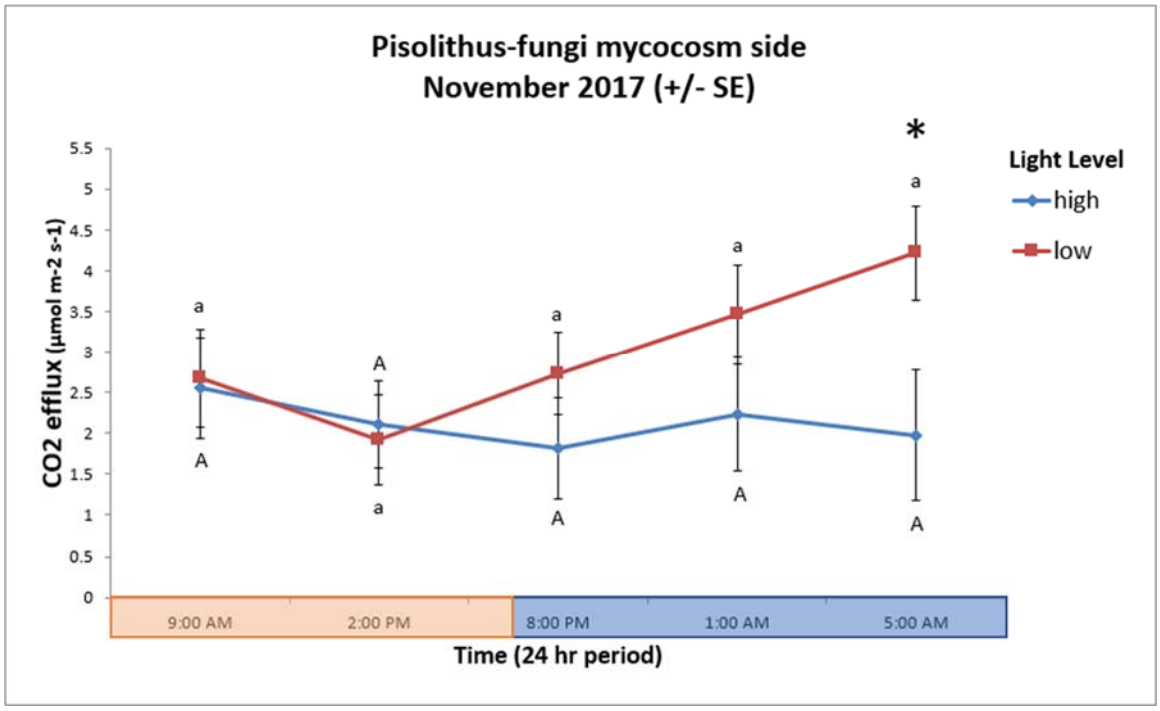

Figure 2: CO_2 efflux rates from the fungus-only side of mycocosms, each containing a *Pinus taeda* seedling inoculated with the ectomycorrhizal fungus *Pisolithus arhizus*, as a function of time and light level. The asterisk (*) indicates a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Standard error bars are shown but are too small to be visible with the current scale.

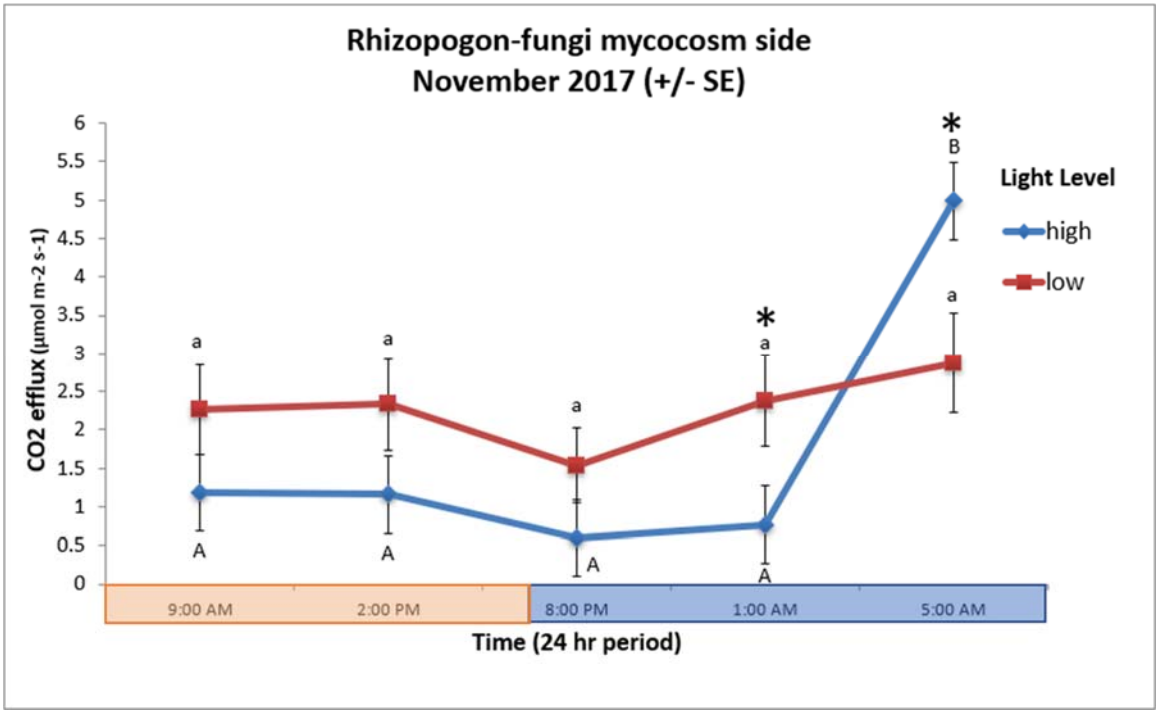

Figure 3: CO2 efflux rates from the fungus-only side of mycocosms, each containing a Pinus taeda seedling inoculated with the ectomycorrhizal fungus *Rhizopogon roseolus*, as a function of time and light level. Asterisks (*) indicate a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Standard error bars are shown but are too small to be visible with the current scale.

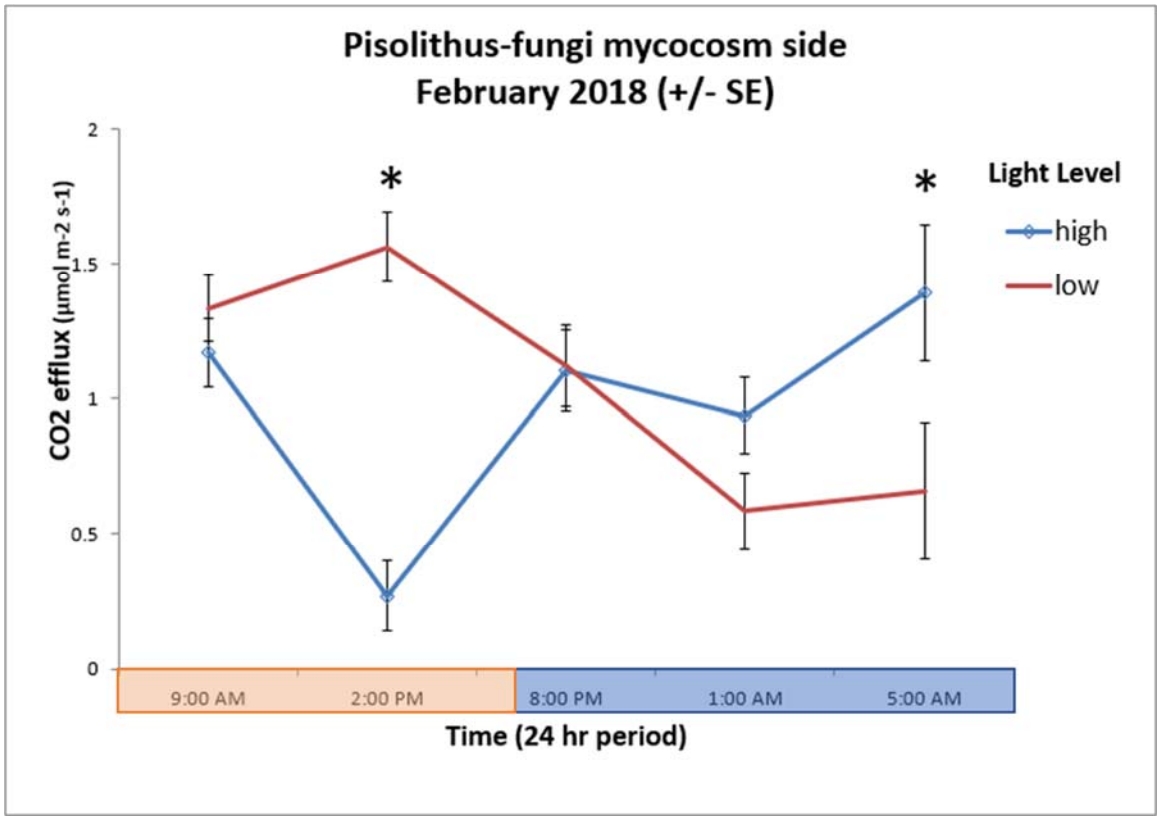

Figure 4: CO2 efflux rates from the fungus-only side of mycocosms, each containing a Pinus taeda seedling inoculated with the ectomycorrhizal fungus Pisolithus arhizus, as a function of time and light level. Means sharing the same letters are not significantly different over time according to Tukey HSD post-hoc tests (high-light: capital letters, low-light: lowercase letters). Asterisks (*) indicate significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Bars represent standard error of the mean.

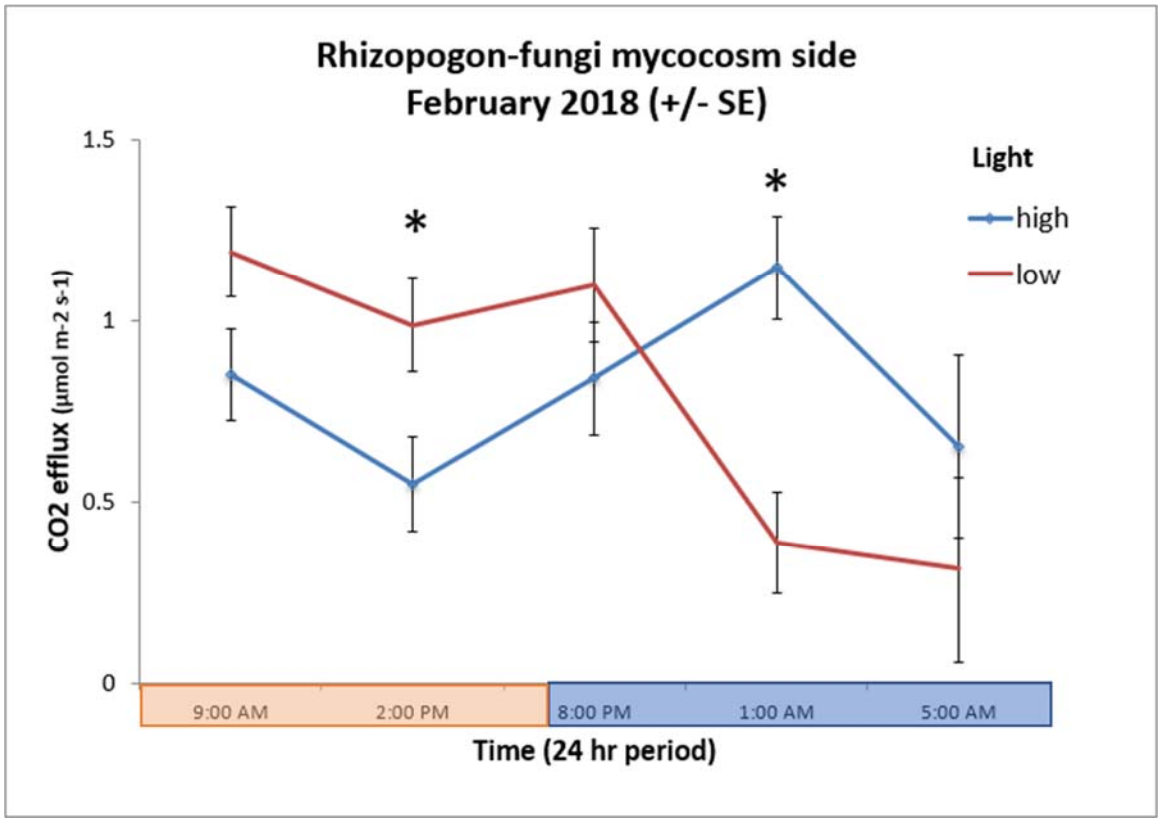

Figure 5: CO2 efflux rates from the fungus-only side of mycocosms, each containing a Pinus taeda seedling inoculated with the ectomycorrhizal fungus *Rhizopogon roseolus*, as a function of time and light level. Means sharing the same letters are not significantly different over time according to Tukey HSD post-hoc tests (high-light: capital letters, low-light: lowercase letters). Asterisks (*) indicate a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Bars represent standard error of the mean.

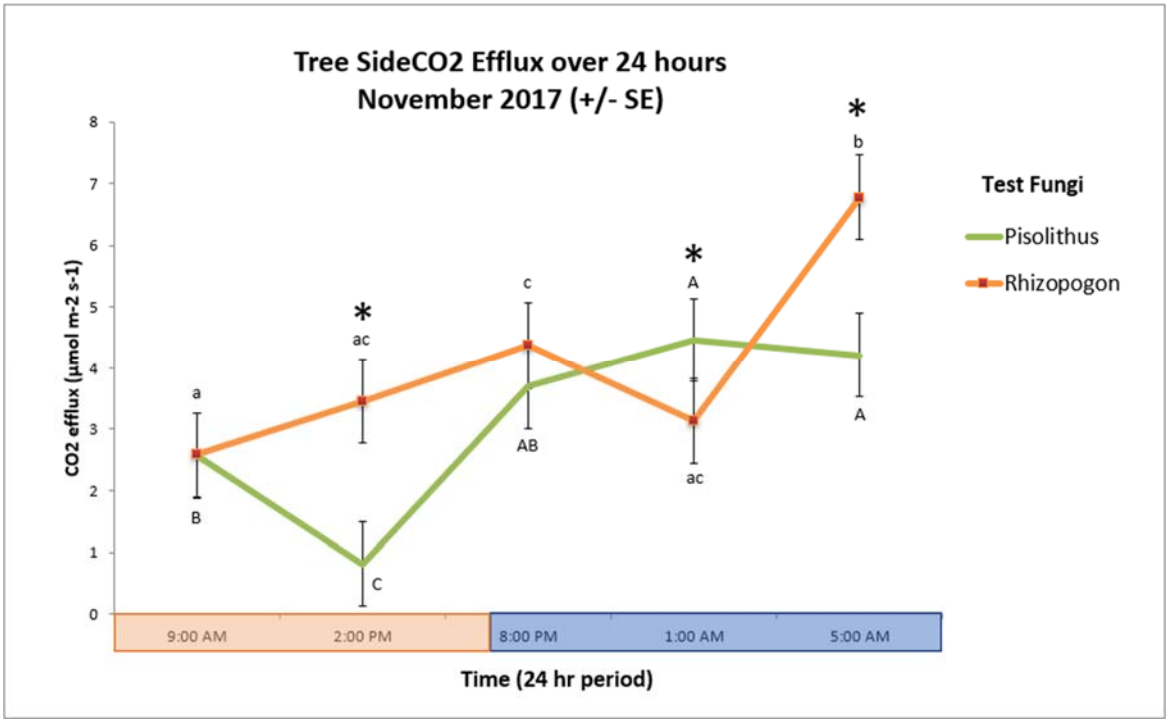

Figure 6: CO_2 efflux rates based on time of day are shown, compared between mycocosms containing soil, a *Pinus taeda* sapling, and either *Pisolithus arhizus* fungi or *Rhizopogon roseolus* fungi. Means sharing the same letters are not significantly different over time according to Tukey HSD post-hoc tests (high-light: capital letters, low-light: lowercase letters). Asterisks (*) indicate a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Bars represent standard error of the mean.

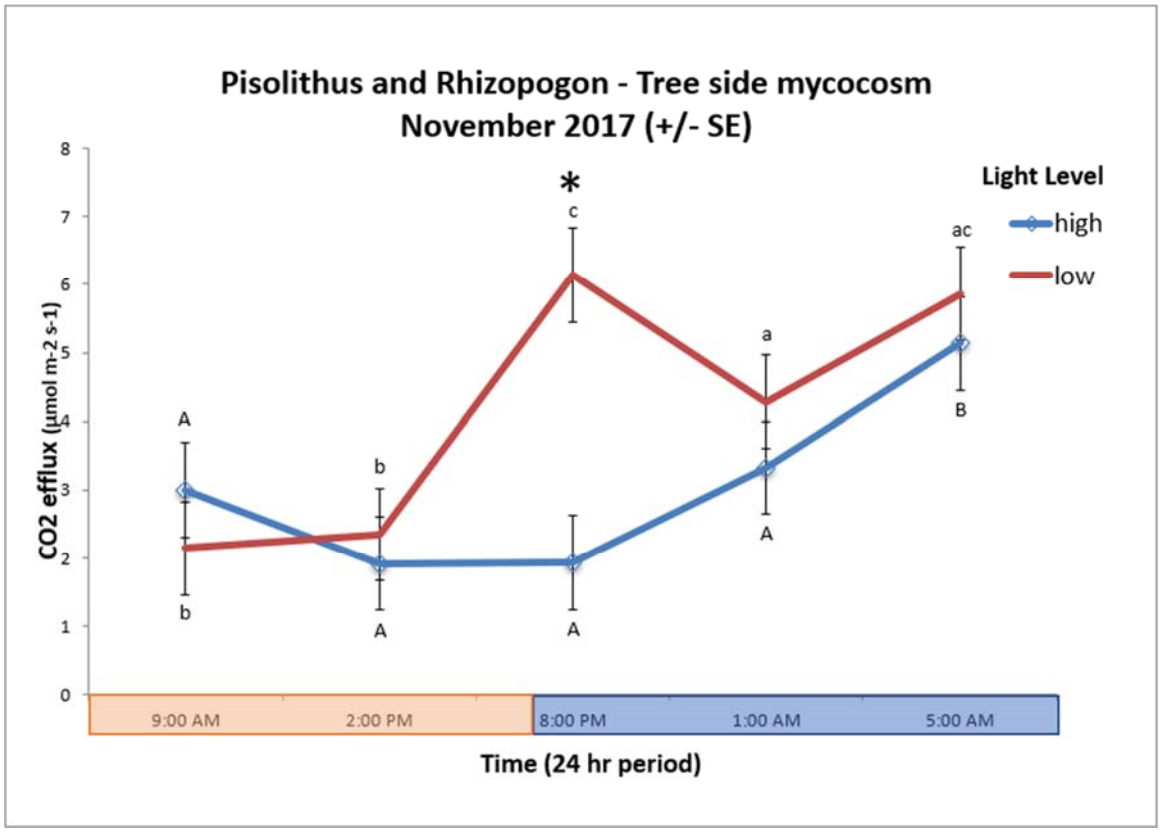

Figure 7: CO_2 efflux rates based on time of day are shown, compared between mycocosms containing soil, a *Pinus taeda* sapling, and either *Pisolithus arhizus* fungi or *Rhizopogon roseolus* fungi with exposure to either high light or low light. Means sharing the same letters are not significantly different over time according to Tukey HSD post-hoc tests (high-light: capital letters, low-light: lowercase letters). The asterisk (*) indicates a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Bars represent standard error of the mean.

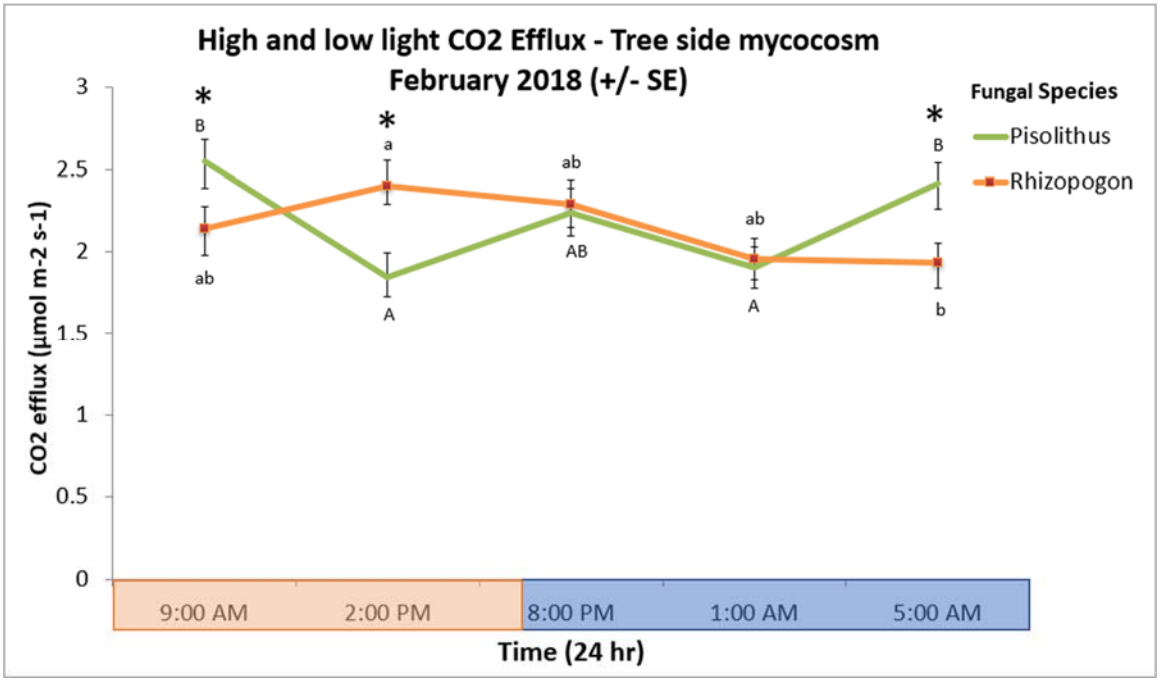

Figure 8: CO_2 efflux rates based on time of day are shown, compared between mycocosms containing soil, a *Pinus taeda* sapling, and either *Pisolithus arhizus* fungi or *Rhizopogon roseolus* fungi. Means sharing the same letters are not significantly different over time according to Tukey HSD post-hoc tests (high-light: capital letters, low-light: lowercase letters). Asterisks (*) indicate a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Bars represent standard error of the mean.

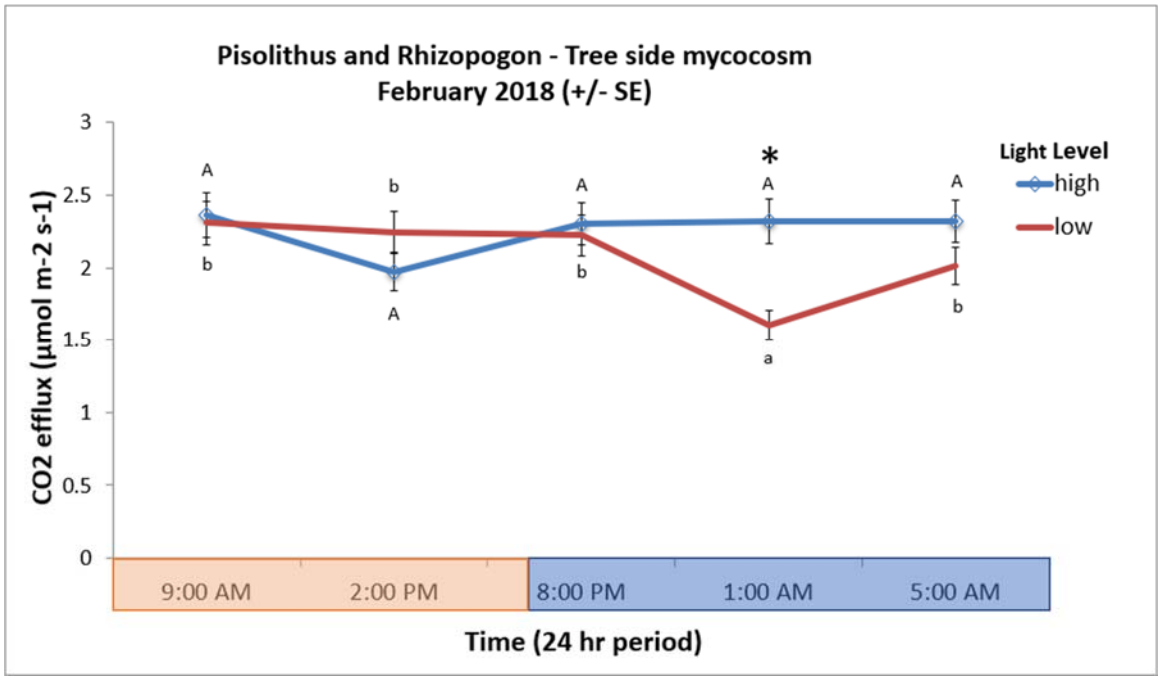

Figure 9: CO_2 efflux rates based on time of day are shown, compared between mycocosms containing soil, a *Pinus taeda* sapling, and either *Pisolithus arhizus* fungi or *Rhizopogon roseolus* fungi with exposure to either high light or low light. Means sharing the same letters are not significantly different over time according to Tukey HSD post-hoc tests (high-light: capital letters, low-light: lowercase letters). The asterisk (*) indicates a significant difference between the two light levels at a particular time point, according to Tukey HSD post-hoc tests. Bars represent standard error of the mean.

Discussion

I found that CO_2 efflux did change over the 24-hour period, but not in a consistent pattern. Specifically, I found different results between the November and February measurements of the fungal side of the mycocosms. Most of our time points for the fungus species had very similar levels of CO_2 efflux, and the time points that were statistically significant were different between the November and February measurements. Even though I did not see an obvious trend over the 24-hour period, I did see variations in respiration over the 24-hour period, which warrants further study into the causes of these variations.

The tree side of the mycocosm did experience some change in the belowground CO_2 output over the 24 hours. For the November measurements on the plant side of the mycocosms, the CO_2 efflux was typically highest during the night hours or early morning (Figures 6 & 7), suggesting a lag between peak photosynthesis and belowground metabolic activity. This trend has been documented before, in a study by Tang et al. (2005) where they measured canopy photosynthesis rates and nearby soil respiration rates in an oak-grass savannah. They saw a 7-12-hour delay in increased root respiration in soil after photosynthesis initiated in a nearby tree; away from the tree, soil respiration increased in correlation with soil temperature. Therefore, it appears that a measurement of soil CO_2 efflux or soil respiration rates at one time during the day is not applicable to an entire 24-hour cycle.

Between the November and February measurements, I saw a few major differences. The average CO_2 efflux was generally substantially greater in November than in February, for both *Pisolithus* and *Rhizopogon* (compare, for example, Figures 2-3 versus Figures 4-5). Part of the reason for this observation could be that as the experiment went on, most of the nutrients in the soil were depleted, and many of the trees began to die or severely reduce their amount of foliage, likely leading to a reduction in photosynthetic activity and reduced allocation of carbon belowground. In this way, it is likely that the November measurements would be more representative of a

natural system, since there were fewer issues with nutrient depletion and dying trees during those measurements. A future experiment could test the effects of soil nutrient content on the respiration rates. This could provide data to further examine the idea that the February and November data differed due to the nutrient depletion in February.

In November, *Pisolithus* primarily had greater CO_2 efflux in low-light than in high-light, particularly during the night hours (for example, see Figures 1 and 3). However, in February, the low-light *Pisolithus* efflux rates decreased during the night and became significantly lower than the high-light *Pisolithus*. I hypothesized that high-light mycocosms would have higher rates of mycorrhizal fungal respiration, and this was seen in the February measurements, but the opposite was seen in November. With the consideration that November is likely a more representative sample, it would seem that this hypothesis was not supported. However, I was unable to ascertain the reason behind why the low-light and high-light mycocosms experienced such differences in their diurnal variations in efflux and why these differences differed between the two observed fungal species.

It is interesting that our plant-side results often depended on which fungal species was associated with the pine seedlings. One possible reason for differences in carbon dioxide efflux is that the different fungi promote different root proliferation in the pine saplings, as has been shown previously for different genotypes of the same fungus (Piculell et al. 2008). Increased root density in the soil would likely lead to increased respiration because there are more plant cells capable of respiration. Another possible explanation could be different carbon utilization rates of the two fungal species (Koide et al. 2014). If one fungus exhibited greater rates of respiration than the other, for example, then that would cause differences in the efflux rates.

A future experiment could add more measurement time points to get more precise data and perhaps reveal new trends. Although I did not have consistent 24-hour effects, I did experience fluctuations in the respiration rate. Based on our data, however, it would appear that this trend has

more to do with the amount of light exposed to the pine seedling and its consequent photosynthetic activity, rather than an independent 24-hour cycle. Therefore, it would seem that in a natural system where light levels vary over a 24-hour period, that *Pisolithus* and *Rhizopogon* fungi would exhibit diurnal fluctuations in their carbon dioxide respiration when temperature is held constant if they were coordinated with a root system.

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