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An Experimental Test of Controls on Resource Exchange in an Ectomycorrhizal Mutualism

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AN EXPERIMENTAL TEST OF CONTROLS ON RESOURCE EXCHANGE IN AN ECTOMYCORRHIZAL SYMBIOSIS

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ABSTRACT

Models of resource exchange mutualisms utilize economic principles to explain how the costs and benefits of these interactions vary with environmental context. All of these models use a ratio of resource exchange (e.g., nitrogen: carbon) as the central variable, and it is unclear whether such exchange ratios predict outcomes of mutualisms in natural systems. Corrêa et al. (2008) hypothesized instead that the absolute flux of the most limiting nutrient, rather than the ratio of the two exchanged resources, best explains the benefits of resource exchange mutualisms. To distinguish between these two competing hypotheses, we measured resource transfers, and their ratios, between *Pinus taeda* seedlings and two ectomycorrhizal (EM) fungal species, *Rhizopogon roseolus* and *Pisolithus arhizus.* We evaluated how carbon availability to plants (manipulated with high and low light exposure) affected those resource fluxes and ratios over 3 time periods (10, 20 and 30 weeks) using mycocosms in environmental chambers. Our results suggest that higher light availability increases resource exchange between mycorrhizal mutualists, and that N:C resource exchange ratios are higher under low light, but that limiting soil nutrients have a stronger effect on plant growth than resource exchange ratios. These results suggest that the "exchange ratio hypothesis," and the "total flux hypothesis" are both correct in their predictions, implying that when mycorrhizal plants have additional C to trade to their mycorrhizal fungi, it has the potential to promote an increase in nutrients to the plant in return, changing the price of exchange without detriment to either mutualist.

LIST OF ABBREVIATIONS AND SYMBOLS

C	Carbon
N	Nitrogen
P	Phosphorus
EM	Ectomycorrhizal

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I INTRODUCTION

Mutualisms are interspecific interactions ubiquitous to all ecosystems on our planet (Bronstein 2015). Mutualists benefit one another through trade of services or resources, such as in plant-pollinator mutualisms in which pollination services are exchanged for nectar resources. In mycorrhizae and rhizobia resource exchange mutualisms, soil microbes provide mineral nutrients to plants in exchange for photosynthates. Resource trade can be costly for organisms in a mutualism, but traded resources may not always be limiting to the species trading them away, with by-product benefits commonly exchanged in some major types of mutualisms (Connor 1995). Moreover, the costs and benefits of traded resources may vary among environments (Johnson et al. 1997, Johnson et al. 2013), and this context dependency can have a variety of important consequences for the ecology and evolution of mutualisms (Hoeksema & Bruna 2015).

Various models have utilized economic theory to predict the outcomes of resource-exchange mutualisms (Schwartz and Hoeksema 1998, Hoeksema and Schwartz 2003, Kummel and Salant 2006, Akçay and Roughgarden 2007, Akçay 2015). Although each economic model differs in approach, the singular common variable is the 'price of resource exchange', defined as the ratio of units of one resource that are traded for one unit of another. These economic models generally assume that the resources being exchanged are costly to each species, and thus the benefits and outcomes of resource-exchange mutualisms are driven by the ratio of resources being exchanged between species, i.e., the exchange price. The comparative advantage model makes predictions for how variable environmental factors (e.g., light) should affect resource exchange and the outcomes of the mutualism (Schwartz and Hoeksema 1998, Hoeksema and

Schwartz 2003). Specifically, those models predict that an increase in availability to one species of the resource they are trading away will increase the price (i.e., exchange ratio) offered by that species, the overall volume of trade, and the growth benefits to both species. Grman et al. (2012) proposed a population dynamics model based on comparative advantage principles which predicts how light affects resource exchange and resource exchange ratios between arbuscular mycorrhizal (AM) plant and fungal mutualists, depending on nutrient availability.

Alternatively, resource exchange prices may be fixed for particular species pairs, potentially varying among different species (Kummel and Salant, 2006) but not varying with environmental conditions or availability of traded resources (Kiers et al. 2011). Despite the importance of resource exchange ratios in mutualism models, they have rarely been quantified in naturally occurring mutualisms. Those studies that measured resource fluxes have typically only provided a short-term snapshot of the relationship or lacked a distinction in the fates (whether to roots or microbial symbionts) of carbon allocated belowground, not accounting for both root and fungal respiration versus assimilation (Douds et al. 1988, Jones et al. 1991, Colpaert et al. 1996, Jones et al. 1998, Qu et al. 2004). As a result, exchange ratios have not been explicitly linked to environmental factors or host/symbiont performance.

In contrast to economic models, Corrêa et al. (2008, 2011, 2012) hypothesized that fitness benefits to plants of ectomycorrhizal (EM) mutualisms are not driven by the ratio of resources being exchanged, but rather by the absolute flux of whatever resource is most limiting to plant growth at a given point in time. Specifically, Corrêa et al (2012) suggested that carbon is only a limiting resource to plant growth when plants are severely light limited (less than 9% of full sun exposure) and that light intensity thus has little effect on plant growth responses to mycorrhizal fungi under most normal conditions. This hypothesis predicts that the absolute

fluxes of truly limiting resources (e.g., nitrogen or phosphorus for plants) exchanged between symbionts better predict outcomes of resource exchange mutualisms, rather than exchange ratios.

Mycorrhizal mutualisms are ideal systems in which to test hypotheses about how resource fluxes are affected by their environmental availability, and how they influence outcomes of resource-exchange mutualisms. Mycorrhizae are mutualisms between plant roots and fungal hyphae in which plants photosynthetically fix carbon (C) compounds into simple sugars and trade them to their fungal symbionts for soil nutrients such as nitrogen (N) and phosphorus (P) (Smith and Read 2008). Mycorrhizal resource exchange is discrete, quantifiable, and occurs over relatively short time scales, which allows feasible nutrient-flux measurements. We measured the cumulative resource exchange between loblolly pine (*Pinus taeda*) and two ectomycorrhizal fungal symbiont species (*Rhizopogon roseolus* and *Pisolithus arhizus*) in order to determine how resource exchange (total fluxes and ratios) differed between fungal species and between high and low light availability, which we assumed influenced C availability to the plant. To do this, we utilized a modified mycocosm approach (Rygiewicz et al. 1994) that allowed us to measure cumulative amounts of exchanged resources over time, and to partition the fate of CO_2 allocated belowground.

Our study aimed to address two key questions regarding *P. taeda* ectomycorrhizal mutualisms: (Q1) How does light availability affect resource exchange? (Q2) Do resource exchange ratios or absolute fluxes better predict pine seedling growth? For Question 1, the "exchange ratio hypothesis" from comparative advantage market models (Schwartz and Hoeksema 1998, Hoeksema and Schwartz 2003) predicts that N:C and P:C exchange ratios would be lower in a high light environment compared to a low light environment, due to pine seedlings in high light having an excess of C to offer their fungal symbionts, and that the price of

exchange should decrease with increased light. The "total flux hypothesis" from Corrêa et al. (Corrêa et al. 2008, Corrêa et al. 2011, Corrêa et al. 2012) predicts that light availability would not affect N:C and P:C exchange ratios since plants are not typically C-limited across most ambient light levels, and absolute fluxes of N and P are controlled by EM fungi without an affect of light. For Question 2, the "exchange ratio hypothesis" predicts that within each ambient light level environment, pine seedling growth would be positively correlated with P:C and/or N:C exchange ratios (i.e., the ratio of P or N received by the plant from the fungus, relative to the C transferred from the plant to the fungus). In contrast, the "total flux hypothesis" predicts that N or P would be most limiting to plant growth, and that the absolute amount of N or P received from EM fungi would be a better predictor of plant growth than N:C or N:P ratios.

II METHODS

Germination of seedlings

Pinus taeda (L.), loblolly pine is a coniferous tree species native to the southeastern United States. Ectomycorrhizal (EM) mutualisms are particularly important in facilitating pine seedling establishment in acidic, nutrient poor soils, which were utilized in this experiment (Brundrett 2009). *Pinus taeda* (*P.taeda)* seeds were obtained from two open-pollinated families selected for *Leptographium* pathogen 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. Seeds were cold stratified at 4 ^{o}C 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 70°, and with the lower half covered in foil, in order to orient the direction of shoot and root growth. Seeds were germinated on a 16-hour photoperiod (400 μmol/m^2/sec) with a consistent temperature of 18°C 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 arhizus* and *Rhizopogon roseolus*) collected from under *P. taeda* trees in Oxford, MS in 2016 and 2014 respectively. *P. arhizus* and *R. roseolus* (hereafter *"Pisolithus"*

and *"Rhizopogon"*) are common in pine forests of the southeastern USA, important for seeding establishment in the soil, and are early and thorough colonizers of pines, making them ideal for this seedling study. Identities of the 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 Rua et al. 2015, Craig et al. 2016, Hoeksema et al. 2018, and Rasmussen et al. 2017).

To make the spore slurries for inoculation, sporocarps were blended with DI water and spore concentrations were adjusted to ~10^7 spores/mL. Pine seedling root systems were dipped in the slurry and planted in cones (21 cm x 4.5 cm) filled with the same sterile soil substrate used in the timed experiment (described below) and allowed to develop for five months, exposed to full light (400 μmol/m^2/sec) on a 16-hour photoperiod in an environmental chamber (Conviron ATC40) at a constant temperature of 25°C. Seedlings were watered to saturation on a weekly basis. After 5 months, 10 mL of background soil microbe slurry (created by filtering 6 L of deionized water through 1 L of fresh soil on a 44 micron sieve) and 10 mL of a 50% diluted MMN media without C source were added to each cone, after which mycorrhizal development was allowed to continue for another 4 weeks before transplanting into experimental mycocosms.

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 to block *Pinus* root growth between sections. 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 resulting soil mixture was low in total carbon (0.088%), nitrogen (0.005%), and phosphorus (0.002%), and contained no detectable ergosterol. The substrate was sieved to 1 mm to remove coarse particles and autoclaved at 121 ^{o}C for 1 hr., twice, 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. 10 mL of microbial filtrate was added to each half of the mycocosms before planting.

Experiment setup

The experiment was a 2 x 2 x 3 factorial design: Two ectomycorrhizal fungal species, crossed with two light levels, crossed with three harvest times (Table 1). Each combination of fungal species, light level, and harvest time was replicated six (*Rhizopogon*) or eight (*Pisolithus*) times. Three trees died during the course of the experiment for a total of 81 treatment mycocosms (see Table 1). The two light levels tested were high light (400 μmol/m^{-2}/s^{-1}) and low light (135 μmol/m^{-2}/s^{-1}) on a 13-hr light cycle. Light treatments are below light saturation for

pine photosynthesis irradiance (PI) curves (Teskey et al. 1994) An additional 24 control mycocosms (four per light level x harvest time combination) containing seedlings with microbial wash, but without mycorrhizal inoculation, were also included, for a project total of 105 mycocosms. Two environmental chambers were utilized in this experiment (both Conviron Models ATC40). Each chamber contained two shelves of growing space, one of which was set at the low light level and one at the high light level per chamber, creating 4 blocks (2 low-light, 2 high-light), each of which contained at least 3 replicates of all combinations of EM fungal species and harvest times, randomized completely. Due to environmental chamber mechanical failure, the third harvest mycocosms were moved to a nearby grow room, 4 weeks before the final harvest, with light exposure levels matching those in the environmental chambers for both high and low light treatments.

Table 1. Experimental design. Two fungal species (*Rhizopogon roseolus* and *Pisolithus arhizus*) were inoculated onto seedlings of *P. taeda*. Six or eight replicates were constructed for each combination of two fungal species, two light levels (high or low), and three harvest times (10, 20, and 30 weeks). Three seedlings died by the end of the experiment and were not included, and treatment groups with a missing seedling are marked with *. An additional 24 mycocosms (four per light treatment per harvest time) of control seedlings with microbial wash, but without mycorrhizal inoculation, were also included, for a project total of 105 mycocosms.

	Pinus taeda* +*Rhizopogon* +*microbial wash	***Pinus taeda* +*Pisolithus* +*microbial wash***	**Control +microbial wash**	
Harvest 1 (10 weeks)	6	8	6	High light
Harvest 1 (10 weeks)	6	8	6	Low light
Harvest 2 (20 weeks)	6	8	6	High light
Harvest 2 (20 weeks)	6	8	6	Low light
Harvest 3 (30 weeks)	5*	7*	6	High light
Harvest 3 (30 weeks)	5*	8	6	Low light

Seedling planting

Mycorrhizal colonization of root tips was verified on each seedling before transplanting from cones into one side of each mycocosm. Five seedlings of each target fungi and non-mycorrhizal controls were rinsed with DI water and frozen for analysis of initial ergosterol, C, P, and N content. Subsamples of soil substrate were collected for initial nutrient content. After planting, mycocosms were treated with a liquid nutrient addition of 10mL of a Hoagland's No.2 (Sigma-Aldrich, St. Louis MO, USA) solution to both sides of each mycocosm (for a total of 20 mL per mycocosm) at the start of the experiment and after each harvest. Mycocosms were watered to saturation on a weekly basis.

Overview of data collection and synthesis

The total C flux from the seedling to the EM fungal symbiont was estimated as the sum of C respired (as CO_2) by the fungi, plus C accumulated in EM fungal biomass on the roots and in the soil (Fig. 1); accumulation in fungal biomass was estimated by measurements of ergosterol from the whole root system and homogenized soil from each half of the mycocosm. Ergosterol is an organic molecule found in fungal cell walls and indicative of living and recently dead fungal biomass (Grant and West 1986, Newell et al. 1987). Recent data suggest that ergosterol would not significantly degrade during the 30-week duration of our experiment (Wallander et al. 2013, Meachum et al. unpublished data). Total fungal biomass was calculated using ergosterol: biomass conversion factors (5.455 μg ergosterol/mg *Rhizopogon roseolus* fungi (dry weight); 1.534 μg ergosterol/mg *Pisolithus arhizus* fungi (dry weight)). Fungal biomass assay analyses of ergosterol were carried out using modified versions of previously described methods (Ekblad and Nasholm 1996, Gessner and Schmitt 1996, Gessner and Newell 2002). We are assuming that

total C in fungal biomass is transferred from the seedling due to the rarity of ectomycorrhizal fungi accumulating C compounds out of the soil on their own (Zak et al. 2019).

Cumulative respired C was estimated using instantaneous measurements just before each harvest with a LI-6400XT (LI-COR Bioscience, Lincoln, Nebraska, USA) infrared gas analyzer (IRGA), using a custom chamber placed over the mycocosms, and respiration rates were integrated over time. The accumulated mass of N and P in plants was estimated from N and P analyses of dry plant biomass from the above and below ground plant parts. Total resource transfers were used to calculate N:C and P:C exchange ratios by dividing the total amount of N and P transferred to the seedling by the total amount of C transferred to the fungus.

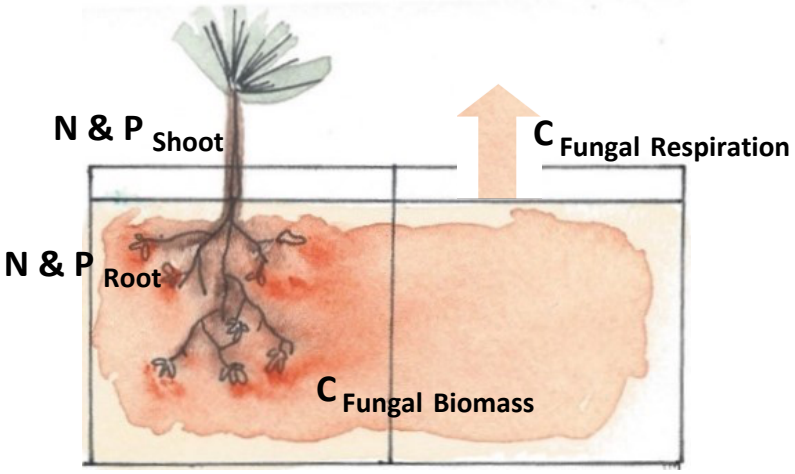

Fig. 1: Fates of resources. Description of fates of carbon (C), nitrogen (N), and phosphorus (P) transferred between tree and fungus. C transferred to the fungus will be incorporated into biomass or respired by the fungus; N and P transferred to the plant will be incorporated into shoot or root biomass.

Respiration measurements

Efflux of CO_2 from soil was estimated using a LI-6400XT (LI-COR Biosciences, Lincoln NE) infrared gas analyzer (IRGA) mounted to a custom polycarbonate box (Fig. 2). LI-COR Soil CO_2 Flux System software was used with a "closed" method, wherein a 'Δ' value, or change in

CO_2 concentration, is selected and 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 minus Δ, at which point the chamber concentration of CO_2 is allowed to rise due to flux from the soil. The software then begins to measure concentration of CO_2 in the chamber over time until target plus 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. To obtain replicate estimates, this measurement process was repeated three times (separated by approximately six minutes) per non-pine (fungus-only) side of each mycocosm. Ambient CO_2 levels were reassessed at the beginning of each set of three measurements and Δ was set at 5 ppm. All CO_2 measurements were taken during the same time period each day (between 11:00 a.m. and 3:00 p.m.).

To test for diurnal fluctuations in soil CO_2 flux from mycocosms, a subset of mycocosms (three from each light x fungal species treatment combination) were measured at 5 time points (9:00 AM, 2:00 PM, 8:00 PM, 1:00 AM 5:00 AM) over a 24-hr period. This test was conducted once between the first and second harvest (12-15 weeks after start of experiment) and then again between the second and third harvest (26-27 weeks after start of experiment). We found no consistent effect of time of day on CO_2 flux rates (data not shown).

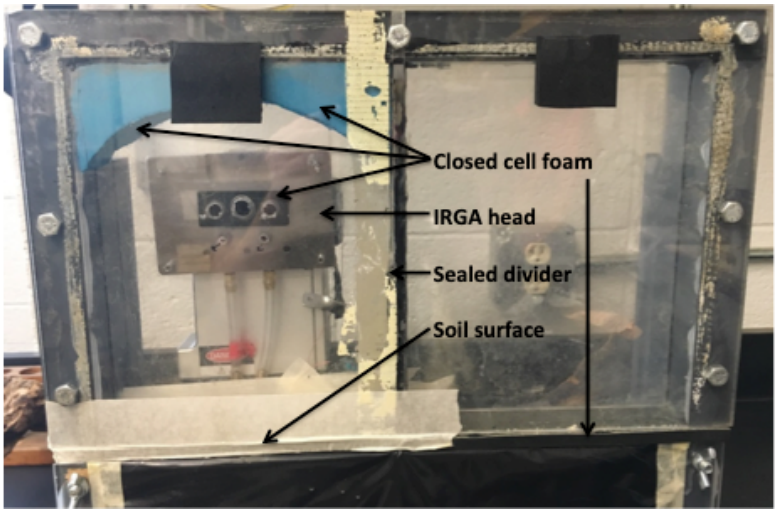

Fig. 2. Custom gas chamber. Photo of custom polycarbonate box gas chamber used for CO_2 flux measurements with the LI-6400XT system. The chamber is separated into two sections by an impermeable barrier and sits flush on the upper surface of the experimental mycocosms. One half of the chamber is used for CO_2 measurements in the non-pine (fungus-only) side of the mycocosm, and the other is sealed off entirely with closed cell foam (CCF) at base. The sensor head is attached to the center of the measurement chamber wall and sealed with CCF, which is also mounted to the base of the chamber to create a seal between the measurement chamber and the upper edges of the experimental mycocosm. The interior corners of the measurement chamber were filled with curved fitted pieces of CCF to prevent pockets of trapped air in the corners, thus promoting thorough mixing during measurement cycles.

Consideration of background values of saprobic C

Despite very low organic matter and nutrients in the experimental soil, we expected small numbers of saprobic bacteria and fungi (introduced with the background microbial slurry) to contribute to soil respiration and (in the case of saprobic fungi) fungal biomass in the experimental mycocosms. One approach we considered to account for this saprobic activity was to assume it was the same in the non-mycorrhizal control mycocosms as in the treatment mycocosms. If so, C in ergosterol and respired CO^2 in non-mycorrhizal controls would represent an estimate of background levels to be subtracted from experimental mycocosms. We attempted to estimate relative abundance of culturable saprobic bacteria and fungi in control versus experimental mycocosms by using dilution plate counts via a modified protocol as described in Vieira & Nahas (2005). Bacterial colonies were grown on trypticase soy agar (TSA) using

cycloheximide as an anti-fungal, and fungal colonies were grown on Martin Agar (MA) using Streptomycin as an anti-bacterial. Three samples of each treatment type at each harvest were counted in replicates of three, and colony-forming units were averaged across replicates. Results from those assays exhibited high variance among replicates and no significant differences between controls and experimental mycocosms ($F_{2,75}$=1.329, p=0.271), although most saprobic microbes are likely not culturable and thus were not detected with this approach. Moreover, in preliminary calculations, subtracting control averages (from non-mycorrhizal controls) from experimental treatment values (from mycorrhizal experimental mycocosms) frequently resulted in values near or below zero, even when seedlings in experimental treatment mycocosms had abundant EM fungal colonization and mycelium, suggesting that saprobic activity was likely inhibited by mycorrhizal fungi in the experimental mycocosms, which is especially likely given the low N levels in our experimental soils (Orwin *et al.* 2011, Averill & Hawkes 2016, Fernandez & Kennedy 2015, Sterkenburg *et al.* 2018). Ultimately, for these reasons, we chose not to subtract control values of C in ergosterol and respired CO_2 from the experimental values.

Estimation of carbon in fungal respiration ($C_{\text{fungal respiration}}$)

To estimate fungal CO_2 efflux from the pine side of each treatment mycocosm, the CO_2 efflux rate (averaged across 3 replicate measurements) from the non-pine side (Fig. 3, $CR1_{(fungi)}$) was divided by the total soil ergosterol content from the non-pine side for each mycocosm to estimate a CO_2 efflux rate per unit ergosterol (Fig. 3, $CB1_{(fungi)}$). This CO_2 efflux rate per unit ergosterol was then multiplied by the sum of the soil and root ergosterol contents from the pine side of each mycocosm (Fig. 3, $CB2_{(fungi)}$) to estimate treatment pine side CO_2 efflux rate (Fig. 3, $CR2_{(fungi)}$). We assumed that respiration of fungi far from plant roots (the non-pine side of

mycocosm) and fungal respiration near plant roots (pine side of mycocosm) was homogenous. Total mycocosm CO_2 efflux rate was calculated as the sum of the estimated pine side and measured non-pine side efflux rates.

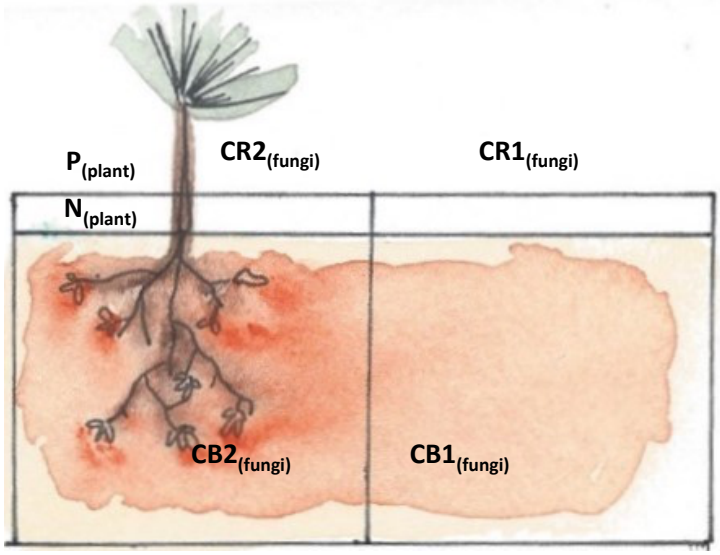

Fig. 3: Calculations for mycocosm fungal C and plant N/P (all values are in μmols):

$CR1_{(fungi)}$ = C in CO_2 respired by non-pine side (averaged across replicate measurements)
$CB1_{(fungi)}$ = C in soil ergosterol content (fungal biomass) of non-pine side
$CB2_{(fungi)}$ = C in soil ergosterol content (fungal biomass) of non-pine side + (C in root ergosterol content of pine seedling – average C in root ergosterol (fungal biomass) content of pre experimental treatment seedlings)
$CR2_{(fungi)}$ = Calculated C in CO_2 respired by the pine side
$((CR1_{(fungi)} / CB1_{(fungi)}) * CB2_{(fungi)})$
$N_{(plant)}$ = Measured N in pine roots and shoots – average pre experimental treatment pine N
$P_{(plant)}$ = Measured P in pine roots and shoots – average pre experimental treatment pine P

Mycocosm CO_2 efflux rates estimated at the three harvest times were fitted with exponential curves to estimate total CO_2 efflux amounts during each of the three growth periods of the experiment One exponential curve was fit to estimate efflux from week 0 through week 10, and another was fit to estimate flux from weeks 10 through 30; this was done for each treatment group of mycocosms separately. To estimate the average amount of CO_2 respired by a mycocosm within a treatment group, the area beneath the curve was calculated using Simpson's

1/3 method for integration. We then used bootstrapping (with replacement) of individual estimates within each growth period to generate a series of estimates of C in respiration for each treatment group, which were randomly assigned to individual mycocosms. Although this random assignment likely added noise to the data, it allowed us to incorporate individual values of C efflux for each mycocosm into analyses, even though all values within a treatment group were used for curve fitting to estimate an overall average value. Mycocosm values for C respired were added to C in fungal biomass to calculate total C transfer from plant to fungus. Week 0 CO_2 efflux values were estimated by regressing harvest 3 root ergosterol values against colonized root-tip counts, for each fungal species separately and using the resulting linear equations ((√ *Pisolithus* root ergosterol) ^{2} = 0.0567+0.0004(# of *Pisolithus* tips), (√ *Rhizopogon* root ergosterol) ^{2} = 0.1499+0.0003(# of *Rhizopogon* tips)) to predict root ergosterol content from pre-experimental seedling total root-tip counts, then multiplying ergosterol content by the average CO_2 efflux rate per unit ergosterol at harvest 1 (for each fungal species separately).

Estimation of carbon in fungal biomass ($C_{\text{fungal biomass}}$)

Accumulation of C into fungal biomass was estimated using cumulative ergosterol content as in Hendricks et al. (2016) for ectomycorrhizal mycelia production. At each harvest, we assumed ergosterol values to represent total ergosterol having accumulated to that point, i.e., we assumed no ergosterol degradation during the course of the experiment, consistent with previous observations (Stahl and Parkin 1996, Wallander et al. 2013). C accumulated in ergosterol during the entire experiment was calculated as the sum of C in soil ergosterol from both the pine and non-pine sides at the final harvest, plus C in root ergosterol at the final harvest, after subtracting an average (for each fungal species separately) of pre-experimental treatment

seedling root ergosterol (Fig. 3, $CB1_{(fungi)} + CB2_{(fungi)}$). Root ergosterol values for harvests 1 and 2 were estimated using EM colonized root tip counts and the same regression equations (described above) used to estimate root ergosterol from root tip colonization of pre experimental treatment seedlings. Cumulative ergosterol contents were converted to total biomass C using ergosterol:C conversion factors developed from analysis of sporocarp and mycelial culture tissue from each fungal species.

Total carbon transferred to fungus (C_{fungus})

Total amounts of C transferred from the seedling to each fungus at each light level, over a given time period, is equal to the sum of the amounts of C incorporated into fungal biomass and C respired ($C_{\text{fungal respiration}} + C_{\text{fungal biomass}}$).

Nitrogen and phosphorus transferred to plant (N_{plant} and P_{plant})

Ectomycorrhizal colonization of pine seedling root tips was approximately 100% across the experiment, so we assumed in our calculations that all N and P acquired by seedlings was transferred by EM fungi as all nutrient acquisition is through root tips. Calculations of cumulative N or P transferred from fungus to plant for each seedling was determined by the amounts of nutrient in the shoot and root system of a given pine seedling after subtracting average N and P contents from pre-experimental treatment seedlings (Fig. 2, N_{plant} and P_{plant}). Total nutrient transfer values were used with total amounts of C transfer to determine resource exchange ratios. The total amounts of C were divided by the total amount of N and P, respectively, to determine resource exchange ratios in μmols (N:C and P:C).

Data analysis

To test effects of light and fungal species on absolute amounts and ratios of transferred resources (Question 1), data on C, N and P fluxes, and N:C and P:C exchange prices were analyzed in separate univariate analyses using linear mixed effects models using the *lmerTest* package in R version 3.5.2, with growth periods (1,2, and 3), light levels (high and low), EM fungal species, and their interactions as fixed main effects (repeated-measures modeling was not required, since separate replicates were destructively harvested at each sampling point). Because the light treatment was applied to whole growth chamber shelves, and to account for variation among *P. taeda* genetic families, we included "shelf" and "family," respectively, as random effects. Highly non-significant 3-way interactions were removed from models. In the case of significant effects of harvest, light treatment, EM fungal species, or their interactions, means were separated using Tukey HSD adjustment of pairwise p- values using the *emmeans* package.

To test for effects of resource transfer on plant mass (Question 2), similar univariate mixed models were used, additionally containing covariates representing effects of total resource transfer and exchange ratios (C, P, N, N:C, N:P) and interactions with categorical predictors. Models with and without all combinations of these covariates were compared using Akalike's Information Criterion corrected for small sample sizes or AICc using the AICc() function in the *MuMIn* package. Models were fitted using both REML (restricted maximum likelihood) and ML (maximum likelihood) approaches, and AICc scores for each set of models revealed the same top model selection. Normality of residuals was confirmed using inspection of histograms for all models. Results of all analyses were used to distinguish among alternative hypotheses for Questions 1 and 2.

III RESULTS

How does light availability affect resource exchange?

Total N:C ratios were significantly affected by an interaction between light and EM fungal species (Light x Fungus Interaction: $F_{1,70}$=4.8261, p=0.0313) (Fig. 4). N:C ratios were significantly higher in the low light treatment for seedlings inoculated with *Rhizopogon* (p=0.0387) compared to the high light treatment, but did not significantly differ between light treatments for seedlings inoculated with *Pisolithus* (p=0.6205). Affects of EM fungal species also changed over time (Harvest x Fungus interaction: $F_{2,70}$ = 8.1580, p=0.0006)(Fig. 5). N:C ratios were more than doubled for seedlings inoculated with *Pisolithus* over seedlings inoculated with *Rhizopogon* at the first harvest (p<0.0001), but reduced to values equal to seedlings inoculated with *Rhizopogon* through the second and third harvest (p=0.8626 and p=0.6521 respectively).

The P:C ratio of transfer between seedlings and fungi was not affected by light treatment (Appendix, Table 5), nor fungi (Appendix, Table 5); only a main effect of harvest was detected whereby P:C ratios decreased throughout the experiment (main effect of Harvest: $F_{2,70}$= 3.0551, p=0.05345) (Fig. 6a).

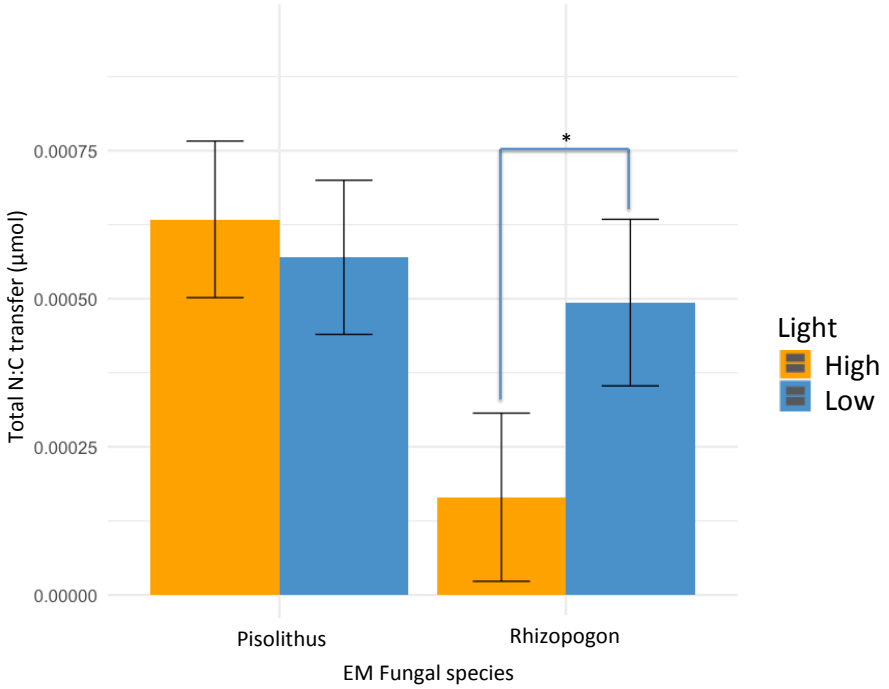

Fig. 4 Relationship between EM fungal species, light treatment, and N:C ratio (mean µmol, +/- SE). Asterisk denotes significant differences between high and low light treatment means within an EM fungal species

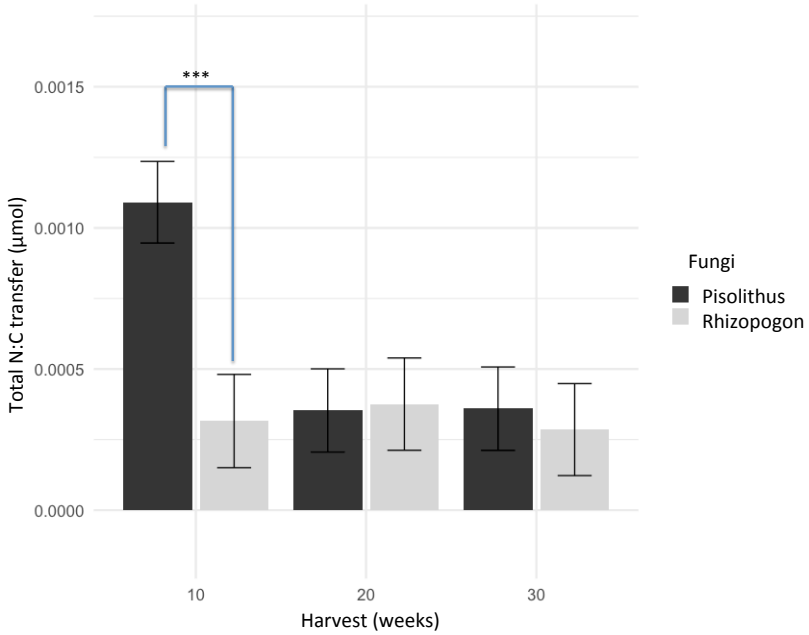

Fig. 5 Relationship between EM fungal species (black bars = *Pisolithus*, grey bars = *Rhizopogon*), and harvest (10, 20, 30 weeks) on N:C ratio (mean µmol, +/- SE).

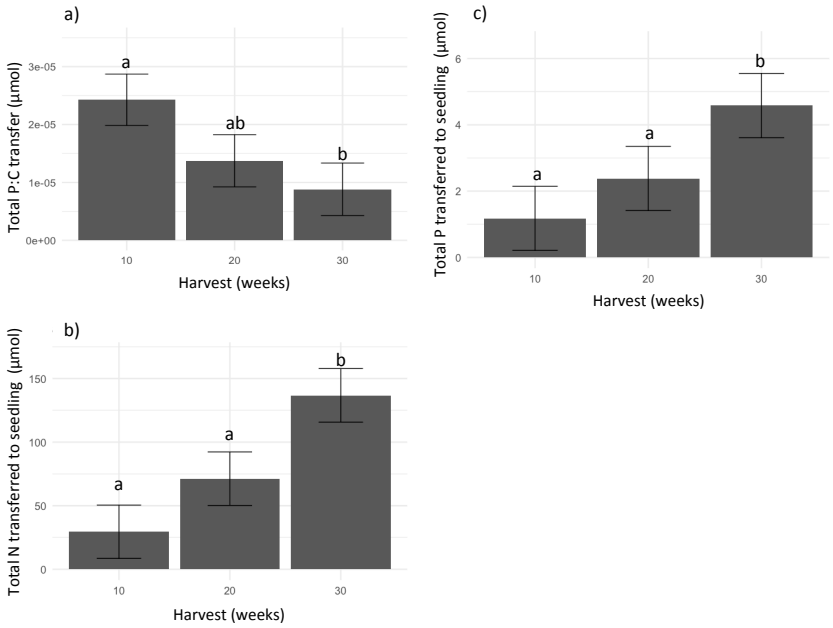

Fig. 6 P:C ratio, N and P transfer by harvesta) P:C exchange ratios across harvests (10, 20, 30 weeks) mean µmol, +/- SE. b) Total N transferred from fungi to seedling across harvests (10, 20, 30 weeks) mean µmol, +/- SE. c) Total P transferred from fungi to seedling across harvests (10, 20, 30 weeks) mean µmol, +/- SE. Letters signify significant pairwise differences between harvest means according to Tukey HSD post-hoc tests

The change in the amount of C transferred over time in relation to light levels depended on EM fungal species (Light x Harvest x Fungus interaction: $F_{2,68}$=50.922, p<0.0001) (Fig. 7). Cumulative C transferred to *Pisolithus* increased throughout the experiment, but this increase was only significant in the low light treatment. The pairwise effect of light treatment was not significant at week 10 or week 20, but by week 30, seedlings in the low light treatment had a nearly significant increase in total fungal C compared to the high light seedlings (p=0.0608). Seedlings inoculated with *Rhizopogon* showed no change in total fungal C transfers to fungi for low light throughout the experiment. Cumulative C transferred to *Rhizopogon* was substantially larger than to *Pisolithus* throughout the experiment, and occurred mostly during the 1st and 3rd growth periods. Cumulative C transfers more than tripled during the 3rd growth period, and was higher under high light (p<0.0001).

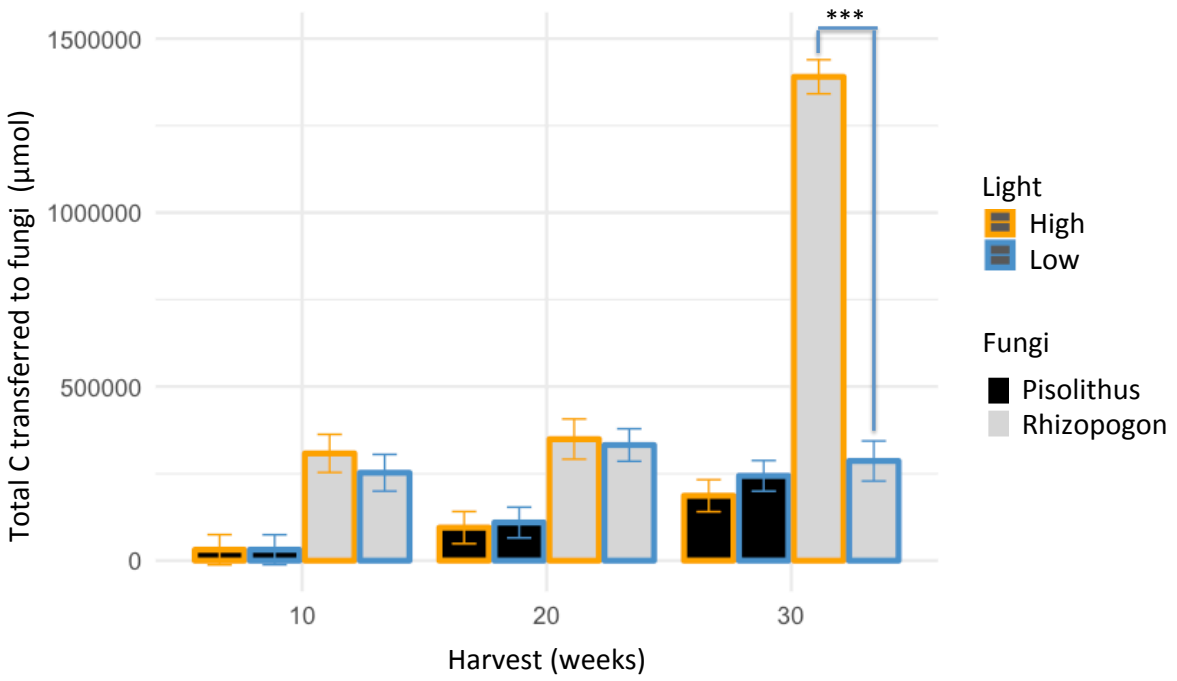

Fig.7 Relationship between EM fungal species (black bars = Pisolithus, grey bars = *Rhizopogon*), light treatment (orange framed = high light, blue framed = low light) and harvest (10, 20, 30 weeks) on total C transfer (from seedlings to EM fungi mean µmol, +/- SE). Asterisk denotes significant pairwise difference in total C transfers between high and low light for *Rhizopogon* at the 3rd harvest.

Total N transferred from EM fungi to the seedling was significantly affected by an interaction between light and EM fungal species (Light x Fungus Interaction: $F_{1,70}$=4.8490, p=0.03096) (Fig. 8). Total N transfer was more than double in the high light treatment compared to the low light treatment for seedlings inoculated with *Rhizopogon* (p=0.0334), but did not differ between light treatments for seedlings inoculated with *Pisolithus* (p=0.6803). Total N transfer increased over time, irrespective of EM fungi, exhibiting a significant increase by the 30 week harvest (main effect of Harvest: $F_{2,70}$= 23.7357, p<0.0001) (Fig. 6b).

Total P transfer from both fungi to seedlings was significantly higher in the high light treatment (main effect of Light: $F_{1,70}$=5.4842, p=0.02204) (Fig. 9), significantly higher for seedlings inoculated with *Rhizopogon* (main effect of Fungus: $F_{1,70}$= 6.9719, p=0.01019, (*Pisolithus*, marginal mean=1.91, SE=0.929)(*Rhizopogon*, marginal mean = 3.50, SE=0.967),

and increased significantly by the 30 week harvest (main effect of Harvest: $F_{2,70}$=12.3550, p<0.0001) (Fig. 6c).

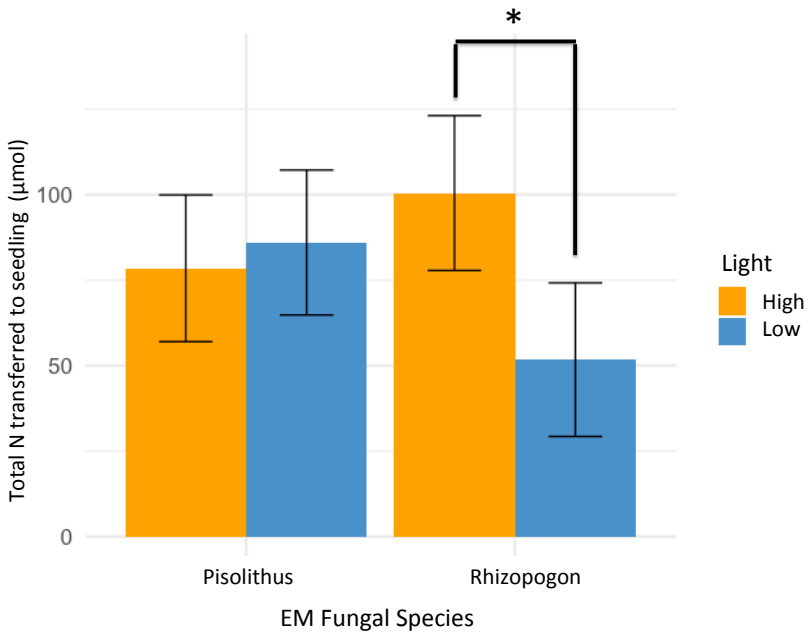

Fig. 8 Relationship between EM fungal species, light treatment, and total N transfer (from fungi to seedling mean µmol, +/- SE). Asterisk denotes significant differences between high and low light treatment means within an EM fungal species.

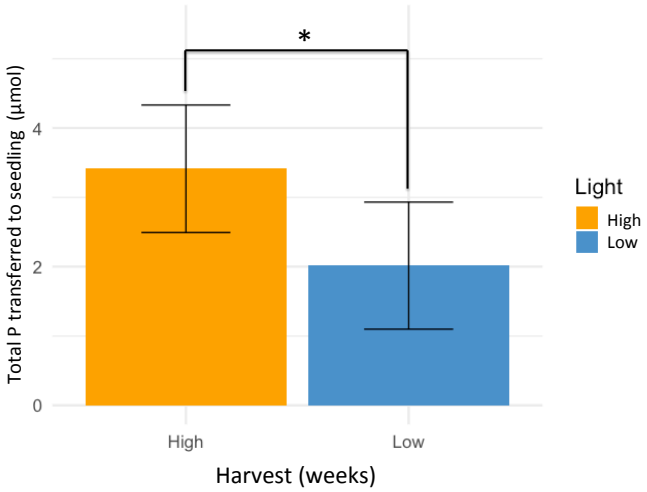

Fig. 9 Relationship between light treatment, and total P transfer (from fungi to seedling (mean µmol, +/- SE)). Asterisk denotes significant differences between high and low light treatment mean

Do resource exchange ratios or absolute fluxes better predict pine seedling growth?

Resource exchange ratios did not affect final biomass of *P. taeda* seedlings. Rather, total fluxes of N and P from EM fungi were the best predictors of final seedling biomass. These effects of total N and P flux were dependent on each other, and on EM fungal species (Light x Harvest Fungus interaction: $F_{1,66}$=9.2990, p=0.0033). Overall, Rhizopogon inoculated seedlings had a larger final plant mass (Fig. 10) Biomass of seedlings inoculated with *Rhizopogon* was positively related to total P transferred to plants, regardless of total N transferred (Fig. 10a), whereas seedlings inoculated with *Pisolithus* showed a reduction in the positive relationship between total P and final plant mass as total N in plants increased (Fig. 10b). Positive plant growth occurred during the first two growth periods, but was slightly negative on average during the 3rd growth period, after accounting for all model effects (main effect of Harvest: $F_{2,66}$=18.6409, p<0.0001) (Fig. 11).

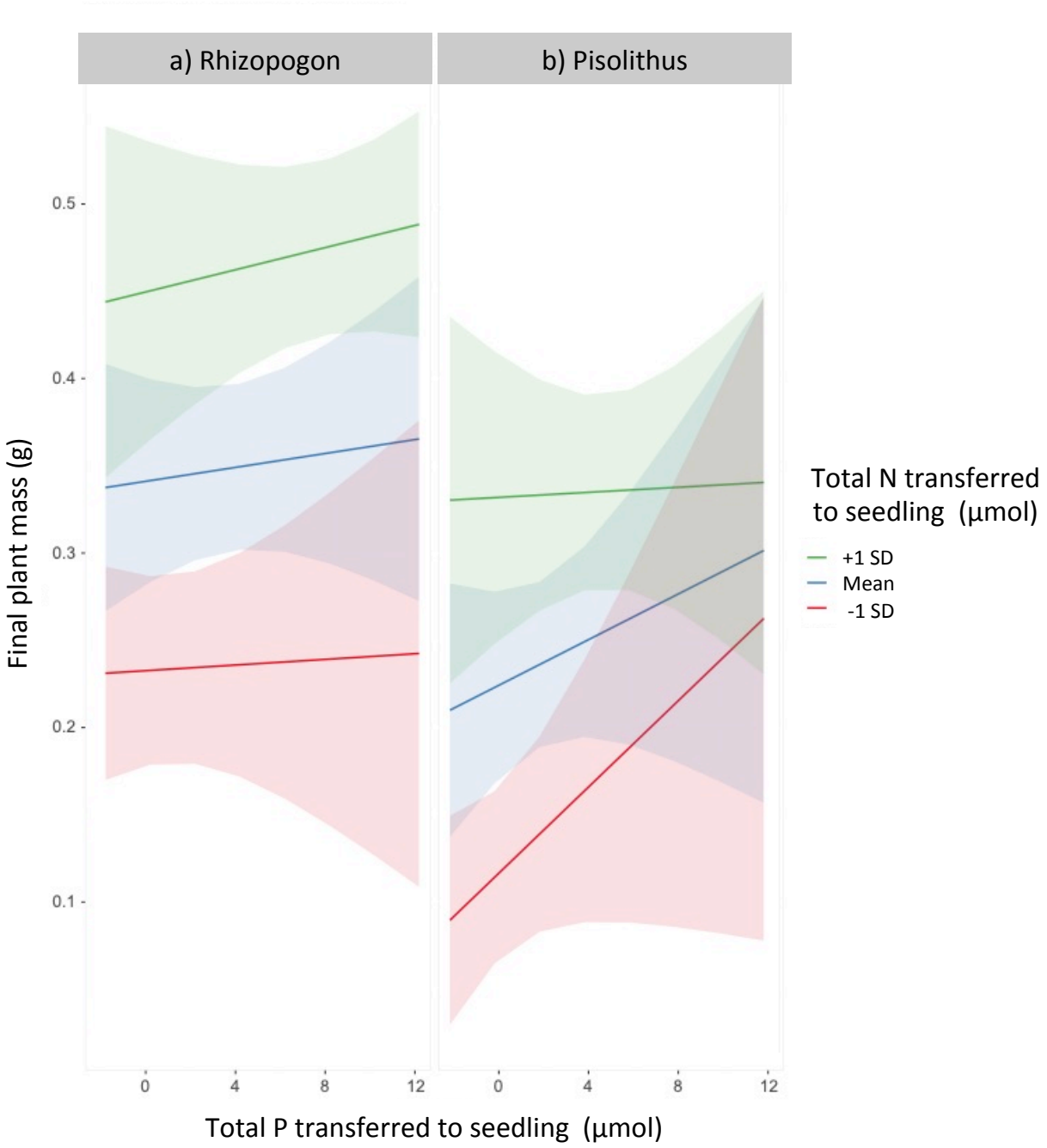

Fig. 10 Relationship between total N, total P, and final plant mass by EM fungal species. a) *Rhizopogon* relationship between total P transfer from fungi to seedling (µmol), total N transfer from fungi to seedling (µmol), and final plant mass (g). Slopes of lines are reported at the mean reported level of N and +/- 1 SD. b) *Pisolithus* relationship between total P transfer from fungi to seedling (µmol), total N transfer from fungi to seedling (µmol), and final plant mass (g). Slopes of lines are reported at the mean reported level of N and +/- 1 SD

IV DISCUSSION

We asked how light availability affects resource exchange and in turn how resource exchange affects plant growth in an attempt to test the assumptions of two alternative hypotheses on the outcomes of resource exchange mutualisms. Our data suggest that overall higher light availability increases resource exchange between mycorrhizal mutualists (Fig. 7, 8, and 9), and that N:C exchange ratios are lower in high light for *Rhizopogon*, i.e., C transferred from seedling increased with increased light availability, therefore reducing the N:C exchange ratios (Fig. 4), but that fluxes of limiting soil nutrients (N and P) have a stronger effect on seedling growth than resource exchange ratios (Fig. 10). These results suggest that the "exchange ratio hypothesis" and the "total flux hypothesis" are both correct in their predictions, and reconciliation of these ideas is needed.

How does light availability affect resource exchange?

Resource fluxes (of C, N, and P) to and from the pine seedlings increased with increased light available to the *Rhizopogon* inoculated seedlings (Fig. 7, 8, and 9). This result supports the predictions of the Grman et al. (2012) model, which suggested no effect of light on exchange ratios when nutrient levels are very low, but predicted differences in total nutrient fluxes between high and low light treatments. However, that model only accounted for P availability (and not N availability), and it is difficult to determine how our nutrient and light conditions correspond to

the parameter space explored in that model. Moreover, N:C resource exchange ratios were also affected by light in our experiment, being higher in a low light environment (Fig. 4). This result suggests that seedlings are somewhat C-limited under low light and are willing to pay a higher price of C (relative to N) when more C is available, as predicted by economic models of resource exchange, but this prediction is dependent on identity of fungal inoculum (Schwartz and Hoeksema 1998, Hoeksema and Schwartz 2003, Kummel and Salant 2006, Akçay and Roughgarden 2007, Akçay 2015).

Previous analyses of the effects of light on mycorrhizal resource exchange are rare. However, multiple studies have found that there is a shift in EM (Pena and Polle 2014) and AM (Knegt et al. 2016) mycorrhizal communities with reduced light (Johnson et al. 1997, Shi et al. 2014), which potentially implies shifts in nutrient flux. Arguably analogous to light manipulation treatments, elevated CO_2 studies have commonly found an increase in C allocation to mycorrhizal fungal biomass with increased C availability (Treseder 2004, Näshalom et al. 2013). Those results imply that plant trade with mycorrhizal symbionts is C limited, and is consistent with our results on C transferred to fungi, specifically by seedlings inoculated with *Rhizopogon* in third harvest (Fig. 7).

Our results imply that light is limiting to resource exchange (Fig. 4, 7, 8, and 9), at least for *Rhizopogon*, and therefore must be limiting to the mutualism as a whole, as light was not limiting to the host plant growth on its own (Appendix, Table 1). We suggest that because EM fungi are C limited, they may be indirectly light limited through the host plant. It is widely agreed that EM fungi acquire a majority, if not all, of their C from host plants and therefore rely on a plants access to C for their own biomass production (Zak et al. 2019). Kiers et al. (2011) found that arbuscular mycorrhizal fungi can discriminate among hosts with varying C supply and

allocate more nutrient transfer to those root tips with higher C supply, which could correspond to plant hosts with a higher light availability.

Both EM fungi tested (*Rhizopogon* and *Pisolithus*) are considered high biomass exploration types (Agerer 2001, Agerer 2006), but showed a distinct difference in resource exchange responses to light treatments (Fig 4, 7 and 8). It is therefore unclear how our results may have been altered if we had an additional low biomass type of EM fungi included in the study, and if these results are applicable to high biomass exploration biomass types in general. Some have suggested that high biomass types may require more C for long distance growth (Goldbold et al. 1997). However, higher biomass exploration types may provide more efficient nutrient uptake and long-distance transport (Koide et al. 2014), and there is also evidence for a higher C demand from low exploration biomass types (Bidartondo et al. 2001). Our results imply that traits other than exploration type may be important for driving variation in nutrient fluxes between different EM fungi.

Do resource exchange ratios or absolute fluxes better predict pine seedling growth?

Absolute fluxes of limiting nutrients (N and P), rather than resource exchange ratios, were found to better predict seedling growth, consistent with the "total flux hypothesis" (Fig. 10), although the effects of N and P transfer on plant growth were interdependent (Table 1 in Appendix), and differed between the two EM fungal species. Seedlings inoculated with *Rhizopogon* were growth limited by P transferred from fungi at all levels of N transferred from fungi (Fig. 10a), whereas seedlings inoculated with *Pisolithus* had a stronger limitation of P on plant growth when N transferred was low and became less limiting as N increased, suggesting that an increase in N correlates with an increase in P (Fig. 10b). The latter results for *Pisolithus*

inoculated seedlings are supported by a previous study in an AM system, which found that when overall nutrient availability is low, P uptake is limited by N uptake (Johnson 2009). Presumably, this interdependence is due to N being an essential limiting nutrient for photosynthesis, thus limiting C transfer to fungi, which thus limits P uptake and transfer (Johnson et al. 2010). It is additionally possible that because we only measured N and P in plant tissue, that the truly most limiting nutrient may be co-limited with N and or P (ref).

Changes in resource exchange over time

Although not a primary question in this study, we observed that nutrient fluxes changed over time in different ways for seedlings inoculated with the two different EM fungal species. *Pisolithus* inoculated seedlings had sharply decreasing N:C ratios after the first harvest (Fig. 5), while both N (Fig. 8a) and C (Fig. 6) total nutrient fluxes increased with harvest period, apparently because C transfer to *Pisolithus* was increasing over time at a faster rate than were nitrogen transfers to the plant. This pattern may have occurred due to the overall low N availability in the soil, leading the seedling to invest increasingly more C in fungal growth in the attempt to gain more soil nutrients (Treseder and Allen 2002, Hobbie 2006, Corrêa et al. 2008, Hasselquist et al. 2016). In contrast, *Rhizopogon* inoculated seedlings had stable N:C ratios throughout the experiment (Fig. 5), even though both N (Fig. 6b) and in particular, C (Fig. 7) increased throughout the experiment, suggesting a consistent price of trade of resources between mutualists (Kiers et al. 2011). Overall plant mass decreased slightly during the third growth period (Fig. 11), likely from increased needle mortality due to C allocation to fungal symbionts in response to very low availability of limiting soil nutrients.

Methodological considerations

Several aspects of the methods and assumptions used here to evaluate resource exchange are worth noting for their potential effect on our conclusions. For example, our "high" and "low" light treatments of 400 and 150 μmol/m^2/sec likely represented approximately 20% and 7.5% of full sunlight at mid-day (Bresinsky et al. 2013), respectively. However, our light treatments were similar to those used in other studies in environmental chamber systems (Corrêa et al 2012), and to test our hypotheses, we wanted to use low enough light levels so that light limitation would be a possibility. In addition, our light intensity was consistent during the 13-hour "daylight" cycle, unlike in a natural system in which irradiation would increase during morning hours and decline during afternoon hours. Both the specific light intensities chosen, and their patterns during a 24-hour period, could affect the results of experiments testing how light may affect resource exchange in mycorrhizal symbiosis.

Nutrient fluxes in pairings of seedlings with a single EM fungal symbiont species, as utilized here, may not be indicative of how those same fungi would interact with plant hosts when in competition with each other or other fungi. For example, economic models predict that when plants can choose among multiple symbiont species they will shift resources to those symbionts offering a better price of exchange (Kummel and Salant 2006). It is also possible that when in competition with other fungal species, a particular EM fungus may invest resources in competitive interactions, passing along less to the host plant, as predicted by virulence models of symbiont superinfection (Nowak and May 1994). However, measuring resource exchange between individually paired plants and mycorrhizal fungal species is a first step in understanding discrete resource exchange between mutualists.

The soil substrate used here was, by design, more nutrient poor than would be found in

many natural pine-EM systems, with low nutrient and organic matter content, and then we supplemented the system with inputs of mineral nutrients periodically. We used this approach in order to limit the activity of saprobic fungi, while allowing for low background levels of other soil microbes, such as mycorrhiza helper bacteria (Garbaye 1994) to improve realism of the system. It is possible that supplying more nutrients in an organic form would change resource exchange fluxes for any particular combination of plants and EM fungi, since EM fungal species vary in their abilities to use mineral versus organic forms of nutrients (Koide et al. 2014). Also, future studies would ideally directly account for abundance and respiration of saprobic organisms, e.g., by supplying organic matter derived only from a C_4 plant that could allow detection of a unique ^{13}C signature from saprobic respiration compared to respiration from a C_3 plant or its mycorrhizal symbionts (Bol et al. 2003). In addition, the mycocosm system used here would likely be inappropriate for testing nutrient fluxes between plants and short-distance, low-biomass exploration types of EM fungi, such as *Lactarius* and *Amanita*, due to our method requiring that fungi grow into the non-plant side of the chamber to measure C in respiration.

Future Directions and Conclusions

Future mycocosm studies of resource exchange fluxes in ectomycorrhizal systems could benefit from evaluating a larger variety of EM fungal mutualists in order to compare groups of EM fungal species differing in their exploration morphotypes, successional status, propensity to form common mycorrhizal networks (CMNs), and other traits. Such data could help to clarify interpretation of results from field experiments manipulating the presence and absence of EM CMNs, which tend to also change the community composition of the EM fungi, favoring early-stage EM fungi in non-CMN treatments (Hoeksema 2015), and making it difficult to know

whether changes in plant performance are due to CMNs *per se* or to changes in EM fungal functional traits.

Evaluating central assumptions and predictions of economic and other models, in addition to evaluating key effects of contextual variables, are crucial to fully understanding resource exchange mutualisms. Our study suggests that light availability is important for nutrient fluxes and N:C exchange ratios between EM mutualists, but limiting nutrient fluxes, not light and exchange ratios, are most not important in predicting plant growth. These results suggest that the predictions of the "exchange ratio hypothesis" and the "total flux hypothesis" are not necessarily in conflict with each other, and imply that when mycorrhizal plants have additional C to trade to their mycorrhizal fungi, it has the potential to promote an increase in nutrients to the plant in return, changing the price of exchange without detriment to either mutualist.

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APPENDIX

Table 1. Analysis of Variance (anova) for nutrient and environmental effects on plant growth using the model(NP<-lmer(plantmass~Harvest+Light+Fungi+total.P+ total.N+Light:Fungi+Light:total.P+Fungi:total.P+Light:total.N+total.P:total.N+ total.N:Fungi:total.P+(1|Shelf)+(1|Tree),data=fulldata))
TypeIIIAnalysisofVarianceTablewithSatterthwaite'smethod

	SumSq	MeanSq	NumDF	DenDF	Fvalue	Pr(>F)
Harvest	0.052788	0.026394	2	66.393	18.6409	3.757e-07***
Light	0.001347	0.001347	1	10.928	0.9514	0.350462
Fungi	0.088557	0.088557	1	66.276	62.5442	3.762e-11***
total.P	0.014325	0.014325	1	66.045	10.1175	0.002238**
total.N	0.057442	0.057442	1	66.479	40.5691	2.055e-08***
Light:Fungi	0.002828	0.002828	1	65.892	1.9975	0.162268
Light:total.P	0.006868	0.006868	1	65.859	4.8502	0.031148*
Fungi:total.P	0.003687	0.003687	1	66.233	2.6037	0.111372
Light:total.N	0.002780	0.002780	1	65.785	1.9632	0.165868
total.P:total.N	0.013398	0.013398	1	66.740	9.4625	0.003039**
Fungi:total.P:total.N	0.013167	0.013167	1	66.255	9.2990	0.003294**

Table 2. Analysis of Variance (anova) for nutrient and environmental effects on N:C resource exchange ratios using the model (lightNC<-lmer(N.C~Harvest+Light+ Fungi+ Harvest:Light+ Light:Fungi+ Harvest:Light:Fungi+ Harvest:Fungi + (1|Shelf)+ (1|Tree), data=fulldata))
TypeIIIAnalysisofVarianceTablewithSatterthwaite'smethod

	SumSq	MeanSq	NumDF	DenDF	Fvalue	Pr(>F)
Harvest	2.3092e-06	1.1546e-06	2	70.004	7.3433	0.0012732**
Light	3.4352e-07	3.4352e-07	1	70.061	2.1848	0.1438628
Fungi	1.4321e-06	1.4321e-06	1	70.631	9.1079	0.0035379**
Harvest:Light	4.6086e-07	2.3043e-07	2	70.117	1.4655	0.2379427
Light:Fungi	7.5882e-07	7.5882e-07	1	70.036	4.8261	0.0313427*
Harvest:Fungi	2.5654e-06	1.2827e-06	2	70.101	8.1580	0.0006528***

Table 3. Analysis of Variance (anova) for nutrient and environmental effects on Ntransferred to the fungi using the model (lightN<-lmer(total.N~Harvest+ Light+ Fungi+ Harvest:Light+ Light:Fungi+ Harvest:Fungi + (1|Shelf)+ (1|Tree), data=fulldata))
TypeIIIAnalysisofVarianceTablewithSatterthwaite'smethod

	SumSq	MeanSq	NumDF	DenDF	Fvalue	Pr(>F)
Harvest	154567	77283	2	70.003	23.7357	1.351e-08***
Light	8401	8401	1	70.043	2.5802	0.11271
Fungi	702	702	1	70.477	0.2156	0.64386
Harvest:Light	245	122	2	70.084	0.0376	0.96313
Light:Fungi	15788	15788	1	70.025	4.8490	0.03096*
Harvest:Fungi	500	250	2	70.072	0.0768	0.92611

Table 4. Analysis of Variance (anova) for nutrient and environmental effects on C transfer from seedling to fungi using the model (lightC<lmer(total.C~Harvest+ Light+ Fungi+

Harvest:Light+ Light:Fungi+ Harvest:Fungi + Harvest:Light:Fungi+ (1|Shelf)+ (1|Tree) , data=fulldata)
TypeIIIAnalysisofVarianceTablewithSatterthwaite'smethod

	SumSq	MeanSq	NumDF	DenDF	Fvalue	Pr(>F)
Harvest	1.9864e+12	9.9322e+11	2	67.730	75.834	<2.2e-16***
Light	4.4357e+11	4.4357e+11	1	1.996	33.867	0.02841*
Fungi	2.6965e+12	2.6965e+12	1	67.016	205.882	<2.2e-16***
Harvest:Light	1.0926e+12	5.4631e+11	2	68.175	41.711	1.477e-12***
Light:Fungi	8.5117e+11	8.5117e+11	1	67.093	64.988	1.848e-11***
Harvest:Fungi	6.1522e+11	3.0761e+11	2	68.519	23.486	1.706e-08***
Harvest:Light:Fungi	1.3339e+12	6.6694e+11	2	68.225	50.922	2.939e-14***

Table 5. Analysis of Variance (anova) for nutrient and environmental effects on P:C resource exchange ratios using the model (lightPC<-lmer(P.C~Harvest+ Light+ Fungi+ Harvest:Light +Light:Fungi + Harvest:Fungi + (1|Shelf) +(1|Tree) ,data=fulldata))
TypeIIIAnalysisofVarianceTablewithSatterthwaite'smethod

	SumSq	MeanSq	NumDF	DenDF	Fvalue	Pr(>F)
Harvest	2.7693e-09	1.3847e-09	2	70.019	3.0551	0.05345.
Light	1.0317e-09	1.0317e-09	1	70.271	2.2763	0.13585
Fungi	7.1477e-10	7.1477e-10	1	70.180	1.5770	0.21335
Harvest:Light	6.5490e-10	3.2745e-10	2	70.450	0.7225	0.48911
Light:Fungi	6.3547e-10	6.3547e-10	1	70.163	1.4021	0.24037
Harvest:Fungi	1.3543e-09	6.7717e-10	2	70.432	1.4941	0.23148

Table 6. Analysis of Variance (anova) for nutrient and environmental effects on P transferred to fungi using the model (lightP<-lmer(total.P~Harvest+ Light+ Fungi+ Harvest:Light+Light:Fungi+ Harvest:Fungi + (1|Shelf) +(1|Tree) ,data =fulldata)
TypeIIIAnalysisofVarianceTablewithSatterthwaite'smethod

	SumSq	MeanSq	NumDF	DenDF	Fvalue	Pr(>F)
Harvest	170.675	85.337	2	70.003	12.3550	2.538e-05***
Light	37.880	37.880	1	70.041	5.4842	0.02204*
Fungi	48.156	48.156	1	70.452	6.9719	0.01019*
Harvest:Light	15.940	7.970	2	70.079	1.1539	0.32134
Light:Fungi	16.996	16.996	1	70.024	2.4607	0.12123
Harvest:Fungi	20.091	10.045	2	70.068	1.4544	0.24051

VITA

EDUCATION

B.S., Biology, Kent State University, Kent, Ohio, Magna cum laude (May 2012)

EXPERIENCE

Assistant Curator (June 2017- May 2019)
Pullen Herbarium with Curator Lucile McCook | University of Mississippi
Department of Biology, Oxford, MS

Responsible for stabilizing and archiving plant and fungi collections primarily located in the southeastern United States. Skills include maintaining, troubleshooting, and updating all protocols related to imaging and digitization of specimens, organizing and mounting voucher specimens, training and overseeing student employees and volunteers, guiding tours and giving presentations related to the herbarium, regularly examine collection for pests, and assist local botanists and naturalists in navigating herbarium use and associated online plant and mycological portals.

Lab Manager / Research Technician (May 2012 – May 2015)
Laboratory of Dr. Christopher Blackwood | Kent State University
Department of Biological Sciences, Kent, OH

Responsible for maintenance of lab and research equipment, ordering lab supplies, overseeing undergraduate students, training graduate students, caring for greenhouse study plants, conducting long term field sampling and lab research including ergosterol extractions, HPLC, 454 next generation sequencing of environmental samples, fluorometric/colorimetric extracellular enzyme assays, DNA extraction, PCR and DNA purification.

Individual Investigation and Undergraduate Volunteer (March 2011-May 2012)
Laboratory of Dr. Christopher Blackwood | Kent State University
Department of Biological Sciences, Kent, OH

Examined arbuscular mycorrhizal colonization in woody angiosperms via root staining and quantified via root staining and light microscopy. Assisted graduate students in tasks related to their dissertation research including processing leaf samples, gel electrophoresis, DNA extraction, Acid hydrolysis and root dissection.

PUBLICATIONS

Hoeksema, J., M. Roy, G. Łaska, A. Sienkiewicz, A. Horning, M.J. Abbott, et al. 2018. *Pulsatilla patens* (Ranunculaceae), a perennial herb, is ectomycorrhizal in northeastern Poland and likely shares ectomycorrhizal fungi with *Pinus sylvestris*. *Acta Soc Bot Pol*.; 87(1):3572.

Valverde-Barrentes, O.J, A.L, Horning, K.A, Smemo, C.B, Blackwood. 2016. Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. *Plant and Soil* 404:1-12.

PRESENTATIONS

Horning A.L, J.D. Hoeksema, Effects of light on resource exchange rates in *Pinus taeda* ectomycorrhizal mutualisms. 2018. Ecological Society of America annual meeting. New Orleans, Louisiana.

Horning A.L, J.D. Hoeksema. Resource exchange prices and context dependency of *P. taeda* ectomycorrhizal mutualisms. 2017. University of Mississippi Graduate Student Symposium. Oxford, Mississippi

Horning A.L., M.J. Abbot, C.T. Tran, J. Mattox, J.D. Hoeksema, J.K. Zjawiony. Study of species interactions in the Primeval Forest of Białowieża, Poland. 2016. University of Mississippi School of Pharmacy's Poster Session. Oxford, Mississippi

Horning A.L, O.J. Valverde-Barrentes, C.B. Blackwood, Angiosperm root evolution: the relationship between root anatomy and mycorrhizal colonization among angiosperm trees. 2012. Soil Science Society of America annual meeting. Cincinnati Ohio

Horning A.L, O.J. Valverde-Barrentes, C.B. Blackwood, Angiosperm root evolution: the relationship between root anatomy and mycorrhizal colonization among angiosperm trees. 2012. Kent State University Science Symposium. Kent, Ohio.