Background: Bacterial symbionts play a variety of major roles in plants and animals, from the regulation of critical metabolic functions to the mediation of multitrophic interactions in complex communities[1]. Symbiotic interactions also occur between microbes, such as bacterial symbioses within fungi, yet these relationships are less well understood than microbial associations in larger organisms. As fungi are critical to the productivity and diversity of plant communities and the movement of energy through the biosphere, a poor understanding of **endohyphal bacteria (EHB)** (Fig.1) and how they regulate fungal function has left a key gap in our understanding. In my dissertation research, I propose to determine the functional contribution, genetic mechanisms, and biochemical modes of interaction between EHB and one of the most widespread and economically important groups of symbiotic microorganisms, the ectomycorrhizal fungi. Currently, EHB have been identified in a wide variety of fungi, spanning at least three phyla and representing diverse lifestyles from pathogens to mutualists[2]. EHB have been implicated in a number of critical functions such as sporulation[3], phosphate transport[4], and the production of toxins[5] and phytohormones[6]. Although **Ectomycorrhizal fungi (ECM)** play crucial roles in carbon cycling and forest ecology[7], significant gaps remain in our understanding of ECM metabolic function, including the critical cross-talk that takes place between plant and fungal partners at the onset of colonization. This process is thought to involve the production of fungal secondary metabolites, including volatile signaling molecules, morphogens and plant hormones[8]. However, researchers have been largely unable to identify the genetic mechanisms responsible for the production of these molecules in fungi. If EHB regulate ECM function, it may fundamentally change our understanding of ECM biology, ecology and their associated ecosystem processes.

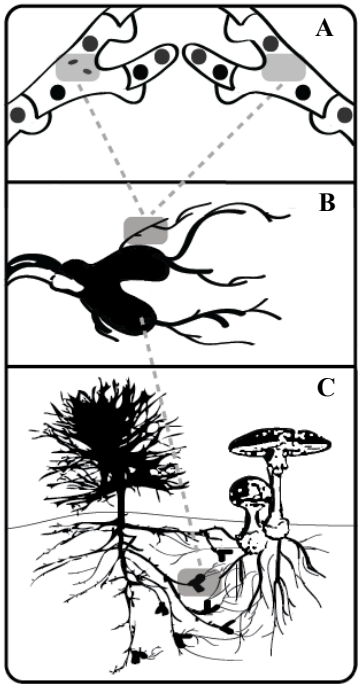

Fig.1 **A:** ECM+EHB (Left) and ECM-EHB (right) strains will undergo comparative analysis of secondary metabolites (H_1). **B:** Gene regulation will be evaluated during mycorrhizal formation (H_2). **C:** Plant growth would be altered by metabolite deficiencies.

Study system: Using two species of ECM fungi previously shown to be colonized by EHB, *Laccaria bicolor* (with *Paenibacillus* sp.)[9] and *Tuber borchii* (with *Bacteroides* sp.)[10], a replicate culture of each species will be "cured" of EHB using antibiotics[6], resulting in the creation of pared cultures that are identical except for the presence (ECM+EHB) or absence (ECM-EHB) of bacteria. Curing will be confirmed using both molecular and visual methods[6]. To assess EHB/ECM interactions at the plant level, two hosts, *Pinus banksiana* and *Quercus rubra*, will be used, as both are known associates of *L. bicolor* and *T. borchii*.

H_1: EHB mediate secondary metabolite production in ECM. Metabolite analysis will be preformed on the paired cultures using Solid-Phase Microextraction GC/MS. If EHB mediate the production of the metabolites, then ECM+EHB will have a different metabolic profile than ECM-EHB. Analysis will focus on the secondary metabolites (phytohormones and morphogens) reported to influence mycorrhizal colonization, including strigolactones[11], auxins[12], jasmonates[8], ethylene[8], cytokinins[13], hydrophobens[14] and a group of SR-proteins known as ectomycorrhizins[14]. While this method can identify differences between EHB+ and EHB- fungal strains, it does not resolve whether EHB influence fungal functionality directly (bacterial synthesis of metabolites) or indirectly (by modulating fungal gene expression).

H_2: EHB influence secondary metabolite production via changes in fungal gene expression. Because the genes involved in plant colonization may be expressed only in the presence of a suitable host, it is necessary to evaluate metabolite production within this context. *P. banksiana* and *Q. rubra* seedlings will be grown in microcosms and their root systems inoculated with ECM+EHB or ECM-EHB. To quantify fungal gene expression, RNA-Seq will be employed at successive steps during colonization from the initiation of directed growth to the establishment of functional mycorrhizas. If there are no differences in gene expression between the paired cultures, it would suggest that the metabolites produced are fungal in origin. Alternately, if ECM+EHB show evidence of the up regulation of genes responsible for the metabolites listed in H_1, but ECM-EHB do not, it would suggest that EHB mediate the production of metabolites, either directly or by the obligate initiation of fungal gene cascades. To determine which of these processes might be taking place, genomic analysis will be carried out following genome sequencing of *Paenibacillus* sp., *Bacteroides* sp. and *T. borchii* (the genome of *L. bicolor* is already publicly available). Genomes will be evaluated using antiSMASH to predict each organism's potential to synthesize the metabolites found in H_2.

H_3: Due to effects on secondary metabolite production, EHB moderate ECM-plant interactions by affecting colonization rates and plant biomass accumulation. *P. banksiana* and *Q. rubra* seedlings will be grown for 2 months, inoculated with ECM+EHB or ECM-EHB and grown for an additional 6 months. Eight-month old seedlings will be harvested, their root tips sequenced to confirm fungal and bacterial identity, and root systems quantified for percent colonization and root/shoot biomass accumulation. If colonization rates or biomass accumulation are significantly higher in plants inoculated with ECM+EHB than in plants inoculated with ECM-EHB, it would support the functional contribution of EBH in ECM-host plant interactions.

Project Merit & Feasibility: The research proposed in this study will fill a key gap in our current understanding of ECM fungi and microbe-microbe interactions. I expect the proposed study to reveal exciting, novel information that will significantly contribute to our understanding of microbial ecology. The University of Minnesota is the ideal location to conduct this research, possessing all the necessary equipment and computing resources, as well as a highly skilled network of collaborators to provide experiential support. My experience as an undergraduate researcher working on ECM systems and as a Junior Research Scientist working on fungal secondary metabolites has fully prepared me to execute all aspects of this proposal.

Project Impact: I know, from personal experience, the difference that mentorship can have in making diverse peoples feel wanted, welcomed, and inspired to be a part of the scientific community. The *in-planta* assays in H_3 provide an ideal opportunity to connect junior investigators with the scientific process. I am currently designing an outreach program, SciNorth, to bring North High School students into UMN laboratories for 8-week rotations (see personal statement). Students will engage in experimental design, hands-on participation in established research projects and student-designed independent research. This program is intended to engage students from demographics underrepresented in STEM fields, to demystify science and excite young investigators about the process of scientific inquiry.

References: [1]Rai, A. *et al. New Phyt.*(2000). [2]Bianciotto, V. *et al. Appl. Environ. Microbio.*(1996). [3] Partida-Martinez. *et al*. *Curr. Bio.*(2007). [4]Ruiz-Lozano. *et al. Bacter.*(1999). [5]Partida-Martinez. *et al. Nature*(2005). [6]Hoffman, M. *et al PLoS One*(2013). [7]Högberg *et al. New Phytol.*(2002).[8]*Signaling and Communication in Plant Symbiosis*.(Springer Berlin,2012).[9]Bertaux, J. *et al. Appl. Env. Microbio.*(2003). [10]Barbieri, E. *et al.Appl. Env. Microbio.*(2000). [11]Gutjahr, C. *Curr. Opin. Plant Bio.*(2014). [12]Frankenberger, W. *et al. Appl. Env. Microbio.*(1987). [13]Gogala, N. *Experientia*(1991).[14]Duplessis, S. *et al. New Phyt.* (2005). [15] Hilbert J. *et al*. *Plant Phys.*(1991).