Annual Report for Period: 11/2001 - 11/2002 **Submitted on:** 02/18/2004

Principal Investigator: Bledsoe, Caroline S. **Award ID:** 9981711

Organization: U of Cal Davis

Title:

BIOCOMPLEXITY:Common Mycorrhizal Networks - Active or Passive Channels? Interacting Roles of Mycorrhizal Fungi, Plants and Soil Resources in Carbon & Nutrient Transfers

Project Participants

Senior Personnel

Name: Bledsoe, Caroline

Worked for more than 160 Hours: Yes

Contribution to Project:

Project Principal Investigator who provides overall supervision and oversight of the project. She also guides students in research on the physiology of nutrient transfer among plants in oak woodlands. She also develops synthetic activities and plans meetings of the larger group.

Name: Zasoski, Robert

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Horwath, William

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Rizzo, David

Worked for more than 160 Hours: Yes

Contribution to Project:

Dr. Rizzo supervises one postdoc and one graduate student who are working on molecular identification of mycorrhizal fungi on plant roots.

Post-doc

Name: Idol, Travis

Worked for more than 160 Hours: Yes

Contribution to Project:

Dr. Idol's research interests focus on the transfer of 15-N and 13-C between plants via a common mycorrhizal network.

Name: Douhan, Greg

Worked for more than 160 Hours: Yes

Contribution to Project:

Dr. Douhan conducted molecular work on the project.

Name: He, Xinhua

Worked for more than 160 Hours: Yes

Contribution to Project:

Dr. He is a postdoctoral associate on the project. His expertise is the study of nutrient cycling in oak woodlands, particularly the use of stable isotopes of carbon and nitrogen. He is conducting studies of nutrient transfer in the field and in the greenhouse.

Graduate Student

Name: Cheng, Xiaomei

Worked for more than 160 Hours: Yes

Contribution to Project:
Ms Cheng's research focuses on oak root systems and their ability to acquire nitrogen.

Name: Aanderud, Zach
Worked for more than 160 Hours: Yes
Contribution to Project:
Mr. Aanderud's research focuses on mycorrhizal links between annual and perennail grasses.

Name: Meding, Steven
Worked for more than 160 Hours: Yes
Contribution to Project:
Mercer Meding is focusing on transfer of nitrogen, potassium, and analogs of calcium by common mycorrhizal networks. His work is based in growth chambers using forbs, grasses and oak seedlings.

Name: Smith, Matt
Worked for more than 160 Hours: Yes
Contribution to Project:
Mr Smith began studies of the sporocarp production at our field site.

Name: Lum, Quenby
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms. Lum assisted with fungal cultures.

Name: Verdi, Elisha
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms. Verdi investigated nutrient transfer among grasses and forbs.

Name: Morris, Melissa
Worked for more than 160 Hours: Yes
Contribution to Project:
Melissa Morris is conducting research on the spatial distribution of roots of specific individual oak trees using molecular methods (microsatellites), as well as one ectomycorrhizal fungus, Cenococum geophilum.

Name: Hynes, Meagan
Worked for more than 160 Hours: Yes
Contribution to Project:
Research assistant working on nutrient leakage from oaks.

Name: Stout, Deb
Worked for more than 160 Hours: Yes
Contribution to Project:
Research assistant conducting experiments on effects of plant phenology on nutrient transfer, especially in oaks and grasses. Both field and lab experiments.

Name: Collins, Kimber
Worked for more than 160 Hours: Yes
Contribution to Project:
Research assistant studying nitrogen fractionation by mycorrhizal fungi in pure culture and in field grown mycorrhizal roots.

Undergraduate Student

Name: Beaudette, Dylan
Worked for more than 160 Hours: Yes
Contribution to Project:

General lab duties and project webmaster.

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Southern Oregon University

SOU and UCDavis are collaborators in this project. We held three joint meetings at UC Riverside, Southern OR U and at UCD during the year 2003.

University of California Riverside Foundation

UCR and UCD researchers collaborate on this joint project. We held 1 joint meeting and 1 field campaigns in 2003.

Other Collaborators or Contacts

Dr. Barbara Cade-Mendum, a research faculty member at Stanford University interacted with our group and provided expertise in soil chemistry and NMR analyses.

Activities and Findings

Research and Education Activities:

Activities for Nov 2001-Oct 2002: We constructed and designed root exclusion boxes to evaluate the transfer of nutrients between plants with mycorrhizal connections. During this year we constructed boxes with 45-micron mesh screen glued to the inside of the box to prevent growth of roots outside the box. Two boxes were taped together, filled with a 3:1 mixture of fine sand:field soil and a young, uninoculated blue oak seedling was planted in each box. Seedlings were inoculated with a culture of a Cortinarius spp.; Cenococcum geophilum, and Lacarria amethysteo-occidentalis. The seedlings were placed in a growth chamber in October 2001 and watered to facilitate plant growth and fungal inoculation.

An experiment was conducted, as part of an on-going study, to examine the transfer of macronutrients between plants restricted to mycelial connections. Specialized growth containers, designed to separate plants with double root restrictive screens and an air-gap, were used to prevent the movement of nutrients between plants by root transport or soil solution mass flow. Nutrient labels applied to the foliage of donor plants were then analyzed for detection in the foliage of receiver plants. In order to examine the potential movement for an array of plant macronutrients through a CMN, a stable isotope and rare element nutrient analogs were used as tracers. 15Nitrate was used to trace nitrogen movement. Arsenic, cesium, rubidium, and strontium were used as tracers for phosphorous, potassium, and calcium respectively. Treatments included separated monocots, dicots, and a mixture of grass and forb species common to a California Sierra Foothill research site. The treatments were set up to encompass two levels of plant biocomplexity.

We examined the transfer of C and N between grasses in the field. During the spring of 2002, we conducted a grass-labeling experiment at the Koch Natural Area in the Sierra Foothill Research and Extension Center. The objective was to investigate the movement of C and N among grasses in a CMN in the field. The design consisted of 1 x 1 m plots laid out in an open area containing both perennial and annual grasses. A 30 cm row of grasses was pressed between two acrylic plates to which layers of tissue paper saturated with a 1% solution of 90%13C- and 10% 15N-labeled urea were attached. This foliar labeling scheme allowed for direct uptake of the C and N inside the leaves of the targeted plants. On Day 0, 1, 3, 5, and 7, leaves from grasses on the upslope and down slope sides of the labeled row of grasses were collected. On Day 7, leaves of the labeled grasses and roots from grasses in the labeled row and upslope and down slope from the labeled row were collected. Labeling was carried out in different plots in early March, April, May, and June 2002. All leaves were analyzed for atom % 13C and 15N. All roots were analyzed for mycorrhizal infection.

During the spring of 2002, we conducted a tree-labeling experiment at the Koch Natural Area in the Sierra Foothill Research and Extension Center. The objective was to investigate the movement of a nutrient and water analog among trees, shrubs, and grasses in a CMN in the field. Pairs of tree saplings and tree sapling-shrubs were located in the field and selected for labeling. The pairs consisted of foothill pine (Pinus

sabiniana) saplings with foothill pine saplings, foothill pine saplings with blue oak saplings, and foothill pine saplings with Ceanothus shrubs. Rubidium was chosen as an exotic element tracer for mobile nutrients such as K and for water. Five leaves or needles of one of the saplings in each pair was dipped in a vials containing RbCl solution. Samples of leaves from each of the trees or saplings in the pair and samples from surrounding grass leaves were collected on Day 0, 4, 7, 11, 18, and 24. On Day 24, roots from the tree saplings and shrubs in the pairs were collected as well. On Day 11 and 24, leaves from representative tree saplings and shrubs from plants distant from the labeled plots but within the same general area were collected as control samples to check for background changes in Rb content. All leaves and roots were analyzed for total Rb content.

In order to examine whether there were differences in transfer among oak species, either ammonium or nitrate was supplied as 15N as to the leaves of small blue, white and live oaks seedlings that had been planted at the Sierra Foothill Research Center. Adjacent seedlings were harvested and the isotopic analysis of the seedlings was carried out to ascertain the extent of 15N transfer.

We in investigated the 15N natural abundance in fungal fruiting bodies collected at our three research sites in order to investigate differences among functional and taxonomic groups of fungi in their N use and transformation. Comparisons were made between mycorrhizal and saprotrohpic fungi, among fungi of the same genus or species at different research sites, among fungi of different genera or species at the same site, and among fungi of the same species fruiting at different times at the same site

We grew eight species of mycorrhizal fungi in culture on NH4+, NO3-, glycine, arginine, alanine, glutamic acid, and bovine serum albumin. Growth rate and biomass of each species was examined. Using a compartmentalized pot we investigating the loss of nutrients from mycorrhizal fungi. Quercus douglasii in both pots were labeled with 15N by foliar application. Half the pots with the labeled seedling will receive an injection of glucose into the soil. Samples of soil, roots, and foliage were analyzed for 15N enrichment to see if the glucose decreased the amount of 15N translocation. We obtained roots that grew near Quercus douglasii at our field site and we morphotyped the roots and determined if there is enough biomass available to do a 15N analysis and molecular identification

We continued collecting and attempting to culture EM fruiting bodies. We began collecting EM root tips to ascertain which time of the year was best for sampling. Fungal collection were expanded to include another species of Hygrophorous (H. roseiburneus), and two hypogeous fungi; Rhizopogon ellenae and Melanogaster spp..

In the area of molecular biology we developed a molecular marker technique to study population genetics of AM fungi & 2) Population genetic structure of Boletus dryophilus.
We worked to create RFLP profiles for known species of EM fungi. However, later in the collection season of 2002, we decided to move away from large scale use of RFLP's and instead began to focus on DNA sequence data. We devised a protocol to extract DNA and obtain PCR products.

The effect of phenological stage on the transfer of nitrogen from an annual grass (Polypogon monspeliensis) to neighboring plants was investigated in a California oak woodland. Eighteen plots (3 treatments, 6 reps) were chosen to include a young blue oak seedling. A solution of 0.5% 15N2-urea was applied to a donor leaf at three phenological stages û vegetative (March 27), flowering (May 10) and seed filling (June 4). After two weeks, plots were harvested and plants (forbs, grasses, and oak seedlings) and soils analyzed for 15N.

Findings:

Findings for this year (Nov 2001 to Oct 2002) are as follows:
We developed a simple and flexible method for labeling plants in the field with 13C/15N dual-enriched urea that we evaluated early in 2002. In March, a small but significant amount of 13C was transferred to labeled plants but not to unlabeled plants. 15N transfer to labeled and unlabeled plants was highly variable (Table 1).In April, both 13C and 15N were transferred to labeled plants. Significant amounts of 15N were transferred to unlabeled plants, but again it was highly variable. In March and April, roots were not cleared and stained sufficiently to detect significant VAM infection or colonization. In May, colonization was low (~3%). Hyphae and vesicles/spores were both present. In June, colonization was higher (~10%) and consisted mainly of vesicles/spores.

An experiment was conducted, as part of an on-going study, to examine the transfer of macronutrients between plants restricted to mycelial connections. Results showed positive transfer of the nitrogen isotope and the phosphorous and potassium analogs within most treatments. Receiver foliage concentrations averaged up to 887%, 164%, 2898%, and 80% above control concentrations for 15nitrogen, arsenic, cesium, and rubidium respectively. The calcium analog did not show significant transfer for any of the treatments. Behavior of the tracers differed based on the nutrient each represented, with the exception that each positively transferred tracer showed high transfer concentration gradients for the treatment with highest level of plant biocomplexity.

In our field study of effects of grass phenology on transfer of 15N to neighboring plants, the donor's phenological stage strongly influenced

15N retention by the donor and the redistribution to receiver plants within the plots. While uptake of 15N by donor grasses increased significantly at each labeling period, the amount of 15N acquired by neighboring plants decreased. Among receiver plants, there were few differences in amount of 15N acquired from the donor grasses. When compared within each harvest, forbs acquired significantly more 15N than oaks only at the first harvest (vegetative stage). In all cases, the amount of N transferred from the donor to receivers was small (<0.14% total plant N), and results do not support the redistribution of 15N via direct transfer through a CMN. First, acquisition of 15N by receiver plants was positively correlated with plant biomass; a similar correlation would be expected in the absence of direct transfer. Second, grasses did not acquire more 15N from the donor grass than other plants, although grasses should have similar mycorrhizal types.

The collection of fungal species grew to a total of approximately 45 species. We discovered that Hygrophorous eburneus was a dominant fruiter in both 2001 and 2002. We learned that members of the family Thelephoraceae, a dominant ectomycorrhizal group in many conifer dominated ecosystems, were present in abundance at the Koch site. With the help of collaborators at UCR and SOU, I was able to prepare a poster on EM fungal species overlap between the three CMN sites.

Developing microsatellite markers for organisms with limited amounts of DNA can be difficult because sequence information is needed. To overcome this problem in the arbuscular mycorrhizal (AM) fungi Glomus etunicatum and Gigaspora gigantea, global amplification of the genomes of each species were performed using linker-adaptor-PCR from single spores. Amplified fragments were enriched for microsatellite motifs using 5'-biotinylated oligonucleotides and recovered by magnetic streptavidin beads. The recovered fragments were reamplified, run on denaturing polyacrylamide gels, and sixteen selected bands were excised, cloned, and sequenced. Seven microsatellite motifs were detected from six clones (efficiency rate of 43.8%). Primers were designed for all putative microsatellite loci and most were successfully amplified from three single spore preparations and from pools of 5, 10, and 20 spores after global amplification. This approach, termed amplified fragment length micosatellites (AFLM), may be useful for organisms that cannot or are not readily cultured in vitro and where DNA is a limiting factor for genetic studies. However, the technique can also be used to isolate microsatellite loci in any organism.

15N as either ammonium or nitrate was supplied to the leaves of small seedlings of blue, white and live oaks at the Sierra Foothill Research Center. Biomass in the live oak was about 2-times higher than that in the blue and white oaks. Similar 15N was accumulated by different oak seedlings irrespective of NO3- or NH4+ supplied. Live oak seedlings accumulated significantly higher amount of total nitrogen, especially in the roots when NO3- was the source.

Training and Development:

The project supported 7 graduate students, who gained research experience from the project. Students gave presentations of their research design and preliminary data to lab groups, thus giving them experience in presentations and teaching. We accompanied students to regional and national meetings and facilitated their interaction with other students, faculty and researchers in their fields.

Outreach Activities:

Graduate students and PI's attended the Mycological Society of America meetings in Corvallis OR in June 2002. Presentations were made by Dr. Greg Douhan (oral and poster), Dr. Xinhua He (poster) and by graduate student Matt Smith. Matt Smith also gave 2 talks for amateur mycological societies. At the Sonoma Mycological Association (SOMA), he presented preliminary findings about my research. At the Davis Mycological Society, he gave a talk about general ecology of mycorrhiza.

Journal Publications

Cheng, Xiaomei and Caroline S. Bledsoe., "Effects of season and site on fine root production by blue oaks and annual grasses in a Northern Californian oak woodland.", Plant and Soil, p. 263, vol. 240, (2002). Published

Ishikawa, C and CS Bledsoe., "Seasonal and diurnal patterns of soil water potential in the rhizosphere of blue oak: evidence for hydraulic lift. ", Oecologia, p. 459, vol. 125, (2000). Published

Wurzburger, N., Martin I. Bidartondo, and Caroline S. Bledsoe., "Characterization of Pinus ectomycorrhizas from mixed conifer and pygmy forests using morphotyping and molecular methods.", Canadian J. Botany, p. 1211, vol. 79, (2001). Published

Cheng, Xiaomei and Caroline S. Bledsoe., "Seasonal and site effects on oak fine root production and mycorrhizal colonization in California oak woodland.", In: (McCreary and Standiford, Eds.) Proc. 5th Symp. Oak Woodlands in California's Changing Landscape, USDA PSW Gen. Tech. Rpt PSW-GTR 18499-106, p. 263, vol. 184, (2002). Published

Denison, R. Ford, Caroline Bledsoe, Michael Kahn, Fergal O'Gara, Ellen L. Simms, and Linda S. Thomashow., "Cooperation in the rhizosphere and the "free rider" problem.
", Ecology.
, p. , vol. , ( ). Submitted

Aanderud, Z.T., C. S. Bledsoe and J. H. Richards., "Contribution of relative growth rate to root foraging of annual and perennial grasses from California oak woodlands.
", Oecologia, p. , vol. , ( ). Submitted

Douhan, G. W. and D. M. Rizzo., "Host-parasite relationships of bolete infecting Sepedonium species in California oak woodlands
", Mycological Research, p. , vol. , ( ). Accepted

Idol, Travis W., William R. Horwath, Elisheva C. Verdi., " A simple method for following 13C and 15N movement in mycorrhizal plants in the field.
", Plant and Soil, p. , vol. , ( ). Submitted

Cheng, X. And C. S. Bledsoe., "Competition for inorganic and organic N competition by blue oak (Quercus douglasii) seedlings, an annual grass and soil microorganisms in a pot study.", Soil Biol. Biochemistry, p. , vol. , ( ). Submitted

Douhan, G. W. and Rizzo, D. M.
, "Amplified Fragment Length Microsatellites (AFLM): A method to develop microsatellite markers in organisms with limited amounts of DNA applied to Arbuscular Mycorrhizal (AM) fungi.", Mycologia, p. , vol. , ( ). Accepted

Books or Other One-time Publications

Web/Internet Site

URL(s):
www.spodo.ucdavis.edu
Description:

Other Specific Products

Contributions

Contributions within Discipline:
Our research is within the disciplines of soil science, plant ecology and soil microbiology. We continue to contribute to these disciplines by increasing knowledge about fungal biodiversity of oak woodlands. This information is available on our web site (www.fungus.ucdavis.edu). Oak woodlands have worldwide distribution but there is little research on the belowground oak woodland ecosystems and how to manage belowground resources for oak woodlands.

Contributions to Other Disciplines:
This multi-disciplinary project is contributing information to disciplines of ecology, soil science, mycology, pathology, plant physiology and molecular biology.

Contributions to Human Resource Development:
Our project contributed to human resource development by training undergraduates, graduate students and postdocs.

Contributions to Resources for Research and Education:
We have begun a collection of sporocarps (fungal fruit bodies) from our oak woodland sites. The collection is housed in the herbarium of the Plant Pathology Department, University of California Davis, Davis CA. We purchased a flow injection system for atomic absorption analyses of arsenic, selenium and other hydride elements. This extended our institutional capabilities to detect very low levels of these elements.
We have developed a web site that is the main platform for disseminating information within the group and to the public. The website contains educational information, project summary, list of participants with correspondence information, updates on project activities, and a

shared files section where results and information germane to the CMN research group can be shared. Only this last section is not available to the general public. In the future, the website will contain a bulletin board section for open discussions and a list of publications that result from CMN research activities.

Contributions Beyond Science and Engineering:

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: less than 20 percent of current funds

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Any Book

Any Product

Contributions: To Any Beyond Science and Engineering