2013 Final Report

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"Final Harvest of Above-Ground Biomass and Allometric Analysis of the Aspen FACE Experiment"

PI: Mark E. Kubiske, USDA Forest Service, Institute for Applied Ecosystem Studies, Rhinelander, WI, 54501; phone: 715-490-5815; email: mkubiske@fs.fed.us

FINAL REPORT

Our objectives in year 3 were to 1) process fine root samples for DNA fingerprinting 2) complete the data analysis and publish results.

DNA fingerprinting

During the 2009 field harvest, fine root samples were collected from **6** soil cores from each of 12 treatment rings. After sampling, the soil cores were cut horizontally into 10 cm slices. The fine roots from the 0-10 cm depth were placed on a 1 x 1 mm sieve and rinsed gently with water to separate soil and root material. The root material was placed in plastic bags, moistened with water and stored at 4 C until the fresh tissue could be transported to the laboratory. Sample were sorted carefully using forceps and frequent washing to separate the individual fine root fragments. The fine roots of trembling aspen (Populus tremuloides Michx.) were separated from those of other plant species based on morphology and color. Individual root fragments were preserved for molecular analysis in CTAB buffer (2% hexadecyltrimethylammonium bromide (w/v), 100 mM Tris-HCL (pH 8), 1.4 M NaCl, 20 mM EDTA and 1% polyvinyl pyrrolidone and stored at -20 C until DNA extraction.

DNA isolation and amplification has been completed using protocols adapted from Marquardt et al. (2007), and Marquardt and Epperson (2004). Total DNA was purified from the fine root tissues using Qiagen Plant DNeasy isolation kits (Qiagen Inc., Valencia, CA). Stock DNA solutions were diluted to 2.0 ng/ul in T_{10} E_1 (10 mM Tris-Cl pH 8.0, 1 mM EDTA) for amplification by the polymerase chain reaction.

Reference fingerprints will be generated from genomic DNA isolated from aspen leaf-tissue collected during the 2008 growing season.

Using the polymerase chain reaction, genomic simple-sequence-repeat variation will be surveyed with ten-microsatellite markers isolated from trembling aspen (Dayanandan et al. 1998; Rahman et al. 2000). Genotyping will start with the most variable of the 10 loci and proceed until we obtain discriminating fingerprints for each clone. The amplification reaction for each primer pair will be conducted

separately. Reaction mixtures will contain 2 ng/ul DNA template in 10 ul of reaction buffer. The reaction buffer will consist of 20 mM Tris-Cl pH 8.75, 10 mM $(NH_4)_2SO_4$, 2.0mM $MgCl_2$, 10 mM KCL, 2 mM $MgSO_4$, 0.1% Triton X-100, 100 ug/ul BSA, 6% sucrose, 0.1 mM cresol red (Routman & Cheverud 1994), 200 uM each dNTP, 200-800 nM each primer, and 0.025 U/ul Platinum TAQ DNA Polymerase (Invitrogen, Inc.). A touchdown-amplification protocol (Echt et al. 1999) with a modified target-annealing temperature of 55 C will be performed using an icycler thermocycler (BIO-RAD Laboratories, Inc.). Amplified products will be diluted in de-ionized water, and two or more loci will be pooled for electrophoresis in the same lane, to generate a unique DNA fingerprint. Appropriate DNA size standards will be run in separate lanes. The DNA size standard will be a mixture of lambda DNA digested with HindIII and Phix174 DNA digested with HaeIII (New England Biolabs). In addition, 10 percent of the DNA fingerprints will designated for random retesting. For the reference fingerprints, PCR fragments will be sized fractionated on 3% trevigel agarose-gels (Trevigen, Inc.), visualized with ethidium bromide (1 ug/ml) and photographed with a gel-doc EQ system (BIO-RAD Laboratories, Inc). The balance of the fingerprinting amplification reactions will be sent to a core facility for fragment analysis.

Allometric Equations

Allometric equations were used to determine total tree biomass of non-harvested trees using diameter at breast height (DBH, 1.3 m) as the predictor variable. The DBH's were measured at the time of harvest in each ring. The equations were weighted, double *ln*-transformed least squares linear regression functions, using the correction of Baskerviller (1972) for the back transformation. The Y variables (dry mass of leaves, branches, stems or tree total) were weighted to correct for heteroscedacisity. A single weighting function was determined for each Y variable and species (across treatments, replicates, and aspen genotypes).

For scaling ^{15}N uptake by taxon, a separate allometric equation was used for each plant part mass x taxon x treatment combination pooled by replicates. For calculating NPP of each community type, a separate allometric equation was used for each total tree mass x treatment ring combination.

Results

We compared annual relative growth increments to key environmental variables that are known to be affected by the ENSO, and to the summer mean *Nino*-4 index, which describes sea surface temperatures in a region of the Tropical Pacific that is sensitive to the ENSO cold phase (*La Nina*). Growth responses to the treatments varied with year-to-year variation in atmospheric vapor pressure deficit (*D*), and photosynthetic photon flux density (PPFD), and autumn temperature. Relative

growth response to elevated CO_2 and O_3 were suppressed in years in years with low *D* and low peak summer PPFD. Relative annual growth responses to both gasses, *i.e.*, increase relative growth under elevated CO_2, and decreased relative growth under elevated O_3, were greatest in years with high peak summer PPFD that were preceded by warm autumn temperatures. The canonical effects of ENSO cold phase on regional weather patterns were supported by our data in that *La Nina* years were marked by dry, high PPFD summer conditions. Annual relative growth responses to both gasses were related to the summer mean *Nino*-4 index: the strongest growth responses to CO_2 and O_3 occurred during *La Nina* years. The environmental factors most closely associated with interannual changes in growth were those known to directly affect photosynthetic responses to both CO_2 and O_3. Our results indicate that significant and predictable feedbacks exist between changing carbon uptake of northern forests and changing climatic conditions.

However, feedbacks between C and N cycles in terrestrial ecosystems, as well as other agents of global change (e.g., elevated O_3), can counteract the enhancement of plant productivity by elevated atmospheric CO_2. We found enhanced forest productivity (~26% increase) under elevated CO_2 was sustained by greater root exploration of soil for growth-limiting N, as well as more rapid rates of decay and N release from forest floor and soil organic matter. Despite initial declines in forest productivity under elevated O_3, compensatory growth of O_3-tolerant genotypes and species resulted in equivalent NPP under ambient and elevated O_3 during the final years of the experiment. We found no interaction between forest community type and either elevated CO_2 or O_3, indicating that each community responded similarly to these trace gases. Forest productivity has been sustained under elevated CO_2 and has recovered under elevated O_3 by mechanisms that remain un-calibrated or not currently considered in coupled climate-biogeochemical models used to simulate interactions between the global C cycle and climate warming.

Plant growth responses to rising atmospheric CO2 and O3 varied among genotypes and between species, which influence the strength of competitive interactions for soil N. Ascribable to the size-symmetric nature of belowground competition, we reasoned that differential growth responses to CO2 and O3 should shift as juvenile individuals mature, thereby altering competitive hierarchies and forest composition. We used tracer 15N and whole-plant N content to assess belowground competitive interactions among five Populus tremuloides genotypes, between a single P. tremuloides genotype and Betula papryrifera, as well as between the same single P. tremuloides genotype and Acer saccharum. Under elevated CO2, the amount of soil N and 15N obtained by the P. tremuloides genotype common to each community was contingent on the nature of belowground competition. When this genotype competed with its congeners, it obtained equivalent amounts of soil N and tracer 15N under ambient and elevated CO2; however, its acquisition of soil N under elevated CO2 increased by a significant margin when grown in competition with B. papyrifera (+30%) and A. saccharum (+60%). In contrast, elevated O3 had no effect on soil N and 15N acquisition by the P. tremuloides genotype common in each community, regardless of competitive interactions. Under elevated CO2, the rank

order of N acquisition among P. tremuloides genotypes shifted over time, indicating that growth responses to CO2 change during ontogeny; this was not the case under elevated O3. In the aspen-birch community, the competitive advantage elevated CO2 initially conveyed on birch diminished over time, whereas maple was a poor competitor for soil N in all regards. The extent to which elevated CO2 and O3 will shape the genetic structure and composition of future forests is, in part, contingent on the time-dependent effects of belowground competition on plant growth response.

We found that both CO2 and O3 exert a selective effect on the tree populations. This, in turn, may lead to changes in ecosystem properties, such as carbon sequestration. W developed a hierarchical Bayesian model of survival. We also examined how survival differences between clones could affect pollutant responses in the next generation. Our model predicts that the relative abundance of the tested clones, given equal initial abundance, would shift under either elevated CO2 or O3 as a result of changing survival rates. Survival was strongly affected by between-clone differences in growth responses. Selection could noticeably decrease O3 sensitivity in the next generation, depending on the heritability of growth responses and the distribution of seed production. The response to selection by CO2, however, is likely to be small. Our results suggest that the changing atmospheric composition could shift the genotypic composition and average pollutant responses of tree populations over moderate timescales.

Following complete tree removal in 2010, we studied the effect of elevated O3 concentration on the regenerating aspen (Populus tremuloides) and maple (Acer saccharum) trees. This study is the first of its kind to examine the effects of acute O3 exposure on aspen and maple sprouts after the parent trees, which were grown under elevated O3 and/or CO2 for 12 years, were harvested. Acute O3 damage was not uniform within the crowns of aspen suckers; it was most severe in the mature, fully expanded photosynthesizing leaves. Young expanding leaves showed no visible signs of acute O3 damage contrary to expectations. Stomatal conductance played a primary role in the severity of acute O3 damage as it directly controlled O3 uptake. Maple sprouts, which had lower stomatal conductance, smaller stomatal aperture, higher stomatal density and larger leaf surface area, were tolerant of acute O3 exposure. Moreover, elevated CO2 did not ameliorate the adverse effects of acute O3 dose on aspen and maple sprouts, in contrast to its ability to counteract the effects of long- term chronic exposure to lower O3 levels.

Products

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