Powell River Project 2020 – 2021 Annual Research Report

Revegetation strategies to build soil organic matter: the foundation of reclamation sustainability and success

Principal Investigator:

Brian D. Strahm, Associate Professor, Forest Resources and Environmental Conservation

Co-Principal Investigators:

Brian D. Badgley, Associate Professor, School of Plant and Environmental Sciences

Jacob N. Barney, Associate Professor, School of Plant and Environmental Sciences

Stephanie A. Duston, Ph.D. Student, Forest Resources and Environmental Conservation

Proposal Summary:

Soil organic matter (SOM) is a critical component of any ecosystem, with important implications for the infiltration and storage of water, retention and supply of nutrients, and microbial community composition and function. As such, development of SOM is critical to reclamation success, yet can only be achieved indirectly. While much work has focused on quantifying rates and potentials of soil carbon sequestration on reclaimed mined lands, far less attention has been paid to the mechanisms of SOM development and means of optimizing it through reclamation practice. We believe that revegetation species selection, or more specifically, the selection of a diverse suite of plant traits, is an important opportunity to manage SOM. Here we propose to test that hypothesis by using isotopically labeled $^{13}CO_2$ to quantify the belowground allocation and fate of plant carbon based on revegetation species selection and characterize the carbon utilization patterns of the microbial communities that are recruited by different revegetation choices in order to make inferences about the long-term fate of SOM. We believe that the results of this work will directly contribute to efforts like those of the Forestry Reclamation Approach and the overall success of reclamation practice.

Introduction:

The development and maintenance of soil organic matter (SOM) is central to the delivery of ecosystem services from any piece of land. Thus, measuring the quantity and dynamics of SOM is often central to increasingly popular soil health/quality assessments designed to characterize the sustainability and production potential of cropland (Stott 2019) as well as forests and rangelands (Amacher et al. 2019). This is largely due to the widely recognized relationships between SOM and important ecosystem attributes such as the infiltration and storage of water, retention and supply of nutrients, and microbial community composition and function. Collectively, these attributes are responsible for the ecological, economic, and social benefits that are derived from all terrestrial ecosystems.

Though these arguments undoubtedly extend to the post-mining landscape, one fundamental difference exists. Whereas most conversations of soil health center on the preservation of existing SOM, the challenge of mined land reclamation is the need to rapidly develop SOM. This is especially relevant regionally, where organic-poor topsoil substitutes are commonly utilized. While we have shown that SOM pools scale with properties like plant biomass and time since reclamation (Avera et al. 2015), very little work has been done to optimize SOM development in the context of current reclamation practice, despite its role as the cornerstone of soil and ecosystem health.

The most significant pathway for SOM development occurs with belowground carbon allocation from plants. The subsequent exudation or deposition of that carbon, and its cycling by the microbial community, converts labile plant compounds into more stable SOM. Specifically, by adapting to metabolize relatively labile root exudates as primary C sources, soil microbial communities may in turn decrease degradation of more recalcitrant SOM (Strickland et al. 2019). Furthermore, SOM stored deeper in the soil profile is more likely to remain stable over long time periods (Rumpel et al. 2011, Fontaine et al. 2007). Thus, plant traits, such as patterns in belowground carbon allocation and rooting depth, can play an important role in determining where and how quickly SOM is likely to be formed in the soil profile (Slessarev et al. 2020). As a result, vegetation selection during reclamation can have a significant impact on SOM development.

We believe that the Appalachian Regional Reforestation Initiative's (ARRI) Forestry Reclamation Approach (FRA; Adams 2017) provides an excellent set of best management practices to both study and facilitate SOM development in the post-mining environment. Specifically, we hypothesize that the recommended use of diverse mixtures of "tree-compatible ground covers" (Burger et al. 2017), with discernable differences in traits (e.g., perennial vs. annual, deep vs. shallow rooted, leguminous vs. non-leguminous), represents an ideal set of circumstances to more rapidly build SOM by diversifying the type, timing, and location of belowground carbon inputs. We further believe that the impacts of vegetation selection on SOM will affect important functional attributes of the microbial community (e.g., metabolic preference) with implications for overall reclamation sustainability and success.

Project Objectives:

Our overall objective is to evaluate the role of post-mining plant species selection and diversity in building SOM and cultivating a microbial community with functional attributes consistent long-term SOM stabilization. Using mesocosms with different types/levels of plant diversity, we will:

1. quantify the belowground allocation and fate of plant carbon based on revegetation species selection and the associated traits of these plant communities, and

2. characterize the carbon utilization patterns of the microbial communities that are recruited by different revegetation choices in order to make inferences about the long-term fate of SOM.

Methods:

Broadly stated, we have used isotopically labeled $^{13}CO_2$ to track the flux and fate of C belowground in various greenhouse-grown plants and assay the carbon acquisition strategies of the resulting soil microbial community. A group of 30 different plant species were chosen to represent predominant differences in functional traits (i.e., N-fixation, rooting depth) that are expected to impact C storage and cycling (e.g., C:N ratio) and are already present in seed mix suggestions presently advocated by the FRA (Burger et al. 2017).

Seeds were germinated and grown in 5 L deep tree pots filled with a mix of washed quartz sand and locally sourced homogenized topsoil. A series of bi-weekly $^{13}CO_2$ labeling pulses were introduced to the growing plants during the experiment in order to ensure increased homogeneity and allocation of $\delta^{13}C$ throughout the plant (Slaets et al. 2019, Bromand et al. 2001, Kuzyakov et al. 2000). Bi-weekly, plants were moved to a sealed labeling chamber (Fig. 1), $^{13}CO_2$ was generated and introduced to the chamber via mixing labeled $NaH^{13}CO_3$ and 0.5 M HCl, and then mixed with a small battery-powered fan to homogenize $^{13}CO_2$ in the chamber atmosphere (Denis et al. 2019, Moore-Kucera et al. 2008). Plants were exposed for 2-hour intervals and then moved from the chamber back to the greenhouse (Bromand et al. 2001).

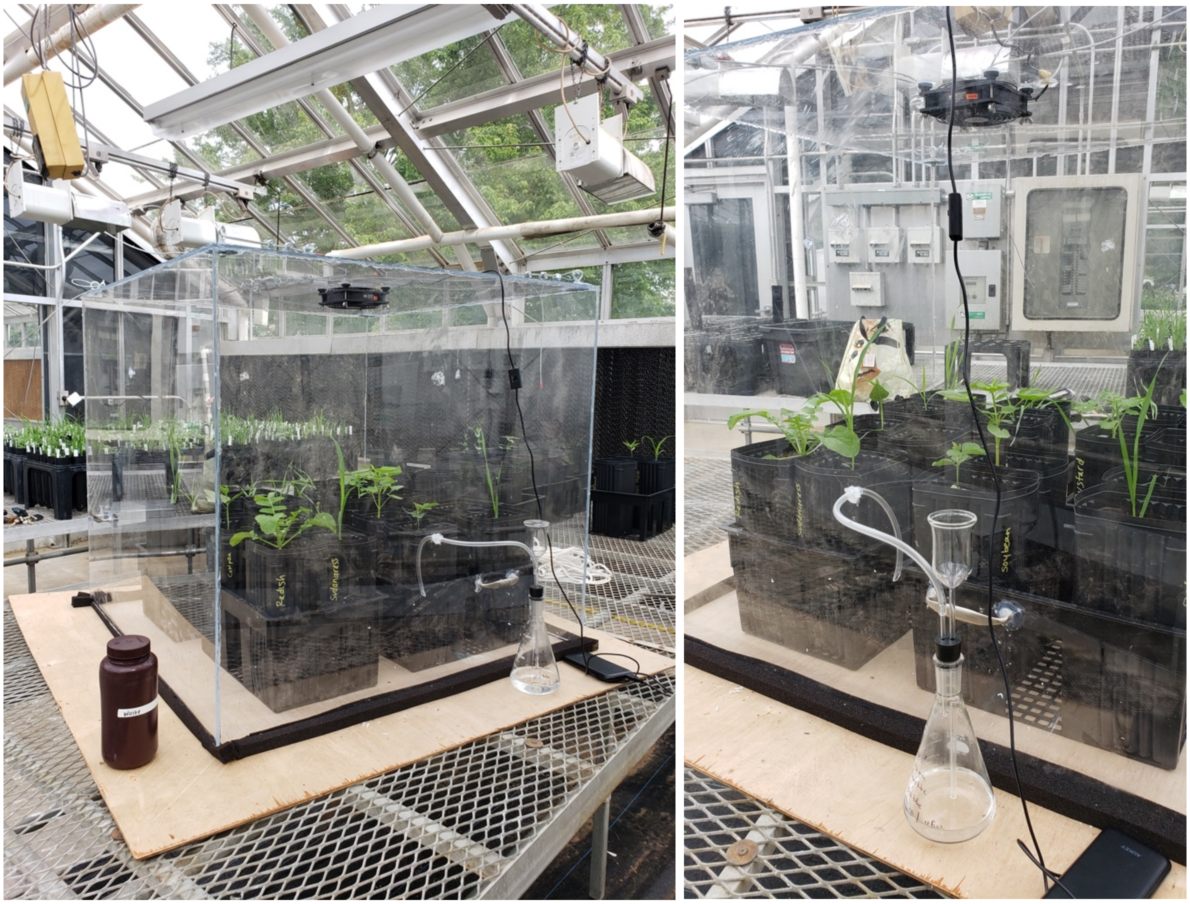

Figure 1. $^{13}CO_2$ labeling chamber used in greenhouse gas experiment.

After 10 weeks of growth, plants were harvested and separated into stem, leaf, and root fractions (Bromand et al. 2001). Fresh roots are being scanned and mapped using WinRHIZO software to obtain quantitative data on physical attributes (Himmelbauer et al. 2004). Soil in the whole pot was homogenized and sub-sampled for microbial and SOM characterization (Kuzyakov et al. 2001). Soil and plant samples were oven-dried and are in the process of being analyzed for $\delta^{13}C$ abundance using a continuous flow isotope ratio mass spectrometer with an elemental analyzer front end (EA-IRMS). Additionally, soil samples will be oxidized with potassium permanganate to determine biologically active C which relates to biological properties of soil such as respiration and microbial biomass (Weil et al. 2003; Culman et al. 2012). Further, to characterize spectral qualities of the dissolved organic fraction, we will employ spectral analysis (UV-VIS) of 0.01 M $CaCL_2$ extracted soluble organic matter (Gabor et al. 2015). This will give us information on the degree of aromatic character and whether the soluble organic matter fraction is more characteristic of plant exudates or microbial constituents (Cory & McKnight 2005).

DNA sequencing combined with DNA-stable isotope probing (SIP) will enable us to assess assimilation and cycling of plant derived carbon in active soil microbial communities that reflects likelihood of long term C storage (Dunford et al. 2010; Youngblut et al. 2018). In DNA-SIP, extracted DNA undergoes Cs gradient centrifugation which retrieves labeled and unlabeled DNA. These samples are presently being extracted and analyzed.

Results and Discussion:

Numerous analyses are currently in process. Thus, quantitative data and analyses are still forthcoming. That said, the greenhouse experiment has been terminated and appears to have been successful in creating diverse mesocosms with a gradient in plant functional traits (e.g., total biomass, belowground C allocation, root length/surface area; Fig. 2).

Figure 2. Visible differences in aboveground biomass following greenhouse experiment (left) and evidence of successful excavation of belowground biomass (right).

Future analysis will include development of quantitative relationships between plant functional traits and belowground carbon accumulation that will be directly transferable to post-mining reclamation with regard to guiding species selection.

Leveraged Funding:

Based on historically funded work from the Powell River Project, specifically our 2016-2017 proposal titled "The role of plant diversity in promoting recovery of soil microbial communities during ecosystem restoration on reclaimed mine lands," our team was recently successful in competing for extramural funding from the USDA National Institute of Food and Agriculture (NIFA):

Badgley, B.D., Barney, J.N., and Strahm, B.D. 2020-2024. Manipulating the soil microbiome through cover crop diversification for added agroecosystem benefits. USDA-NIFA. $497,881.

In a more rapid return on investment, this team has continued to build on this line of inquiry, and specifically on this Powell River Project investment, to again successfully compete for extramural funding from the USDA-NIFA:

Badgley, B.D., Barney, J.N., and Strahm, B.D. 2021-2025. Elucidating soil microbiome traits that predict long-term accumulation of stable soil organic matter. USDA-NIFA. $749,998.

Conclusions:

The Powell River Project has the explicit objective of enhancing the restoration of environmental quality of mining-influenced lands and waters, and to benefit communities and businesses in southwestern Virginia's coalfield region. Quite simply, we see the rapid development of SOM following surface mining disturbances as the cornerstone of the biophysical system that ensures this environmental quality. Further, it confers benefits to critical microbially mediated ecosystem functions that enhance vegetative productivity (e.g., habitat regeneration and C sequestration) and other ecosystem services with direct benefits to social (e.g., clean water) and economic (e.g., forestry) priorities in the region. Additionally, our hypothesis is that existing reclamation best management practices (i.e., FRA), specifically, revegetation with diverse mixtures, is an important yet underrecognized means of achieving these goals. With a successful greenhouse experiment under our belt, we anticipate using the forthcoming data and the extramural support it has enabled, to provide tangible guidance for the optimization of reclamation practice where SOM development and C sequestration are prioritized.

References:

Adams, Mary Beth, ed. 2017. The Forestry Reclamation Approach: guide to successful reforestation of mined lands. Gen. Tech. Rep. NRS-169. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 128 p. https://doi.org/10.2737/NRS-GTR-169

Amacher, Michael C.; O'Neil, Katherine P.; Perry, Charles H. 2007. Soil vital signs: A new Soil Quality Index (SQI) for assessing forest soil health. Res. Pap. RMRS-RP-65WWW. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 12 p.

Avera, B.N., B.D. Strahm, C.E. Zipper, and J.A. Burger. 2015. Development of ecosystem structure and function on reforested surface-mined lands in the Central Appalachian Coal Basin of the United States. New Forests 46:5 DOI 10.1007/s11056-015-9502-8.

Bromand, S.; Whalen, J.; Janzen, H,; Schjoerring, J.; Ellert, B.H. 2001. A pulse-labelling method to generate 13C- enriched plant materials. Plant and Soil. 235, 2533-257.

Burger, J.; Davis, V.; Franklin J.; Zipper, C.; Skousen, J.; Barton, C.; Angel, P. 2017. Chapter 6: Tree-compatible ground covers for reforestation and erosion control. In: Adams, Mary Beth, ed. The Forestry Reclamation Approach: guide to successful reforestation of mined lands. Gen. Tech. Rep. NRS-169. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 6-1 – 6-8.

Cory, R. M., & McKnight, D. M. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environmental Science and Technology. 39(21), 8142–8149. https://doi.org/10.1021/es0506962

Culman, S., Snapp, S., Freeman, M., Schipanski, M., Beniston, J., Lal, R., Drinkwater, L., Franzluebbers, A., Glover, J., Grandy, A. S., Lee, J., Six, J., Maul, J. E., Mirsky, S., Spargo, J., Wander, M. 2012. Permanganate Oxidizable Carbon Reflects a Processed Soil fraction that is Sensitive to Management. Soil Biology & Biochemistry. 76(2), 494-504. https://doi.org/10.2136/sssaj2011.0286

Denis, E.; Ilhardt, P.; Tucker, A.; Huggett, N.; Rosnow, J.; Moran, J. 2019. Spatially tracking carbon through the root-rhizosphere-soil system using laser ablation-IRMS. Journal of Plant Nutrition and Soil Science. 182, 401-410.

Dunford, E. A., & Neufeld, J. D. 2010. DNA stable-isotope probing (DNA-SIP). Journal of visualized experiments : JoVE. 42:2027. https://doi.org/10.3791/2027

Fontaine, S.; Barot, S.; Barré, P.; Bdioui, N.; Mary, B.; Rumpel, C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature. 450, 277-280.

Gabor, R. S., Burns, M. A., Lee, R. H., Elg, J. B., Kemper, C. J., Barnard, H. R., & McKnight, D. M. 2015. Influence of leaching solution and catchment location on the fluorescence of water-soluble organic matter. Environmental Science and Technology. 49(7), 4425–4432. https://doi.org/10.1021/es504881t

Himmelbauer, M., Loiskandl, W. & Kastanek, F. 2004. Estimating length, average diameter and surface area of roots using two different Image analyses systems. Plant and Soil. 260, 111–120.

Kuzyakov, Y.; Domanski, G. 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science. 163, 421-431.

Kuzyakov, Y.; Ehrensberger, H.; Stahr, K. 2001. Carbon partitioning and below-ground translocation by Lolium perenne. Soil Biology and Biochemistry. 33, 61-74.

Moore-Kucera, J; Dick, R. 2008. A pulse-chase method to 13Carbon-label Douglas-fir seedlings for decomposition studies. Soil Science. 173, 46-53.

Rumpel, C.; Kögel-Knabner, I. 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant and Soil. 338, 143-158.

Slaets, J.; Resch, C.; Mayr, L.; Weltin, G.; Gruber, R.; Dercon, G. 2019. Laser spectroscopy steered 13C-labelling of plant material in a walk-in growth chamber. Rapid Communications in Mass Spectrometry. 34, 1-8.

Slessarev, E.; Nuccio, E.; McFarlane, K.; Ramon, C.; Saha, M.; Firestone, M.; Pett-Ridge, J. 2020. Quantifying the effects of switchgrass (*Panicum virgatum*) on deep organic C stocks using natural abundance 14C in three marginal soils. GCB-Bioenergy. 12, 834-847.

Strickland, M.S., Thomason, W.E., Avera, B., Franklin, J., Minick, K., Yamada, S. and Badgley, B.D., 2019. Short‐Term Effects of Cover Crops on Soil Microbial Characteristics and Biogeochemical Processes across Actively Managed Farms. Agrosystems, Geosciences & Environment, 2(1), pp.1-9.

Stott, D.E. 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures. Soil Health Technical Note No. 450-03. U.S. Department of Agriculture, Natural Resources Conservation Service.

Weil, R., Islam, K., Stine, M., Gruver, J., & Samson-Liebig, S. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture. 18(1), 3-17. doi:10.1079/AJAA200228

Youngblut, N., Barnett, S., & Buckley, H. 2018. SIPSim. A Modeling Toolkit to Predict Accuracy and Aid Design of DNA-SIP Experiments. Frontiers in Microbiology. 9:570.