Powell River Project (2022 – 2023) Annual Report

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

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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 have:

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

2. characterized traits of soil microbial communities that are affected by different revegetation choices related to differences in the long-term fate of SOM.

This past year, we have taken these individual plant observations at the mesocosm scale and:

3. quantified the belowground allocation and fate of plant carbon, as well as the traits of soil microbial communities, in mixed plant communities more like reclaimed field environments.

Methods:

Plants will be selected from earlier experiments binned into 3 groups: group A will be a mix of 4 plants that all, individually, exhibited increase in SOM; group B will be a mix of 4 plants that were somewhat neutral; group C will be a mix of 4 plants that showed SOM decrease (i.e., microbial priming). Plants will be grown in 25 gal grow bags. An acrylic chamber, modified for $^{13}CO_2$ labeling as used in objectives 1 and 2, will be utilized to trace plant derived carbon. There will be one control group with no plants or labeling, and then three treatment groups in replicates of 5. Within each treatment, there will be multiples of each plant to maximize plant density in the bag. Plants will be grown over 24 weeks in order to allow for interactions between species. Soil will be sampled at the start of the experiment, then at 10 weeks which was standard for objectives 1 and 2, allowing to compare 10-week growth to previous experiments, and finally at 24 weeks.

Whole soil and mineral associate organic matter (MAOM) will be analyzed for: %C, %N, $\delta^{13}C$, and $\delta^{15}N$ (EA-IRMS, Elementar, Ronkonkoma, NY, USA). To determine changes in the microbial community energy activities, we will use qPCR to identify microbial functional genes for bacteria and fungi that are strongly correlated with C-mineralization rates for Acidobacteria, Bacteroidetes and other community species according to methods described by Trivedi et al. (2012). In addition, using assays, we will quantify extracellular enzyme activity for four enzymes involved in c-mineralization processes: β-D-cellulosidase (CB), β-Xylosidase (XYL), α-Glucosidase (AG) and *N*-acetyl-β-Glucosaminidase (NAG).

Results and Discussion:

In the 2020-2021 funding cycle, we used reduced funding from the Powell River Project to accomplish our first objective. Briefly, we 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 currently 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 (archived) and SOM characterization (Kuzyakov et al. 2001). Soil and plant samples were oven-dried and analyzed for $\delta^{13}C$ abundance using a continuous flow isotope ratio mass spectrometer with an elemental analyzer front end (EA-IRMS).

Results demonstrate that root traits (diameter, left; total belowground biomass, right) significantly increase bulk SOM, with differential responses between leguminous (green) and non-leguminous (red) species (Fig. 2). However, interestingly, the trends are opposite when considering the mineral-associated organic matter fraction, a sub pool of SOM often shown to have a longer residence time (Fig. 3). These results raise two interesting follow-up questions regarding the need to fractionate the SOM pool to better understand long-term carbon dynamics and potential interactions with nitrogen (as mediated by legumes here) on these SOM pools.

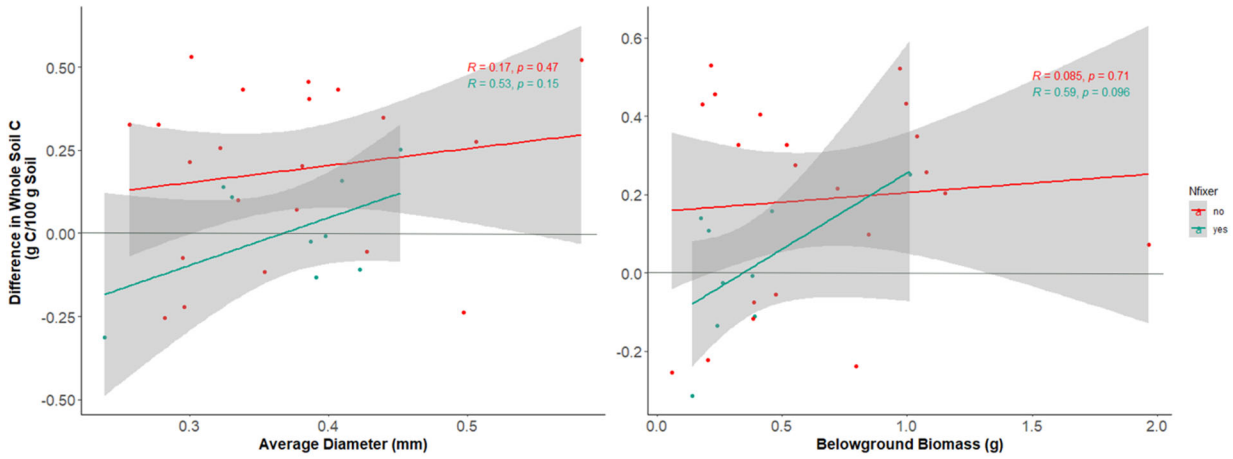

Figure 2. Changes in SOM after 10-weeks as a function of root diameter (left) and belowground biomass (right) by leguminous (green) and non-leguminous (red) species.

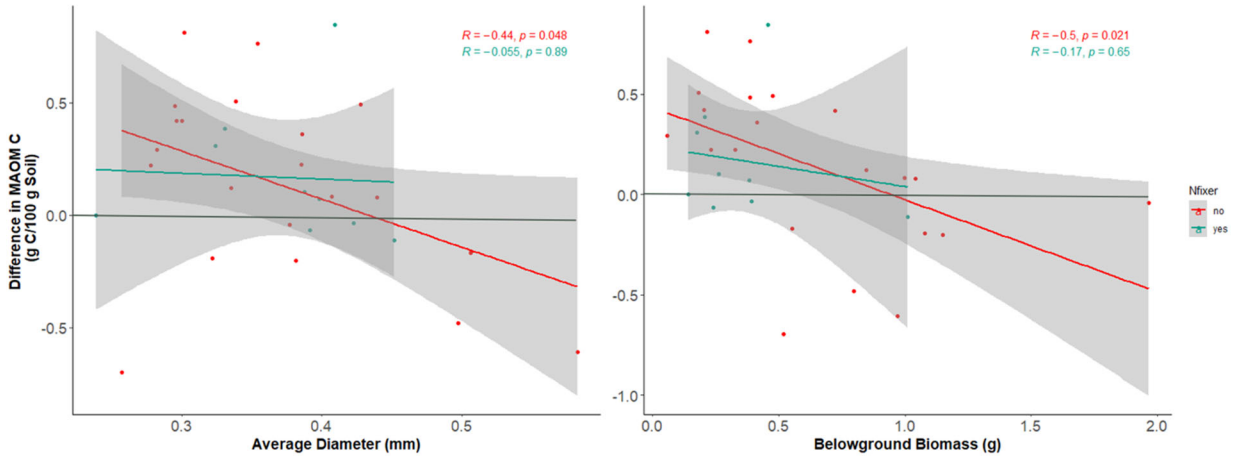

Figure 3. Changes in mineral associated organic matter (MAOM), shown to have a longer residence time in soil systems, after 10-weeks as a function of root diameter (left) and belowground biomass (right).

In the 2022-23 cycle, we tackled the third objective through a new collaboration with Dr. Mark Reiter at the Eastern Shore AREC where a unique opportunity arose to test plant diversity through a series of historically managed cover crop diversity trials. While not directly using FRA plant materials, this collaboration is critical in allowing us to resolve the cumulative impacts of plant mixtures on SOM and will provide a mechanistic basis to further inform planned experiments with PRP/FRA species. These samples are presently being analyzed for total carbon, mineral associated organic matter fraction, and particular organic matter fraction with results to be incorporated with those of Obj. 1 and 2 and presented at this fall's Soil Science Society of America Meeting.

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. Thus, we foresee this work providing novel support and opportunities for further optimization of practices currently being recommended to reclamation professionals.

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