U.S. Department of Energy
Office of Biological and Environmental Research
Subsurface Biogeochemical Research

Final Report

Mechanistic and predictive understanding of needle litter decay in semi-arid mountain ecosystems experiencing unprecedented vegetation mortality

Project Award #: DE-SC0016451
Award Amount: $200,000
Completion Date: Aug 14, 2020

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Acknowledgement: This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Subsurface Biogeochemical Research under Award Number DE-SC0016451.

Executive Summary: This research compared needle litter decay rates and underlying mechanisms between litter released from insect-killed trees to that of naturally senesced litter from healthy trees across climate variables with elevation and snowmelt rates as variables. Our research was designed to address questions of whether there is a significant impact to C and N cycling at the terrestrial-atmospheric interface in association with pine needle decomposition and the role of changes in soil moisture and temperature as a function of these processes. Field activities were primarily conducted within the Rocky Mountain Biological Laboratory's (RMBL) East River watershed across a spectrum of ecoregions that spanned approximately 700 vertical meters. Forested catchments at these elevations in southwestern Colorado are threatened by heat-induced drought, altered snowmelt patterns, and invasive insect infestation that have all been associated with climate change. Needle litter from two members of the Pinaceae family experiencing large-scale insect infestations (spruce and lodgepole pine) were collected, redeployed and then sampled over the course of three years. We experimentally tested three tractable parameters that are known to impact litter decay and biogeochemical cycling and are relevant to bark beetle-induced tree mortality: (1) moisture content, (2) temperature by using elevation as a surrogate, and (3) changes to litter compositional chemistry that result from beetle infestation. We examined decay processes and monitored the migration of soluble and gaseous nitrogen and carbon species as well as microbiological signatures of decay such as biodegradable fractions, metal availability and the temporal succession of associated microorganisms at the terrestrial interface.

Over approximately three years of field experimentation, we have gained further understanding of the processes associated with needle litter decomposition under various aspects of climate, season, and environmental perturbations from enhanced snowmelt. In our work to accelerate snowmelt, we developed an effective protocol that induces snow melt nearly three faster than ambient controls. This aligns with similar timing plots deployed about 400 vertical meters lower in elevation (Leonard et al. 2020a). Accelerated snowmelt additionally resulted in increased dissolved organic carbon (DOC) mobilization in subsurface porewater in contrast to the control, revealing implications for enhanced carbon release with increased temperatures causing early snowmelt and fewer total frost days per year. With the additional variable of conifer needle litter decomposition, we found that under the same environmental conditions tree species exerted a larger role in needle decomposition dynamics than bark beetle impact. Additionally, needle decomposition enhanced soil organic nitrogen and carbon concentrations, heterotrophic respiration, and exerted selective pressures on the resident microbial ecology. These biogeochemical signatures were enhanced during high moisture conditions, indicating the importance of moisture events on microbial processes and carbon nitrogen cycling (Leonard et al. 2020b). The comparison of these needle plots across elevations allowed for further analysis of decomposition dynamics on a broader scale. Drought conditions during the study revealed that sustained periods of dry soil moisture significantly altered the soil microbial communities as evidenced by lower CO_2 production and heterotrophic respiration across elevations. The most significant impacts to soil microorganisms and respiration were observed at the Lower Subalpine elevation, likely an at-risk ecoregion for DOC release as temperatures increase. Microbial communities exhibited resilience to this dry period by returning to a similar structure after snowmelt in the subsequent year (Leonard et al in preparation).

Introduction, Objectives, and Hypotheses: Mountainous regions across the globe are experiencing major disturbances associated with a changing and variable climate. These disturbances alter ecosystem functioning with potential effects and feedbacks to interdependent carbon and nitrogen cycling. Of particular interest and concern, insect infestations have been identified as a large-scale, climate-related disturbance that have the potential to switch the Rocky Mountain region from a carbon sink to a source through the defoliation and degradation of millions of acres of evergreen trees. While it is understood that this will lead to cascading effects in both the hydrosphere and atmosphere, current predictive models are not equipped with the information to incorporate these dynamic and comparatively sudden disturbances related to carbon decay and release.

The objective of this research was to improve fundamental understanding of how a changing climate will alter plant decomposition processes in montane and subalpine watersheds. In complement to LBNL SFA efforts, the emphasis of this work was on coniferous needle litter decay and its relationship to ongoing climate-induced tree stresses and mortality within the region. The work was guided by an overarching hypothesis that changes in litter decomposition after bark beetle attack will alter biogeochemical inputs to the atmosphere and hydrosphere. Research was further structured to address the following three research questions.

1. How does needle litter decay change as a result of coupled perturbations to: (a) needle chemistry content, (b) soil temperature and moisture levels and, (c) precipitation regimes associated with bark beetle infestation?

2. Are changes to decay rates from these processes associated with linear or non-linear correlations in the flux of N and C to the atmosphere and subsurface?

3. Can process-relevant microbial signatures, such as keystone guilds, clade ratios, functional genes, and respiratory processes correlate to litter decay and soil parameter changes?

Research Approach, Tasks and Results: Laboratory activities were primarily conducted at our home institution of Colorado School of Mines in Golden, CO with additional analytical support from Lawrence Berkeley Laboratory co-investigators in Berkeley, CA. Site specific support further came from LBNL collaborators at our site in Crested Butte, Colorado. Field deployments were conducted at the nearby RMBL field station's East River watershed of Colorado, which is within driving distance of the Colorado School of Mines campus. As bark beetles had not infested the immediate Crested Butte area at the onset of the project, spruce needles were collected from Monarch Pass, CO and lodgepole pine needles were collected from Officer's Gulch Colorado. The research design focused on the collection of needle litter from two different tree species in the region (i.e. spruce and lodgepole) followed by deployment and analysis over several years to enable a series of parallel investigations.

The field-based experiments were deployed for approximately 3 years due to the need to understand the longer-term process of needle decay in a field environment. Needle litter is substantially more recalcitrant to degradation than leafy plant matter and even more so in the arid montane and subalpine region of focus. Hence, this field incubation time was needed to ensure adequate needle decay necessary to document changes in needle chemistry, microbial shifts and associated C and N transport. Statistically significant results that differentiate between selective environmental pressures such as accelerated snowmelt and elevation also benefited from the increased timeframe of study.

Task A: Litter deployment and sampling. We tested three tractable parameters that are known to impact litter decay and biogeochemical cycling and are of relevance to large scale bark beetle induced tree mortality: (1) moisture, (2) temperature by using elevation as a surrogate, and (3) changes to litter compositional chemistry that result from beetle infestation. We selected these parameters due to their relevance to climate change and human activity as well as their intertwined relevance to microbial functionality during litter decay. Importantly, they may also contribute to feedback signals impacting climate change. Approaching this overlapping framework of parameters in parallel allowed us to address our research questions and better understand mechanisms and predict outcomes.

The experiments included litter from both Lodgepole pine and Englemann spruce. Both of these populations have been widely impacted by the Mountain Pine beetle and the Ips spruce beetle, respectively. We established our field sites and deployed harvested spruce and lodgepole needle litter within gas flux rings instrumented with lysimeters in Washington Gulch, CO in the fall of 2016. The selected field deployment entailed a matrix of 3 different elevations with an accelerated snowmelt plot contained within the middle elevation for a total of 72 different rings. Plots were established in selected meadows in upper subalpine (3,475 m), lower subalpine (3,170 m) and lower montane (2,774 m) ecoregions (**Figure 1**).

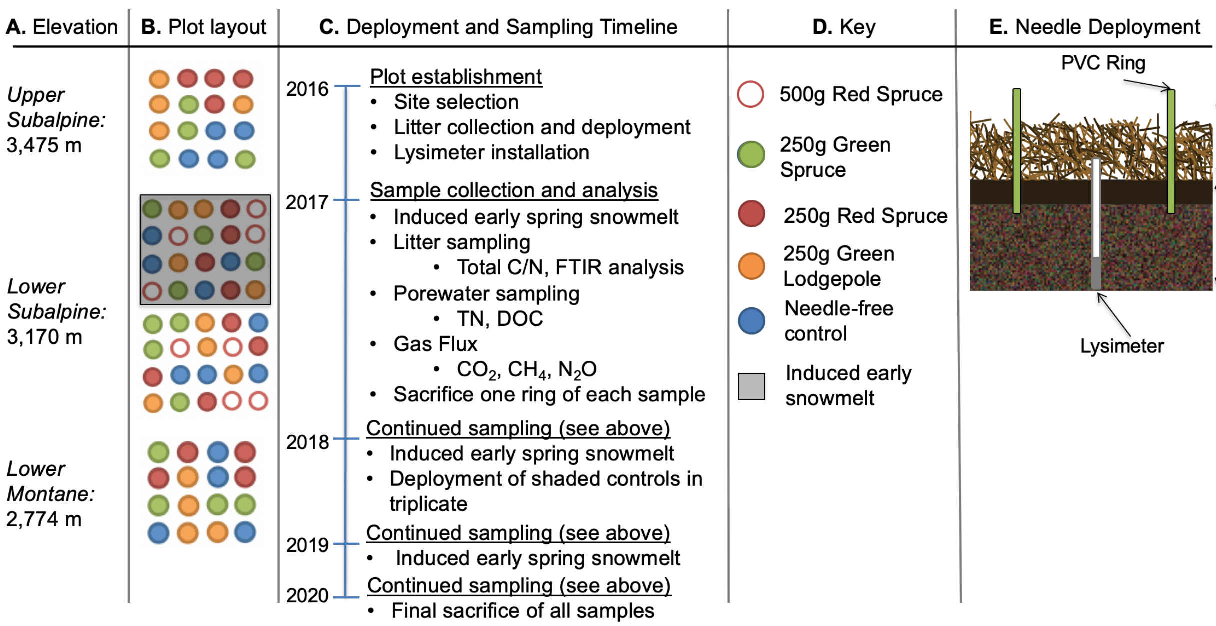

Figure 1. Research plot layout and timeline. The research plot layout at (A, B) each of the three elevations in Crested Butte, CO with (C) timeline of establishment and sampling and (E) a cross-sectional schematic of the needle deployment.

After one year, needles were removed from select rings and indicated that more time is needed to incubate our results in the field setting. In the fall of 2017, we deployed litter "bags" that contained known masses of needles to ensure more robust mass quantification of loss over subsequent years. A parallel plot in the lower subalpine plot was exposed to an accelerated snowmelt protocol in the Spring of 2017, 2018 and 2019. This protocol was published for the scientific community as a model for future work as described in Leonard et al 2020a. The accelerated snowmelt efforts during the three study years accelerated snowmelt by 14-23 days, resulting in temperature and moisture profiles that mapped more closely to the lower montane region than it did for the natural lower subalpine plot across all three of those years (Leonard et al. 2020a).

Task B: Biogeochemical processes and decay: Past studies have shown microbial respiration is sensitive to temperature fluctuations. It is anticipated that warming trends will increase carbon availability and rates of heterotrophic microbial activity, ultimately releasing more CO_2 to the atmosphere and exporting increased amounts of DOC via leaching. Less is known about how the suite of climate-associated variables identified in this proposed study will impact microbial decay of needles and the resultant response across boundaries into bulk soils and the atmosphere at our target elevation in the East River watershed. Hence, we need an improved fundamental mechanistic understanding to better extrapolate across systems, prepare for, and help predict relevant biogeochemical outcomes.

Monitoring and sample collection was confined primarily to the snow free months in the summer and fall from 2017-2019 with less frequent sampling in 2020. We also maintained a focus on capturing the snowmelt season and dynamics associated with that cycle. Aqueous samples from shoulder seasons (which was the only time when the soils were sufficiently hydrated) were analyzed for pH, C-N content and species. In addition, we collected gas flux measurements of N_2O, CH_4, and CO_2 above litter deployment at the lower subalpine and lower montane plots. Climatic measurements of precipitation, temperature and peak snow water equivalent were recorded for each plot. Litter samples were harvested temporally during the experiment for compositional shifts associated with decay with reliance on C:N composition and ratios, FTIR analysis and mass balance. Biogeochemical processes were measured as a function of our experimental variables that in addition to the elevation deployments and accelerated snowmelt, encompass needle litter depth and needle litter chemistry (via tree species and bark beetle impact).

By combining total C, N, and FTIR analyses, we determined that the deployed lodgepole needles were a higher quality substrate than the spruce, with the beetle impacted spruce needles of the lowest quality. This differentiation in litter quality was maintained throughout two years of decomposition, indicating functional differences in decomposition processes of the lodgepole and spruce needles. Specifically, nitrogen content increased in the spruce needles, but was reduced in the lodgepole needles, revealing more complex decomposition dynamics associated with nitrogen species. This was further elucidated with gas flux measurements, in which only ambient N_2O flux was observed with minimal presence of inorganic nitrogen in the soil. Organic nitrogen dominated in the soils associated with needle decomposition, a sign of enhanced microbial N accumulation with little mineralization.

Needle decomposition enhanced heterotrophic microbial respiratory activity and as a result, increased near-surface carbon cycling associated with seasonal CO_2 production and higher magnitudes of extractable DOC in the soil, with the largest CO_2 production rates associated with lodgepole needles, especially during high moisture events (**Figure 2**). C:N ratios decreased at a similar rate across the three years for all needle samples at each elevation, revealing similar processes across each location. While correlations between decomposition rates and geochemical fluxes were difficult to quantify without disturbing the long-term needle deployments, significant linear correlations were determined between CO_2 production and soil moisture at the Lower Subalpine site, and soil temperature at the Lower Montane site. This indicates the importance of soil water saturation and temperature dynamics during dry-wet cycles and how a decoupling of the two during seasonal or climatic changes could occur, with one acting as the primary selective pressure on heterotrophic activity and needle decomposition (Leonard et al, in preparation).

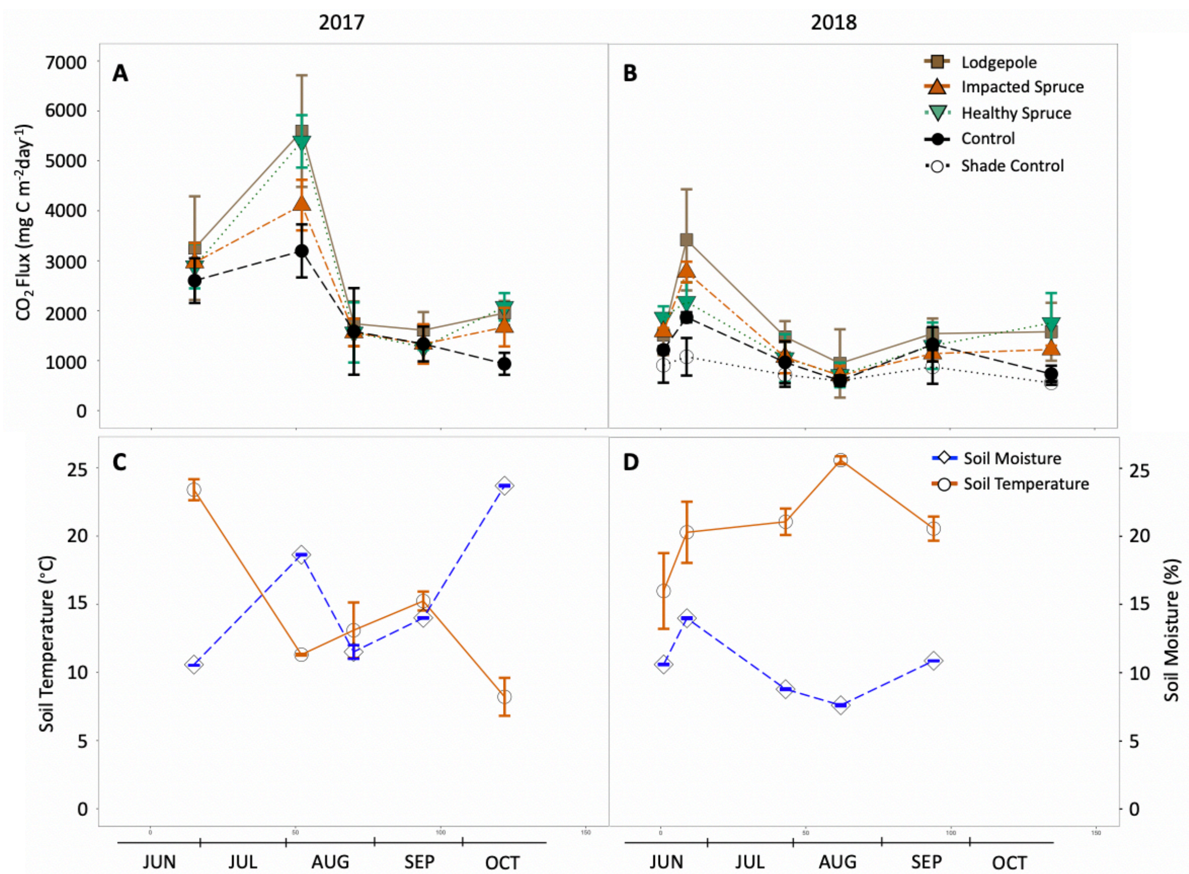

Figure 2. Gas flux as a function of season and needle decomposition at the Lower Subalpine Elevation. Measured carbon dioxide above needles (A,B) and on-site soil moisture and temperature during the sampling events of 2017 (A, C) and 2018 (B, D) (*figure reprinted from Leonard et al. 2020b*).

Task C: Diagnostic microbial signatures: The focus of this task was on microbial markers that serve as indicators of systemic biogeochemical cycling changes. Specifically, we focused our analyses on shifts over time on clades involved in litter decomposition, dominant guilds, and soil respiration rates as a proxy for microbial activity to query driving forces underlying litter decay and their interface with biogeochemical measurements and experimental variables. 16S and 18S region sequencing were used to identify bacterial and fungal phylogeny and correlate manipulated variables and outcomes to changes in potential system drivers.

Microbiological signatures associated with decay at the needle/soil horizon were collected in the snow free seasons of 2017, 2018 and 2019. Beta diversity differed as a function of needle type and not as a function of bark beetle infestation for both fungal and bacterial soil communities. Similar to the soil respiration rates, microbial activity is both temperature and moisture dependent, with soil moisture driving community differences during dry summer seasons, and soil temperature driving communities in the early spring snowmelt seasons. As a result, significant differences in community structure were observed during or after high moisture events (**Figure 3**). Communities associated with lodgepole needle decomposition were most different from those associated with the controls with higher abundance of potential nitrogen fixers and lower abundance of chemoorganotrophs.

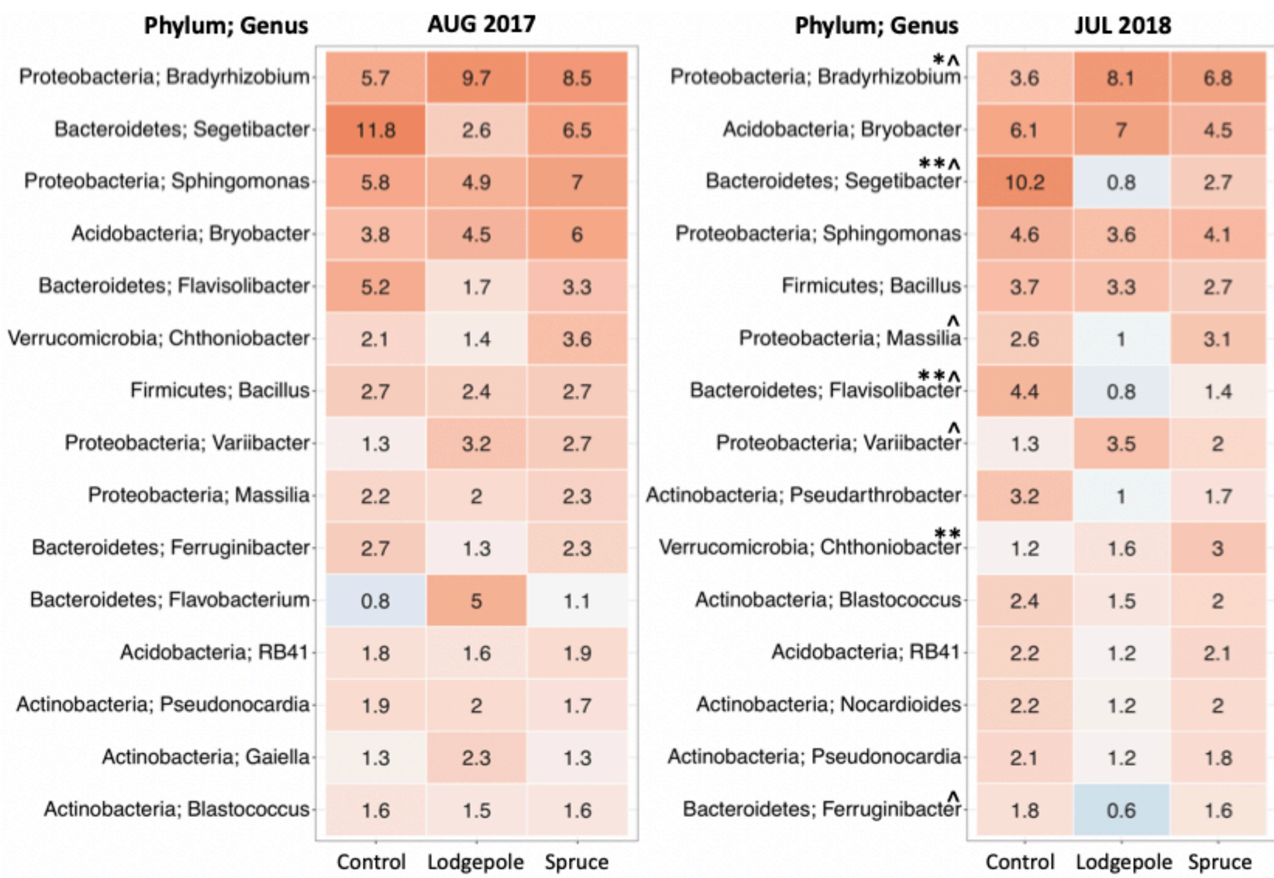

Figure 3. Bacterial genera differ as a function of sample type. A heat map representing numeric values of the percent read abundance at the Lower Subalpine elevation. Symbols indicate significant differences determined by differential abundance analysis as follows: ^Lodgepole and controls *Impacted spruce and controls, **Binned spruce and controls. (*figure reprinted from Leonard et al. 2020b*)

Conclusions: Funding was primary used to support PhD student Laura Leonard who plans to graduate with a doctorate in Environmental Engineering in 2021. Findings have formed the foundation of her dissertation with two publications in print and two more in preparation. Partial support was also provided for postdoctoral scholar Dr. Kristin Mikkelson in the early phase of work and was associated with field-based publications that enable us to contrast tree-scale processes with isolated needle litter decay to understand implications for larger watershed scale processes.

The results of this study shed light on the abiotic and biotic factors associated with temporal needle decomposition. Over the study period we have gained further understanding of the expected impacts to soil biogeochemistry during the first stages of litterfall for healthy and beetle disrupted ecosystems. In a situation where conifer needles fall to the forest floor, it is likely increased CO_2 production from heterotrophic activity will occur, while enhanced inorganic nitrogen cycling will occur depending on the C:N ratio of the litter and the state of the rhizosphere. As more noticeable biogeochemical responses were observed during high moisture events, the biogeochemical signatures from increased decomposition rates will be most pronounced in high moisture events. Finally, the expected outcomes of litter decomposition on soil biogeochemistry will differ as a function of tree species with lodgepole forest soil communities contrasting from those of a spruce forest. During bark beetle infestation, needle decomposition alone will likely exert a modest pressure on soil biogeochemistry with contrasted with relevant processes such as evapotranspiration, canopy interception, energy fluxes, and rhizodeposition.

Research further illuminated mechanisms and behavior of needle decomposition when isolated from complex processes that occur at the tree scale. This has highlighted the key aspects of the forest that influence biogeochemical cycling during healthy stages of tree life, and during periods of stress from bark beetle infestation. Additionally, the accelerated snowmelt experimentation and resulting protocol provides access to others for future work in a variety of disciplines to further understand the hydrological, ecological, and geochemical impacts of regional warming in snowpack dependent ecosystems.

Scholarly Outputs: The research has been associated with **5 publications** to date that acknowledge this funding source: DE-SC0016451. We further anticipate the submission of ~2 additional publications within the next year on topics related to 1) biogeochemical pressures on needle litter decay across elevations and snowmelt regimes and 2) ecosystem perturbation pressures on dissolved organic carbon release and subsequent disinfection byproduct formation during water treatment. The work has also been disseminated through 10+ conference abstracts and presentations at conferences including the Rocky Mountain Geobiology Symposium, Front Range Microbiome Symposium, Graduate Research and Discovery Symposium, and Watershed Science Collaboration Workshops. Journal article titles (and links where appropriate) are provided in the list below.

Publications Supported by this Grant (where Colorado School of Mines **student advisees** and ***postdocs*** are highlighted in addition to the **PI** and **collaborators**):

1. **Leonard LT**, ***Mikkelson K***, Hao Z, Brodie EL, Williams KH, **Sharp JO**. (2020b) A comparison of lodgepole and spruce needle chemistry impacts on terrestrial biogeochemical processes during isolated decomposition. *PeerJ* 8:e9538 https://doi.org/10.7717/peerj.9538
2. **Leonard LT**, Wilmer C, Steltzer H, Williams KH, **Sharp JO**. (2020a) Accelerated snowmelt protocol to simulate climate change induced impacts on snowpack dependent ecosystems. *Bio-protocol*. 10(06): e3557. *https://doi.org/10.21769/BioProtoc.3557*
3. ***Mikkelson***, **Brouillard, Bokman**, **Sharp**. (2017) Ecosystem resilience and limitations revealed by soil bacterial community dynamics in a bark beetle-impacted forest. *MBio* 8:6 e01305-17. *https://doi.org/10.1128/mBio.01305-17.*
4. **Brouillard, Bokman**, ***Mikkelson***, **Sharp**. (2017) Extent of tree mortality influences compensatory biogeochemical responses in bark beetle infested mountain pine forests. *Soil Biol Biogeochem* 114:309-18. *https://doi.org/10.1016/j.soilbio.2017.06.016*
5. ***Mikkelson KM***, **Bokman CM**, **Sharp JO** (2016) Rare taxa maintain microbial diversity and contribute to terrestrial community dynamics throughout bark beetle infestation. *Appl. Environ. Microbio*. 82(23) 6912. *https://doi.org/10.1128/AEM.02245-16*
6. **Leonard et al** ***(in preparation)*** Selective pressures on soil biogeochemistry during litter decomposition in shifting forested ecosystems.
7. **Leonard et al** ***(in preparation)*** Historic water quality and climate data as forensic tools to understand the impact of climate change on disinfection byproduct formation in drinking water.