The role of plant diversity in promoting recovery of soil microbial communities during ecosystem restoration on reclaimed mine lands

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Summary: Plant establishment is a key component of land reclamation and restoration, but ultimately full recovery of other beneficial ecosystem services that were provided prior to disturbance is desired for successful restoration. Many of these ecosystem services, primarily related to nutrient cycling and other biogeochemical processes, are regulated by microorganisms. However, the factors that promote the return of beneficial soil microorganisms – and the ecological functions they perform – to restored ecosystems are not well understood. Our previous work in the Powell River Project has discovered that the type of vegetation used to reforest reclaimed mine soils may not only control the resulting plant community, but also the trajectory of the soil microbial community and the processes they mediate related to important ecosystem services. In this work, we experimentally tested the hypothesis that carbon substrate diversity, provided by plant root exudates, is an important mechanism in shaping soil microbial communities as ecosystems recover. In a controlled greenhouse experiment, we analyzed microbial community structure and diversity of dissolved organic carbon substrates in experimental PRP soil mesocosms planted with one of five plant diversity treatments: chicory, clover, foxtail, rye, or an even mix of all four. Results indicate that plant species has significant effects on soil dissolved organic carbon substrate quantity and quality, as well as soil microbial community structure, with clover and chicory resulting in the most distinct changes. In addition, among the non-leguminous plant species, patterns of carbon substrate and microbial diversity tracked each other, suggesting an important interaction between the two.

Introduction:
Soil microorganisms mediate a multitude of ecosystem services upon which society depends. However, the factors that promote and regulate the diversity and function of the soil microbiome is a key knowledge gap that directly limits our understanding of how to restore managed ecosystems, such as reclaimed mine lands, and ensure the return of valuable services. In the Powell River Project (PRP) and other reclaimed mine soils in central Appalachia, the forest reclamation approach (FRA) can successfully promote establishment and growth of desirable mixed hardwood tree canopies, but it is also imperative to understand how the rest of the ecosystem, including other organisms and beneficial ecological processes – such as regulation of the biogeochemical and water cycles – can best be restored. Fortunately, recent technological advances in DNA sequencing (Sogin *et al.* 2006; Louzopone and Knight 2007) have made it possible to study the diversity and abundance of environmental microorganisms in unprecedented detail, allowing new research into the recovery of the soil microbiome following ecosystem restoration efforts in these systems. As a result, general soil factors such as pH (Kuramae *et al.* 2010, Nacke *et al.* 2011), nutrient pools (Banning et al., 2011; Kuramae *et al.* 2010), and plant species (Berg *et al.* 2009, Prober *et al*. 2015) have been shown to affect the development and successional patterns of soil microorganisms in ecosystems. However, additional knowledge is required to determine how to use these factors to improve soil ecosystem restoration in a predictable manner.

Since 2013 we have used an interdisciplinary approach that combines ecosystem science, biogeochemistry, and microbial ecology in the Powell River Project (PRP) investigating the impacts of ecosystem restoration using FRA practices. Through this work we have documented how most of the soil microbiome in PRP experimental plots recovers surprisingly quickly (<30 yr), with even 5 yr old plots having fairly similar soil microbiomes to unmined reference plots (Sun et al., 2017). At the same time, we observed that even after soil microbiomes have largely recovered, not all ecosystem processes have returned to pre-mining levels in experimental PRP plots (Avera et al., 2015). Finally, preliminary analyses of non-FRA plots with different types of vegetation (i.e., pines, fescue, or mixed hardwoods) indicated that soil microorganisms are distinctly different from FRA plots of any age (unpublished data). This suggests a strong role for vegetation type and diversity in restoring soil microbial communities in Appalachian hardwood ecosystems, which may have as much impact as age or type of parent material. In order to use these important interactions between restoration approach, plant diversity, and the soil microbiome to improve re-establishment of beneficial ecosystem services, additional knowledge of the mechanisms of these interactions is required.

The objective of this work was to address one fundamental knowledge gap in the recovery of these systems by investigating the role of carbon diversity supplied by plant root exudates in controlling diversity and recovery of the soil microbiome in reclaimed soils. Recent paradigm shifts in soil carbon cycling suggest that labile carbon a more important role than previously thought in soil organic matter formation (Lehmann and Kleber, 2015). Specifically, we employed a controlled greenhouse experiment with planted mesocosms containing reclaimed mine soil from the PRP to test the hypothesis that plant diversity, mediated through the variability of carbon substrates supplied by plant root exudates, is an important mechanism in plant impacts on soil microbial community structure. This mechanism is potentially even more important in nascent reclaimed mined lands where soil carbon contents are low.

Objectives

1. Quantify the degree to which vegetation diversity impacts diversity and structure and diversity of the microbiome in reclaimed PRP soils.

2. Characterize how the diversity of carbon substrates is linked to changes in the structure and diversity of the microbiome in reclaimed soils.

Methods

We initially developed the central hypothesis that vegetation type, mediated through changes in carbon substrates supplied to the soil, impacts the recovery of the soil microbiome based on results from long-term field observations. To further this understanding in the current work, we used a highly controlled manipulative greenhouse study with planted soil mesocosms containing reclaimed PRP soil. Previously collected and dried reclaimed soil from the PRP was mixed 1:1 with sand to facilitate later root removal for analysis and homogenized. A total of 72 identical soil mesocosms were constructed in 1 L plastic pots in January 2017, which were housed in greenhouse facilities at the VT Glade Road Research Center in collaboration with Jacob Barney, VT PPWS. Mesocosms were maintained with artificial growth lights and regular irrigation, but were unfertilized. All mesocosms were identical except for plant species, which were divided into one of six treatments, and which were randomized spatially on greenhouse tables to avoid location effects.

Vegetation treatments included unplanted soil controls, single species treatments including chicory (*Cichorium* spp.), clover (*Trifolium* spp.), foxtail (*Setaria* spp.), or ryegrass (*Elymus* spp.), and a high diversity treatment containing an even mix of all four plant species. Plants were started from seed (Ernst Seeds, Meadeville, PA, USA) in planting flats filled with commercial potting mix in December 2016. Once seedlings emerged and began vigorous growth, individual plants of relatively even size were selected, roots were cleaned of potting soil, and seedlings were transplanted to the experimental PRP mesocosms in January 2017. Each pot received four plants: either four of the same species for single species treatments, or one of each species for the high diversity treatment. In addition to automated irrigation, mesocosms were maintained regularly during the growth period by removing weeds and observing plant growth. In March 2017 half of the pots (six for each treatment) were sacrificed for destructive sampling. At this time, above and below-ground plant biomass was measured (dry weight) and soil samples were collected for microbial community structure and carbon substrate analysis. The experiment was ended in April 2017 when plant growth began to exceed pot capacity and the remaining six pots of each treatment were sacrificed for sampling. The same analyses were repeated except for below-ground plant biomass, which could not be reliably separated from the soil matrix for weighing at that time.

Microbial analysis. All soil samples were immediately transported back to the lab, 4 mm sieved, and preserved via freezing at -80 °C within 24 h. Total DNA was extracted from each soil sample using the PowerSoil DNA extraction kit (MoBio, Carlsbad, CA) and then quantified prior to further analysis. Bacterial diversity and taxonomy were characterized by sequencing amplified taxonomic marker genes, using the V4 hypervariable region of the 16S rRNA gene (Caporaso *et al.* 2012). Sequencing was performed at the Virginia Bioinformatics Institute using the Illumina

MiSeq platform. Sequences were quality filtered, aligned, clustered, and classified using QIIME (Caporaso *et al.* 2010) and USEARCH (Edgar, 2013). Sequences were clustered into operational taxonomic units (OTUs) at a similarity cutoff of 97% and representative sequences for each OTU were classified against the Greengenes reference database for bacteria (DeSantis et al., 2006). Diversity measures were analyzed and visualized using the R statistical software platform (www.r-project.org).

Carbon Substrate Analysis. A large aliquot of the remaining soil after microbial sampling and plant removal (~100 g) was air dried in the lab to stabilize dissolved organic carbon substrates. Water extractable dissolved organic matter (DOM) was analyzed for both total dissolved organic carbon (DOC) concentrations as well as fluorescence spectra to evaluate if different plant species, in isolation or in mixture, produce different qualities and quantities of DOM.

Results: A one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc separation, showed that chicory produced significantly higher quantities of DOC than any other individual species (Fig. 1A, $p < 0.001$). With respect to DOM quality, specific ultraviolet absorbance (SUVA) at 254 nm, has been shown to positively correlate with the aromaticity of the organic compounds (Hansen et al. 2016). Here, our results show declining aromaticity of DOM along the lines: ryegrass ≈ foxtail > clover > chicory (Fig. 1B, $p < 0.0001$). In both cases, species mixtures were intermediate in terms of concentration and aromaticity. Thus, it appears that the different plant species produce discernably different quantities and qualities of dissolved organic carbon that are then expected to manifest themselves in differences in microbial community structure and function.

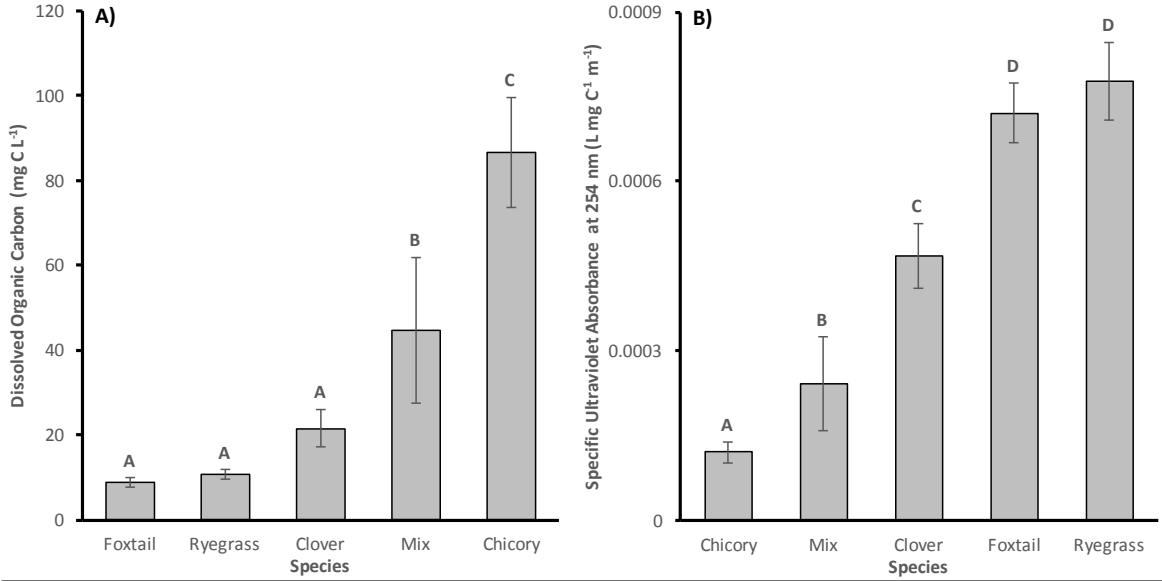

Figure 1. Dissolved organic carbon concertation (A; mg C L^{-1}) and specific ultraviolet absorbance at 254 nm (B; L mg C^{-1} m^{-1}) for the individual species and their mixture. Statistically significant differences are show with different letters using Tukey's HSD following a one-way ANOVA.

For the soil microbial communities, unfortunately an instrument delay at the sequencing center resulted in having access to microbial data only just prior to compiling this report. However, even with only preliminary analyses, some early trends are evident. For example, clover and

chicory treatments provided the strongest differences in total microbial community structure (Figure 2). Given the well-studied symbiosis between legumes and symbiotic N-fixing bacteria, it is not surprising that clover had significant effects on soil microbiome structure. The direct symbioses and nitrogen inputs were both likely mechanisms in causing this change. However, chicory is an interesting finding because among the non-leguminous treatments, it stood out as the most distinct and different, whereas the microbial communities in the foxtail, rye, and mixed treatments had more variable and indistinguishable structures. This general pattern agrees with the findings of the increase in DOC concentration and aromaticity discussed above. There is also the general finding that some plants (clover and chicory) had much stronger impacts on the microbial community by making them more similar within the same treatment than did others (foxtail, ryegrass, and soil blanks). This suggests that there is variation in the degree to which different plant types might be beneficial in shaping soil microbiomes.

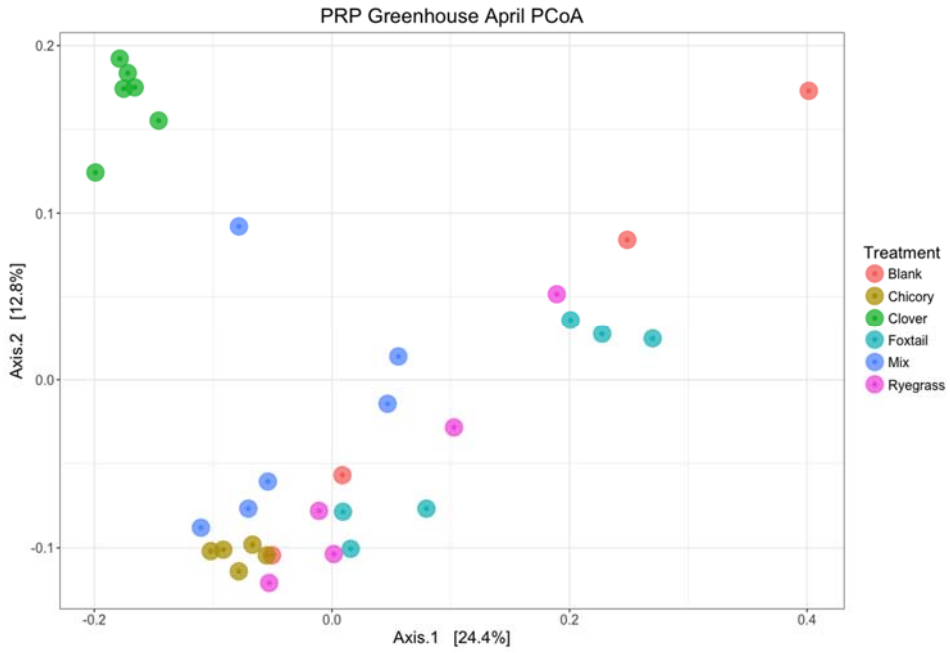

Figure 2. Principal coordinate analysis of all soil microbial community similarities among all samples collected in April at the end of the experiment. Clover (green) planted soils contained the most distinct and dissimilar microbial communities, while among the non-leguminous plants chicory (yellow) was distinct from foxtail and ryegrass and the less variable. Mixed plant samples were intermediate to all of the individual species treatments.

With regard to changes in specific microbial groups, at the phylum level (Figure 3) there are not striking differences in community composition. This is common at such a high taxonomic level, particularly in complex environments like soil, which have many factors that affect microbial growth and function. The largest effects of clover seem to be an increase in Actinobacteria and Verrucomicrobia, with a relative decrease in Firmicutes. Given that both Actniobacteria and Verrucomicrobia are often considered important microbial phyla in established soils, these findings suggest that the inclusion of leguminous plants can help establish these groups early on and are likely beneficial in reclamation approaches. Although the diversity of the soil microbiota is incredibly high and complex at lower taxonomic levels (~200 classes and ~650 families detected in these samples), looking at the mean relative abundance of only the 50 most abundant families by treatment does indicate some potentially important changes occurring in the chicory

treatment at lower taxonomic levels (Figure 4). For example, families that increased in chicory compared to foxtail and ryegrass include Alicyclobacillaceae and an unkown family from Myxococcales, which are organic heterotrophs associated with acidic environments and increased organic matter inputs, which could be responding to chicory inputs. Interestingly, Bdellovibrionaceae also increased in chicory, which includes many predators of other bacteria, suggesting a more developed microbial community in this treatment. In contrast, Geobacteraceae were reduced in chicory compared to foxtail and ryegrass, which includes slower growing anaerobic bacteria that metabolize simple carbon compounds and use iron as an electron acceptor, which agrees with lower inputs of complex organic substrate into these soils. Ongoing data analysis is continuing in attempt to tease out more direct relationships among microbial taxa and carbon substrate diversity.

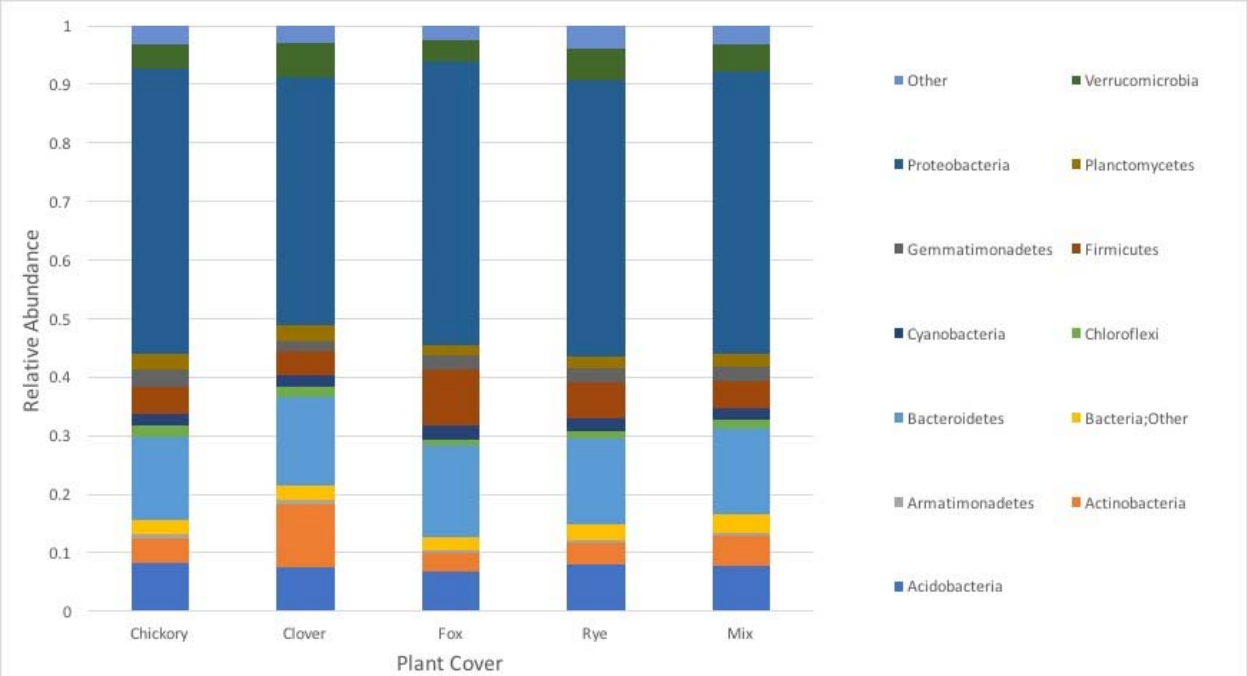

Figure 3. Relative abundances of all microbial phyla that average > 0.5% of total sequence reads averaged for each treatment. Most notable were increase Actinobacteria (orange) and Verrucomicrobia (dark green) in the clover planted soils.

Other Benefits and deliverables:

The primary objective of this work is to investigate a mechanistic linkage between plant diversity and promoting microbial recovery during ecosystem restoration. Based on these promising preliminary results, it appears that the experiment was successful in demonstrating that carbon substrate diversity can play an important role in mediating plant-microbe interactions during ecosystem restoration. Further data analysis is expected to result in significant results that will lead to at least one publication in a peer-reviewed scientific journal. In addition, coPIs Badgley and Strahm are in the process of developing proposals to two NSF programs including Environmental Sustainability (October) and Ecosystem Science (January) based on and including preliminary results from this work. Finally, this work also supported the undergraduate research of one VT Environmental Science student, Stephanie Duston, during the 2016-2017 academic year.

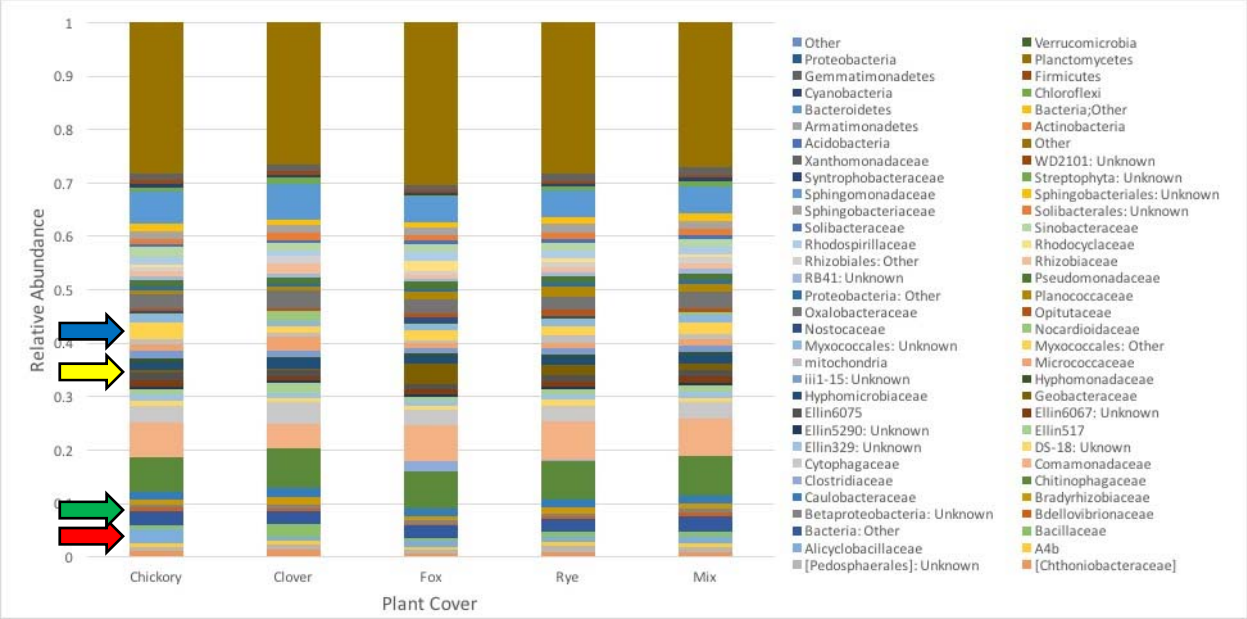

Figure 4. Relative abundances of all microbial phyla that average > 0.5% of total sequence reads averaged for each treatment. Arrows have been used to designate the families Alicyclobacillaceae (red), Myxococcales: Unknown (blue), Bdellovibrionaceae (green), and Geobacteraceae (yellow) that were discussed in the text above.

References:

Avera BN, Strahm BD, Burger JA, Zipper CE (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, 683-702.

Banning NC, Gleeson DB, Grigg AH, Grant CD, Andersen GL, Brodie EL, Murphy DV (2011) Appl Environ Microbiol 77:6158-6164

Berg G, Smalla K (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS microbiology ecology 68:1-13.

Caporaso JG, Kuczynsky J, Stormbaugh J, et al. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335-336.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2012) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108:4516-4522.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069-5072.

Edgar RC (2013) UPARSE: Highly accurate OUT sequences from microbial amplicon reads. Nature Methods 10:996-998.

Hansen AM, Kraus TEC, Pellerin BA, Fleck JA, Downing BD, Bergamaschi BA (2016) Optical properties of dissolved organic matter (DOM): effects of biological and photolytic degradation. Limnol. Oceanogr. 61:1015-1032.

Kuramae EE, Gamper HA, Yergreau E, Piceno YM, Brodie EL, DeSantis TZ, Andersen GL, van Veen JA, Kowalchuk GA (2010) Microbial secondary succession in a chronosequence of chalk grasslands. ISME 4:711-715.

Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. Nature, 528:60-68.

Louzupone CA, Knight R (2007) Global patterns in microbial diversity. PNAS 104:11436-11440.

Nacke H, Thurmer A, Wollherr A, Will C, Hodac L, Herold N, Schoning I, Schrumpf M, Daniel R (2011) Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLoS One 6:e17000.

Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS, ... Cleland EE (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology letters, 18:85-95.

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in deep sea and the underexplored "rare biosphere". PNAS 103:12115-12120.

Sun S, Li S, Avera B, Strahm BD, Badgley BD (2017). Bacterial and fungal communities show distinct succession patterns during ecosystem restoration. Applied and Environmental Microbiology 83, e00966-17.