THE EFFECT OF SOIL MICROBIOTA ON THE GROWTH OF *BOUTELOUA CURTIPENDULA*

by

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DEDICATION

This paper is dedicated to my wonderful wife, Rachel, who has been an essential partner throughout all my academic pursuits.

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ABSTRACT

Urban grassland fragments provide ecosystem benefits ranging from flood mitigation, carbon sequestration, and promotion of biodiversity. The functionality of urban grassland fragments is dependent upon a rich and diverse native plant community and the absence of exotic plants with fewer ecological connections. When mature, the native prairie grass *Bouteloua curtipendula* (Michx.) (sideoats grama) can improve the resilience and functionality of urban grasslands by serving as a green mulch, providing habitat while outcompeting seedlings of undesirable plants. Thus, strategies that promote *B. curtipendula* growth and maturation could improve urban grassland functionality. Little is known about the factors contributing *to B. curtipendula* germination and early growth, but soil microbial communities have been linked to plant germination, growth, and drought tolerance. In this study, we used sterilized soil to examine the impact of soil microbes on *B. curtipendula* growth under greenhouse conditions. *Bouteloua curtipendula* emergence was delayed in sterilized soils, with only 50% of samples emerging by two weeks post-seeding (compared to greater than 90% in non-sterile soils). Also, *B. curtipendula* grown in sterilized soil grew five fewer blades of grass on average than *B. curtipendula* grown in unsterilized soil throughout the seven-week experiment. Cumulative grass-blade length in *B. curtipendula* grown in sterile soil was less than half (10 cm) the length for plants grown in not sterile soil (25 cm), after the first four weeks after seeding. Using high throughput sequencing of the soil, we found *B. curtipendula* grown in sterilized soil also induced a greater proportion of plant pathogens and nitrifying bacteria compared to *B. curtipendula* grown in non-sterilized soil. By seven weeks after seeding, *B. curtipendula* transformed the bacterial community of sterile soil such that it was indiscernible from non-sterile soil. However, fungal communities in sterilized soil were still different from non-sterilized soil seven weeks post-seeding. Last, we found soil sterilization and growth of *B. curtipendula* changed the relative abundance of metabolic subsystem genes in the soil. Collectively, our findings demonstrate a role of soil microbes on the early growth of *B. curtipendula* under controlled conditions. In addition, we demonstrate how *B. curtipendula* growth shapes soil microbial communities. These results may be useful for developing more effective methods for urban grassland installation.

INTRODUCTION

Although classic grassland conservation efforts have focused on expansive nature preserves and relic prairies, recent studies are acknowledging the opportunity for grasslands in cities (Klaus 2013). Isolated islands of native grassland within cities, termed "pocket prairies", support insect diversity (Burghardt et al. 2009; Smith et al. 2015), increase pollinator activity (Winfree et al. 2011; Wratten et al. 2012; Fukase 2016), and increase biological control of pest organisms (Dobbs 2013; Dale et al. 2020). Urban habitat spaces dominated by regionally-native plants can also enhance the diversity and breeding success of native birds (Snyder et al. 2007; Burghardt et al. 2009; Narango et al. 2017). In contrast, non-native plant species reduce the capacity for urban plant communities to support bird and insect biodiversity (Gleditsch 2017; Mata et al. 2021). Thus, the capacity for pocket prairies to promote urban biodiversity depends upon the plant community structure.

Because plant composition is an important indicator of the function of urban pocket prairies, it is important to understand how management efforts could better support diverse, native plant communities. One approach to reducing non-desirable species infiltration to pocket prairies involves a thick planting of native grasses or sedges among forbs, which shade the soil to limit weed germination and photosynthesis of newly emerged weed seedlings. A thick planting of mature grasses and sedges also outcompete new seedlings for water and nutrients. This strategy, referred to as "matrix planting" has been successful in anecdotal reports from landscape designers (Vogt 2023). Matrix planting relies on a robust clumping or spreading groundcover to fill in the gaps between ornamental forms in a plant community. One commonly used matrix plant is *Bouteloua curtipendula* (Michx.), commonly known as sideoats grama, chosen for its bunching form, drought tolerance, easy establishment from seed, and low growth habit (Vogt

2023). A recent study found broadcast seeding of prairie mixes results in more desirable plant species and less invasive plants compared to more labor-intensive methods (such as planting plugs) (Anderson and Minor 2020). However, strategies that promote rapid and stable establishment of *B. curtipendula* and other matrix plants could therefore increase overall functionality of pocket prairies by contributing to native plant diversity and reducing invasion by exotic plant species.

Soil microbial communities are likely an important, yet understudied, aspect of urban pocket prairie functionality. Soil microbes could have intrinsic ecosystem functions which may add to the value of pocket prairies, such as carbon sequestration and reduction of pollutants. For example, soil bacteria mineralize organic nitrogen to more bioavailable forms (Stephanou et al. 2021), break down water pollutants passing through soil, and sequester carbon to lessen the impacts of atmospheric carbon dioxide on climate change (Clarholm 1985; Shah et al. 2013; Schloter et al. 2018). In fact, urban soil likely provides the same ecosystem services as non-urban soil (Baruch et al. 2021), especially in areas revegetated with locally-native plants (Mills et al. 2020; Baldi et al. 2023). These soil microbial processes promoted by native plant habitats are likely critical for urban agriculture, human quality of life, and biodiversity within cities.

Soil microbial communities may also be an important factor in determining the rate of establishment of matrix plants in pocket prairies. To successfully establish, plants need to germinate from seeds, uptake water and nutrients from the soil, gather sunlight for photosynthesis, and survive stressors which perturb these processes. Soil microbes have long been linked to germination and growth of early seedlings, with both inhibitory and stimulatory effects (Harper and Lynch 1980). Endophytic bacteria increased quinoa (*Chenopodium quinoa*) germination rates by activating immune-related signaling pathways within the seed (Pitzschke

2018). A more recent, broad-scale study used high throughput sequencing of soil microbes to investigate a link between soil microbial community and germination across several plant families. The authors found certain microbial taxa were positively correlated with germination in some plant families but negatively correlated with germination in other plant families (Eldridge et al. 2021). Specifically, germination of grasses and sedges was negatively correlated with plant pathogens and Actinobacteria, but positively correlated with Chloroflexi, Cyanobacteria, and Gemmatimonadetes (Eldridge et al. 2021). Thus, it is possible microbial community processes could promote the germination of matrix plants such as *B. curtipendula* and thereby increase the success of pocket prairie plantings.

Soil microbes are also able to promote growth of seedlings and mature plants. One mechanism of soil microbe stimulation of plant growth is through enzymes like 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which interrupts synthesis of the plant stress hormone ethylene (Glick et al. 2007). Soil bacterial metabolites also increase water availability (Joseph and Phillips 2003), which could promote plant establishment during periods of drought. Soil microbial decomposition can also promote plant growth. One study found fungal endophytic symbionts increased plant litter decomposition and subsequent nutrient availability in the soil (Lemons et al. 2005). Soil bacteria also increase nitrogen and phosphate availability for plants, which could be important for plant growth when these nutrients are limiting (Clarholm 1985; Zhang et al. 2003; Stephanou et al. 2021). The stoichiometric ratio of fungi to bacteria is also important for grassland litter decomposition, with low fungi:bacteria ratios corresponding to high nitrogen immobilization by bacteria (Hossain and Sugiyama 2020).

Further evidence for the benefits of soil microbes on prairie plant growth comes from work in remnant prairies and prairie restoration. Plant biomass increased in three herbaceous

legume perennials (*Lespedeza capitata* (Fernald), *Amorpha canescens* (Pursh), *and Dalea purpurea* (Wemple)) when inoculated with remnant prairie soil (Grman et al. 2020). In addition, remnant prairie inoculation increased the number of leguminous root nodules on *A. canescens* and *L. capitata* (but not *D. purpurea*). However, inoculation did not impact root colonization into the soil (Grman et al. 2020). These results are similar to those showing inoculation of prairie restorations with remnant prairie soil accelerated succession due to an increased abundance of late-successional plant species (Middleton and Bever 2012).

The urban setting of pocket prairies makes them unique from prairie remnants, but there is evidence microbes could help plants adapt to these novel conditions. For instance, fitness of *Brassica rapa* L. during experimental drought was largely linked to microbial communities that rapidly adapted to the new conditions (Lau and Lennon 2012). In another study, soil microbial metabolites increased rates of transpiration by triggering stomata opening, thus increasing water uptake and nutrient acquisition from roots (Joseph and Phillips 2003). Soil microbes also reduced the need for fertilizer applications in tomato (*Solanum lycopersicum* L.) (Adesemoye et al. 2009). Thus, soil microorganisms could also increase prairie plant tolerance of limited water and nutrients and in urban pocket prairies compared to remnant prairies.

Urban pocket prairies also differ from native remnant prairies in the degree of soil disturbance. In mammalian intestines, disruption of the microbial community with antibiotics increases relative abundance of opportunistic pathogens (Becattini et al. 2016). Perturbation of soil microbial communities could similarly open niches for less beneficial or even pathogenic bacteria. If so, these disturbances could impede the establishment of prairie plants and reduce the likelihood of urban pocket prairie long-term success. Indeed, soil sterilization reduced the above-

ground biomass of *Bouteloua gracilis L.* (Emam et al. 2014), but it remains unclear which microbial changes were associated with reduced *B. gracilis* growth.

Although the general role of soil microbes in plant germination, nutrient uptake, growth, and stress tolerance has been described, it remains unclear how soil microbial communities could promote the establishment of matrix plants such as *B. curtipendula* following acute soil perturbations. In this study, we investigate the role of microbial communities on the growth of the matrix plant *B. curtipendula* by comparing germination and plant growth with or without acute perturbation of the soil microbes using autoclave sterilization. In addition, we use high throughput sequencing to measure the changes to bacteria and fungi in response to soil perturbation and in the presence or absence of *B. curtipendula* under controlled conditions. These results could have important implications for the efficient establishment of grasses in urban grassland restorations.

MATERIALS AND METHODS

Plant growth measurements

Bouteloua curtipendula was grown from seed under four soil conditions: sterilized planted, not sterilized planted, sterilized not planted, and not sterilized, not planted. The conditions represented our treatments. Miracle-Gro Seed Starting Potting Mix and *B. curtipendula* seeds (obtained from Prairie Legacy; SKU BOU01) were used in a 72-cell seed tray. Sterile conditions were created by autoclaving the potting soil for two complete cycles at 132 C for 30 min. Soil samples of sterile and not sterile groups were collected at the time of planting (week 0). One seed was placed 0.25 cm in each well for each soil condition. Each condition had 6 replicates, constituting a single 72-cell seed tray. The tray containing all replicates was placed in the same bay of a controlled greenhouse environment with temperature range from 19-25º C, relative

humidity from 60-70%, light levels from 150-425 PAR (from natural sunlight supplemented with full spectrum LED lights as necessary to stay within the desired range), and twice daily watering at 0500 and 1600. Blade height and number of blades were recorded weekly for each plant in the tray for seven weeks after planting. Soil samples were collected at 0, four, and 7 weeks after planting. Collecting soil samples involved sacrificing the plant and removing those samples from subsequent plant measuring analysis. Soil pH was measured by resuspending 500 mg of the soil in 5 ml sterile distilled water and using a pH meter. All soil samples had similar pH values (between 8.1 and 8.7). Roots were separated and dried at week 7 to record dry root mass.

DNA extraction

Soil samples were frozen at -20º C, gently homogenized using a metal spatula, and soil DNA was purified from 250 mg soil using the Qiagen DNeasy PowerSoil Pro Kit according to manufacturer's protocol (ref: 47014). The extracted DNA was EtOH precipitated for additional cleanup. Samples were resuspended in 4 mM TrisHCl, pH 7.0.

Next-generation sequencing

Whole genome metagenomics sequencing libraries were prepared using the Illumina Nextera DNA Flex Library Prep kit, where DNA is tagged sample-identifying indexes and adapters for Illumina sequencing. Then, samples were sequenced by an Illumina MiniSeq using 500 µl of a 1.8 pM library with a high-output cassette.

Fungal internal transcribed spacer (ITS) libraries were prepared by using the Fungal Metagenomic Sequencing protocol from Illumina, which uses a pool of 8 forward and 7 reverse

amplicon primers that target the fungal ITS1 region between the 18S and 5.8S rRNA genes. They include the ITS1-F and ITS2 amplicon primers from Bellemain et al. (2010), that are widely used for fungal barcoding studies. Amplicon primers were synthesized by Sigma Aldrich.

Data analysis

Quality control of the raw reads was performed using FASTQC in BaseSpace (Illumina; version 1.0.0). Adapter sequences were removed and reads with low quality scores (average score < 20) were filtered out using the FASTQ Toolkit within BaseSpace (Illumina, version 2.2.0). Taxonomic classification was analyzed using Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST, version 4.0; Meyer et al. 2008). After upload to MG-RAST, data is preprocessed by using SolexaQA (Cox et al. 2010) to trim low-quality regions from FASTQ data. Potential human sequencing reads were removed using Bowtie (Langmead et al. 2009) (a fast, memory-efficient, short read aligner), and only filtered reads passed into the next stage of the annotation pipeline. MG-RAST uses DEseq for normalization.

Fungal ITS samples were all processed in BaseSpace, where primer and adapter sequences were removed and reads with low quality scores (average score < 20) were filtered out using the FASTQ Toolkit (Illumina, version 2.2.0). The Metagenomics app within BaseSpace (version 1.0.1) was used to perform a taxonomic classification using the UNITE v9 Fungal database. Default parameters were used for all software unless otherwise noted.

All statistical analyses were performed in R statistical package (R Core Team 2021). Samples were rarefied to the minimum number of sequence reads using the rrarefy function in Vegan (Oksanen 2020). Diversity analysis was performed on rarefied data using the Vegan package (Oksanen 2020). Statistical significance across multiple categories was determined by

one-way ANOVA, with a Tukey multiple comparisons post-test for intergroup differences. Nonmetric multidimensional scaling (NMDS) analysis was performed using Bray-Curtis distances (computer over 1000 iterations) and statistically compared using multivariate ordination analysis with the adonis function in Vegan (Oksanen 2020). Graphs were generated with the ggplot2 package (Wickham 2016).

RESULTS

Sterilized soil slows growth of B. curtipendula

One week after planting, *B. curtipendula* sown into sterilized soil emerged in 8.3% of samples (n=12). In contrast, one week after sowing *B. curtipendula* into non-sterilized soil, 66.6% of samples had emerged. By two weeks after seeding, we observed emergence in 50% of samples seeded into sterilized soil and 91.6% of samples seeded into non-sterilized soil. *Bouteloua curtipendula* grown in sterilized soil developed 2.5 blades of grass on average compared to the 7.5 blades of grass developed when grown in non-sterilized soil ($P<0.05$ by ANOVA, n=12 per group for weeks 1-4; n=6 per group for weeks 6-7) (Fig. 1A). During the first four weeks after planting, the cumulative blade length of *B. curtipendula* grown in sterilized soil was less than that of *B. curtipendula* grown in non-sterilized soil ($P<0.05$ by ANOVA, n=12 per group for weeks 1-4; n=6 per group for weeks 6-7) (Fig. 1B); however, by week 6 and 7, no difference in cumulative grass length between the two conditions was detected ($P>0.05$) (Fig. 1B).

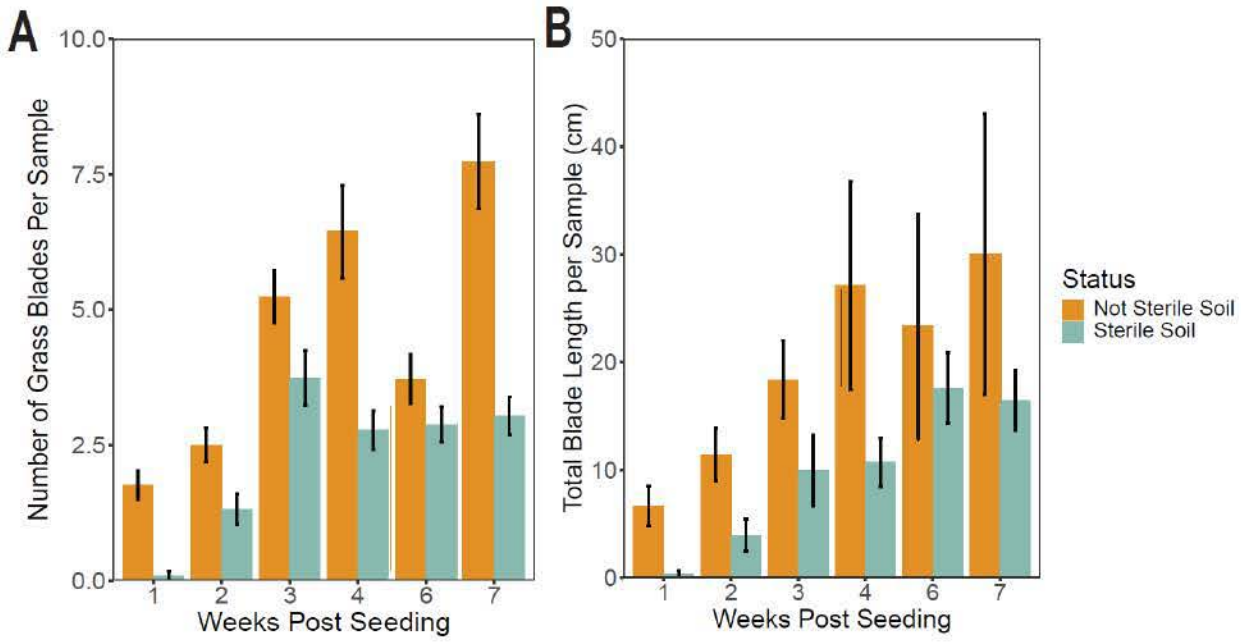

Figure 1. Acute soil perturbation impedes growth of *Bouteloua curtipendula*. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 and the number of grass blades per plug was counted (A) and the cumulative length of all the grass blades was measured (B). Bars represent means of n=12 samples per group (weeks 1-4) or n=6 samples per group (weeks 6-7) +/- SEM. Differences between means was compared using a one-way ANOVA. (A) Comparison between sterile and not sterile, $P<0.001$; comparison across weeks, $P<0.001$; interaction between sterility and weeks, $P=0.0138$. (B) Comparison between sterile and not sterile, $P=0.0006$; comparison across weeks, $P<0.001$, interaction between sterility and weeks, $P=0.866$.

Response of soil bacterial and fungal community acute perturbation and B. curtipendula growth

To study the soil microbes associated with differential *B. curtipendula* growth, we used whole genome high-throughput, short-read sequencing (HTS) and targeted amplicon HTS of fungus-specific ITS elements. To study the soil bacterial community, we used the whole genome sequencing output and selected all reads aligned to the domain Bacteria and performed NMDS

analysis at the taxonomic level of genus. Soil sterilization and planting with *B. curtipendula* both changed the overall soil bacterial community structure (Fig. 2A) (P=0.001, Adonis). In the non-sterilized samples, soil planted with *B. curtipendula* had a similar overall bacterial community to non-planted soil at both four- and seven-weeks post-seeding (Fig. 2A). However, in sterilized samples, soil planted with *B. curtipendula* had a unique bacterial community compared to unplanted soil at four weeks post-seeding. By seven weeks post-seeding into the sterilized soil, the bacterial community was similar between planted and unplanted samples (Fig. 2A) (interaction between soil sterility and time, P=0.001, Adonis; interaction between sterility and planted/not planted, P=0.011, Adonis). The bacterial community of the seed and the sterilized soil at week 0 were highly divergent from the rest of the experimental samples and thus are not represented in Fig. 2A because their inclusion in the graph precluded visualization of differences throughout the experiment. Nevertheless, these results indicate a transient disruption to the soil bacterial community resulting from planting *B. curtipendula* into sterilized soil.

In contrast to what we observed with bacteria, fungal communities in sterilized soil were similar between planted and unplanted samples at week 4 (Fig. 2B). Instead, planting *B. curtipendula* into sterilized soil induced a unique fungal community at week 7 compared to unplanted sterilized soil at the same time point (Fig. 2B). Interestingly, fungal communities in sterilized soil planted with *B. curtipendula* began to more closely resemble the seed-fungal community by week 7, suggesting seed-origin fungi were better able to colonize sterilized soil (Fig. 2B).

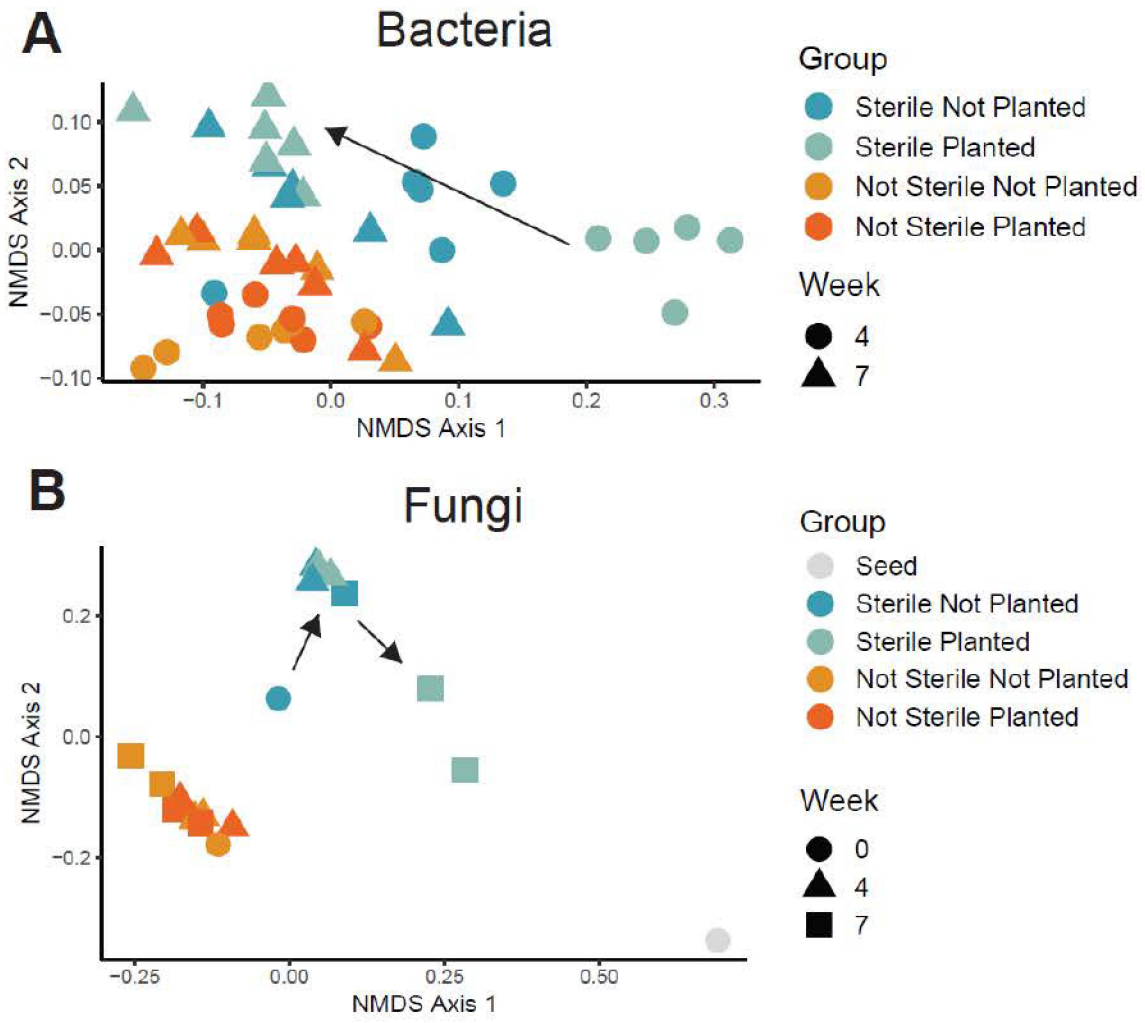

Figure 2. Soil sterilization and planting *Bouteloua curtipendula* shift the soil microbial communities. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At week 4 and seven, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). The *B. curtipendula* seed ("Seed") and soil samples immediately after autoclaving ("Week 0") were taken as a control. Each symbol represents an individual sample. Multi-ordinate variance was compared by Adonis. (A) Comparison between sterile and not sterile, P=0.001; comparison between planted and not planted, P=0.001; comparison across weeks, P=0.001; interaction between sterility and planted/not planted, P=0.011; interaction between sterility and weeks, P=0.001; interaction between weeks and planted/not planted, P=0.001; interaction between sterility, weeks, and planted/not planted, P=0.001. (B) Comparison between sterile and not sterile, P=0.001; comparison between planted and not planted, P=0.002; comparison across weeks, P=0.007; interaction between sterility and planted/not planted, P=0.201; interaction between sterility and weeks, P=0.006; interaction between weeks and planted/not planted, P=0.057; interaction between sterility, weeks, and planted/not planted, P=0.063. Arrows indicate changes from week 4 to week 7 in the sterilized soil microbial community following planting with *B. curtipendula*.

Functional diversity changes in response to soil microbiome perturbation and planting with B. curtipendula

At week 0, sterilized soil had approximately 60% less bacterial diversity and 20% less richness than non-sterile soil (Fig. 3A, 3C). Bacterial diversity and richness of the seed at week 0 was intermediate between sterile and non-sterile soil (Fig. 3A, 3C). We observed slightly more bacterial diversity and richness in sterilized soil planted with *B. curtipendula* compared to unplanted soils at four weeks post-seeding (Fig. 3A, 3C). However, bacterial richness and diversity were equal across all conditions by seven weeks post-seeding (Fig. 3A, 3C). In contrast, we did not observe a decrease in fungal diversity immediately following sterilization (week 0) (Fig. 3B). However, fungal diversity in sterilized soil at four weeks post-seeding was less than in soil that was not sterilized. Although fungal diversity of the sterilized soil increased from four weeks to seven weeks, diversity was not fully restored to levels observed in not sterilized soil. Of note, we observed more fungal diversity and richness at seven weeks in sterilized soil planted with *B. curtipendula* compared to unplanted soil.

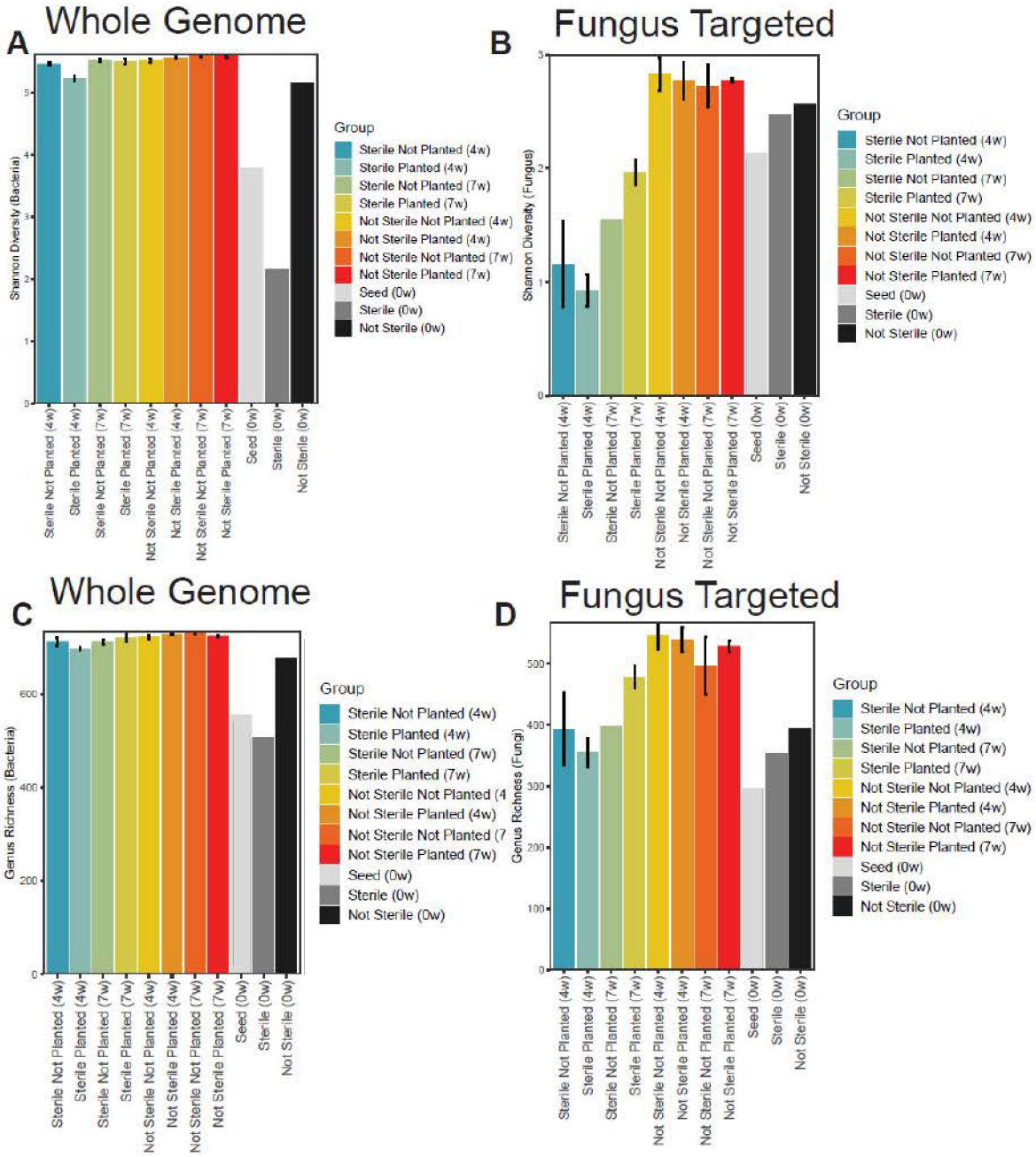

Figure 3. Soil microbial diversity and richness changes in response to soil sterilization and *B. curtipendula* growth. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At week 4 and seven, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Bars represent means +/- SEM of diversity (A, B) or richness (C, D) within whole genome sequencing (A, C) or fungus ITS-targeted sequencing (B, D), n=6 samples per group (week 0 and seed, n=1).

We next analyzed the level 1 subsystem metabolic pathway relative abundance across experimental groups. Carbohydrates, clustering-based subsystems, amino acids and derivatives, and protein metabolism pathways were the most abundant across all soil metabolic pathways, representing greater than 10% of all level 1-mapped sequencing reads. Among the sterilized soil at week 0, carbohydrates and dormancy and sporulation were overrepresented compared to other pathways. In contrast, sterilized soil at week 0 had a lower percentage of protein metabolism and respiration subsystems. Aside from the sterilized soil at week 0, the majority of metabolic pathways were similar between groups ($P>0.05$).

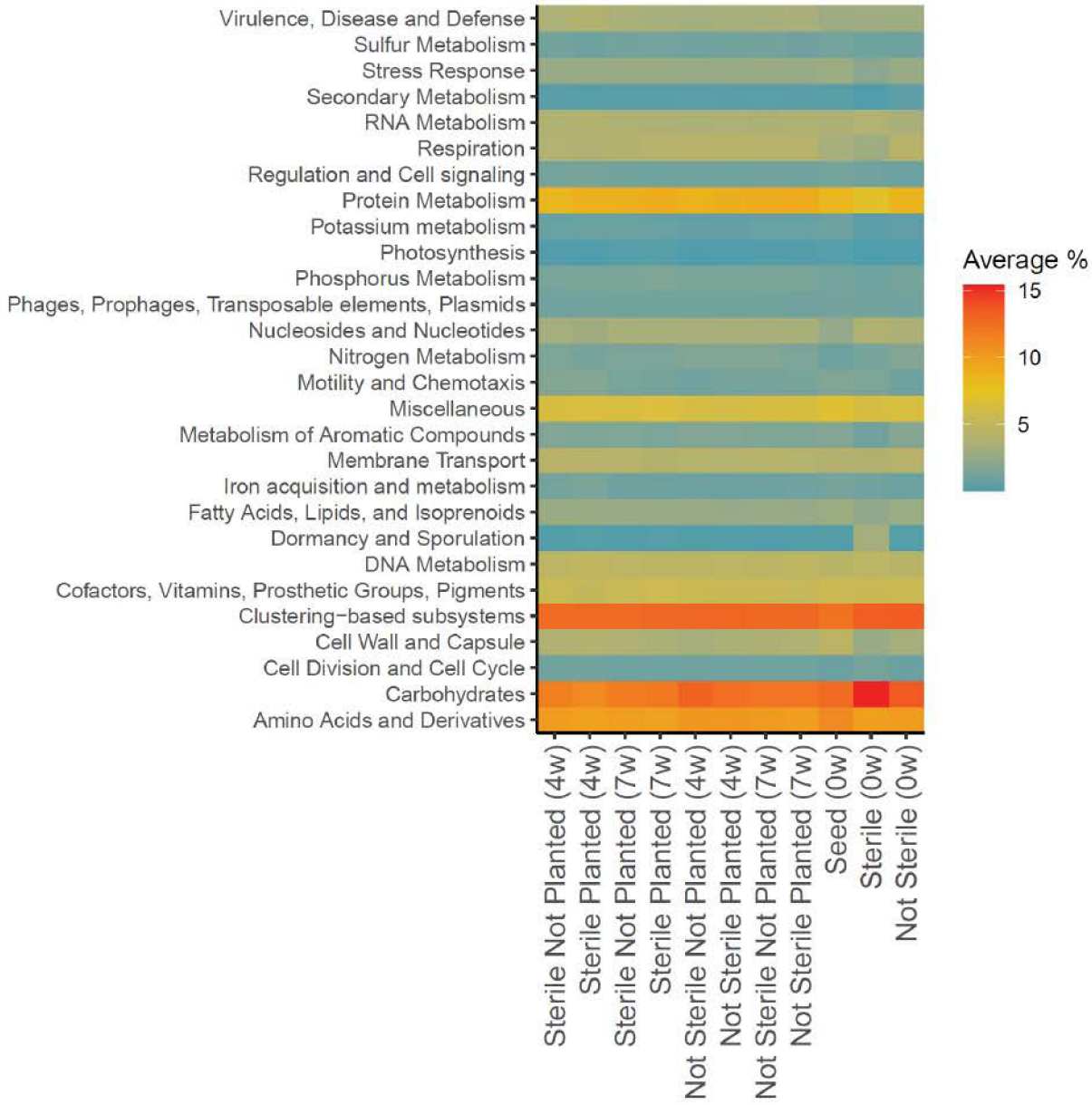

Figure 4. The impact of soil sterilization and planting with *Bouteloua curtipendula* on soil metabolic pathways. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At four and 7 weeks, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Whole-genome sequencing was mapped to metabolic systems using MG-RAST. Colors represent the average percent of each metabolic system within a group (n=6; week 0 and seed, n=1).

We next analyzed more specific metabolic pathways using the MG-RAST level 3 functional subsystems (which fall within the level 1 metabolic analysis) (Fig. 5A). Spore germination (within Dormancy and Sporulation) was overrepresented in sterilized soil at week 0 (Fig. 5B), suggesting spore-forming bacteria were the primary species to survive autoclaving. Although underrepresented in sterile soil at week 0, Ton and Tol transport systems (within Membrane Transport) and iron acquisition (in Vibrio) were overrepresented in sterile planted soil at week 4 compared to all other groups (Fig. 5D, I). Cobalt/Zinc/Cadmium resistance was also elevated in sterile planted soil at four weeks compared to other groups (Fig. 5E). In contrast, nitrogen fixation and carbon monoxide-induced hydrogenase were underrepresented in sterile planted soil at four weeks compared to other groups (Fig. 5G, H). Soil sterilization reduced the relative abundance of multidrug resistance efflux pump at week 0 from approximately 0.4% to approximately 0.2% (Fig. 5J). However, multidrug resistance efflux pump relative abundance was similar across all groups by four weeks post-seeding. By 7 weeks post-seeding, flagellum, iron acquisition, Cobalt/Zinc/Cadmium resistance, Carbon Monoxide-induced hydrogenase, and Ton and Tol transport systems were similar between sterile and not sterile soils in both planted and non-planted groups (Fig 5). However, nitrogen fixation continued to be lower in sterilized soil compared to not sterile soil at 7 weeks post-seeding (Fig. 5H).

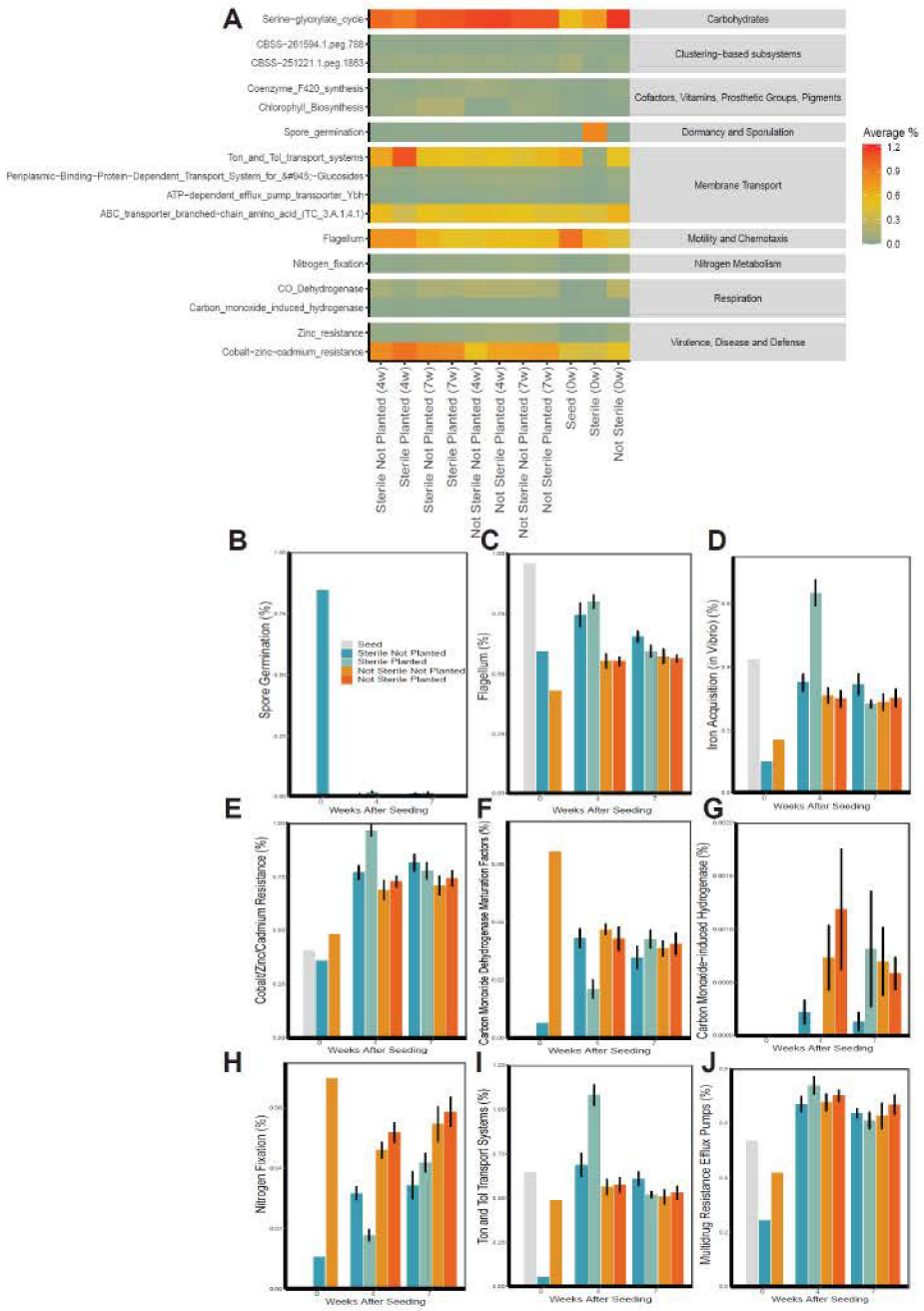

Figure 5. Soil sterilization and planting with *Bouteloua curtipendula* changed the relative abundance of soil metabolic pathway subsystems. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At four and 7 weeks, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Whole-genome sequences were mapped to level 3 functional systems in MG-RAST. (A) Select level 3 functional subsystems for each group with the indicated level 1 metabolic system (gray background, on right). (B) Mean +/- SEM of average percentage of select subsystems at weeks 0, 3, and 7 post-seeding (n=6; week 0/seed, n=1).

Impacts of soil microbiome perturbation and planting with B. curtipendula *on individual bacterial and fungal taxa*

Almost all bacteria detected in the week 0 sterilized soil belonged to the phylum Firmicutes (Fig. 6A). In contrast, not sterilized soil at week 0 was primarily composed of the phyla Proteobacteria, Planctomycetes, Bacteroidetes, and Actinobacteria (Fig. 6A, Fig. 7A, B). At four weeks post-seeding in the sterilized soil, Proteobacteria and Bacteroidetes were over-represented compared to planted not sterilized soil (Fig. 6A, Fig. 7B). The phylum Actinobacteria was less abundant in sterilized planted soil at four weeks compared to not sterilized planted soil (Fig. 6A, Fig. 7A). By 7 weeks post-seeding, the relative abundance of Bacteroidetes, Actinobacteria, and Proteobacteria were similar between all groups (Fig. 6A, Fig. 7A, B).

Among the fungi, Ascomycota, Basidiomycota, and Chytridiomycota were the most abundant in the soil (Fig. 6B). Nearly all fungi on the seed belonged to the phyla Ascomycota or Basidiomycota, with an approximate 2.8:1 ratio of Ascomycota to Basidiomycota (Fig. 6B, 7C, 7D). The fungal phylum Ascomycota was overrepresented in sterilized soil compared to not sterilized soil at both four and 7 weeks, representing greater than 75% of all fungal phyla reads (Fig. 6B, Fig. 7C). In contrast, the phylum Basidiomycota was less abundant in sterilized soils compared to unsterilized soils (Fig. 6B, Fig. 7D). Planting sterilized soil with *B. curtipendula*

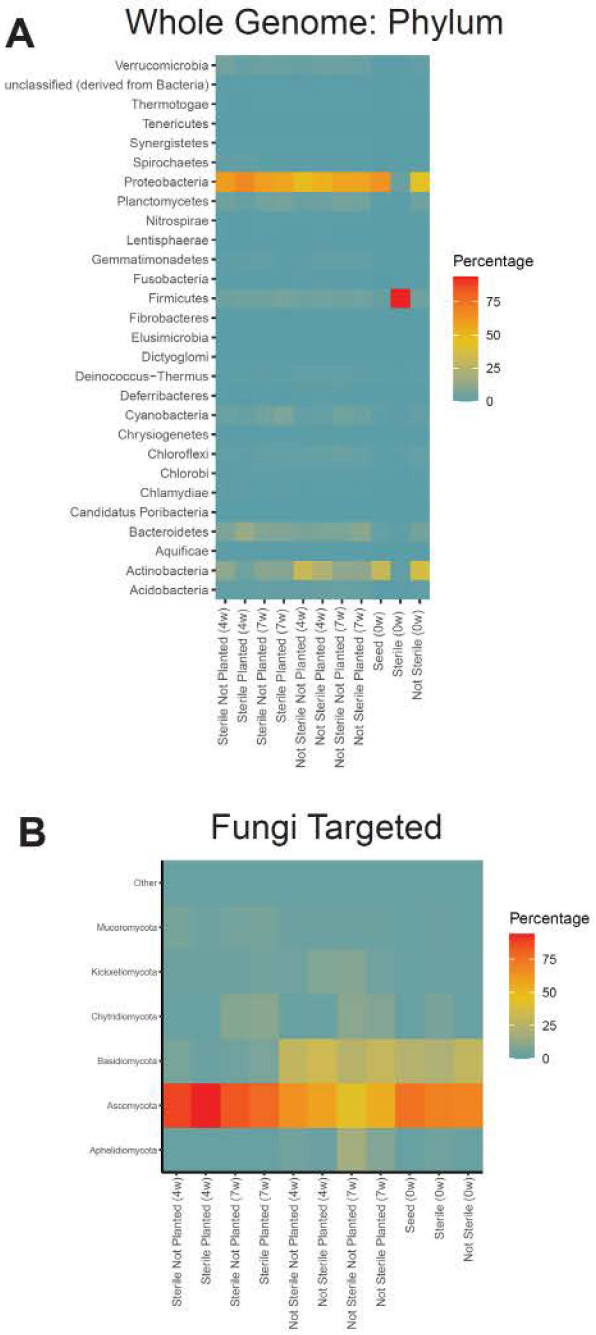

Figure 6. Soil sterilization and planting *Bouteloua curtipendula* alters the relative abundance of soil bacterial and fungal phyla. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At four and 7 weeks, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Whole-genome sequences (A) or fungus ITS targeted sequences (B) of phyla representing greater than the threshold of 2% of the total rarefied reads are represented. Phyla below the threshold were pooled into "other." Colors indicate the average percentage each phylum makes up within a group (n=6; week 0/seed, n=1).

caused a slight increase in the relative abundance of Ascomycota and a slight decrease in the relative abundance of Basidiomycota at four weeks. By 7 weeks, slightly fewer Ascomycota and slightly more Basidiomycota were present in planted soil. Interestingly, planted soil at week 4 had an approximate Ascomycota to Basidiomycota ratio of 30:1. By week 7, the Ascomycota to Basidiomycota ratio decreased to approximately 15.4:1, closer to the seed ratio of 2.8:1. These findings are consistent with the NMDS analysis showing the soil fungal community of sterilized soil planted with *B. curtipendula* resembles the seed more closely at 7 weeks post-seeding compared to four weeks post-seeding.

For Bacteria, at the level of genus, the sterilized soil at week 0 was almost entirely comprised of *Anoxybacillus*, *Bacillus*, *Geobacillus*, and *Paenibacillus*. These genera are all known to form heat-resistant spores and therefore not surprising to be found after sterilization. In contrast, the dominant genera in the not sterile soil at week 0 were *Mycobacterium*, *Streptomyces*, *Rhodopseudomonas*, *Salinispora*, *Mesorhizobium*, and *Bradyrhizobium*.

Soil sterilization affected the relative abundance of several bacterial genera at four weeks post-seeding. The genus *Caulobacter*, which is often associated with plants (Berrios 2022), was only induced by *B. curtipendula* seeded into sterile soil (Fig. 9B). In addition, the plant pathogens Acidovorax, *Cellvibrio*, *Pseudomonas*, and *Xanthomonas* were all overrepresented in sterilized soils planted with *B. curtipendula* (Fig. 9A-D). In contrast, *Rhodopseudomonas*, which is often categorized as a plant growth-promoting bacteria, was significantly reduced in the sterilized soils planted with *B. curtipendula* compared to the not sterilized planted soils (Fig. 9E). However, by week 7, *Rhodopseudomonas* relative abundance greatly increased the sterilized soils planted with *B. curtipendula* and *Rhodopseudomonas* was similarly abundant across all

groups. Likewise, the potential plant pathogens *Acidovorax*, *Cellvibrio*, and *Xanthomonas* were no longer elevated in the sterilized soils by week 7 (Fig. 9A-D).

Cercophora was the predominant fungal genus in the sterilized soil at week 0 (Fig. 8B, Fig. 9G), while *Mycothermus* was the predominant fungal genus in the not sterilized soil at week 0 (Fig. 8B, Fig. 9I). *Epicoccum* and *Alternaria* were the most abundant fungal genera on the seed at week 0 (Fig. 8B, Fig. 9F,H). *Cercophora* continued to be the dominant fungal genus in the sterilized soil at four weeks in both the planted and unplanted conditions (Fig. 8B, Fig. 9G). By 7 weeks in sterilized planted soils, a decrease in *Cercophora* relative abundance coincided with an increase in *Epicoccum, Alternaria,* and *Immersiella*. In the sterilized soils not planted with *B. curtipendula*, Cercophora continued to be highly abundant at 7 weeks, with no apparent increase in *Epicoccum*, *Alternaria*, or *Immersiella*. Interestingly, *Cercophora*, *Immersiella*, and *Epicoccum* remained low in abundance in the not sterile soils throughout the experiment (Fig. 8B, Fig. 9G, H). Instead, *Mycothermus* remained the predominant fungal genus in the not sterile soil through week 7 (Fig. 8B, Fig. 9I). However, while *Mycothermus* decreased in relative abundance over time in the not planted soil, the genus increased in relative abundance from four weeks to 7 weeks in planted soil (Fig. 9I). The observation that seed-associated fungi (Alternaria and Epicoccum) are good at colonizing the sterile soil, but not the not sterile soil is consistent with the earlier NMDS overall community observations.

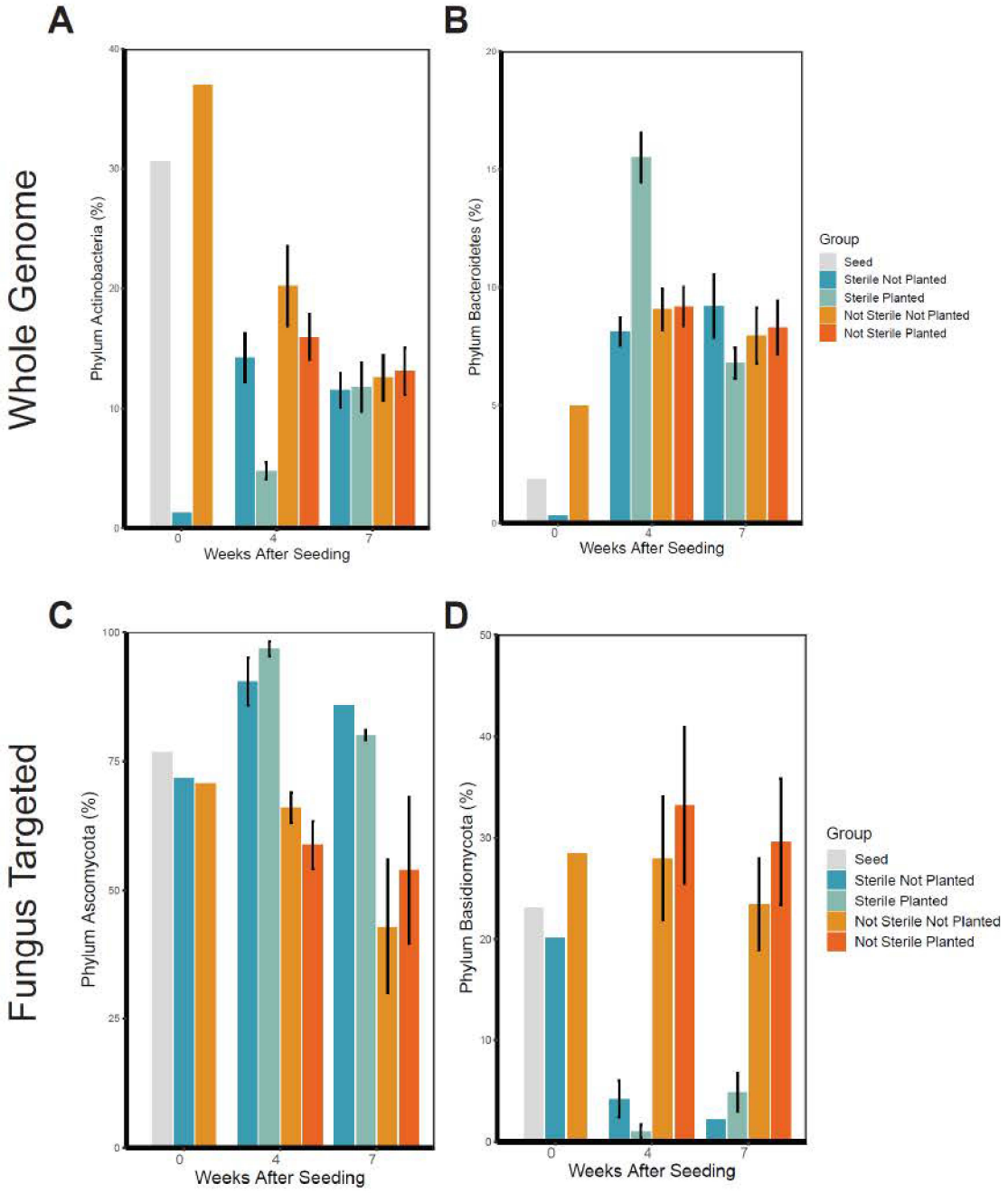

Figure 7. Soil sterilization and planting with *Bouteloua curtipendula* changes the relative abundance of bacterial phyla Actinobacteria and Bacteroidetes and fungal phyla Ascomycota and Basidiomycota. bacterial and fungal phyla. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At four and 7 weeks, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Bars represent mean +/- SEM of bacterial phyla (A-B) Actinobacteria (A) and Bacteroidetes (B) or fungal phyla (C-D) Ascomycota (C) and (Basidiomycota) (D) (n=6; week 0/seed, n=1).

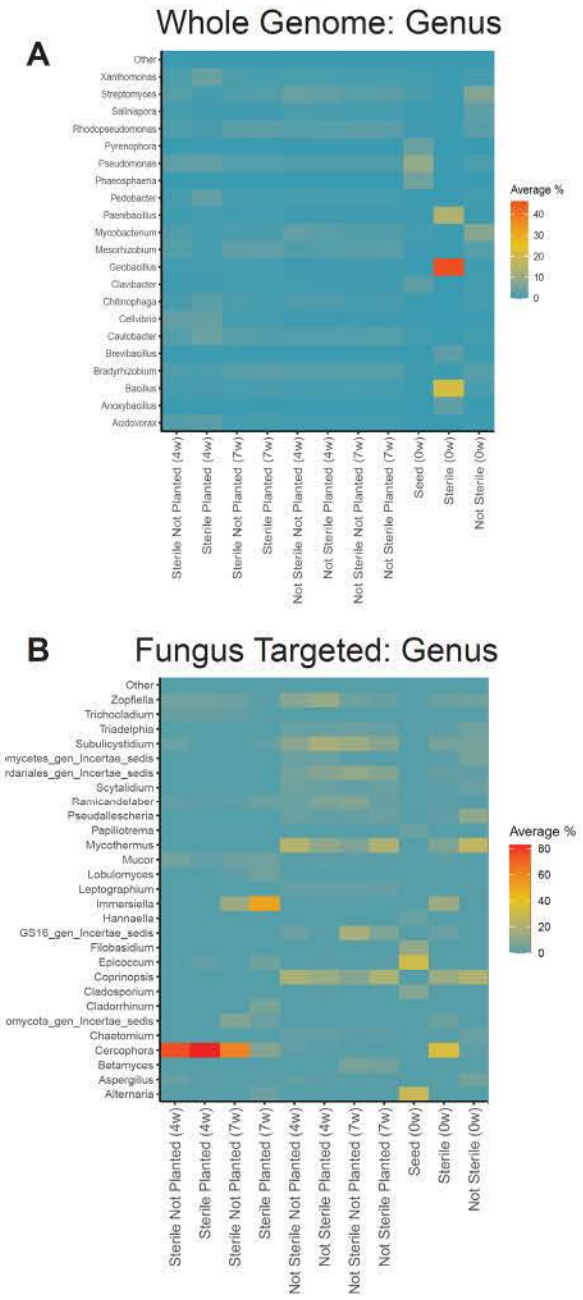

Figure 8. Soil sterilization and planting with *Bouteloua curtipendula* alters the relative abundance of soil bacterial and fungal genera. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At four and 7 weeks, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Whole-genome sequences (A) or fungus ITS targeted sequences (B) of genera representing greater than the threshold of 2% of the total rarefied reads are represented. Phyla below the threshold were pooled into "other." Colors indicate the average percentage each genus makes up within a group (n=6; week 0/seed, n=1).

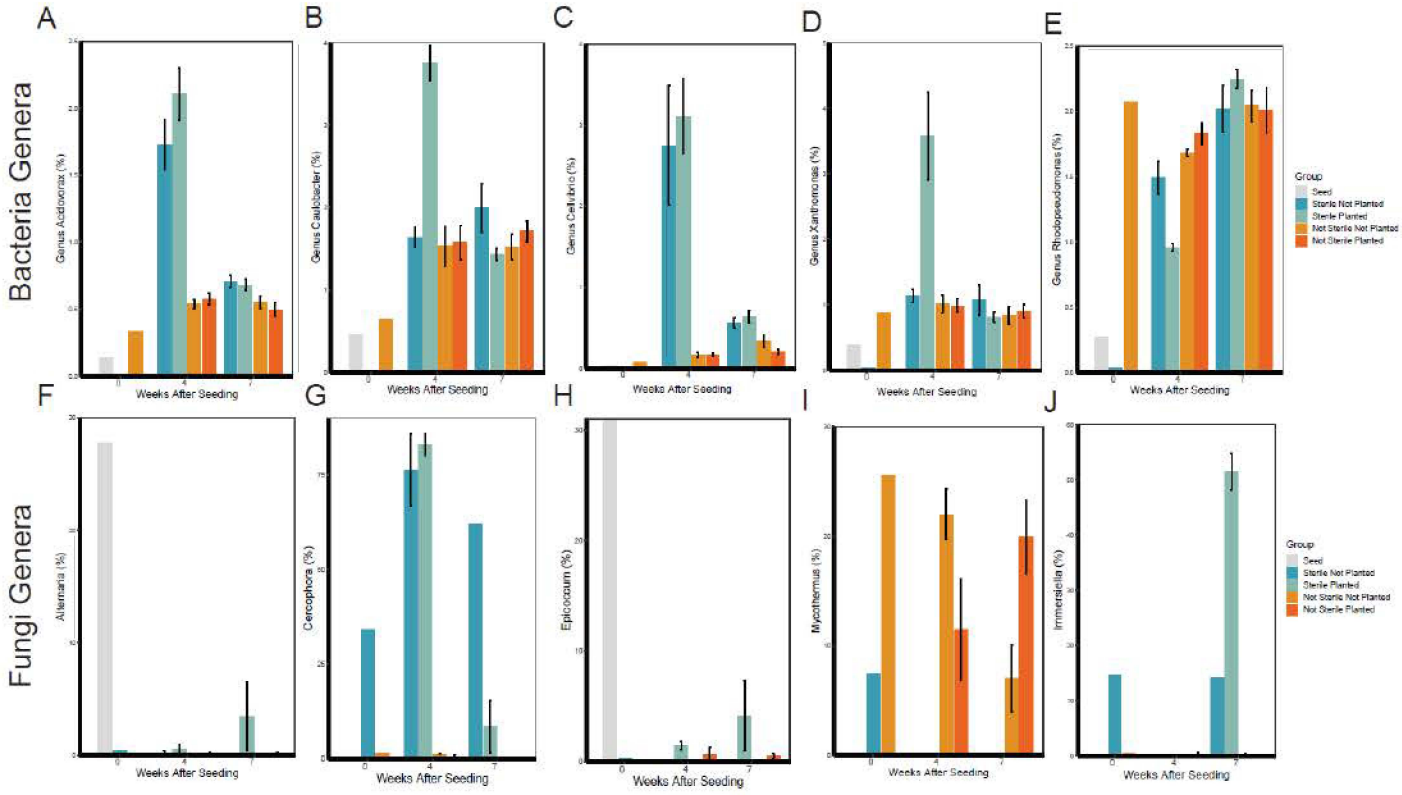

Figure 9. Soil sterilization and planting with *Bouteloua curtipendula* alters the relative abundance of specific bacterial and fungal genera. Potting soil was sterilized via autoclaving (as described in the materials and methods, "Sterile") or left unsterilized ("Not Sterile") and then added to 72 plug trays. *B. curtipendula* was seeded at week 0 ("Planted") or left unseeded as a control ("Not Planted"). At four and 7 weeks, soil was collected for DNA purification and illumine sequencing (as described in the materials and methods). Bars represent mean +/- SEM of bacterial (A-E) or fungal (F-J) genera (n=6; week 0/seed, n=1).

DISCUSSION

In this study, we show how transient perturbation of the soil microbial community through autoclave sterilization impaired the growth of the prairie grass *B. curtipendula*. *Bouteloua curtipendula* seeded into sterilized soil had fewer grass blades and shorter blade length throughout the course of a 7-week controlled experiment, but the difference was most notable in the first four weeks post-seeding. Although sterilization was transient, the soil microbial community did not immediately return to a pre-sterilization state. Instead, planting *B. curtipendula* seed into the sterilized soil caused a strong bacterial community divergence at four weeks that eventually stabilized by 7 weeks post-seeding (Fig. 2A). In contrast, the fungal community divergence in the sterilized soil planted with *B. curtipendula* was slower, not being detectable until 7 weeks post-seeding (Fig. 2B). *B. curtipendula* seeded into sterilized soil also induced more of the potentially pathogenic bacterial genera *Acidovorax*, *Cellvibrio*, and *Xanthomonas* and less of the plant growth promoting *Rhodopseudomonas* at four weeks post-seeding (Fig. 9A-E). This overabundance of bacterial plant pathogens subsided at 7 weeks post-seeding into sterilized soil, giving way to *B. curtipendula*-induced fungal genera such as *Alternaria*, *Epiccocum*, and *Immersiella* (Fig. 9F, 9H, 9I).

Although two rounds of autoclaving did not completely sterilize the soil, it did greatly reduce bacterial richness and diversity (Fig. 5A, C). The remaining bacterial taxa identified through high throughput sequencing were spore forming bacilli (Fig. 8A). Also, the dominant functional subsystem in sterilized soil at time 0 was spore germination (Fig. 4A, B). The genetic data are consistent with the morphology viable bacteria that grew on nutrient agar plates following our sterilization protocols (data not shown). Fungal richness and diversity were only moderately reduced immediately following soil sterilization (Fig. 5B, D). However, the low

diversity and richness of fungi genera at four weeks post-seeding suggests the sequences detected immediately after sterilization arose from non-viable fungi. Indeed, we did not observe significant fungal growth on nutrient agar plates of sterilized soil even after one week of culturing at 20º C (data not shown). Thus, the sterilization could be considered a significant disruption to the soil microbial communities.

Our results demonstrated soil microbial communities were highly dynamic and influenced by *B. curtipendula* following acute destabilization. While the overall bacterial community in sterilized soil was distinct from that of not sterilized soil at four weeks (Fig. 2A), this difference was exacerbated when sterilized soils were planted by *B. curtipendula*. In sterilized soils, *B. curtipendula* decreased the relative abundance of the phylum Actinobacteria and increased the relative abundance of the phylum Bacteroidetes at four weeks post-seeding. In not sterilized soils, the bacterial community was stable and not significantly affected by seeding *B. curtipendula*.

We also show how *B. curtipendula* germinates and establishes more slowly in soils with an acutely disrupted microbiome compared to unperturbed soils (Fig. 1). It is interesting to note how soil sterilization increased the relative abundance of potential plant pathogen bacteria, such as Acidovorax (Li et al. 2011; Burdman and Walcott 2012; Ahmed et al. 2021), Cellvibrio (DeBoy et al. 2008; Gardner and Keating 2012), and Xanthomonas (White et al. 2009; Sujan et al. 2020) at four weeks post-seeding. By 7 weeks post-seeding, the potentially pathogenic bacteria stabilized and returned to levels comparable to that of the not sterilized soil seeded with *B. curtipendula*. These data are consistent with our observation of impaired *B. curtipendula* growth in sterilized soil, especially during the first four weeks post-seeding.

Acute disruption of gut microbiota with antibiotics is known to increase the relative abundance of human pathogens (Becattini et al. 2016). Our findings suggest acute disruption of the soil microbiota could also promote plant pathogens that impede growth of plants, such as the prairie grass *B. curtipendula*. It is interesting that the bacterial communities restabilized by 7 weeks post-seeding and *B. curtipendula* growth in sterilized soil was less deficient at these later time points. It is possible that soil texture, moisture, pH, and organic mineral content (which should be similar between sterilized and not sterilized soil) is the major determinant of long-term soil microbial community, and acute disturbance will always be a transient disruption before returning to the long-term stable community. It is also possible that the fungal species added in stabilization of the bacterial community and the reduction of potentially pathogenic bacterial taxa by 7 weeks. Indeed, we find the *B. curtipendula*-induced fungal community took 7 weeks to establish in sterilized soil (Fig. 2B). Interestingly, one of the fungal genera that increased in *B. curtipendula*-planted sterilized soil by 7 weeks includes *Epicoccum*, which was also highly abundant on the *B. curtipendula* seed (Fig. 9H). Thus, seed-associated fungi could be responsible for reducing the plant pathogens in the soil and thus promoting *B. curtipendula* growth. Indeed, *Epicoccum sp.* associated with other plants produce antimicrobial compounds against phytopathogens (Talontsi et al. 2013). In contrast, the other seed-associated fungi induced by 7 weeks, *Alternaria*, is most commonly associated as a fungal plant pathogen itself (Cho and Yu 2000; Thomma 2003). Future studies will be necessary to determine whether seed-associated fungi promote *B. curtipendula* growth through antimicrobial activity against plant pathogens in the soil. Nevertheless, our results show the soil microbial dynamics associated with inhibition of prairie grass growth following acute soil sterilization.

Our study is consistent with previous studies showing positive interactions between plants and the soil microbial community. Much of this work involves a group of bacteria categorized as plant growth promoting bacteria (PGPB) and fungi (PGPF) (Olanrewaju et al. 2017). Microbes known to be PGPB and PGPF can release secondary metabolites that suppress plant pathogenic bacteria and fungi in the soil, thus promoting plant growth (Narayanan and Glick 2022). Such is the case for the fungus *Mycothermus*, which showed a strong positive correlation with lisianthus (*Eustoma sp.*) growth due to suppression of plant disease (Liu et al. 2021). PGPB can also promote nutrient uptake and increase fertilizer efficiency (Adesemoye et al. 2009). One specific bacteria genus associated with plant growth is *Rhodospseudomonas*. When inoculated into sterilized soil, *Rhodopseudomonas* enhanced germination of tomato plants (Koh and Song 2007). *Rhodopseudomonas* incoculation also promoted growth of Stevia and Brassica plants (Wong et al. 2014; Xu et al. 2018). We found significantly less *Rhodopseudomonas* in sterilized soil planted with *B. curtipendula* compared to not sterilized soil planted with *B. curtipendula* at four weeks post seeding (Fig. 9). In addition, we found sterilization essentially eliminated *Mycothermus* from the soils throughout the course of our experiment (Fig. 9). The reduction of *Rhodopseudomonas* and *Mycothermus* in sterilized soil is associated with increased pathogenic bacterial and fungal taxa (Fig. 9) and impaired *B. curtipendula* growth (Fig. 1). Thus, our results suggest that a diverse and unperturbed microbial community could support prairie grass growth through multiple mechanisms: directly promoting plant growth and reducing the abundance of potential plant pathogens.

Ultimately, our results suggest that functional and diverse microbial soil communities could aid in the establishment of prairie grasses and subsequently increase the success of urban prairie plantings. Native plant species promote broad ecological functions in habitat areas and

these functions are reduced by invasive species. However, microbial communities associated with invasive species can favor invasive grasses and impede native grasses (Batten et al. 2008). Another study found soil sterilization increased the success of invasive *Bromus tectorum* infiltrating into *Bouteloua gracilis* (a plant closely related to *B. curtipendula* used in our study) (Emam et al. 2014). These findings combined with the results of our study emphasize the importance of soil microbial communities in promoting highly functional native plant communities. Thus, efforts to promote native habitat spaces should consider approaches which include native-plant promoting soil microbial communities to ensure the greatest habitat functionality.

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