environmental microbiology

Environmental Microbiology (2022) **24**(9), 4001–4012 doi:10.1111/1462-2920.15970

Under the canopy: disentangling the role of stemflow in shaping spatial patterns of soil microbial community structure underneath trees

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Summary

Stemflow is a spatially concentrated input of rainwater at the base of trees, resulting from precipitation draining down tree branches to the stem. Depending on tree shape, stemflow can represent a significant fraction of the total rainfall that contacts the tree's canopy area, and can become chemically enriched along its drainage path. As a result, stemflow has been hypothesized to influence microbial communities in the receiving soil proximal to the stem. However, previous studies have (i) yielded conflicting results on the significance of stemflow as a driver in bacterial community composition, and (ii) not directly compared communities in soils with and without stemflow receipt. In this study, a stemflow diversion system was employed on *Quercus virginiana* trees in Skidaway Island (Georgia, USA) to directly compare soil bacterial communities receiving no stemflow to those beneath trees with no diversion system in place. In both treatments, sample distance from the stem significantly influenced bacterial community structure. However, the absence of stemflow resulted in increased bacterial community diversity across all samples. Stemflow diversion also significantly altered longitudinal patterns in the abundance of multiple taxonomic groups. These results support the hypothesis that *Q. virginiana* stemflow has a significant impact on bacterial soil inhabitants and is a key factor in taxon selection in stem-proximal communities.

Received 9 July, 2021; revised 3 March, 2022; accepted 3 March, 2022. *For correspondence. E-mail professor.vanstan@gmail.com.

Introduction

Above-ground plant surfaces significantly alter the amount and pattern of mass and energy inputs to land surfaces (Bonan and Doney, 2018). One such alteration occurs during rain events, as plant canopies intercept and redistribute rainfall into highly spatially heterogeneous patterns of throughfall and stemflow – two 'hydrologic highways' that transport materials from the atmosphere and canopy to the surface (Van Stan *et al*., 2021). Throughfall is the portion of rainwater that drips from the canopy or passes through gaps, while that which drains from outlying crown areas to the stem base is stemflow. Globally, most trees' mean annual stemflow represents <2% of rainfall (Van Stan and Gordon, 2018); however, even a small amount of rainfall (e.g. ~0.1 cm) over a single tree's canopy (e.g. ~500,000 cm^2) can result in a large volume (50 L) concentrated around the stem base (e.g. ~0.1–10 m^2) in a single storm (Van Stan and Allen, 2020). Because stemflow drains down substantial bark surface area, it can also become significantly chemically enriched from materials on and within the bark (Van Stan and Gordon, 2018). Thus, for over a century [since Riegler, 1881] the stemflow process has been reported to be able to magnify small fractions of rainwater across a tree canopy by 10–100 times at the surface surrounding tree stems (Herwitz, 1986; Friesen and Van Stan, 2019).

This voluminous and chemically enriched stemflow has been hypothesized to influence soil physicochemical properties and bacterial populations for decades (Bollen *et al*., 1968; Tarrant *et al*., 1968). To date, investigation of this hypothesis with regards to soil microbial communities has been limited to comparative observations of near-stem soil areas, where stemflow was assumed or

observed to infiltrate, (i) between species of differing stemflow generation (Nacke *et al*., 2016; Rosier *et al*., 2016), (ii) with distance from the stem (Bollen *et al*., 1968; Nacke *et al*., 2016), or (iii) with microbial communities from the bark surfaces that stemflow must flow over before reaching the soil (Ceccherini *et al*., 2008). Results from these studies in unmanipulated systems are contradictory, with some suggesting stemflow may influence soil microbial community structure (Ceccherini *et al*., 2008; Rosier *et al*., 2016); while others found no evidence of this (Bollen *et al*., 1968; Nacke *et al*., 2016). The only study to date to use high-throughput sequencing technology (Nacke *et al*., 2016) found no detectable influence of distance from stem on fungal or bacterial community structure in soils (as close as 50-cm) near the stem base of a voluminous stemflow-generating species, *Fagus sylvatica*, suggesting a limited importance of stemflow in driving microbial community structure under this tree. Interestingly, Nacke *et al*. (2016) did observe a detectable change in soil bacterial community in near-stem soils for *Picea abies*, a species whose stemflow is reported to represent 0.4% (Delfs, 1967) and, sometimes, 'negligible' amounts of rainfall across its canopy (Jost *et al*., 2004).

These inconclusive comparative observations are not surprising when one considers that many other factors besides stemflow will alter near-stem soil physiochemistry and soil microbial communities – something noted and discussed by Nacke *et al*. (2016). For example, fine root biomass can vary significantly near stems (Petritan *et al*., 2011) which can, in turn, influence soil compaction and hydrophobicity patterns (Clemente *et al*., 2005). pH has also been suggested to significantly influence soil community composition under tree canopies (Nacke *et al*., 2016; Dukunde *et al*., 2019; Habiyaremye *et al*., 2020), although change in soil pH from stemflow has not yet been shown to impact microbial communities (Nacke *et al*., 2016; Rosier *et al*., 2016). Under these circumstances, manipulation experiments have become a touchstone method for hypothesis testing in ecology – particularly in testing precipitation impacts on ecosystems (Beier *et al*., 2012). This study describes results of a manipulation experiment in which we test the null hypothesis that redirection of stemflow away from the tree will not affect soil bacterial community composition in close proximity to the tree. Rejection of this null hypothesis indicates that mechanisms exist for stemflow to influence soil bacterial communities, meriting further research on this topic.

Results

The study year (2017) resulted in an average amount of rainfall for the study site: 1165.6 mm, compared to the range in 30-year mean annual rainfall, 750–1200 mm y^{-1}. Estimated stemflow was extrapolated from eight oak trees in the nearby (approximately 3.4 km south) Skidaway Island State Park, GA, USA (Fig. S1). This was done because (i) stemflow volumes from the control trees could not be collected or diverted without preventing stemflow from entering the soil, (ii) installing large water collection bins beside the manipulated trees to store redirected stemflow for measurement would cover, compact and otherwise disturb the near-stem soils and (iii) installing tipping buckets beside manipulated trees to monitor redirected stemflow would require some stabilizing structure (i.e. driving posts into soils or pouring small concrete pads) in addition to disturbing the near-stem soil microclimate via shading. Situating collection/monitoring devices to collect diverted stemflow via gravity also required the gauges to be too close to the stem. As our aim was solely to isolate the stemflow effect, great care was taken to avoid (or at least minimize) disturbance to all other processes acting on the near-stem soils, including other precipitation inputs like throughfall dripping through the surrounding canopy. For these reasons, the stemflow was redirected from the tree stem by a small-diameter pipe to a distance of 3 m from the stem base (see methods for further details). The nearby trees where stemflow was measured were of similar stem diameter at breast height, or ~1.4 m above ground, (average 28 vs. 31 cm) and carried similarly low epiphyte biomass (as the trees whose soils were sampled). Total annual stemflow yield (mm per unit canopy area) was measured as 97.0 mm (or only 8.3% of rainfall) for the comparison of oak trees. The coefficient of variability for total stemflow yield between the eight trees, despite similar canopy and stem diameter, often exceeded 100% (44% of all 10-min stemflow observations), but was approximately 10% or less for a few large storms: 59.9 mm on Aug-09-2017, 87.6 mm on Aug-24-2017 and 112.7 mm during Hurricane Irma on Sep-10-2017 (Fig. S1). Maximum stemflow volume for any of the trees was observed during Hurricane Irma (a quantitatively impressive 839.4 L $tree^{-1}$), while minimum observed stemflow volume per tree was 1.4 L.

To test the impact of stemflow on microbial community composition, a stemflow collection collar and redirection system was installed on two oak trees to eliminate stemflow with minimal disruption of water inputs from throughfall. After a period of 7 months, soil samples were collected along transects proceeding outward from the stem for these experimental trees as well as three size-matched control trees, and soil microbial community structure was assessed through 16S rRNA gene sequencing. A total of 4 401 596 reads representing 12 295 OTUs were obtained from the 173 total soil samples. Lateral trends in community composition were

generally less striking in soils with diverted stemflow. Communities from soil samples collected in close proximity to control trees exhibited an abundance of OTUs in the Actinobacteria family, with distinct shifts in community composition with increasing distance from the tree stem base (Fig. 1). Experimental trees were enriched in Proteobacteria and depleted in Actinobacteria relative to control trees. Average relative abundance of Proteobacteria was significantly elevated in experimental samples (*t*-test, $p < 0.001$), ranging from 11.3% to 36.4%, compared to 9.2%–28.1% in control samples. Actinobacteria, however, were significantly depleted in experimental samples, with relative abundance ranging from 3.8% to 47.6% compared to 17.4%–51.0% in control samples (*t*-test, $p < 0.001$). Experimental soil samples exhibited significantly higher microbial diversity (Shannon Index) than those that received undisturbed stemflow (*t*-test, Wilcoxon test, $p < 0.001$) (Fig. S3, left panel).

Principal coordinate analyses (PCoA) were performed to investigate the similarity between microbial communities collected from different trees, directions and locations (Fig. 2). Each treatment group was analysed separately to understand within group variation. The control samples formed distinct clusters along both axes based on distance from the tree stem, with distances 0–15, 25–75, 100–150 and 200–250 cm each grouping together (Fig. 2A). The experimental group separated along the first dimension according to distance when analysed independently (Fig. 2B). This was also observed when all samples were analysed together; however, some of the control samples distance groups were localized to the lower *y*-axis (Fig. 2C). A PERMANOVA found significant differences in population composition between sample groups (Table 1). The distance of a sample from the stem base and the stemflow treatment were significant factors as singular explanatory variables as well as when their interaction was included ($p \leq 0.001$). No other factors (tree, transect direction and all models of combined variation) were found to be significant.

Control versus experimental groups showed distinct differences in the number and types of microbial taxa exhibiting lateral patterns in bacterial abundance. The 100 most abundant OTUs (across all samples) were tested for significant correlation with proximity to the stem under each treatment (Figs 3 and 4). In these figures, positive or negative correlations indicate that taxa increase or decrease in relative abundance with proximity to the stem respectively. Total abundance of taxa that decreased in relative abundance with proximity to the stem ranged from 1.0% to 32.1% across distances in the control (undisturbed) samples (Fig. 3). This range was smaller in the experimental samples, 0.2%–5.6%. Taxa that increased in relative abundance with proximity to the stem comprised 0.6%–47.9% of control samples and 1.0%–28.3% of experimental samples (Fig. 4). The abundance of 21 and 39 out of the 100 most abundant OTUs were significantly negatively and positively correlated with proximity in undisturbed samples respectively, while seven and 28 OTUs showed significant negative and positive correlations among samples with relocated stemflow respectively. Of these, four OTUs were found to decrease in relative abundance with proximity to stems in both treatments, while 17 OTUs exhibited this pattern in the control but not the experimental treatments (Table S2). Twenty-two OTUs were found to increase in relative abundance with closer proximity to stems in both treatments, while 17 OTUs showed this trend in undisturbed but not experimental samples (Table S3).

For both treatments, fewer OTUs showed significant increases in relative abundance with increasing proximity to the stem, and the number of overlapping OTUs was limited to one OTU from each of the following phyla: Acidobacteria, Actinomycetes, Firmicutes and Proteobacteria (Fig. 3). Of these, only *Massilia timonae* was more abundant in the control samples. In general, Actinomycetes such as Arthrobacter (OTU1) and *Streptomyces mirabilis* (OTU2) rose significantly in abundance in the control samples, comprising over 10% of the community in some of the furthest samples from the stem (Table S2). In contrast, OTUs whose relative abundance decreased closer to the stem made up a small relative fraction of communities in soils near experimental trees, with unique OTUs belonging to phyla Bacteroides and Proteobacteria. Only three OTUs in this category comprised more than 1% relative abundance: *M. timonae* (2.79%), Chitinophagaceae (1.78%) and Syntrophobacteraceae (1.20%).

Over half of all taxa where relative abundance significantly increased with proximity to stems were shared between treatments (Fig. 4). Together, these taxa showed large changes in abundance, making up approximately 38% of the taxa at 0 m of the control trees and only 22% of sequences recovered at 0 m in the experimental trees. However, given the higher numbers of correlated taxa, the difference in relative abundance of individual taxa (Fig. 4) was not as striking as with OTUs which decreased with closer stem proximity (Fig. 3). Actinomycetales OTU 36 was the most abundant overall in both treatments, with a maximum relative abundance of 9.15% and 7.99% in the control and experimental treatments respectively (Table S3). Most of the unique and significantly correlated OTUs in each treatment were in orders, and often families, that were shared between treatments. For example, Solirubrobacterales (193) was observed in the experimental group and three unique OTUs in this order (1216, 482 and 492) also increased in relative abundance at distances closer to control tree stems. Several OTUs whose abundance correlated with

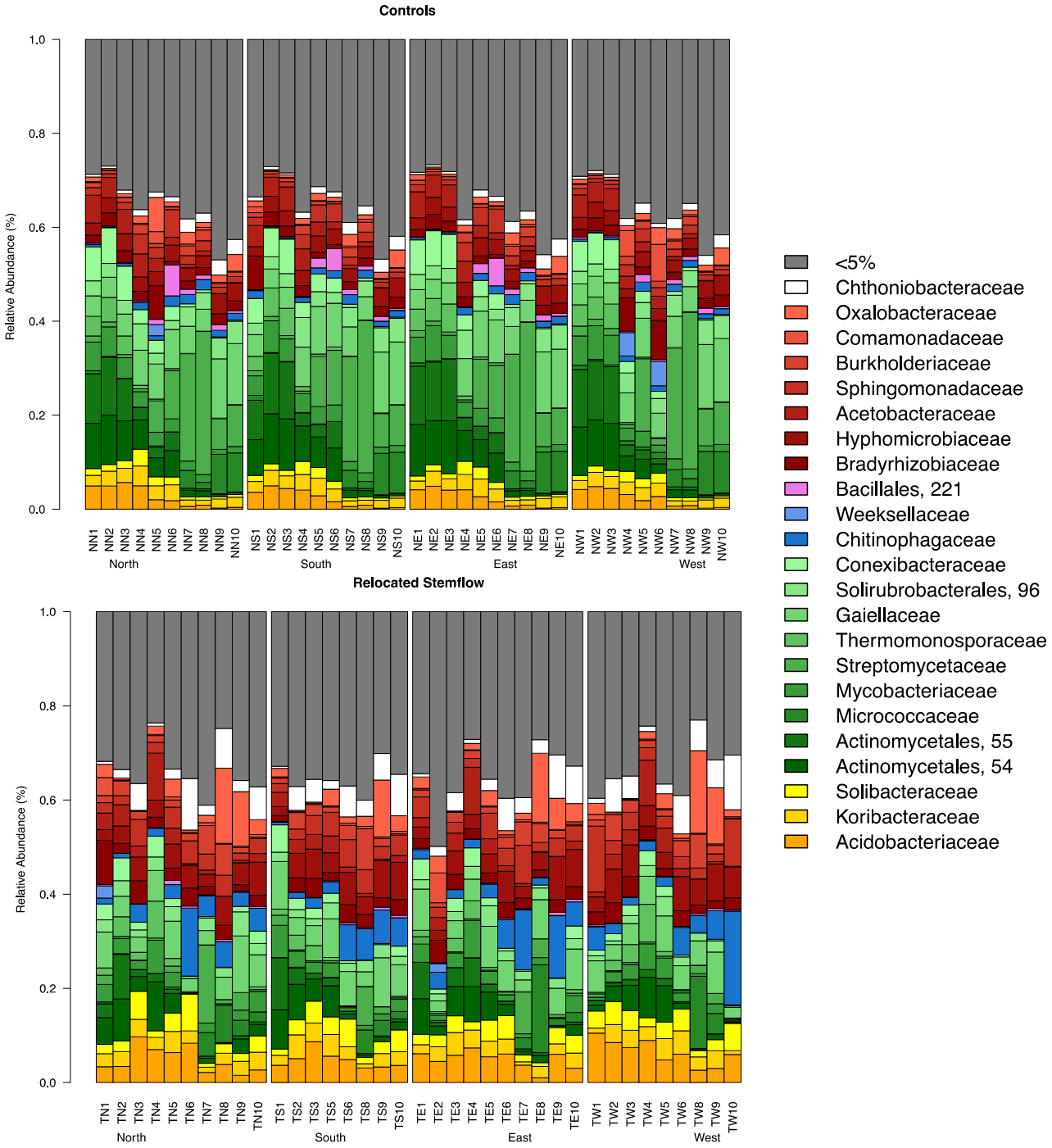

Fig. 1. Community composition shifts in soils receiving undisturbed SF and those with relocated SF. The average relative abundance of each family within a distance group was determined for all trees in each treatment group (control samples, panel A, experimental samples, panel B) along each transect. All families representing 5% or more of the community in any sample are displayed in colour while all other taxa below this threshold are shown in grey. Prominent families are listed in the legend and are coloured by phyla (yellows, Acidobacteria; greens, Actinobacteria; blues, Bacteroidetes; purples, Firmicutes; reds, Proteobacteria; white, Verrucomicrobia). If a family is unnamed, the next lowest classification is listed along with family number.

distance in the control group were members of the orders Acidobacteriales and Rhodospirillales; these orders were not observed to significantly correlate in experimental samples (Figs 3 and 4). Burkholderiales was the only order with OTUs unique to samples with relocated stemflow (7495, 7597).

Bray–Curtis dissimilarity was calculated to analyse diversity between samples to further investigate the

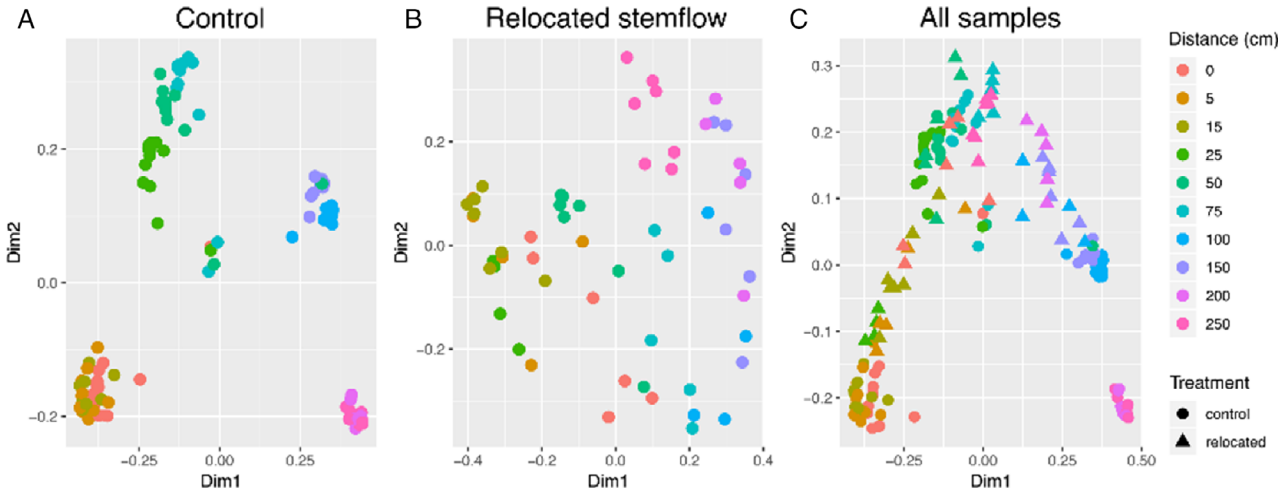

Fig. 2. Soil communities cluster by distance from the tree base. Principle coordinate analyses were completed separately for control samples and those with relocated stemflow, as shown in panels A and B respectively. All samples were included to assess the impacts of distance (cm) regardless of treatment (C). PCoAs were completed using rarified data.

Table 1. PERMANOVA results.

Source of variation	Terms	*F* Model	R^2	*p*-value
Treatment	1	12.138	0.0663	**0.001**
Tree	1	1.2938	0.0150	0.16
Direction	1	0.3669	0.0065	1
Distance	1	15.3890	0.4594	**0.001**
Distance × treatment	2	10.3390	0.1803	**0.001**
Distance × tree	2	0.9999	0.0587	0.487
Distance × direction	2	0.6133	0.0591	1
Treatment × tree	2	0.5766	0.0032	0.837
Treatment × direction	2	0.4775	0.0080	0.999
Treatment × direction	2	0.5464	0.0195	1

Significant results are shown in bold.

significant trends in both treatments observed with distance from the stem base. In the control group, increasing proximity away from the stem has a strong and significant relationship with Bray–Curtis dissimilarity (Mantel statistic = 0.7418, $p \leq 0.0001$) (Fig. 5A). This relationship was also observed in the experimental samples ($p \leq 0.0001$) (Fig. 5B); however, the relationship with dissimilarity and distance was not as strong (Mantel statistic = 0.4387). Outliers in the control group at distances 0 and 5 cm were identified as belonging to sample N1S1. To test that this sample did not alter the results, sample N1S1 was removed and the above analysis was re-run. The increase in dissimilarity observed remained significant when weighted and unweighted dissimilarity was calculated (*p*-value ≤0.0001, Mantel statistic = 0.752). When samples collected at each distance were compared between treatments, median beta diversity ranged from 0.60 to 0.87 (Fig. S4). No consistent or significant trend in dissimilarity was observed with change in sample distance (rho = 0.4667, $p \geq 0.05$); however, the samples furthest from the tree stem (250 cm) were the most dissimilar.

Dissimilarity was also calculated between samples from each treatment group and between samples from different trees in each treatment (Fig. S3 centre, right panels). When comparing within-group dissimilarly between treatment types, median experimental dissimilarity exceeded control dissimilarity significantly ($p < 1 \times 10^{-16}$). Beta diversity in both treatment groups was significantly lower than when comparing between treatment groups ($p = 7.23 \times 10^{-5}$, $p < 1 \times 10^{-16}$, for experimental and control groups respectively). Median dissimilarity between experimental trees was significantly higher than between control trees (*p*-values ranged from 1.81×10^{-8} to 1.85×10^{-11}). There were no significant between-tree differences in beta diversity among control tree combinations ($p \geq 0.05$).

Discussion of stemflow influences over soil bacterial community structure

Stemflow is thought to be a key component of plant and soil microbial interactions as it transports nutrients, microbial organisms and metazoans from the tree canopy to the soil (Bittar *et al*., 2018; Ptatscheck *et al*., 2018; Teachey *et al*., 2018; Magyar *et al*., 2021; Van Stan *et al*., 2021), likely altering the ecology and biogeochemistry of the receiving ecosystem. However, despite the possibility of intense water influx to receiving soil via stemflow for some ecosystems, no work to date has altered stemflow routing to determine the impacts of periodic water flux from tree canopies on soil bacterial community composition. Additionally, there are discrepancies in the literature on this topic (Bollen *et al*., 1968;

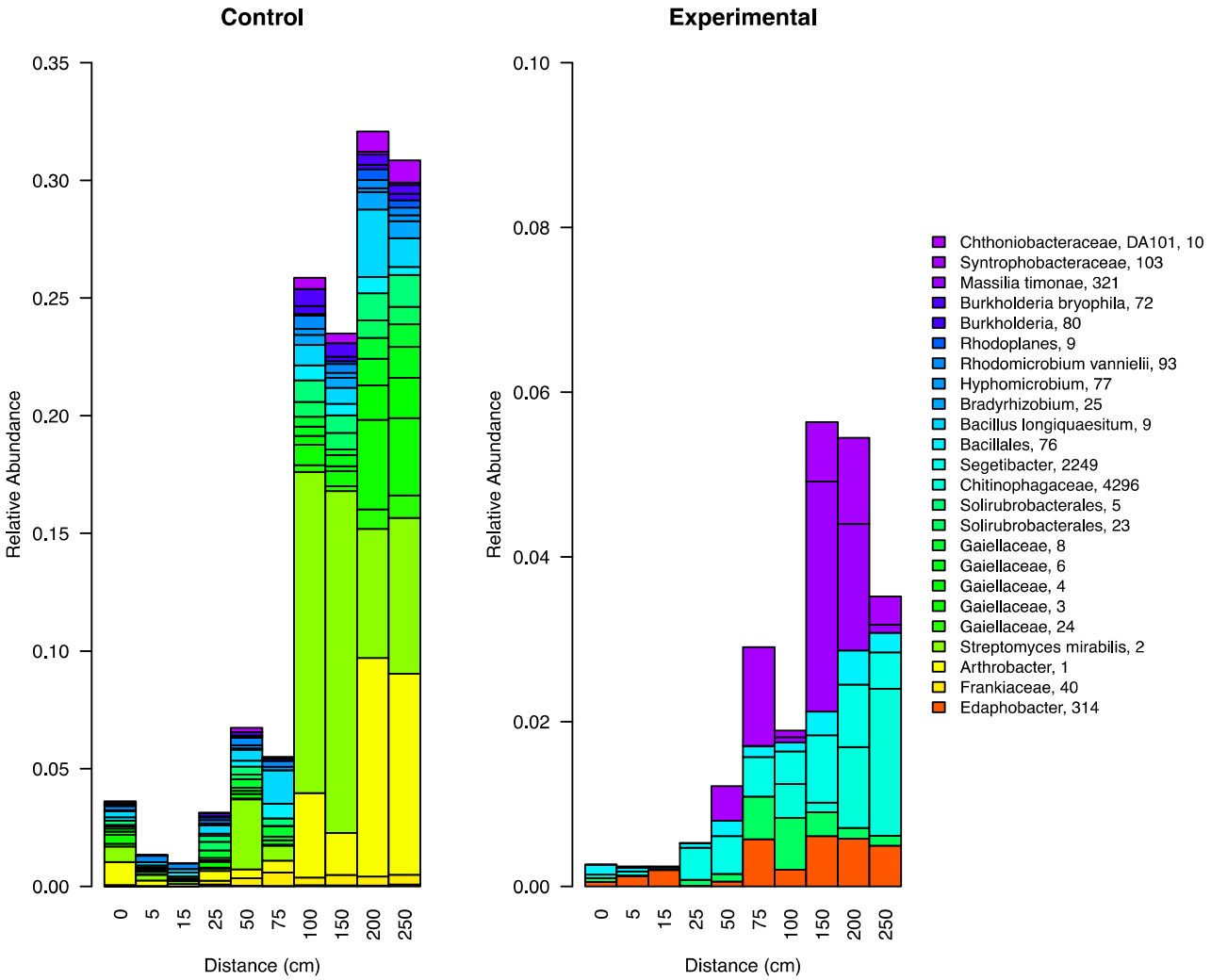

Fig. 3. Taxa negatively correlated with proximity to the stem differ between stemflow treatments. Samples were randomly selected from each set of control distance groups so that sample count per distance was the same as the experimental samples. Counts for the 100 most abundant OTUs were summed for each distance within a treatment before relative abundance (within the entire bacterial population) was calculated. Relative abundance of the negatively correlated (Spearman's) taxa in the control samples is shown in the left panel and experimental sample results in the right.

Ceccherini *et al*., 2008; Nacke *et al*., 2016; Rosier *et al*., 2016) which are possibly caused by the lack of stemflow manipulation and differing soil physiochemistry between experimental designs. In this work, we aimed to address the former variable, stemflow, in *Q. virginiana* by directing all stemflow away from experimental tree stem bases for 7 months before sampling microbial soil communities. The results of this work reject the null hypothesis, indicating that the disruption of stemflow can significantly alter soil bacterial communities near the base of *Q. virginiana* trees. We observed both (i) that stemflow homogenizes bacterial communities, driving down overall diversity when compared to soils deprived of stemflow in which we observed an increase in community variation; and (ii) that proximity to the stem base has significant impacts on community composition regardless of stemflow presence; however, these effects vary between treatments.

First, significant changes in communities along an outward transect from the stem were observed in both control and experimental trees, implying that proximity to the tree base consistently impacts bacterial community structure regardless of stemflow manipulation. Nacke *et al*. (2016) also observed significant shifts in bacterial community composition with increasing distance from spruce and beech stem bases. Measured soil characteristics did not correlate with distance from stem bases in their study, and they hypothesized that change in root mass may influence the lateral community patterns observed. Other studies have also reported significant

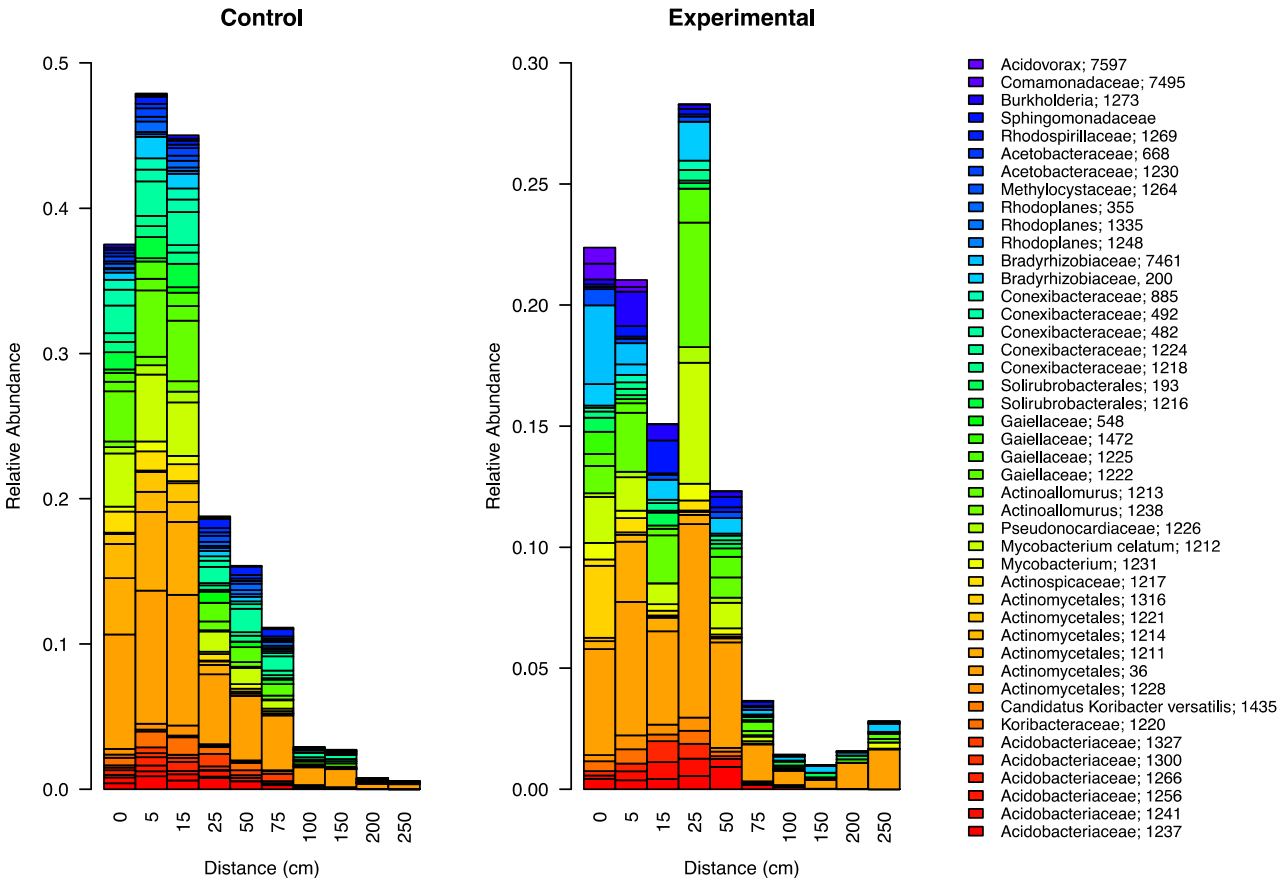

Fig. 4. Taxa that significantly positively correlate with proximity to the stem base overlap between stemflow treatments. The methodology applied to generate Fig. 3 was applied for negatively correlated taxa. Relative abundance of positively correlated taxa in control and experimental samples are shown in the left and right panels respectively.

differences in local diversity observed in the same soils with and without oak roots (Habiyaremye *et al*., 2020). While the authors are not aware of studies that describe the lateral root density of oak trees, changes in *Q. virginiana* root mass may also act as driver of microbial community structure. Additionally, soil community diversity has been demonstrated to be highly variable at the μm and mm scales (Ettema and Wardle, 2002; Grundmann, 2004). Thus, the high beta diversity levels between distances in the experimental samples are not surprising even between samples taken 10 cm apart. Given the experiment design solely manipulated stemflow, differences in these spatial variations in beta diversity between treatments are likely due to the removal of stemflow.

Similarly, substantial overlap was noted in taxa that increased with proximity to the stem base in both treatments (Fig. 4). While the moisture levels may vary between treatments, many other environmental conditions remain the same: tree root presence, light, air and soil temperature, receipt of throughfall, and ground cover. Therefore, we expected to observe some level of overlap in lateral community trends between treatments regardless of stemflow status. Tree species also shape local microbial communities (Nacke *et al*., 2016; Dukunde *et al*., 2019), and in one study, oak and beech trees were shown to select for bacteria with mineral weathering functionality, specifically linking Burkholderia isolates to beneficial iron mobilization (Calvaruso *et al*., 2010). The Burkholderiaceae family was abundant at notable levels in both treatments in our study; relative abundance of Burkholderiaceae OTU 1273 increased with closer stem proximity in both treatments, while OTU 80 and *B. bryophila* (OTU 72) relative abundances decreased with closer stem proximity in control samples, again reflecting the potential for versatile bacterial families to occupy specific niches within similar environments.

Importantly, however, control samples showed stronger positional trends in overall community composition than experimental samples deprived of stemflow. Specifically, near-stem control communities were more similar to a 0 cm reference sample than more distant samples. In

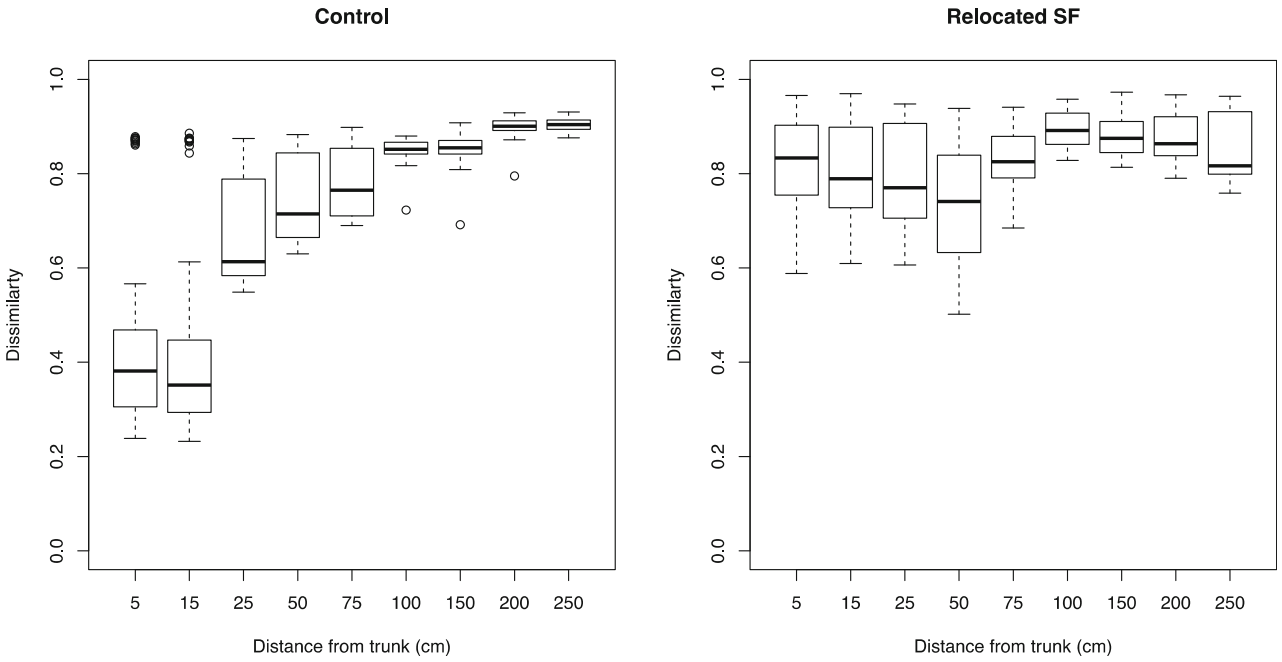

Fig. 5. Dissimilarity significantly increases between tree base communities and more distant communities when stemflow is present. Weighted Bray–Curtis values were calculated between samples collected at the tree base (0 cm) and all other distances within the control (left) and experimental treatments (right). Analysis of unweighted Bray–Curtis values yielded highly similar results.

contrast, near-stem communities in trees without stemflow did not show strong trends in community similarity with distance from the 0 cm reference sample. In addition, control samples exhibited lower alpha (Shannon) diversity and overall median dissimilarities, again suggesting a homogenization of the soil microbial community by stemflow. Results also reveal that more microbial taxa (that represented a larger fraction of the community) showed significant positive and negative trends in relative abundance with proximity to the stem in control trees, indicating stemflow can influence these lateral trends in soil microbial community. While the taxa which tended to increase in relative abundance with closer proximity to the stem were similar whether stemflow was present or diverted from soils, these taxa were more abundant in the control samples (Fig. 4).

We hypothesize that the growth of bacteria that are enriched with proximity to the stem in both treatments is further promoted by stemflow, or that these bacteria are supplied to the soils via stemflow. What is known about stemflow, and even stemflow from our study tree species and site, suggests both hypotheses are plausible. To begin with, *Q. virginiana* stemflow from this site is not only capable of voluminous water inputs; it can transport large quantities of labile nutrients, up to 16 mg-C of biolabile-dissolved organic matter m^{-2} mm^{-1} of rainfall (Van Stan *et al*., 2017; Howard *et al*., 2018), and cells to the soil, 3.8×10^7 cells m^{-2} mm^{-1} of rainfall (Bittar *et al*., 2018). On the other hand, there was little overlap among taxa which tended to decrease in relative abundance with closer proximity to the stem when comparing between treatments – although again these bacteria represented a larger fraction of the community surrounding trees that received stemflow (Fig. 3). Hypothetically, microbial taxa that decrease in abundance with proximity to the stem may represent bacteria that are either outcompeted by stemflow-favoured bacteria or killed by stemflow, perhaps by hydrolysis or salinity-related lysis – both of which are plausible given chemically concentrated and/or voluminous stemflow (Van Stan and Gordon, 2018). Additionally, if stemflow promotes the growth of other microbes (i.e. those taxa in Fig. 4 which sum to 40%–50% of the community in the control) some of the taxa that appear to decrease in abundance may be apathetic to stemflow, yet decrease as a *fraction* of the community due to the compositional nature of the dataset. Although insufficient DNA was recovered in this study to facilitate such analyses, future studies aimed at quantitative analysis of microbial abundance in these soils could aid in disentangling these two possibilities.

The above hypotheses assume that stemflow infiltrates into soils very close to the stem; however, stemflow has been just as often observed to runoff for some distance from the stem, and infiltrate over large areas of the soil surface (Herwitz, 1986; Van Stan and Allen, 2020). In this case, stemflow may mechanically transport taxa to distances further from the stem and promote growth in taxa in more distant soils. Stemflow infiltration areas have

been observed both within millimetres of distance from the stem, and to extend as far as 4 m from the stem (Van Stan and Allen, 2020). Stemflow may also hit an impermeable subsurface soil layer (like observed in Herwitz, 1986) or a large root (which are often near tree stems), then flow laterally through the shallow subsurface as far as 2.8 m from the stem (Guo *et al*., 2020). Bradyrhizobium OTU 25 may be an example of such transport, as the relative abundance of this taxon increased with distances up to 2 m from the stem in the control but not in experimental (stemflow-deprived) samples (Fig. 3, Table S2). This nitrogen-fixing genus was also identified in rainfall and stemflow samples of *Q. virginiana* trees at this site (Teachey *et al*., 2018), and is known for its genetic diversity, which contributes to the range of metabolic traits encapsulated in this genus (Ormeño-Orrillo and Martínez-Romero, 2019). This last point may also explain why the abundance of another member of Bradyrhizobium, OTU 200, significantly increased with stem proximity in both treatments, perhaps filling a different metabolic niche than its close relative.

This study was not designed to directly test the mechanisms by which stemflow can influence soil bacterial communities; however, our results justify the discussion of hypotheses for future testing. Habiyaremye *et al*. (2020) demonstrated that soil pH, organic matter and temperature were significant factors in structuring community composition in microbial oak root populations (*Quercus robur*) of clonal trees. Although these soil characteristics were not measured in our study, it is possible that the same factors influenced the observed bacterial community structure. Observations reported here, in conjunction with previous work, suggest that the hypothetical alteration of these factors by stemflow may indirectly alter soil bacterial community structure. The significant influence of stemflow on soil moisture, physiochemistry and the composition of soil solutions has been reported for trees generating similar amounts of stemflow as observed at our site. Recently, in German temperate forests, Metzger *et al*. (2021) found near-stem microsites of unique physiochemistry, including increased soil organic C content and macropore proportion, and Jochheim *et al*. (2022) found higher macronutrient ion and heavy metal concentrations in soil solutions within the stemflow infiltration area. At a forest site on the Canary Islands, Aboal *et al*. (2015) studied a range of tree species with differing stemflow generation, finding multiple soil chemical variables, pH, conductivity, and available phosphorus, correlated with the corresponding tree's stemflow inputs. As mentioned earlier, stemflow from the study species at the study site has been found to be strongly enriched in solutes. It may be that the removal of stemflow at our site would have resulted in decreased soil moisture and solute supply to near-stem soils, thereby indirectly influencing bacterial community compositional patterns around the stem.

Conclusions and future directions

By diverting stemflow away from soils at the base of *Quercus virginiana* stems, we were able to observe direct and significant impacts of stemflow on soil bacterial community structure at the stem base for this site. Removal of stemflow significantly impacted bacterial community structure, resulting in higher diversity across all samples compared to communities in soils receiving stemflow. These data also suggested that stemflow can significantly alter the spatial patterns of relative abundance in taxa up to 2.5 m from the stem base. The relative abundance of some taxa correlated with distance from the stem and between treatments. These patterns showed both differential responses to stemflow removal from (i) different families and (ii) from different OTUs in the same family (e.g. Bradyrhizobium and Burkholderiaceae). While we have demonstrated that stemflow can be a driving factor in bacterial community structural patterns near stem bases and discussed various hypothetical mechanisms underlying this result, the specific parameters contributing to diversity loss in these stemflow-related patterns remain unknown. Future work in which the chemical composition of both the soil, stemflow and rainfall (i.e. pH, salinity, nutrient concentration) is measured may shed light on the explanatory variables correlating with stemflow-related community change, and with distance from the stem base. The findings of this work could also be expanded by conducting similar stemflow diversion experiments in which soil samples are taken periodically. For example, examining soil community composition before and directly after storm events would capture the immediate impacts of stemflow, while intermediate sampling over multiple months would explore the long-term impacts of stemflow diversion. As this study manipulated stemflow for a single tree species, further work including additional species is merited to compare root structure and density differences in relation to moisture penetration (and therefore interspecific influences on near-stem bacterial community structure/patterns). Finally, as we observed shifts in the enrichment of closely related bacterial taxa between treatments, developing genomic and transcriptional profiles of soil communities receiving and deprived of stemflow would likely shed light on the functional traits supporting enriched bacterial groups in each treatment.

Experimental procedures

Study site description

All sampling was done in a forested site at the Skidaway Institute of Oceanography campus, University of Georgia, Savannah, GA, USA (31.99° N, 81.02° W). Climate is subtropical humid (Köppen *Cfa*) and the range of 30-year mean annual precipitation is 750–1200 mm y^{-1} (University of Georgia Weather Network, 2012). The site is flat (0–10 m amsl) with 0%–5% slopes on Chipley fine sandy soils (Natural Resources Conservation Service-Web Soil Survey, 2015) and dominated by *Quercus virginiana* Mill. (southern live oak), representing 315 stems ha^{-1} and 90% of the basal area (59.3:65.8 m^2 ha^{-1}). The *Q. virginiana* canopy hosts dense epiphytic vegetation – *Tillandsia usneoides* L. (Spanish moss) and *Pleopeltis polypodioides* (resurrection fern) – but trees with little-to-no epiphytic cover were selected for stemflow monitoring, relocation and soil sampling to permit maximum drainage of rainfall via stemflow.

Hydrometeorological monitoring

Total rainfall (mm) during the study period (December 2016–December 2017) was 1165.6 mm per the Skidaway weather station (http://www.georgiaweather.net/?variable=HI&site=SKIDAWAY). Stemflow was monitored for eight individual oak trees comparable in size and canopy condition to the trees whose stemflow was relocated and the control trees. Stemflow monitors consisted of polyethylene tubing collars wrapped about the trunk at 1.4 m height, nailed to the tree stem, sealed with silicon, and connected to tipping buckets (Texas Electronics TR-525I, Dallas, TX, USA) shielded by an HDPE plastic bowl to prevent throughfall from entering. Tipping buckets were interfaced with a datalogger (Campbell Scientific CR3000, Logan, UT, USA) that recorded observations at 10 min intervals. Stemflow collars and tipping buckets were maintained weekly to prevent clogging.

Stemflow relocation experiment and soil sampling

Five oak trees were involved in this experiment, where three control trees were not disturbed and two trees' stemflow was relocated. More control trees were included in the study to gather greater data on the characterization of the undisturbed soil bacterial community near stems that received stemflow. Devices for the relocation of stemflow to nearby open areas consisted of a stemflow collar attached to ~3 m long PVC conduits that emptied into a ~20 L PVC bucket whose bottom has been removed and has a diameter (30.5 cm) similar to that of the trunk (Fig. S2). The bottomless bucket was pushed ~10 cm deep into the soil to allow all stemflow to infiltrate in the localized area (Fig. S2). This was necessary to prevent stemflow from running over the land surface toward the near-stem soils. The bucket was sized similarly to the stem basal area as it is generally observed that stemflow tends to preferentially infiltrate over areas roughly equivalent to stem bases (Johnson and Lehmann, 2006). Stemflow relocation devices were observed to function properly during storm intensities ~6 mm over 5 min, as stemflow did not overflow the bucket.

Relocation devices were installed on October 31, 2016, and maintained after every storm (that exceeded 5 mm after a 24 h antecedent dry period) over 7 months, until May 26, 2017 – when soils were sampled. Soils were sampled along four transects oriented north, south, east and west. Each transect was sampled at the following distances from the stem base: 0, 5, 15, 25, 50, 75, 100, 150, 200 and 250 cm. Due to constraints on available DNA sequencing runs, we selected a subset of these soil samples for analysis (as apparent in figures and tables).

DNA extraction, PCR, sequencing and analysis

Soil samples (0.3 g of homogenized soil) were obtained from the topsoil (top 10 cm) then placed on ice for transport (a few minutes away) to the lab on site (at the Skidaway Institute of Oceanography), where the DNA was immediately extracted. Roots, leaves and visible non-soil materials (bugs, etc.) were removed from the soil samples. DNA extraction was performed using Mo-BIO PowerSoil® Kits (Mo-BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's protocols. After DNA extraction, the samples were kept at −20°C until further processing.

The methods of Tinker and Ottesen (2016) were followed to amplify the V4 region of the 16S rRNA gene for each extracted DNA sample. Samples were pooled into a library which was submitted to the Georgia Genomics Facility for sequencing (Illumina MiSeq 250 × 250 bp; Illumina; San Diego, CA). The returned raw reads associated with this work have been deposited in the Sequence Read Archive of the National Center for Biotechnology Information under accession number PRJNA732720.

Sequences were processed using the QIIME2 software package (Bolyen *et al*., 2018) using the following protocol: reads were imported in the Phred33 format then paired using vsearch (Rognes *et al*., 2018); quality filtering was completed with the paired quality scores using the QIIME default settings; reads were denoised and assigned to OTUs using Deblur (Amir *et al*., 2017) and a trim length of 250 bp; classification was completed using

the Sklearn classifier provided by QIIME2 which was pretrainer on Greengenes 13_8 (McDonald *et al*., 2012). Sequence reads classified as 'unknown' or 'chloroplasts' were removed using the filter-features function in QIIME2.

Statistical analysis was performed in R using the vegan (Oksanen *et al*., 2018) and ggpubr (Kassambara, 2020) packages. Samples with less than 1000 reads were discarded, yielding 4 401 596 total sequences in 173 quality-filtered samples. On average, each sample had 25 443 sequences and 786 unique OTUs. All samples were rarified to the depth of the sample with the lowest number of reads, 3463. These rarified data were used for all analyses excluding those exploring relative abundance patterns between samples and taxa. Ordination analysis used the rarefied community dataset and the wcmdscale() function in vegan. PERMANOVA was performed using the adonis() function; three-way interactions were not tested.

To determine which OTUs were significantly negatively and positively correlated across distance in each treatment, samples from the control group were randomly selected so that an even number of samples were analysed for each treatment at each distance. Within each distance and treatment group, counts for each OTU were then added together. The 100 most abundant OTUs were selected and Spearman correlations for each across distance were determined.

Acknowledgements

This work was performed with the sequencing support of the Georgia Genomics and Bioinformatics Core at the University of Georgia. Thanks to Jamal Koledoye for his assistance in sample extraction and preparation for sequencing. Thanks to the staff at Skidaway Institute of Oceanography for maintaining and securing the research site, and to the students who assisted in site maintenance or sample processing (Elliott Lewis, Julius Lewis and Ansley Whitetree).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information.