Fagus **Graphical Abstract**

grandifolia:
High angle
Smooth bark

*Liriodendron
tulipifera*:
Low angle
Rough bark

Seasonal
variability in
SMC
(per NMDS)

Alters soil microbial community
(SMC) near trunks via:
Stemflow water/nutrient dynamics
Stemflow nutrient constituency
Stemflow pH

Seasonal
variability in
SMC
(per NMDS)

Seasonal dynamics of the soil microbial community structure within the proximal area of tree boles: possible influence of stemflow

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Abstract

Soil microbial community (SMC) structure affects several ecosystem services (soil-carbon mineralization and stabilization), yet responds to edaphic conditions. Stemflow, water that drains over the exterior surface of trees, concentrates precipitation to soils near the trunk, thereby altering edaphic conditions. While recognizing that a suite of factors can affect soils, our research investigates the potential linkages between soil moisture, chemistry, and SMC structure within near-trunk soils from two species of contrasting stemflow production (*Fagus grandifolia* Ehrh. [American beech, AB] and *Liriodendron tulipifera* L. [yellow poplar, YP]) across seasons. Variations in SMC structure were determined by Nonmetric MultiDimensional Scaling (NMDS) analysis of Denaturing Gradient Gel Electrophoresis (DGGE) banding patterns. Sequencing/BLAST analysis of dominant DGGE-bands were conducted for shared and unique bands occurring in both AB and YP stemflow-influenced soils. Findings suggest species-specific differences in stemflow potentially alter moisture dynamics, pH, mineral nutrients, and soil-C near-trunk soils. SMC structure also increases in variability under low stemflow flux (i.e., for YP). However, SMC structural variability decreases for near-stem soils across individual trees and seasons when stemflow flux is consistently high (i.e., for AB). Differences in canopy structure that govern stemflow production may be a plant trait capable of altering SMC structure. Variation in SMC structure may be related to tree species stemflow input fluctuation response to seasonal change. Future investigations should consider intricate interrelationships among stemflow and species composition of the SMC in near-trunk soils in order to better contextualize the effect of stemflow on SMC vis-à-vis other factors, such as litter quality.

Keywords: Stemflow; Soil Microbial Community (SMC); Soil Moisture; Soil Chemistry; PCR-DGGE; Multidimensional Scaling

1. Introduction

Diversity of the soil microbial community (SMC) significantly influences several soil ecosystem services, including nutrient cycling [1], carbon (C) mineralization [2], and stabilization/formation of soil structure [3]. Since the SMC represents a critical component of forested ecosystems, several studies have investigated how aboveground plant species affect SMC diversity and function [4,5]. The general consensus suggests that numerous plant traits can alter SMC structure, including leaf litter quantity/quality [6], specific nutrition requirements [7], the development of understory vegetation [8], and pedoclimate [9]. Tree canopy cover has also been hypothesized as a possible plant trait capable of directly or indirectly influencing several variables contributing to the spatial patterning of SMCs [10]. For example, an individual tree canopy is able to capture and funnel precipitation down its trunk to produce a spatially-concentrated hydrologic input to near-trunk soils called stemflow [11]. Depending on species-specific canopy structural features and meteorological conditions, stemflow can input large quantities of chemically enriched water to less than 0.5 m² of soil area in a single storm [11], potentially altering the SMC structure by shifting soil moisture and nutrient dynamics [12].

Stemflow from voluminous-generating genera (e.g., *Fagus*, *Stewardia*, or some *Quercus* spp.) has repeatedly been shown to alter horizontal and vertical soil moisture patterns [13,14], even to the extent of contributing to aquifer recharge [15]. Moreover, stemflow contains high concentrations of micro- and macronutrient ions as well as dissolved organic matter (DOM) [16], and can significantly alter soil pH, chemistry and solution chemistry [17]. Despite stemflow's prodigious impact on soils surrounding the tree trunk, this is among the first studies to link stemflow and SMC's [18,19]. Earlier work still leaves many knowledge gaps unaddressed, for

example: measurements on a single alder tree by Bollen et al. [18] found that *Streptomyces* in the soil were influenced by stemflow. Yet, there are a multitude of other clades, and even other Actinobacterial genera, of significance within SMCs that have not been investigated with regards to stemflow influence. Moreover, how might the influence of stemflow on the SMC vary between tree species of differing stemflow generation? Ceccherini et al. [19] attempted to determine whether stemflow contributed microbes to the SMC, but was unable to definitively say whether stemflow transported Bacteria to the forest soil or whether the same communities inhabited both the canopy and soil. Addressing these knowledge gaps is a critical step forward to determining whether interspecific stemflow differences can ultimately affect microbial diversity in forest soils. Such a study should begin, at a minimum, with comparison of two species representing differing ends of the continuum of canopy structures which control stemflow generation and chemical enrichment. The major canopy structures controlling stemflow variability include: branch angle, bark thickness and structure, and canopy thickness [20].

In this study we evaluate SMC structure near the trunk (< 0.5 m) of two tree species with differing canopy structures— *Fagus grandifolia* Ehrh. (American beech, AB) and *Liriodendron tulipifera* L. (yellow poplar, YP) across three seasons. These two species represent each end of the canopy structural continuum controlling stemflow generation and chemical enrichment as AB is a voluminous stemflow generator of steeply inclined branches covered in smooth bark with a deep canopy, and YP canopies exhibit the opposite qualities. Previous research at a field site where these two species are among the co-dominant species (Fair Hill, MD, USA) clearly demonstrated that these species significantly differ in quantity and quality of stemflow input to near-trunk soils. Specifically, AB produces 2-4 times the stemflow generated by YP trees depending on tree size and storm conditions [21]. Nutrients dissolved in these species' stemflow

also differ substantially, with YP stemflow being much more enriched up to 10 and even 30 times that of SO_4^{2-} and NO_3^- , respectively, for example than observed for AB [22]. Even the DOM transported by these species' stemflow is strikingly dissimilar, with AB stemflow DOM flux being an order of magnitude greater (and of lower aromaticity and molecular weight) than that of YP [16]. Based on this understanding, we tested the following hypotheses: (i) soils experiencing differential stemflow inputs as a function of species will differ significantly in moisture content and soil chemistry (e.g. soil OM, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and nutrients); (ii) SMC structure in soils near YP trunks will differ significantly from near-trunk AB soils due to lower stemflow water and nutrient flux; and (iii) SMC seasonal variations will be less pronounced in AB soils as a result of continual water and nutrient supply.

2. Methods

2.1. Site Description

Soil samples were collected from an experimental forest located within Fair Hill Natural Resource Management Area (NRMA), a satellite site of the Christina River Basin Critical Zone Observatory (39° 42'N, 75° 51'W). AB and YP co-dominate this forest site, representing 46% and 32% of all identified trees, respectively. The understory is sparsely vegetated with the majority of understory vegetation existing in wetland areas that were avoided in this study. The litter layer is well mixed per visual inspection, does not differ substantially in quantity or quality between near-trunk soils for AB or YP. Annual mean precipitation is approximately 1,200 mm for the 30-year period from 1981-2010 [23]. Overall average annual unadjusted potential evapotranspiration is approximately 670 mm yr⁻¹. Lowest average monthly air temperature typically occurs in January (-0.1 °C) and highest average summer temperature occurs in July (25.7 °C [23]). A continuously-monitoring meteorological station is located near the study site at

Fair Hill NRMA, operated by the Delaware Environmental Observing System (DEOS), provides precipitation observations every 5 minutes. Soils are classified as a Glenely loam series [24], pH 6.8, organic matter (OM) content 10.3%, texture: 51.0% sand, 29.0% silt, 20.0% clay, and CEC is 22.5 meq 100 g⁻¹ (K. Yoo, personal communication).

2.2. Soil moisture monitoring and soil sampling

Soil moisture was monitored from Sep-2011 to Aug-2012. Three Decagon 5TE soil moisture probes (Pullman, WA, USA) were installed at a depth of ~ 3.0 cm within the mineral soil profile at a distance of 0.5 m from the trunk of one AB and one YP. Each of the three probes per tree were installed on the up-slope, down-slope, and zero-slope sides. Soil moisture measurements were logged by an Arduino-based datalogger at 10 min intervals. Soil moisture was also monitored at an open canopy site by a single Campbell Scientific CS650-L (Logan, UT, USA) at a 10 min temporal resolution at the DEOS station.

Soil sampling was conducted at three peak seasonal times (i) summer (August/2011-foliated); (ii) winter (February/2012-leafless); and (iii) spring (May/2012-leafing) near the trunk (at 0.5 m) of six trees of similar diameter at breast height (dbh = 1.37 m). The six-sampled trees (3-AB; 3-YP) were situated on a moderate slope of the catchment and, as such, three soil core samples (100 g) per tree were taken from the 0-10 cm soil layer after litter removal on the up-slope, down-slope, and zero-slope sides of the trunk (Fig. 1). After the 100g sample was manually mixed in the sample bag to ensure homogeneity, a representative subsample (0.5 g soil) from each soil core was placed into a microfuge tube (2 ml) and stored at -20 °C until DNA extraction. Bulk soil cores were dried at 60 °C for 48 h and stored prior to soil physiochemical analysis.

2.3. Soil chemistry

Soil chemical analysis was conducted on pooled samples from each tree at the University of Delaware Soil Testing Laboratory (Table 1). Total organic C (TOC) and total nitrogen (TN) were measured by combustion using an Elementar Vario Max CN Analyzer. Soil total organic matter (OM) content was measured by loss on ignition, and soil pH was measured in a 1:1 soil/distilled water suspension. Plant available nutrients (P, K, Ca, Mg, Mn, Zn, Cu, Fe, and Al) were extracted by Mehlich-1 and analyzed by inductively coupled plasma optical emission spectroscopy using a Thermo Iris Intrepid II XSP Duo View ICP. The natural abundance of carbon-13 ($\delta^{13}\text{C}$) and nitrogen-15 ($\delta^{15}\text{N}$) were determined by simultaneous elemental analysis-isotope ratio mass spectrometry (EA-IRMS, CE Instruments NC2500 EA coupled to a Thermo-Finnigan Delta Plus XL IRMS). Stable isotopes were reported using the delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) vs. Pee Dee Belemnite (PDB) and atmospheric N_2 standards.

2.4. Soil DNA extraction and Polymerase Chain Reaction (PCR)

Total genomic DNA was extracted from soil samples using MO BIO PowerTM Soil DNA Kit (Carlsbad, CA, USA) following manufacturer's instructions. DNA concentrations were estimated based on 260 nm absorbance using a Nanodrop Spectrophotometer ND-2000 (Wilmington, DE, USA). Bacterial PCR amplification was performed in a 50 μl reaction containing approximately 25 ng of template DNA, 25 μl PCR Mastermix (2x, Qiagen), 1.0 μl (each) primer, 0.5 μl 1% bovine serum albumin (BSA), and distilled water. Eubacteria-specific primers 1070f (ATGGCTGTCGTCAGCT) and GC-clamped 1392r (ACGGGCGGTGTGTAC) were used to amplify the 16S rRNA genes [28]. PCR reactions were performed using an automated Eppendorf Mastercycler Thermal Cycler (Perkin-Elmer, Norwalk, CT) and followed the PCR program as previously described [25]. Fungal DNA was amplified using a semi-nested approach [26]. Initially primers NS1 (GTAGTCATATGCTTGTCTC) and EF3

(TCCTCTAAATGACCAAGTTTG) were used to amplify a portion of the 18S rRNA gene [27]. Amplicons from the first PCR were diluted 1:100 and used as template in second PCR using primers NS1 and FR1-GC (AICCATTC AATCGGTAIT) [28]. Fungal PCR cycling program included a 5 min denaturation step at 94 °C; 25-cycles of 30 s at 94 °C, 45 s at 47 °C, 3 min at 72 °C, and final extension at 72 °C for 10 min. Second PCR program using primers NS1 and FR1-GC was similar to the first program with the exception that the cycle number was lowered to 20 and the annealing temperature was increased to 48 °C. PCR products for bacterial and fungal communities were confirmed by agarose gel electrophoresis.

2.5. DGGE and banding pattern analysis

In order to reduce sample numbers and minimize the variation of replicates, PCR amplicons from soil samples were pooled before loading (Fig. 1). Prior to pooling, however, a series of DGGE gels were run on the individual samples to ensure acceptable reproducibility (banding patterns were similar, >80%, across pooled samples per analysis of similarity as described in Section 2.7). Bacterial DGGE was performed using a DcodeTM Universal Mutation Detection System (Bio-Rad) and similar amount of PCR products were loaded on a 1.5 mm-thick vertical polyacrylamide (acrylamide:bisacrylamide ratio of 37.5:1) and a linear gradient of the denaturants urea and formamide, which increased from 50% at the top of the gel to 65% at the bottom. Electrophoresis was performed at 60 °C in 1 × TAE buffer, and a voltage of 70 applied for 16 h [28]. Fungal DGGE was completed using a linear gradient of denaturants 18-45%, while electrophoresis conditions were similar to bacteria with the exception of 120 V for 20 h [29].

The DGGE gel was stained with SYBR Gold and photographed [30] with a CCD camera mounted on a UV transilluminator (UVP). Gel images were analyzed using GelCompareII v.5.10 (Applied Maths, Austin, TX, USA). Initially, gel images were normalized by classifying a

reference lane which contained bands that span the gradient range; bands within the lane were then assigned a relative reference position. Spectral analysis was conducted across all remaining lanes using the normalized reference positions. Bands in each lane were counted and quantified automatically, generating data matrices for downstream statistical analysis.

2.6. Band excision and sequencing

Dominant and unique bands were excised from DGGE gels with a sterile razor blade, placed in a clean microfuge tube, and incubated in diffusion buffer (0.25M ammonium acetate, 10mM magnesium chloride, and 0.1% SDS) at 50 °C for 30 min. One microliter of supernatant was used to reamplify the band. PCR products were purified by USB ExoSAP-IT (Cleveland, OH, USA) and sequenced with no-GC clamp primers 1070f (Bacteria) and NS1 (Fungi) using Bigdye-terminator chemistry by ABI PRISM3100 Genetic Analyzer (Foster City, CA, USA). Dominant bands were considered those which were present and of high intensity across both species' and all seasons. Several bands existed that were unique to AB or YP proximal soils, yet only those which showed seasonal variability in presence/absence or band intensity were chosen for analysis as this manuscript sought to identify seasonal shifts in SMC structure. Nucleotide sequences obtained from this study have been assessed for presence of chimera using DECIPHER [31] and deposited in the GenBank with assigned accession numbers: Bacteria KP061796 – KP061804; Fungi KP061805 – KP061811. All sequences were compared with the GenBank database using BLAST, and the closest bacterial/fungal strain matches were obtained (Tables 2 and 3).

2.7. Statistical and phylogenetic analysis

Data normality and homogeneity were reviewed for soils prior to analysis of variance (ANOVA). No data transformations were required. One-way ANOVA was used to test for

differences in soil chemistry between AB and YP. When F -ratios were significant ($p < 0.10$), treatment means were compared via Tukey-Kramer HSD using SAS-JMP (Cary, NC, USA). GelCompareII v.5.10 (Applied Maths NV) was used to normalize and analyze similarities of the bacterial and fungal community DGGE profiles. Normalized DGGE banding patterns were then compared using analysis of similarities (ANO-SIM). Based on the band data matrix extracted from DGGE gels, nonmetric multidimensional scaling (NMDS) was performed with the MDS procedure of the SAS system (SAS/STAT, 1992). The differences between bacterial and fungal community DGGE patterns, individually, were calculated by intensity of similarity and illustrated in two-dimensional NMDS plots. Band patterns with higher similarity were appear closer, and band patterns with the lower similarity were located further apart. In order to evaluate the degree to which the ordination matches the distance matrix, the stress value of NMDS was determined. Stress value (S) < 0.1 indicated a good ordination, with little risk of misinterpreting banding patterns [32].

3. Results

3.1. Soil properties

Soils within 0.5 m of the AB and YP trunks produced several significant differences in soil properties (Table 1). Mean soil pH was approximately 0.13 units lower in AB soils ($p = 0.10$) compared to YP (Table 1). OM content was larger in soils near YP ($p < 0.10$), directly contributing to a 40% increase in total C and N in contrast to soils in the proximal area of AB (Table 1). In the upper 10 cm of mineral soil, ^{13}C differed significantly ($p < 0.01$) between the two tree species (Table 1). ^{15}N enrichment was observed in the YP mineral soil layer, while soils under AB were depleted in ^{15}N (Table 1). In general, mineral soil plant-available nutrients differed between tree species as follows: Al, Cu, and K were significantly greater in soil around

YP, however; Mn and Zn concentrations were greater in soils near AB (Table 1). Finally, no significant differences were observed between the tree species for concentrations of Ca, Fe, Mg, or P (Table 1).

3.2. Soil Moisture Status

A time series of soil volumetric water content (SVWC, in %), spanning a total of 44 individual storm events, within the proximal area of the tree trunk of an American beech and yellow poplar were shown in Figure 2A. The seasonal storm-driven SVWC cycle is divided into three periods (I, II, and III) representing seasonal leaf states: defoliated-to-foliating, foliating-to-foliated, and foliated-to-defoliated, respectively. Each period spans the following Julian days: (PI-2012) 1-114, (PII-2012) 115-210, and (PIII-2011) 211-365. During period I (defoliated-to-foliating), the SVWC status beneath AB and YP canopies remained relatively stable (30 and 25%, respectively) however, open canopy SVWC significantly decreased ($> 30\%$) in early March-2012 (Julian day 62) continuing to first storm event (Julian day 114 [Fig. 2A]). In period II (foliating-to-foliated), a steady decrease in SVWC (%) was measured in all soils (open $>$ YP $>$ AB), yet AB soils remained 5-10% wetter compared to YP canopy influenced soils (Fig. 2A). Through period III (foliated-to-defoliated), a steady increase in SVWC was observed, where SVWC in the YP soils slightly exceeded that of AB soils (Fig. 2A).

In order to evaluate the SVWC response to storm intensity and stages of canopy foliation, we assessed hourly SVWC responses to 3-storm events with differing intensities during three observation periods within each season defoliated-to-foliating (Fig. 2B), foliating-to-foliated (Fig. 2C), and foliated-to-defoliated (Fig. 2D). The initial storm event during period I (i.e. defoliated) increased SVWC (%) in both AB and YP soils however, soils in AB remained 5.0%

wetter > 20 hrs. after rain event (Fig. 2B). During period II (i.e. foliated) an initially low intensity storm later increased its rainfall intensity to 2.4 mm h^{-1} , resulting in a doubling of AB SVWC (16%) compared to YP SVWC (8% [Fig. 2C]). During this same storm event, SVWC remained approximately 8% greater in AB soils, despite a substantial increase in rainfall intensity to 5.4 mm h^{-1} (Fig. 2C), similar to period I results AB soils remained ~ 8.0% wetter than YP soils after storm event ceased. Lastly a high intensity storm event (averaging 4.5 mm h^{-1}) during period III (i.e. foliated-defoliated) increased SVWC in AB and YP soils by 10% and 6%, respectively (Fig. 2D), YP soils remained ~5.0% wetter during this seasonal period compared to AB soils.

3.3. Comparison of AB versus YP SMC structure

Similarity analysis of DGGE images suggested that soils experiencing differing stemflow dynamics maintained moderate (> 50%) similarity across seasons for both the bacterial and fungal communities (Fig. 3A-B). Initial analysis of the YP bacterial dendrogram suggests that the largest variability in bacterial community occurred mainly within spring and summer seasons (Fig. 3A). A similar increase in bacterial community variability was observed within the AB spring samples however, this trend was not detected within either the summer and winter season samples (Fig. 3A). Dendrogram analysis of the fungal community indicates high similarity (> 90%) within each seasonal grouping for both AB and YP soils (Fig. 3B). Similar to the observations of the YP bacterial dendrogram we observed increasing variability within the YP fungal community as a function of season (summer > spring > winter [Fig. 3B]). In the AB fungal community we observed complete clustering of the DGGE band profiles with similarity approaching 90%, especially in soils experiencing the same season, suggesting the community profile is dominated by seasonal effects (Fig. 3B).

To better visualize the similarity or dissimilarity between the different DGGE profiles, NMDS plots were generated using band presence/absence (Fig. 4 and 5). The NMDS analysis of bacterial and fungal seasonal DGGE banding profiles for AB and YP soils produced several two-dimensional plots with low stress value, thereby suggesting good ordination. Bacterial community structures between AB and YP soil were very similar within winter samples (Fig. 4A) however, structural differences increase between AB and YP soils in the spring (Fig. 4B) and summer seasons (Fig. 4C). The NMDS ordination comparing AB and YP fungal communities across all seasons maintained low stress value (Fig. 5A-C) and tight grouping of the AB fungal communities separate from the YP communities suggests distinct differences in AB and YP fungal community structure.

3.4 Seasonal influence effecting AB and YP community

In order to determine if seasonal factors influenced microbial community structure within the near-trunk soils of each tree species, we constructed NMDS plots of AB and YP SMCs of all canopy leaf states for Bacteria (Figs. 4D-E) and Fungi (Figs. 5D-E). The AB bacterial community profile produced a low stress value ($S = 0.07$), and moderate grouping between individual tree communities (Fig. 4D). NMDS results from the YP bacterial profile ($S = 0.10$) suggest larger heterogeneity and greater seasonal influence on YP bacterial communities, specifically between spring and summer (Fig. 4E). Additionally we observed significant changes in the fungal community as a result of seasonal changes (Fig. 5D-E). NMDS results from the AB fungal profile ($S = 0.02$) suggest tight grouping between individual trees that diverge as seasons change (Fig. 5D). Analyses of the YP fungal community profile suggest moderate stress value ($S = 0.14$) which were largely driven by community structural difference during the summer sampling (Fig. 5E).

3.5. Identification of sequenced bacterial and fungal bands

Nine bacterial bands and seven fungal bands were sequenced and identified from the DGGE profiles (Fig. 3). Four bacterial bands (Fig. 3A, Table 2) and two fungal bands (Fig. 3B, Table 3) were common across AB and YP soils regardless of season. The remaining selected bands were unique and well represented within AB and YP profiles. The majority of identified bacterial bands belonged to uncultured bacterial clones (45%), followed by Actinobacteria (33%), then Proteobacteria (22%). BLAST analysis of the bacterial community revealed that two shared bands (Fig. 3A, S1 and S2) were identified as uncultured bacteria similar to the sequences identified in N-amended soil from Harvard forest (Table 2). Shared bacterial band S3 (Fig. 3A) was closely related to *Bradyrhizobium* sp., and shared bacterial band S4 (Fig. 3A) was genetically similar to an uncultured *Actinobacterium* clone (Table 2). Bacterial sequences unique to AB were AB5 (*Burkholderia* sp.) and AB6 (an uncultured endophytic bacterium clone found in Artic soil) (Fig. 3A, Table 2). YP proximal soils hosted several bacterial bands that were unique compared to AB, two bands were identified as *Actinobacterium* sp (YP7 and 9), and one (YP8) was similar to uncultured bacteria clone found in N-amended soil from Harvard forest (Fig. 3A, Table 2). Sequences of all identified fungal bands (Fig. 3B) belonged to the phyla *Basidiomycota* (Table 3). Shared fungal band S1 (Fig. 3B) represented *Russula exalbicans* and band S2 (Fig. 3B) was most closely resembled *Lactarius hygrophoroides*—both ectomycorrhizal fungi of the *Russulales* order. Unique AB fungal sequences included species typically associated with mycorrhizal function (AB4-5) (Table 3). Further BLAST analysis of the fungal community suggests that several sequences (S1-2, AB3-4, and YP-5) were closely related to members of the order *Russulales* (Table 3). The remaining sequences (YP6-7) were identified common

ectomycorrhizal spp. including *Lactarius volemus*. (YP5), *Piloderma fallax* (YP6), and *Hypholoma subviride* (YP7) (Table 3).

Seasonal trends were observed in the DGGE banding profiles of sequenced species. AB bacterial bands AB5, AB6 and all YP-unique bacterial bands, maintained strongest intensity in the summer and weakest in the winter samples (Fig. 3A). In terms of fungi the *Clavulina sp.* band (AB3) was most intense during summer and winter, but light-to-absent during the spring (Fig. 3B). During spring, the *Russula compacta* band (AB4) was strongest across all locations, diminishing to the lowest intensity for two of the three sampling locations during winter (Fig. 3B). All the unique fungal bands for YP were most intense from the summer sampling and least from the winter sampling (Fig. 3B). Band (YP5) showed the smallest variability in band intensity across seasons, while the *Hypholoma subviride* band (YP7) varied the most in intensity across seasons (Fig. 3B). The fungal YP7 band was nearly absent in all winter samples (Fig. 3B).

4. Discussion

4.1. Stemflow influence on soil C and N cycling

It is important to note that C and N dynamics within forest soils are controlled by numerous environmental factors (*e.g.*, moisture, litter quality, pH, and nutrients) affecting SMC decomposition efficiency. Our results indicate that soils receiving greater stemflow production (AB) maintained significantly lower total C and N when compared to limited (YP) stemflow soils (Table 1). Stemflow-related increases in AB soil moisture and nutrient status ([*e.g.*, Mn] Table 1) potentially influenced both physiochemical (*i.e.*, soil aggregation) and biochemical (enzymatic) mechanisms that stabilize soil C and N [33,34]. Physiochemical controls on soil C and N cycling, rapid rewetting pulses observed within AB soils (Fig. 2) have been shown to disrupt soil micro- and macroaggregates, exposing protected C and N to SMCs and

microclimatological conditions favoring rapid mineralization [35]. Moreover, it is possible that increased nutrient leaching observed in AB stemflow soils ([e.g., Al, Cu, K] Table 1) resulted from aggregate disruptions [34]. Soil moisture status plays a significant role in affecting microbial enzymatic function [36,37]. The efficiency of microbially-generated extracellular enzymes to process complex soil C is critical to the cycling and recovery of vital nutrients from litter inputs [36]. Our soil moisture observations suggest that increases in soil moisture in near-trunk AB soils compared to YP, potentially favored a surge in enzyme activity especially during spring months (Fig. 2) leading to significant decreases in AB soil C and N. In support of this, other research efforts suggest that decreases in soil moisture comparable to what was observed in our study (10-15%) can significantly reduce soil enzymes responsible in C (β -glucosidase) and N (protease and urease) cycling [33]. Ushio et al. [38] showed different tree species can exert a significant influence over soil enzyme activity as a result of soil moisture status, and the quality of soil C and N (which differs significantly between AB and YP stemflow at this site [16]). Rapid wetting-drying of AB stemflow soils could cause hydration/dehydration events that release substantial intercellular enzymes into the soil due to microbial biomass turnover temporarily increasing soil C and N pools [39]. Additionally, elevated Mn within AB soils (Table 1) potentially aided the decrease of soil C and N as research suggests that elevated Mn concentrations will favor late stage litter decomposition [40].

Isotopic enrichment analysis of C^{13} and N^{15} provides further insight into differing soil C and N cycling mechanisms. Initially, we hypothesized that soils experiencing increased stemflow volumes (i.e., AB) would maintain greater soil moisture status, in turn decreasing C^{13} and N^{15} enrichment due to greater leaching. Our results support this hypotheses as soils near drier YP trunk were significantly enriched in C^{13} ($P < 0.01$) and N^{15} ($P < 0.09$) compared to wetter AB

soils (Fig. 2). Studies suggest that isotopic enrichment results either from microbial discrimination against heavier isotopes or increased mobility of dissolved organic matter (DOM) with variable isotopic values [41,42]. Observations from our investigation suggest that microbial discrimination did not lead to increase in isotopic enrichment of AB soils since soil OM was significantly lower (Table 1), this finding is supported by several studies suggesting no isotopic enrichment during microbial organic matter decomposition [43,44]. The depletion of C^{13} and N^{15} within AB soil was potentially the result of DOM leaching due to the: (1) high sand content (>50%) limiting sorption of DOM; and (2) increased soil moisture status within soils near AB (Fig. 2). Kaiser et al. [45] proposed that differential sorption and leaching of enriched DOC drives the observed enrichment of C^{13} and N^{15} with depth. Future studies should consider stemflow as a possible mechanism influencing the enrichment of C^{13} and N^{15} within subsoils.

4.2. Near-trunk soil microbial community structure varies with stemflow

Stemflow potentially effects the chemistry (Ca, K, P, pH) (Table 1), moisture content (Fig. 2) and physical complexity of the soil environment [46] within the trunk (< 0.5 m) of individual trees. However, SMC structure has not yet been linked to species specific differences in stemflow production and or changes in soil chemistry. Research at Fair Hill NRMA site has assessed differences in solute fluxes due to stemflow generation between AB and YP [22]. AB stemflow flux can represent ~15% of rainfall, which is considerably elevated compared to a maximum YP stemflow flux of ~5%. Compared to YP, AB produces greater soil moisture surpluses via stemflow (Fig. 2B-D) that quickly enter the soil draining along subsurface root preferential flow pathways [e.g., 11]. Thus, significant alterations to near-trunk soil moisture dynamics (Fig. 2) and nutrient status (Table 1) by interspecific differences in stemflow supply/quality at this site [21,22] appear to be a possible mechanism affecting SMC structure

(Figs. 3-5). Soils surrounding YP trunks hosted a greater number of bacterial and fungal bands (Fig. 3). Sequenced bands suggest greater variability of bacterial species in near-trunk YP soils (Table 2), possibly driving the dissimilarity of DGGE banding patterns (Figs. 3A-B). Greater SMC variability in YP near-trunk soils may be due to YP's rougher-bark producing less frequent and lower magnitude stemflow than AB [16,22]. Studies show high spatiotemporal resource supply/quality (particularly moisture, pH, salinization, and nutrient constituency) can increase bacterial and fungal community variability [e.g., 47,48] thus varying selection pressures and dispersal limitations [49,50]. The higher TC, TN, and C:N observed in near-trunk YP soils may contribute to differences in community structure, as these have been linked to presence and abundance of many microbial clades (e.g., *Alpha*- and *Gammaproteobacteria*, *Actinobacteria*, gram-positive Bacteria, and saprophytic fungi) in other forest soils [51].

Sequenced bands lend support to the hypothesis that stemflow's influence over soil moisture and nutrient status is a possible driver of differing SMC structure (Tables 2-3). For example, unique band AB5 was identified as *Burkholderia* sp. (Table 2), which is known for their ability to release iron from biotite [52], perhaps explaining the higher soil Fe values in soils receiving AB stemflow (Table 1). Band YP7 was identified as a *Mycobacterium* sp., which prefer soils comparable to YP stemflow-affected soils: high in OM and low in pH [53]. Furthermore, *Mycobacterium* spp. belong to the phylum *Actinobacteria* which are intolerant to high soil moisture conditions such as those found in AB soils [54]. Within AB stemflow-affected soils, two unique fungal bands were identified (AB3-AB4) associated with *Clavulina* sp. and *R. compacta* respectively (Table 3), both have been found to prefer soil moisture and pH conditions comparable to AB soils [55]. *Clavulina* spp. have also been recognized as saprophytic [56],

which lend support to soil C results indicating greater turnover of C and N in AB soils (Table 1; Fig. 2).

4.3. Seasonal variation in SMC structure

The paucity of investigations studying the effect of tree species on SMC structure through seasonal cycles represents a significant knowledge gap [57]. However, the few studies examining seasonal SMC structural trends yield dynamic profiles of the bacterial and fungal community, greatly illuminating our understanding of the mechanisms influencing dominant and minor SMC members [e.g., 58,59]. Studies suggest that significant SMC structural shifts are initiated by seasonal fluctuations in soil moisture and temperature associated with tree metabolic activity (dormancy v. vegetative growth) [57,60]. To investigate seasonal variability in SMC structure for AB and YP we selected PCR-DGGE knowingly understanding this method's limitations: *i*) only dominant SMC members are detected, *ii*) bias of SMC structures due to primer selection and PCR, and *iii*) same bacteria/fungi may produce multiple bands [26]. To address these limitations, we analyzed multiple gels under varying operating conditions, used multiple primer systems (i.e., fungal DGGE), and excised and sequenced several gene fragments. Nevertheless, PCR-DGGE still provides only a “snapshot” of SMC structural dynamics in this study and next generation sequencing is recommended in future investigations to explore more detailed SMC structure.

NMDS plots comparing bacterial (Fig. 4A-C) and fungal (Fig. 5A-C) SMCs between AB and YP supported our second hypothesis: that different tree species will harbor dissimilar SMC's regardless of seasonal influence. The wide spread across seasonal YP bacterial and fungal SMCs (Figs. 4E and 5E) suggest that their banding patterns were tightly linked to seasonal changes rather than tree specific factors (i.e., stemflow generation), specifically diminished moisture

reaching soils as rainfall is prevented from becoming stemflow by increasing canopy cover [20]. Conversely, AB bacterial, and especially fungal, SMCs clustered distinctly across sampled trees for all seasons (Figs. 4D and 5D) suggesting a tree-mediated effect, possibly stemflow, influencing seasonal SMC structure. Another comparison from the sequencing results further highlights the differences between YP and AB: bacterial bands closely identifying with an uncultured *Actinobacteria* clone (S4 [Fig. 3A, Table 2]) and *Mycobacterium* sp. (YP7 [Fig. 3A, Table 2]) showed no seasonal effect in YP soils; however, within AB soils the band intensity diminished in the winter months (Fig. 3A). Stemflow may account for this tree-mediated effect on seasonal SMC structure as strong seasonal shifts in stemflow water and solute supply have been observed at this site [20,22]. During winter months, increased stemflow from AB [22] produces wetter soils compared to YP (Fig. 2), potentially limiting the growth of these slow-growing, strict aerobic bacteria [54]. Canopy phenological alterations further alter stemflow volume and chemistry via physiological controls (e.g., leaf out and nutrient retention) which could combine with seasonal temperature and moisture changes to further alter SMC structure [11].

5. Conclusions

Our results suggest that stemflow potentially alters several edaphic conditions near individual tree trunks, additionally temporal soil moisture trends, soil pH, and mineral plant nutrients (Al, Cu, K, Mn) appear to vary in response to species specific stemflow yield as well. This, in turn, may explain the SMC structural differences in the near-trunk soils of tree species whose canopy architecture produces differing stemflow quantity and chemical quality. Specifically, SMC diversity (particularly bacterial) may increase in the proximal areas of tree species where stemflow inputs are more variable (i.e., the rougher barked, thinner YP canopy

structure) compared to spatiotemporally consistent pulse like stemflow application during storms (produced by thin-barked, thick AB canopy structure). Soil chemistry and microbial community differences between species' near-trunk soils also suggest the potential for stemflow processes to influence soil pH, and therefore nutrient status. The decreased enrichment of ^{13}C and ^{15}N in AB soils suggests that the soil moisture pulses produced by AB may have increased SOM turnover via aggregate disruption and microbial enzymatic upregulation. Seasonal patterns in DGGE banding patterns and intensity further suggest that as stemflow yield changes with canopy phenology and meteorological conditions, specific microbial clades or species (*e.g.*, ectomycorrhizal and saprophytic fungi, or Proteobacteria and Actinobacteria) appear to shift in relation to different stemflow fluxes. Future work should further clarify the intricate interrelationships among stemflow, soil moisture, and detailed composition of microbial communities in soils near tree stems.

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Figure Captions

Fig. 1: Diagram of the experimental sampling protocol used to determine tree species and seasonal influences on microbial community structure. Bulk soil was sampled at the base of three tree subjects' American beech (AB) and yellow poplar (YP). DNA was extracted from each soil sample and pooled prior to PCR amplification; pooled PCR products were then subjected to DGGE analysis in order to determine composition of the bacterial and fungal community.

Fig. 2: (A) American beech (AB) and yellow poplar (YP) daily soil moisture content (%) assessed by DECAGON 5TM probes from Sept. 2011 (PIII Julian days 211-365) – Aug. 2012 (PI & PII Julian days 1-210), including daily rainfall (mm day⁻¹) and open canopy soil moisture (%) determined by Delaware Environmental Observing System (DEOS). (B-D) Hourly soil moisture response (%) to individual rainfall events representing the three temporal points of seasonal soil collection. Hourly soil moisture was evaluated 12 h prior to and 24 h post rainfall event in order to determine soil response to rainfall under different canopy structures.

Fig. 3: Analysis of similarity dendrogram depicting community % similarity and denaturant gradient gel electrophoresis banding patterns for (A) bacterial 16S rRNA, (B) fungal 18S rRNA from both American beech (AB) and yellow poplar (YP) stemflow-influenced soils. Gray bar indicates the standard deviations between each comparison. Bold-numbered bands are those excised and sequenced.

Fig. 4: Nonmetric multidimensional scaling (NMDS) ordination comparing American beech (AB) and yellow poplar (YP) bacterial DGGE banding profiles isolated across 3-sampling periods (A-C). Plots (D-E) evaluate seasonal influences of the bacterial community per individual tree species. Symbols of the same color indicate samples selected from same tree (i.e., Blue = tree 1, Red = tree 2, and Green = tree 3).

Fig. 5: Comparison of nonmetric multidimensional scaling (NMDS) ordination for (A-B) fungal DGGE banding profiles isolated from American beech (AB) and yellow poplar (YP) across all seasons. Plots (D-E) assess seasonal influences on the fungal community per individual tree species. Symbols of the same color indicate samples selected from same tree (i.e. Blue = tree 1, Red = tree 2, and Green = tree 3)

Table 1: Summary of Soil Properties within the Proximal Area (< 0.5m) of Both American beech and yellow poplar Trees

Soil parameter	American Beech (AB)	yellow Poplar (YP)	F	P
pH ^a	3.81 (0.09)	3.94 (0.02)	2.38	0.10
OM (%) ^b	4.98 (0.98)	7.10 (1.20)	3.55	0.05
TC (%) ^c	2.23(0.45)	3.85 (0.44)	5.56	0.03
TN (%) ^c	0.14 (0.04)	0.22 (0.03)	5.41	0.03
C/N	13.6 (0.03)	17.5 (0.02)	6.11	0.03
C ₁₃ ^d	-27.8 (0.15)	-27.2 (0.11)	9.50	0.01
N ₁₅ ^d	-0.06 (0.37)	0.75 (0.28)	3.40	0.09
Al (mg kg ⁻¹) ^e	445.0 (11.6)	533.0 (34.1)	7.50	0.02
Cu (mg kg ⁻¹) ^e	0.60 (0.02)	0.73 (0.06)	3.40	0.08
K (mg kg ⁻¹) ^e	69.7 (4.13)	82.9 (3.90)	4.00	0.01
Mn (mg kg ⁻¹) ^e	10.7 (1.13)	7.12 (1.23)	3.89	0.08
Zn (mg kg ⁻¹) ^e	1.98 (0.20)	1.20 (0.12)	9.75	0.01
Ca (mg kg ⁻¹) ^e	43.0 (4.90)	41.7 (4.50)	0.03	0.90
Fe (mg kg ⁻¹) ^e	54.3 (7.78)	46.3 (5.10)	0.78	0.40
Mg (mg kg ⁻¹) ^e	41.0 (5.00)	41.6 (4.74)	0.01	0.90
P (mg kg ⁻¹) ^e	6.60 (0.50)	7.20 (0.44)	0.76	0.40

^a(1:1 v:v) measured in deionized water-Accumet pH meter

^b(LOI) loss on ignition-Blue-M High Temperature Furnace

^cmeasured by combustion using an Elementar Vario Max CN Analyzer

^dEA-IRMS, CE Instruments NC2500 EA and Thermo-Finnigan Delta Plus XL IRMS

^esoil extracted using the Mehlich-1and analyzed for plant available nutrients via ICP-OES

Table 2: Identification of selected 16S rRNA fragments generated from excised bands of DGGE profiles of yellow poplar and American beech soils

ID	Most closely related organism (based on partial 16S rRNA gene)	Identity (%)	Accession no.	Phylogenetic group	Source
Band 1 (Shared)	Uncultured bacterium clone	97	JQ057666	N/A	N-amended soil Harvard Forest
Band 2 (Shared)	Uncultured bacterium clone	98	JQ058784	N/A	N-amended soil Harvard Forest
Band 3 (Shared)	<i>Bradyrhizobium</i> sp.	97	HE584529	Protoebacteria	Legume Root
Band 4 (Shared)	<i>Actinoalbmurus</i> sp.	92	KF226038	Actinobacteria	Biochar amended soil
Band 5 (AB)	<i>Burkholderia</i> sp.	97	DQ777734	Proteobacteria	PCB contaminated soil
Band 6 (AB)	<i>Uncultured Endophytic bacterium clone</i>	99	HE815201	N/A	Artic soil
Band 7 (YP)	<i>Mycobacterium</i> sp.	96	DQ986506	Actinobacteria	Clinical samples
Band 8 (YP)	Uncultured bacterium clone	97	JQ053209	N/A	N-amended soil Harvard Forest
Band 9 (YP)	<i>Actinobacterium</i> sp.	99	FJ182141	Actinobacteria	Rock samples

Table 3: Table 2: Identification of selected 18S rRNA fragments generated from excised bands of DGGE profiles of yellow poplar and American beech soils

ID	Most closely related organism (based on partial 18S rRNA gene)	Identity (%)	Accession no.	Phylogentic group	Source
Band 1 (Shared)	<i>Russula exalbicans</i>	99	AY293156	Basidiomycota	N/A
Band 2 (Shared)	<i>Lactarius hygrophoroides</i>	99	JN940335	Basidiomycota	Soil (Great Smokey Mt National Park)
Band 3 (AB)	<i>Clavulina sp.</i>	99	AY757265	Basidiomycota	N/A
Band 4 (AB)	<i>Russula compacta</i>	99	AF026582	Basidiomycota	NA
Band 5 (YP)	<i>Lactarius volemus</i>	99	JN940346	Basidiomycota	N/A
Band 6 (YP)	<i>Piloderma fallax</i>	99	GU187644	Basidiomycota	N/A
Band 7 (YP)	<i>Hypholoma subviride</i>	99	HQ832424	Basidiomycota	Soil Great Smokey Mt National Park

Figures

Figure: 1 Experimental Sampling Protocol for Analysis for Soil Microbial Community Structure within the Proximal Area of American beech (AB) and yellow poplar (YP).

Figure 2

Figure 3

A)

YP-Spring-2
YP-Summer-2
YP-Spring-3
YP-Summer-1
YP-Summer-3
AB-Spring-2
AB-Spring-3
YP-Winter-1
YP-Winter-2
YP-Winter-3
AB-Winter-1
AB-Winter-2
AB-Winter-3
AB-Spring-1
AB-Summer-1
AB-Summer-3
AB-Summer-2

B)

YP-Summer-2
YP-Winter-2
YP-Winter-3
AB-Summer-1
AB-Summer-2
AB-Summer-3
YP-Spring-1
YP-Summer-3
AB-Spring-2
AB-Spring-3
AB-Spring-1
AB-Winter-2
AB-Winter-3
AB-Winter-1
YP-Spring-2
YP-Spring-3
YP-Summer-1

Figure 4

Figure 5