

Soil bacterial diversity based on management and topography in a silvopastoral system

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Abbreviations: PLS, pure live seed; AU, animal units; ANOVA, analysis of variance; LSD, least significant difference; SOM, soil organic matter; OTU, operational taxonomic unit; PERMANOVA, permutational analysis of variance; PCoA, principal coordinate analysis; PCA, principal component analysis; VWC, volumetric water content; TFUs, topographic functional units; LiDAR, light detection and ranging.

Abstract

Soil microorganisms play crucial roles in nutrient cycling and provisioning ecosystem services. However, little is known about how soil microbial communities are affected by soil management and landscape position in silvopastures. The current study aimed to understand effects of forage species [non-native, cool season orchardgrass (*Dactylis glomerata* L.) and a warm-season native grass mix (*Andropogon gerardii* L. and *Schizachyrium scoparium* L.) planted in strips between hedgerows], soil fertility (poultry litter and a control), and soil moisture regime (*aquic* and *udic*) on soil bacterial communities in a factorially arranged design with 3 replications; and, to evaluate linkages between terrain attributes and soil bacterial assemblages. Thirteen terrain attributes representing topographic variability were clustered into 4 topographic functional units (TFUs) using the k-means method, and their impact on soil microbial diversity was evaluated. Illumina sequencing results identified a soil moisture regime x forage species interaction, with native grass species under wet (*aquic*) conditions resulting in the most diverse microbial assemblages relative to dry (*udic*) and wet soil conditions for the non-native forage (orchardgrass). These results suggest an enhanced soil microbial diversity under native grasses with greater available soil water. Overall, microbial diversity was negatively correlated with elevation, suggesting niche differentiation and microbial preference for lower elevations. Overall, TFUs and selected terrain attributes may be useful for predicting microbiota dynamics in integrated tree-livestock systems.

Key words: soil microbial diversity; forage systems; poultry litter; terrain attributes; metagenomics; soil moisture.

1. Introduction

The importance of soil microorganisms are gaining attention, particularly in agricultural systems, as they interact with the mineral surface to govern availability of plant nutrients (Zhu et al., 2016) and overall ecosystem balance (Fierer, 2017). Although they have other unknown ecological attributes, they can be identified with state-of-the-art genomic approaches and manipulated or managed to increase ecosystem services (Sathya et al., 2017) and plant productivity (Fierer, 2017). In agroecosystems, different management practices could change soil microbial structure. For instance, increased aboveground diversity may bequest belowground ecosystems with greater species diversity that mitigates risk (Zak et al., 2003) and enhances climatic resiliency (Naeem, 1998). Unlike sole pastoral systems, silvopastoral systems are endowed with all these attributes.

Silvopasture, an agroforestry management practice that integrates trees and animal production under one system, is widely practiced in North America (Orefice and Carroll, 2017). It is generally considered a sustainable livestock production system (Jose and Dollinger, 2019) as it can address the three pillars of sustainability: planet, people, and profit (Tedeschi et al., 2015). It is also often more profitable (Broom et al., 2013) and preferable for improving forage quality (Ford et al., 2019; Jose and Dollinger, 2019; Neel et al., 2016) and minimizing cattle heat stress during hot summer months (Kay et al., 2018) than sole pastures. Apart from the system itself, management activities within, including selection of appropriate tree (Broom et al., 2013) and grass species (Jose, 2009), can improve grazing system (Xu et al., 2017), while the use of manure and/or fertilizer (Blazier et al., 2008; Lindgren and Sullivan, 2014) plays a crucial role in improving the net productivity of silvopasture. Such management practices improve productivity by enhancing CO₂ assimilation and improving nutrient availability (Lindgren and Sullivan,

2014). The latter is regulated by soil biophysical processes where soil microbes play an essential role. On the other hand, microbial composition and community structure, in turn, are influenced by soil nutrient status and ecological associations with plant roots (Zhang et al., 2019).

The effect of silvopasture system on soil microbial activity, diversity, and abundance was previously studied and found to have advantages over sole pasture systems (Barros et al., 2018; Cubillos et al., 2016; Vallejo et al., 2012, 2010). The role of pasture manure applications on soil bacterial diversity was also previously evaluated and indicated that an increased animal manure distribution results in enhanced microbial diversity (Yang et al., 2019). A study by Zhao et al. (2015) revealed that fertilizer applications and forage species altered microbial structure. Moreover, soil pH, C/N ratio, and available P were reported to be drivers of abundance of some specific groups of bacteria in soils (Hermans et al., 2017; Kaiser et al., 2016). Soil bacterial community structure also responds to changes to local topography that can be represented by terrain attributes, the elevation reportedly being the most strongly correlated with soil microbial richness and diversity (Peng et al., 2020; Singh et al., 2014; Yin and Yan, 2020). Further, recent studies revealed that microbial community structure showed strong correlation with soil moisture regime, temperature, pH, and P content along the elevation (Peng et al., 2020; Shen et al., 2019; Singh et al., 2014; Yin and Yan, 2020; Zhang et al., 2019).

Despite knowledge on the influence of management on soil microbial community, evidence on the effects of poultry litter application, soil moisture regime, grass species, and their interaction with terrain attributes on diversity and abundance of soil bacterial community are lacking. Specifically, Adhikari et al. (2018) identified topographic influences on soil nutrient distribution, however, studies evaluating microbial abundance linkages with terrain attributes are few. Therefore, the current study is aimed to i) evaluate bacterial diversity and phylogenetic

abundance in response to forage species, poultry litter applications, and soil moisture regime, and to ii) understand the interaction of these practices with terrain attributes in a silvopastoral system characterized by soils with a fine texture and neutral pH.

2. Materials and Methods

2.1. Site description

This study was conducted in a 4.25-ha paddock located at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, AR (36.09°N, 94.19°W). The site is located in the Ozark Highlands, Major Land Resource Area 116A (Soil Survey Staff, 2019a). Information on previous site history is described by Sauer et al. (2015). Briefly, soil in most of the experimental area is mapped as Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudult) with some Pickwick silt loam (fine-silty, mixed, semiactive, thermic Typic Paleudult) and small areas of Johnsburg silt loam (fine-silty, mixed, active, mesic Aquic Fragiudult), and Nixa cherty silt loam (loamy-skeletal, siliceous, active, mesic Glossic Fragiudults) soils (Soil Survey Staff, 2019b).

Adhikari et al. (2018) provides details about the derivation of terrain attributes, and topographic functional units (TFUs) in the study area and details on their derivation are provided in section 2.7. Briefly, TFUs are derived using terrain attributes where the individual units are more homogeneous in terms of terrain properties and behave as a single functional unit within a landscape and can be used to describe functional behavior or soils. Based on terrain attributes, the study site could be divided into four TFUs, namely A, B, C, and D; TFU A had the highest nutrients present, whereas TFU B had the lowest P, K, Zn, Cu, Fe, and Ca but highest Na content. However, Mn, Mg, and B did not vary among TFUs (Adhikari et al., 2018).

Topographic functional units are the landscape units that show more homogenous terrain properties within and less homogeneous properties between the units in terms of soil-terrain relationship (Adhikari et al., 2018). The wetter location within the study site has a fine, mixed, active, thermic Typic Endoaqualf and *aquic* soil moisture regime (virtually free of dissolved oxygen because it is saturated by ground water or by water of the capillary fringe), whereas the better drained soils have an *udic* soil moisture regime (classified as no evidence of saturation or reduction within 50 cm of the surface; Soil Survey Staff, 1999c). The site has a mean (30-yr mean) annual precipitation of 1,232 mm and a mean annual air temperature of 14.5°C (NCDC, 2019a, 2019b). The mean and normal temperature and daily precipitation of the study area from January 2018 to July 2019 is reported in Supplementary Fig. 1.

2.2. Tree hedgerow and grass strip management

The hedgerows layout and the soil types of the experimental site were mapped and well described by Sauer et al. (2015) and Adhikari et al. (2018). In the year 2000, hedgerows of a total of fifteen rows of three species, namely, northern red oak (*Quercus rubra* L.), eastern black walnut (*Juglans nigra* L.), and pecan (*Carya illinoensis* Wangenh K. Koch) were established. Each species had five rows, and the rows were oriented east-west at 15-m spacing (Sauer et al., 2015). In 2014, the eastern black walnut trees rows were replaced with rows containing three species: American sycamore (*Platanus occidentalis* L.), cottonwood (*Populus deltoides* W. Bartram ex Marshall), and pitch/loblolly pine (*Pinus rigida* x *Pinus taeda*).

Two forage species treatments were seeded in between the tree rows, and the experimental unit was randomly allocated to grass species and soil type. The grass treatments were: 1) a cool-season orchardgrass (*Dactylis glomerata* L., var. Tekapo), and 2) a mix of native warm-season grasses (*Andropogon gerardii* Vitman and *Sorghastrum nutans* L.) in 8:1 ratio. The

orchardgrass was planted fall 2015 at 17 kg pure live seed (PLS) ha⁻¹, whereas the mix species were seeded spring in 2016 at 10 kg PLS ha⁻¹. Grasses were planted with a Haybuster 107C no-till drill (DuraTech, Jamestown, ND). Prior to establishment, Cornerstone[®] Plus (N-[phosphonomethyl] glycine) was used to kill existing vegetation at a 2.2 kg ha⁻¹ rate (41% a.i.). Heifers (*Bos taurus* L.) grazed the site at a rate of 2.20 animal units (AU) ha⁻¹ from May 24 to July 6 in 2018, and at rate of 2.42 AU ha⁻¹ grazed the site from May 29 to July 11, 2019.

2.3. Treatment implementation and field management

Treatments included moisture regime (*udic* and *aquic*), forage species (native grass mixture and non-native orchardgrass) and fertility (poultry litter applied and not applied), set up in a factorial design. The primary treatment (forage species) was implemented as described above with three replications. The second main effect (fertility) was implemented on orchardgrass and native grass receiving locally sourced poultry litter and applied at a rate of 84 kg N ha⁻¹ on March 21, 2018 and April 12, 2019 (fresh weight basis). Poultry litter in 2018 had a pH of 6.2 and contained 1.98% N, 0.58% P, and 1.02% K on dry basis, while that in 2019 had a pH of 5.2 and contained 2.48% N, 0.69% P, and 0.94% K. The third main effect (soil moisture regime) was determined by random placement of volumetric water content (VWC) TEROS 11 sensors (METER Group, Pullman, WA) at two soil depths (15 and 60-75 cm). Water content measurements were recorded every 4 h and logged on a Decagon EM50 data logger (METER Group, Pullman, WA) throughout the experimental period from May to July in 2018-2019. Soil moisture data were averaged each day and expressed as daily mean volumetric water content for further analysis (Supplementary Fig. 2). Weather variables were measured by a micro-meteorological weather station approximately 500 m away from the experimental site.

2.4. Soil sample collection and analysis

Per replicate, four soil samples (per species, fertility, and soil moisture regime) were collected by auger in triplicate on March 6, 2018 and May 17, 2019 from the Ap horizon (0 to 15 cm) in the center of grass alleys between two hedge rows (experimental unit). The sample points were georeferenced. To prevent contamination, each soil sample was taken using an auger sterilized with 70% ethanol between experimental units. Samples from each treatment combination (species, fertility, and soil moisture regime) and topographic position (depression and top slope) were collected and stored in a cooler for transport to the laboratory, where they were stored at -20°C until DNA extraction.

After removing plant materials manually, soil samples were dried in a forced-air oven at 70°C for 48 hours. Dried samples were then ground and sieved to pass a 2-mm mesh. The pH was determined potentiometrically in deionised water (1:2.5 solid:liquid ratio). Weight-loss-on-ignition was used to determine soil organic matter (SOM) concentration after 2 hrs at 360°C (Schulte and Hopkins, 2015). Total C and N were determined via combustion using a VarioMax CN analyzer (Elementar Americas, Mt. Laurel, NJ). Mehlich-3 extractable soil nutrients were determined using a 1:10 solid: liquid ratio (w:v) (Tucker, 1992) and analyzed by inductively coupled argon-plasma spectrometry (ICP, Agilent Technologies, Santa Clara, CA).

2.5. DNA extraction and Illumina sequencing

DNA was extracted from each soil sample using the extraction kit of MpBio FastDNA Spin Kit for Soil (MpBio Laboratories, SKU 116560200-CF) according to the manufacturer's directions. Extracted DNA was quantified using Quant-It™ PicoGreen® (Invitrogen) dsDNA quantitation assay and stored at -20°C.

Bacterial community composition was determined using Illumina Miseq sequencing of 16S rRNA gene amplicons. Extracted DNA was sent to the University of Tennessee Genomic Services Laboratory, where the V4 region of the 16S rRNA gene was amplified with barcoded primers 515F and 806R (Caporaso et al., 2011). Amplicon libraries were pooled, and 291 base-paired end sequences were obtained on the Illumina MiSeq Platform, resulting in a total of 4,196,620 sequence reads. Reads were processed using the open source bioinformatics software Mothur V 1.40.0 following the Miseq SOP protocol (Kozich et al., 2013). Sequences that did not match the primers were eliminated from demultiplexed sequence reads. Ambiguous base sequences with a length less than 100 bp were deleted and chimeric sequences were removed using the UCHIME algorithm implemented in Mothur. After the quality control pipeline, 3,321,000 sequence reads remained using a 97% similarity threshold to define ribotypes in Mothur (7.91% were deleted).

2.6. Estimation of diversity and evenness indices

The greengenes database was used to classify the operational taxonomic unit (OTU) at the genus level using the Bayesian method (Cole et al., 2014); thereafter, relative abundance of all OTUs were summed within phylum and analyzed for relative abundance at both phylum and OTU levels. Except for the analysis of bacterial community structure, all other analyses were conducted based on OTU level. Based on this subsampled dataset, richness was calculated using the Chao index (Hughes et al., 2001) and diversity was calculated using the indexes of Simpson and Shannon from Mothur output files (Schloss et al., 2009). Beta-diversity was measured by using Bray-Curtis index, weighted and unweighted UniFrac distance metrics (Schroeder and Jenkins, 2018).

2.7. Topographic evaluation on microbial properties

The relationship between topography and soil microbial properties was explored using Pearson's correlation analysis. Topographic information was provided with terrain attributes derived from a digital elevation model compiled from light detection and ranging (LiDAR). A 1-m grid digital elevation model was downloaded from the USDA-Geospatial Data Gateway site (<https://datagateway.nrcs.usda.gov>) and down sampled to 10 m resolution for developing the 13 terrain attributes: altitude above channel network, aspect, elevation, flow accumulation, mid-slope position, multi-resolution ridge top flatness index, multi-resolution valley bottom flatness index, normalized height, wetness index, slope percent, slope height, slope-length factor, and valley depth in SAGA GIS environment (Conrad et al., 2015). These terrain attributes represent topographic landscape variability and are related to water flow and distribution. Altitude above channel network measures the vertical distance of a point to the nearest channel. Elevation represents land surface elevation above mean sea level and its normalized value is the normalized height. Flow accumulation gives the number of upland pixels draining to a given raster, whereas wetness index determines a potential of a pixel to retain moisture. Similarly, valley depth determines the relative height difference to the immediate adjacent channel network, whereas the difference to the crest lines is the slope height. Slope percent and slope-length factor show a maximum rate of change between pixels and neighbors, and the length of the slope is calculated per the universal soil loss equation. Aspect shows the direction of the steepest angle from the north direction. Multi-resolution ridge top and valley bottom flatness index identifies high and depositional areas in the landscape, respectively (Guo et al., 2019). Once the terrain attributes were derived, the raster values at soil sample locations were extracted

and used to calculate Pearson's correlation coefficient between bacterial properties (richness and diversity, 2018-2019) and terrain attributes at an alpha level of 0.1.

To characterize functional relationships between topography and bacterial properties, the study area was divided into functional zones, topographic functional units, which in a previous study of Adhikari et al. (2018) showed similar terrain functional properties in terms of moisture and energy flow and distribution across the landscape. First, the terrain attributes were converted into principal components or factors, and the factors with eigen value >1 were used as inputs to k-means clustering technique to derive TFUs that divided the study area into 4 TFUs (MacQueen, 1967). Principal component analysis of 13 terrain attributes provided 7 principal components or factors without losing much information in the data, and they were clustered into 2 to 10 potential clusters using JMP software (SAS Institute, 2016). To identify the optimum number of clusters, a cubic clustering criterion was calculated for each cluster and the one with the highest value was identified as an optimum cluster representing the TFUs in the study area. Detail statistical procedures followed in the derivation of TFUs in the study area including principal component analysis and k-means clustering techniques and are provided in Adhikari et al., 2018. The TFUs were named A, B, C, and D in which A TFU represents an accumulation zone where soils potentially tend to remain moist due to the higher values of wetness index, valley depth, and flow accumulation (aquic). Similarly, B TFU is an area with higher elevation and slope, but with lower values of wetness index, valley depth, and flow accumulation, indicating the dryer part of the study area (udic). While the TFU C and D are intermediate in terms of moisture retention compared to TFU A and TFU B, TFU D tends to remain slightly drier compared to TFU C. Once the TFUs were identified, the distribution of richness and diversity among TFUs were compared using student's t-test for their significant difference. The

TFUs were grouped into *aquic* and *udic* soil moisture regimes for purposes of statistical analyses assuming the differences in water dynamics would affect the microbial responses.

2.8. Statistical analysis

Data on soil physiochemical properties were analyzed with a Mixed Model (V9.4; SAS Institute, Cary, NC) consisting of the random effects of replication and year, with fixed effects being forage species, fertility, and soil moisture regime. When main effect differences were found, pair-wise post hoc comparisons were performed by the SAS macro ‘pdmix800’ (Saxton, 1998); with Fisher’s least significant difference (LSD) at a Type I error rate of 5% (SAS Institute Inc, 2009).

To evaluate differences in microbial diversity and evenness, ANOVA was carried using the statistical software R 3.5.1 (R Core Team, 2012) and JMP R 12 (SAS Institute Inc, 2015). Principal coordinate analysis (PCoA) plots were generated based on weighted and unweighted UniFrac distance metrics using MicrobiomeAnalyst (Dhariwal et al., 2017). Bacterial community structure quantified in a matrix of Bray-Curtis similarities was analyzed in a permutational analysis of variance (PERMANOVA) to compare bacterial communities at the OTU level in PRIMER-E (Clarke and Gorley, 2006).

3. Results and Discussion

3.1. Soil physiochemical variation based on treatments

Soil P was greater ($P<0.05$) under the fertilized native warm-season grass mix compared to fertilized or unfertilized orchardgrass but did not differ from the unfertilized native grass mix. Similarly, the greatest soil P and K occurred under the dry (*udic*) native grass mix (Table 1), likely owing to less loss due to overland flow and plant P and K uptake in drier areas.

Concentrations of K and Mg were also greater under native grass species regardless of poultry litter application. In contrast, there were no three-way (forage species × poultry litter fertility treatment × soil moisture regime) interactions for any soil physiochemical properties ($P>0.05$; Table 1). However, there was a two-way interaction for soil P and K (species × fertility; species × moisture regime). In addition to the absence of grass species effects, neither the soil moisture regime nor poultry litter impacted soil C and N concentrations. Similar findings were also reported previously by Bloor (2015) who evaluated the effects of manure amendments under different grass species and found little impact on soil N and C. This could be associated with cattle manure deposition across the site masking any treatment-induced changes in silvopastoral systems.

3.2. Bacterial community composition based on fertility, moisture regime, and forage species

The PERMANOVA test on the Bray-Curtis dissimilarity showed differences in soil bacterial community structure at the phylum level based on the interaction of moisture regime and forage species ($P<0.05$); however, there were no differences in bacterial communities based on the single factors of manure amendment, soil moisture regime, and forage species [$(P>0.05)$; (Table 2)]. The following top ten phyla dominated soil bacterial communities: Proteobacteria (mean relative abundance of all libraries was Proteobacteria (37%), Acidobacteria (25.53%), Actinobacteria (12.07%), Verrucomicrobia (7.65%), Chloroflexi (5.50%), Bacterioidetes (4.37%), Firmicutes (2.58%), Nitrospirae (2.09%), Planctomycetes (1.64%), and Gemmatimonadetes (1.56%, Fig. 1A). Even though the differences in terms of the relative abundance, for instance Proteobacteria, can be comparable across the treatments, the order in the level of composition is similar. The top 20 soil bacterial communities at the OTU level are presented in Fig. 1B.

The findings by Estendorfer et al. (2017) on bacterial relative abundance under orchardgrass were consistent with the current findings. Protobacteria generally dominate the soil bacterial community under grasslands (Arenz et al., 2014; Singh et al., 2007; Spain et al., 2009), but the level of relative abundance of a bacterial phylum can be affected by soil management practices, which was also evident in this study (Fig. 1). In the current study, the interaction of *aquic* soil moisture regime and native grasses mix increased the relative abundance of protoebacteria compared to other treatments. However, whole community evenness and diversity, expressed with Simpson's and Shannon indices, respectively, were reduced (Table 3). On the other hand, it is interesting that these diversity indices were greater in *aquic* than *udic* soils under orchardgrass pastures, implying greater microbial preference to wet soil moisture regimes for these systems.

3.3. Bacterial community alpha diversity based on fertility, moisture regime, and forage species

Alpha diversity was not influenced by fertility (poultry litter vs. the control), moisture regime (*udic* vs. *aquic*), and forage species (native vs. non-native) as estimated by three different algorithms (Chao1, Shannon, and Simpson's index) (Table 3). It was hypothesized that poultry litter applications would drive soil microbial community abundance, although this was rejected, given fertility did not impact community structure ($P>0.05$). This was likely due to cattle grazing in silvopastures masking any fertilizer effect from poultry litter. The Chao index was used to calculate the richness of soil bacterial community in this study. There was no influence from individual main effects, as well as for two and three-way interactions of these effects. The Simpson's index was used to calculate the evenness of soil bacterial community in this study. This result indicates that the moisture regime x forage species interaction affected bacterial community evenness ($P<0.05$). Fig. 2A illustrates the influence of moisture regime and forage

species on soil bacterial richness using Chao1; Fig. 2B shows bacterial evenness (calculated using Simpson index), and Fig. 2C presents the bacterial diversity (via Shannon index). The Shannon index was used to measure community diversity, including both richness and evenness. Overall, moisture regime x forage species interacted to affect bacterial community diversity ($P < 0.05$).

Richness, evenness, and diversity indices illustrate the alpha diversity of bacterial community in response to changes in edaphic or anthropogenic factors. Unlike orchardgrass, the native grass mix was less effective in maintaining evenness and diversity of alpha diversity in *aquic* soils. Moreover, the variability of these diversity indices within a treatment (Fig. 1) shows some bacterial phyla may be more sensitive to changes in soil conditions among the sampling points across the landscape. This may be reflected mainly in variabilities of soil nutrients (Peralta et al., 2010) or extremophile presence. This is demonstrated in the boxplot (Fig. 2). The highest proportion of Protoebacteria may have reduced the evenness of bacterial community distribution under the same treatment. The absence of difference in richness due to the different management practices or their interactions might be attributed to niche differentiation of the bacterial species (Lennon et al., 2012).

The positive interaction observed under the native prairie grass mix with *udic* moisture regime may be linked to the increased root biomass and the exudates from native grasses (Eisenhauer et al., 2017). Plant root exudates and biomass turnover are sources of amino acids, organic acids, and sugars that are essential food sources for soil microbes (Haichar et al., 2014). Since different plant species have different root-structures, the mixed native grass species roots assumedly cover a wider surface area, thus providing a more conducive environment for microbes compared to the sole non-native orchardgrass species.

3.4. Bacterial community composition based on treatments

Community structure was compared across moisture regimes, fertility treatments, and forage species to determine if moisture regime (*udic* vs. *aquic*) and forage species (native vs. non-native) impacted the bacterial community structure. Pairwise distances were calculated from all samples using the Bray-Curtis distance metric. These distances were visualized by principal coordinate analysis (PCoA), which showed overlaps between bacterial communities based on moisture regime and forage species. No significant shifts in microbial composition were observed in PCoA plots based on Bray-Curtis (PERMANOVA $P > 0.05$) (Fig. 3). This result illustrates short-term management practices are less likely to alter soil bacterial composition, as previous studies reported shifts in soil microbial composition following 10+ years of continuous poultry litter applications (Ashworth et al., 2017; Yang et al., 2019).

3.5. Topographic linkages to soil microbiota

Among 13 terrain attributes used in the study, only 7 attributes were found to be significantly correlated with bacterial properties (Table 4). Richness was positively correlated with elevation ($r = 0.47$), and mid-slope position ($r = 0.52$) in 2019 and the relationship was significantly, negatively correlated with flow accumulation ($r = -0.22$) and slope percent ($r = -0.16$). Moreover, richness was weakly correlated with multi-resolution ridge top flatness index, valley depth, and altitude above channel network in both 2018 and 2019. In general, richness in 2018 had a weak correlation with terrain attributes, compared to 2019 except for aspect, multi-resolution valley bottom flatness index, and wetness index. These results indicate terrain attributes play a large role in soil microbial community abundances spatially and temporally.

Diversity had a significantly positive correlation with flow accumulation ($r = 0.51$), slope-length factor ($r = 0.56$), and altitude above channel network ($r = 0.42$) in 2018. In 2019,

diversity was significantly negatively correlated with elevation ($r = -0.59$) and mid-slope position ($r = -0.47$); while slope height, multi-resolution ridge top flatness index, and normalized height showed a weak negative correlation with diversity (Table 4). The strong correlation between the Shannon index and elevation agrees with a recent finding by Merino-Martín et al. (2020). Elevation is among the most important terrain attributes and has been widely studied for its effect on soil microbial composition and structure (Merino-Martín et al., 2020; Zhang et al., 2019), although interacting effects from management and inherent soil properties and elevation may occur (Xu et al., 2020; Zhang et al., 2019). Terrain attributes alone illustrated little correlation with diversity; therefore, the TFU's which were developed based on combinations of multiple terrain attributes, were evaluated.

The distribution of richness and diversity in different TFUs are shown in Fig. 4. Average richness in the study area was 3,803 (± 1764), where it was above average in TFU A, B, and C. TFU A had the highest (5,804), and D had the lowest (2,626) richness. On the other hand, TFU B, C, and D had diversity lower than its overall mean (231) in this silvopasture study. TFU A had the greatest (373), and D (188) had the smallest diversity. Results showed that the richness was influenced by TFUs. For example, richness in TFU A was different than from TFU B and D, and that from D was different from TFU A, and C. Richness in TFU B and C were not different. However, diversity among the TFU B, C, and D were not different, except for TFU A which was different from the rest of the TFUs.

Unlike management practices, richness was influenced by TFU, suggesting topographic effects may define soil microbial niches. The greatest soil bacterial richness was found in TFU A (Fig. 4) and may be attributed to this topographic position remaining moist for long periods (Adhikari et al., 2018). Moisture gradients along the landscape was also previously reported to

be a determinant for variations in soil bacterial composition and abundance (Lennon et al., 2012). The effect of topography on soil microbial composition and diversity of bacterial communities has also been reported by Liu et al. (2007), (2020).

4. Conclusions

This study evaluated the soil phylogenetic response to forage grass species, poultry litter applications, and soil moisture regimes, as well as soil microbiota interactions with topography using next generation sequencing to understand the interaction of management with terrain attributes in a silvopasture system. Soil moisture regime x forage species interacted to effect bacterial community diversity, with native grass species under dry (*udic*) soil moisture regime resulting in the most diverse microbial assemblage relative to *udic* and wet (*aquic*) soil moisture regimes for the introduced forage (orchardgrass). In addition, a strong correlation was observed between soil bacterial diversity and terrain attributes (elevation and flow accumulation), thus suggesting the significance of considering TFUs to understand soil microbial diversity dynamics. Therefore, TFUs controlled water distribution, with the unequal distribution of water controlling biologic responses. Hence, studies evaluating richness and diversity of soil microbes should consider the sample location within the context of landscape position.

Acknowledgements

Authors appreciate the sampling support of Taylor Adams with the USDA-Agricultural Research Service and the site maintenance assistance from Dr. Dirk Phillip and Robert Rhine with the University of Arkansas, Animal science Department. Mention of a trade name, proprietary

product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Funding source

This research was supported in part by the Foundation for Food and Agriculture (Agreement 58-6022-9-002).

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Figure Captions

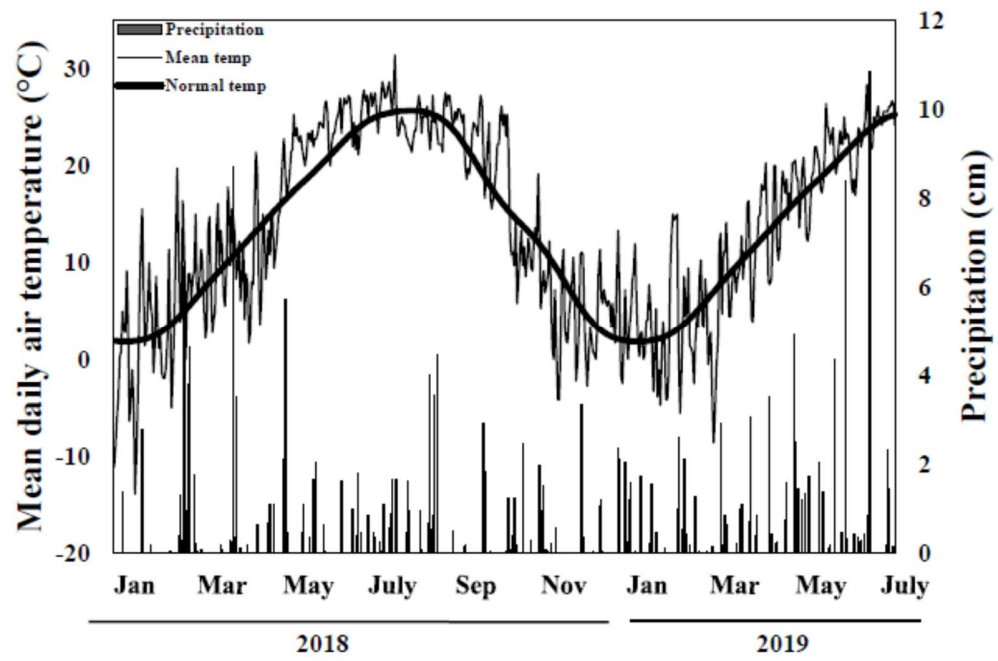
Figure 1. Mean relative proportion of bacteria population in phylum (A) and OTU (B) level.

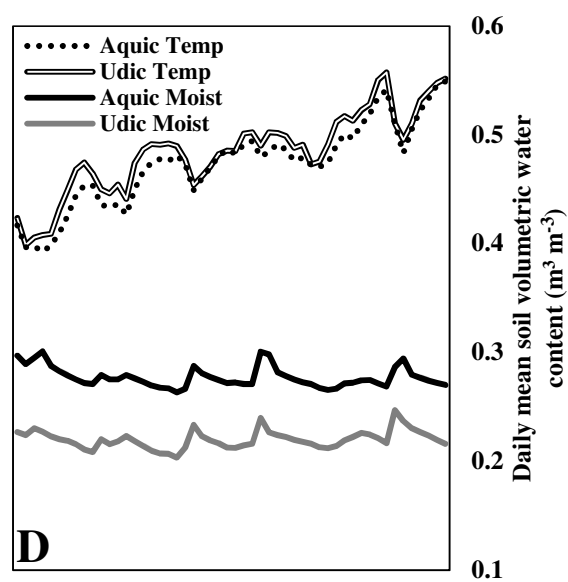
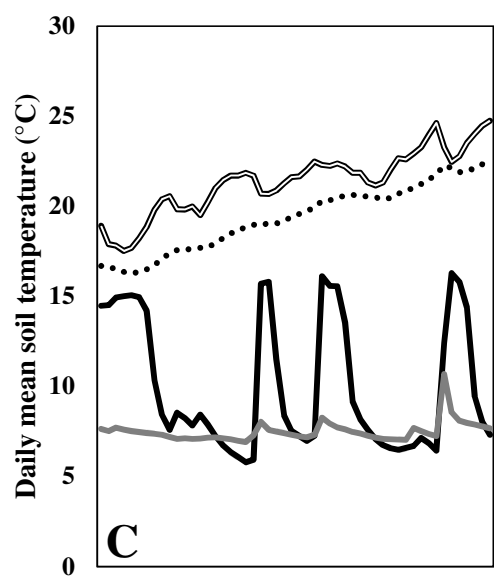
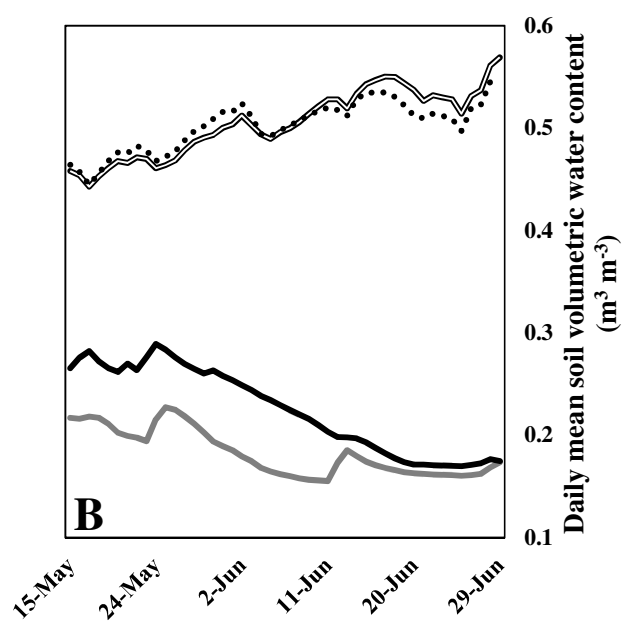
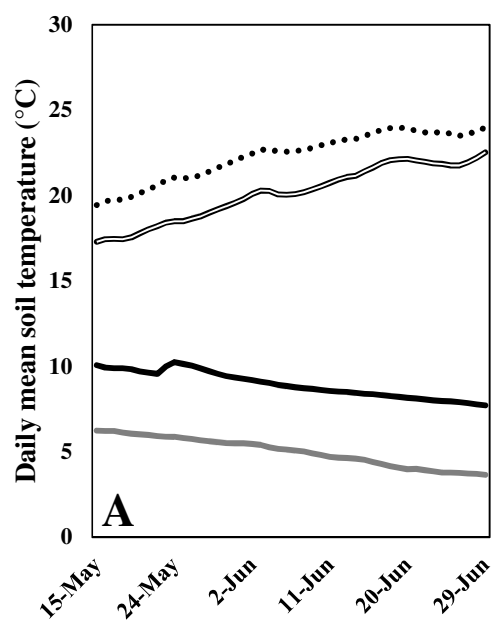
Factors include soil moisture regimes [udic (D) and aquic (w)], and forage species orchardgrass (OG) and native grass (NG).

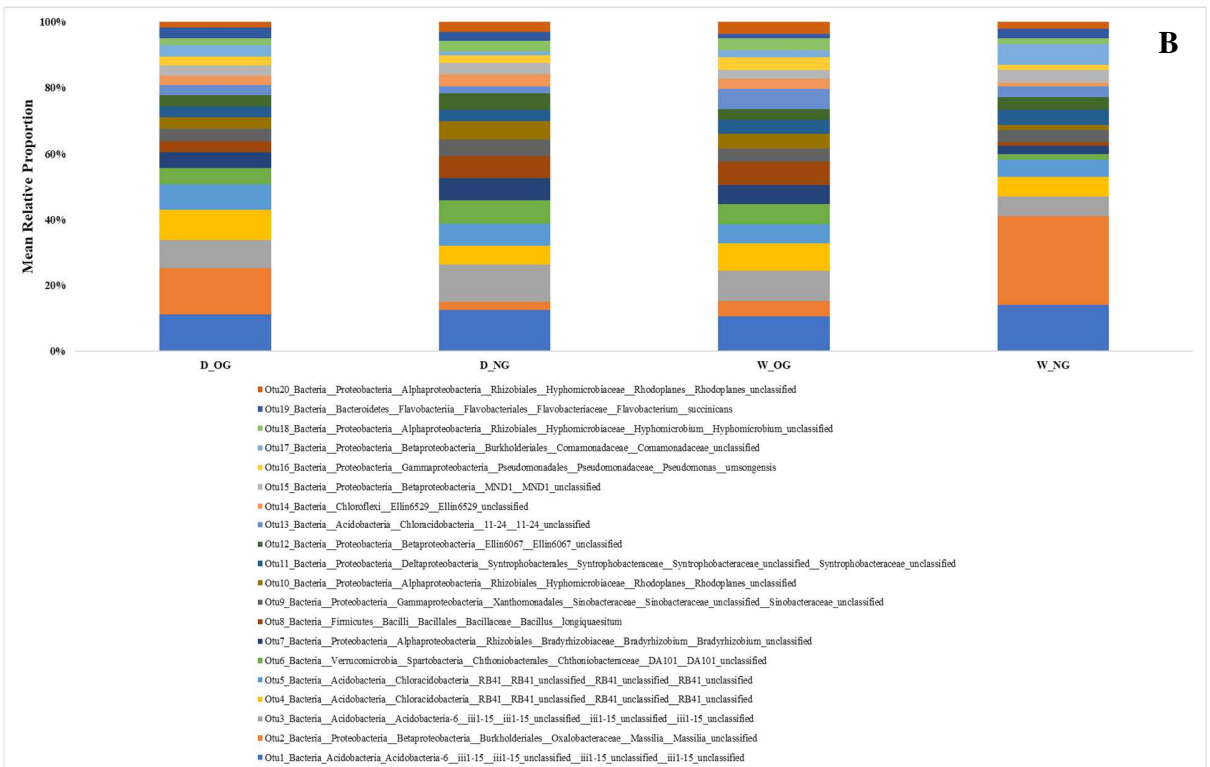
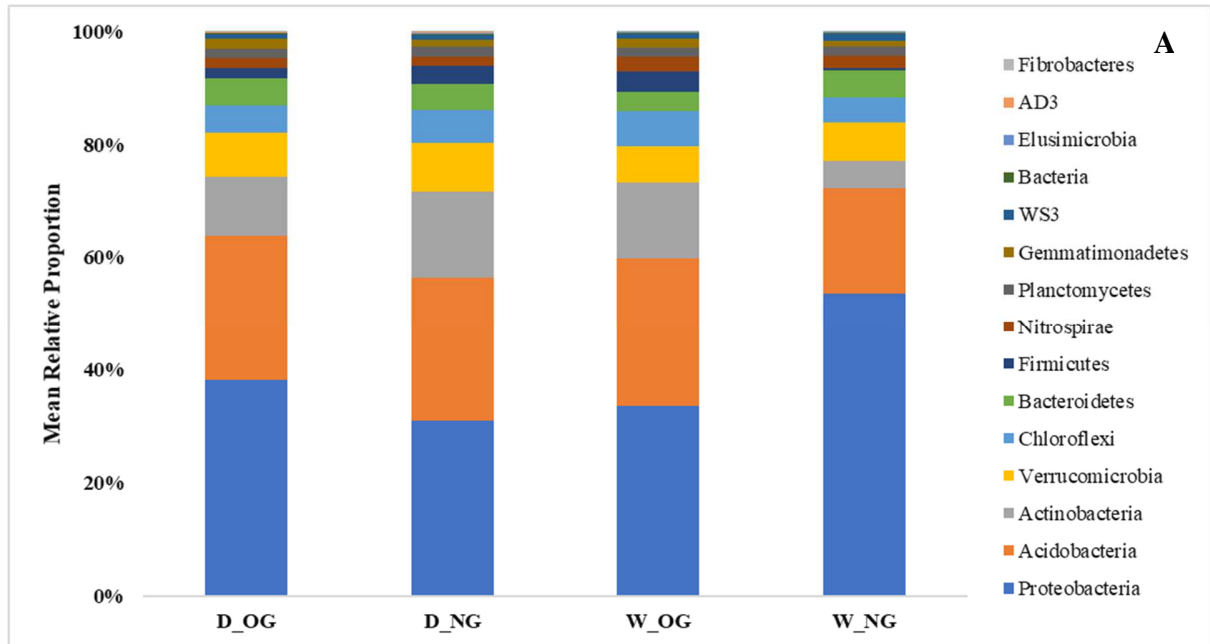
Figure 2. Mean soil bacterial richness, evenness, and diversity [Chao1 (A), Simpson index (B), and Shannon index (C)] in different soil moisture regime (dry or wet) under different forage species (native and non-native) system. Factors include soil moisture regime [dry (D) and wet (w)], and forage species orchardgrass (OG) and native grass (NG).

Figure 3. Principal Coordinate Analysis (PCoA) of Bray–Curtis distances of bacterial community structures in different soil system. Factors include soil moisture regime [dry (D) and wet (w)], and forage species orchardgrass (OG) and native grass (NG).

Figure 4. Richness and diversity as influenced by 4 topographic functional units (TFUs) (A, B, C and D). Letters a, b and c are notations for significance ($P < 0.05$).







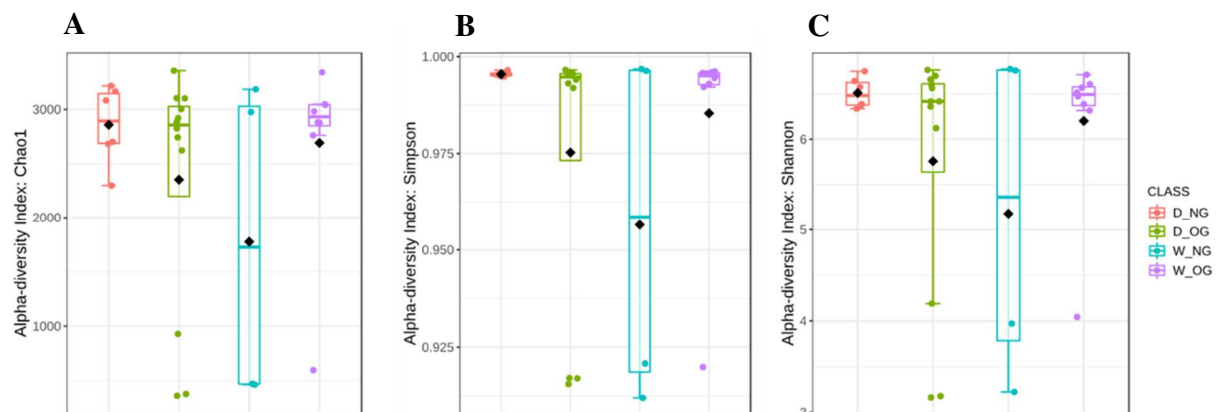


Table 1. Soil properties at a silvopasture site in Fayetteville, AR in 2018 and 2019 (analyzed across years as there were no year effects; $P \geq 0.05$). Samples were collected at 0-15 cm at four factor levels: forage species (NG = native grass mix, and OG = non-native orchardgrass), moisture regime (W = wet/mesic, and D = dry/xeric), and fertility (F = fertilized with poultry litter, and NF = un-fertilized control). [Fert = fertility, OM = soil organic matter].

Forage	Fert/Moist	pH	OM	C	N	P	K	Ca	Mg	S
			%			mg kg ⁻¹				
NG	F	6.75a [‡]	3.48a	1.94a	0.19a	77a	125a	1657a	73a	13.2a
NG	NF	6.73a	3.27a	1.68a	0.16a	59ab	89ab	1646a	57ab	15.0a
OG	F	6.75a	3.17a	1.88a	0.18a	40b	76b	1850a	49b	13.8a
OG	NF	6.69a	3.56a	1.89a	0.19a	52b	68b	1538a	50b	12.6a
NG	D	6.75ab	3.38a	1.97a	0.19a	83a	137a	1741ab	75a	14.3a
NG	W	6.74ab	3.27a	1.65a	0.17a	52b	77b	1562ab	55ab	13.8a
OG	D	6.57b	3.38a	1.79a	0.17a	49b	77b	1470b	57ab	12.6a
OG	W	6.87a	3.45a	1.98a	0.19a	44b	67b	1918a	42b	13.8a

[‡]Different letters indicate significant differences at $P < 0.05$ within a column (among F and NF, or D and W treatments).

Table 2. PERMANOVA in soil bacterial community structure by moisture regime, fertilization, and grass at a silvopasture site in Fayetteville, AR in 2018 and 2019 (analyzed across years as there were minimal year effects; $P \geq 0.05$). PERMANOVA results illustrate differences in bacterial community structure by single factor of soil moisture regime (dry or wet), fertility (fertilized by poultry litter or non-fertilized), and forage species (orchardgrass or native grass), as well as two factors (moisture regime x fertility, forage x fertility, and moisture regime x forage), and three factors (moisture regime x fertility x forage).

Factor	Pseudo-<i>F</i>	<i>P</i>-value
Moisture regime	1.9608	0.148
Forage species	0.73523	0.496
Fertility	1.074	0.322
Moisture regime x Fertility	0.96214	0.351
Forage species x Fertility	2.0381	0.123
Moisture regime x Forage species	3.805	0.042*
Moisture regime x Fertility x Forage species	1.8078	0.166

*Significant at $P < 0.05$.

Table 3. ANOVA of richness and diversity in bacterial community structure. ANOVA results illustrating richness and diversity in bacterial community structure by single factors of soil moisture regime (dry or wet), forage species (orchardgrass or native grass), and fertility (fertilized by poultry litter or non-fertilized), as well as two factors (moisture regime x fertility, forage x fertility, and moisture regime x forage), and three factors (moisture regime x forage x fertility) in a silvopasture site in Fayetteville, AR.

Parameter	Factor	F-value	P-value
Chao1	Moisture regime	1.9448	0.1771
	Forage species	1.3148	0.2638
	Fertility	1.8245	0.1905
	Moisture regime x Fertility	1.2319	0.2790
	Forage species x Fertility	2.1094	0.1605
	Moisture regime x Forage species	4.1263	0.0545
	Moisture regime x Forage species x Fertility	2.9114	0.1020
Shannon	Moisture regime	2.1233	0.1592
	Forage species	1.2300	0.2794
	Fertility	3.5267	0.0737
	Moisture regime x Fertility	3.1843	0.0881
	Forage species x Fertility	3.5564	0.0726
	Moisture regime x Forage species	6.9957	0.0148*
	Moisture regime x Forage species x Fertility	3.9033	0.0609
Simpson	Moisture regime	2.4438	0.1323
	Forage species	1.1360	0.2980
	Fertility	1.9514	0.1764
	Moisture regime x Fertility	1.9529	0.1762
	Forage species x Fertility	2.9865	0.0980
	Moisture regime x Forage species	7.2339	0.0134*
	Moisture regime x Forage species x Fertility	2.8896	0.1033

*Significant at $P < 0.05$.

Table 4. Pearson's correlation coefficients among soil bacterial properties and terrain attributes observed in 2018 and 2019 at a silvopasture site in Fayetteville, AR.

Terrain attribute	2018		2019	
	Richness (Chao1)	Diversity (Simpson)	Richness (Chao1)	Diversity (Simpson)
Altitude above channel network	-0.18	0.42*	0.28	-0.41
Elevation	-0.32	0.34	0.47*	-0.59*
Aspect	0.22	0.29	0.07	-0.09
Flow accumulation	-0.1	0.51*	-0.22	0.29
Mid-slope position	0.18	0.14	0.52*	-0.47*
Multi-resolution ridge top flatness index	0.25	-0.27	0.35	-0.32
Multi-resolution valley bottom flatness index	-0.20	0.12	-0.08	0.03
Normalized height	-0.15	0.08	0.03	-0.21
Wetness index	0.31	0.00	0.15	-0.11
Slope percent	0.14	-0.13	-0.16	0.21
Slope height	-0.12	0.19	0.16	-0.30
Slope-length factor	-0.09	0.56*	-0.15	0.32
Valley depth	0.11	0.22	0.25	0.03

*Different letters indicate significant differences at $P < 0.01$.