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Biyensa Gurmessa[1,2,6], Amanda J. Ashworth[1*], Yichao Yang[2], Kabindra Adhikari[3], Mary Savin[2], Phillip Owens[4], Tom Sauer[5], Ester Foppa Pedretti[6], Stefania Cocco[6], and Giuseppe Corti[6]

[1] USDA-ARS, Poultry Production and Product Safety Research Unit, 1260 W. Maple St. Fayetteville, AR 72701, USA; *corresponding author, Amanda.Ashworth@usda.gov

[2] Department of Crop, Soil, and Environmental Sciences, University of Arkansas, 115 Plant Science Building, University of Arkansas, Fayetteville, AR 72704, USA;

[3] USDA-ARS, Grassland Soil and Water Research Laboratory, Temple, TX 76502, USA;

[4] USDA-ARS, Dale Bumpers Small Farms Research Center, 6883 S. Hwy 23, Booneville, AR 72927, USA;

[5] National Laboratory for Agriculture and the Environment, Agricultural Research Service, United States Department of Agriculture, 1015 N University Blvd, Ames, IA, 5001,1 USA;

[6] Department of Agriculture, Food and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche 10, 60131 Ancona, Italy

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 CO_2 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

92 abundance in response to forage species, poultry litter applications, and soil moisture regime, and
93 to ii) understand the interaction of these practices with terrain attributes in a silvopastoral system
94 characterized by soils with a fine texture and neutral pH.
95
96 **2. Materials and Methods**
97 *2.1. Site description*
98 This study was conducted in a 4.25-ha paddock located at the University of Arkansas
99 Agricultural Research and Extension Center in Fayetteville, AR (36.09°N, 94.19°W). The site is
100 located in the Ozark Highlands, Major Land Resource Area 116A (Soil Survey Staff, 2019a).
101 Information on previous site history is described by Sauer et al. (2015). Briefly, soil in most of
102 the experimental area is mapped as Captina silt loam (fine-silty, siliceous, active, mesic Typic
103 Fragiudult) with some Pickwick silt loam (fine-silty, mixed, semiactive, thermic Typic
104 Paleudult) and small areas of Johnsburg silt loam (fine-silty, mixed, active, mesic Aquic
105 Fragiudult), and Nixa cherty silt loam (loamy-skeletal, siliceous, active, mesic Glossic
106 Fragiudults) soils (Soil Survey Staff, 2019b).
107 Adhikari et al. (2018) provides details about the derivation of terrain attributes, and
108 topographic functional units (TFUs) in the study area and details on their derivation are provided
109 in section 2.7. Briefly, TFUs are derived using terrain attributes where the individual units are
110 more homogeneous in terms of terrain properties and behave as a single functional unit within a
111 landscape and can be used to describe functional behavior or soils. Based on terrain attributes,
112 the study site could be divided into four TFUs, namely A, B, C, and D; TFU A had the highest
113 nutrients present, whereas TFU B had the lowest P, K, Zn, Cu, Fe, and Ca but highest Na
114 content. However, Mn, Mg, and B did not vary among TFUs (Adhikari et al., 2018).

115 Topographic functional units are the landscape units that show more homogenous terrain
116 properties within and less homogeneous properties between the units in terms of soil-terrain
117 relationship (Adhikari et al., 2018). The wetter location within the study site has a fine, mixed,
118 active, thermic Typic Endoaqualf and *aquic* soil moisture regime (virtually free of dissolved
119 oxygen because it is saturated by ground water or by water of the capillary fringe), whereas the
120 better drained soils have an *udic* soil moisture regime (classified as no evidence of saturation or
121 reduction within 50 cm of the surface; Soil Survey Staff, 1999c). The site has a mean (30-yr
122 mean) annual precipitation of 1,232 mm and a mean annual air temperature of 14.5°C (NCDC,
123 2019a, 2019b). The mean and normal temperature and daily precipitation of the study area from
124 January 2018 to July 2019 is reported in Supplementary Fig. 1.

125 *2.2. Tree hedgerow and grass strip management*

126 The hedgerows layout and the soil types of the experimental site were mapped and well
127 described by Sauer et al. (2015) and Adhikari et al. (2018). In the year 2000, hedgerows of a
128 total of fifteen rows of three species, namely, northern red oak (*Quercus rubra* L.), eastern black
129 walnut (*Juglans nigra* L.), and pecan (*Carya illinoinensis* Wangenh K. Koch) were established.
130 Each species had five rows, and the rows were oriented east-west at 15-m spacing (Sauer et al.,
131 2015). In 2014, the eastern black walnut trees rows were replaced with rows containing three
132 species: American sycamore (*Platanus occidentalis* L.), cottonwood (*Populus deltoides* W.
133 Bartram ex Marshall), and pitch/loblolly pine (*Pinus rigida* x *Pinus taeda*).

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

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

144 *2.3. Treatment implementation and field management*

145 Treatments included moisture regime (*udic* and *aquic*), forage species (native grass mixture
146 and non-native orchardgrass) and fertility (poultry litter applied and not applied), set up in a
147 factorial design. The primary treatment (forage species) was implemented as described above
148 with three replications. The second main effect (fertility) was implemented on orchardgrass and
149 native grass receiving locally sourced poultry litter and applied at a rate of 84 kg N ha^{-1} on
150 March 21, 2018 and April 12, 2019 (fresh weight basis). Poultry litter in 2018 had a pH of 6.2
151 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
152 and contained 2.48% N, 0.69% P, and 0.94% K. The third main effect (soil moisture regime) was
153 determined by random placement of volumetric water content (VWC) TEROS 11 sensors
154 (METER Group, Pullman, WA) at two soil depths (15 and 60-75 cm). Water content
155 measurements were recorded every 4 h and logged on a Decagon EM50 data logger (METER
156 Group, Pullman, WA) throughout the experimental period from May to July in 2018-2019. Soil
157 moisture data were averaged each day and expressed as daily mean volumetric water content for
158 further analysis (Supplementary Fig. 2). Weather variables were measured by a micro-
159 meteorological weather station approximately 500 m away from the experimental site.

160 *2.4. Soil sample collection and analysis*

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

169 After removing plant materials manually, soil samples were dried in a forced-air oven at
170 70°C for 48 hours. Dried samples were then ground and sieved to pass a 2-mm mesh. The pH
171 was determined potentiometrically in deionised water (1:2.5 solid:liquid ratio). Weight-loss-on-
172 ignition was used to determine soil organic matter (SOM) concentration after 2 hrs at 360°C
173 (Schulte and Hopkins, 2015). Total C and N were determined via combustion using a VarioMax
174 CN analyzer (Elementar Americas, Mt. Laurel, NJ). Mehlich-3 extractable soil nutrients were
175 determined using a 1:10 solid: liquid ratio (w:v) (Tucker, 1992) and analyzed by inductively
176 coupled argon-plasma spectrometry (ICP, Agilent Technologies, Santa Clara, CA).

177 *2.5. DNA extraction and Illumina sequencing*

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

182 Bacterial community composition was determined using Illumina Miseq sequencing of 16S
183 rRNA gene amplicons. Extracted DNA was sent to the University of Tennessee Genomic
184 Services Laboratory, where the V4 region of the 16S rRNA gene was amplified with barcoded
185 primers 515F and 806R (Caporaso et al., 2011). Amplicon libraries were pooled, and 291 base-
186 paired end sequences were obtained on the Illumina MiSeq Platform, resulting in a total of
187 4,196,620 sequence reads. Reads were processed using the open source bioinformatics software
188 Mothur V 1.40.0 following the Miseq SOP protocol (Kozich et al., 2013). Sequences that did not
189 match the primers were eliminated from demultiplexed sequence reads. Ambiguous base
190 sequences with a length less than 100 bp were deleted and chimeric sequences were removed
191 using the UCHIME algorithm implemented in Mothur. After the quality control pipeline,
192 3,321,000 sequence reads remained using a 97% similarity threshold to define ribotypes in
193 Mothur (7.91% were deleted).

194 *2.6. Estimation of diversity and evenness indices*

195 The greengenes database was used to classify the operational taxonomic unit (OTU) at the
196 genus level using the Bayesian method (Cole et al., 2014); thereafter, relative abundance of all
197 OTUs were summed within phylum and analyzed for relative abundance at both phylum and
198 OTU levels. Except for the analysis of bacterial community structure, all other analyses were
199 conducted based on OTU level. Based on this subsampled dataset, richness was calculated using
200 the Chao index (Hughes et al., 2001) and diversity was calculated using the indexes of Simpson
201 and Shannon from Mothur output files (Schloss et al., 2009). Beta-diversity was measured by
202 using Bray-Curtis index, weighted and unweighted UniFrac distance metrics (Schroeder and
203 Jenkins, 2018).
204

205 *2.7. Topographic evaluation on microbial properties*

206 The relationship between topography and soil microbial properties was explored using
207 Pearson's correlation analysis. Topographic information was provided with terrain attributes
208 derived from a digital elevation model compiled from light detection and ranging (LiDAR). A 1-
209 m grid digital elevation model was downloaded from the USDA-Geospatial Data Gateway site
210 (https://datagateway.nrcs.usda.gov) and down sampled to 10 m resolution for developing the 13
211 terrain attributes: altitude above channel network, aspect, elevation, flow accumulation, mid-
212 slope position, multi-resolution ridge top flatness index, multi-resolution valley bottom flatness
213 index, normalized height, wetness index, slope percent, slope height, slope-length factor, and
214 valley depth in SAGA GIS environment (Conrad et al., 2015). These terrain attributes represent
215 topographic landscape variability and are related to water flow and distribution. Altitude above
216 channel network measures the vertical distance of a point to the nearest channel. Elevation
217 represents land surface elevation above mean sea level and its normalized value is the
218 normalized height. Flow accumulation gives the number of upland pixels draining to a given
219 raster, whereas wetness index determines a potential of a pixel to retain moisture. Similarly,
220 valley depth determines the relative height difference to the immediate adjacent channel
221 network, whereas the difference to the crest lines is the slope height. Slope percent and slope-
222 length factor show a maximum rate of change between pixels and neighbors, and the length of
223 the slope is calculated per the universal soil loss equation. Aspect shows the direction of the
224 steepest angle from the north direction. Multi-resolution ridge top and valley bottom flatness
225 index identifies high and depositional areas in the landscape, respectively (Guo et al., 2019).
226 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 Protobacteria (37%), Acidobacteria (25.53%), Actinobacteria (12.07%), Verrucomicrobia (7.65%), Chloroflexi (5.50%), Bacteriodetes (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.

296 The findings by Estendorfer et al. (2017) on bacterial relative abundance under orchardgrass
297 were consistent with the current findings. Protobacteria generally dominate the soil bacterial
298 community under grasslands (Arenz et al., 2014; Singh et al., 2007; Spain et al., 2009), but the
299 level of relative abundance of a bacterial phylum can be affected by soil management practices,
300 which was also evident in this study (Fig. 1). In the current study, the interaction of *aquic* soil
301 moisture regime and native grasses mix increased the relative abundance of protoebacteria
302 compared to other treatments. However, whole community evenness and diversity, expressed
303 with Simpson's and Shannon indices, respectively, were reduced (Table 3). On the other hand, it
304 is interesting that these diversity indices were greater in *aquic* than *udic* soils under orchardgrass
305 pastures, implying greater microbial preference to wet soil moisture regimes for these systems.
306 *3.3. Bacterial community alpha diversity based on fertility, moisture regime, and forage*
307 *species*
308 Alpha diversity was not influenced by fertility (poultry litter vs. the control), moisture regime
309 (*udic* vs. *aquic*), and forage species (native vs. non-native) as estimated by three different
310 algorithms (Chao1, Shannon, and Simpson's index) (Table 3). It was hypothesized that poultry
311 litter applications would drive soil microbial community abundance, although this was rejected,
312 given fertility did not impact community structure ($P>0.05$). This was likely due to cattle grazing
313 in silvopastures masking any fertilizer effect from poultry litter. The Chao index was used to
314 calculate the richness of soil bacterial community in this study. There was no influence from
315 individual main effects, as well as for two and three-way interactions of these effects. The
316 Simpson's index was used to calculate the evenness of soil bacterial community in this study.
317 This result indicates that the moisture regime x forage species interaction affected bacterial
318 community evenness ($P<0.05$). Fig. 2A illustrates the influence of moisture regime and forage

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

324 Richness, evenness, and diversity indices illustrate the alpha diversity of bacterial community
325 in response to changes in edaphic or anthropogenic factors. Unlike orchardgrass, the native grass
326 mix was less effective in maintaining evenness and diversity of alpha diversity in *aquic* soils.
327 Moreover, the variability of these diversity indices within a treatment (Fig. 1) shows some
328 bacterial phyla may be more sensitive to changes in soil conditions among the sampling points
329 across the landscape. This may be reflected mainly in variabilities of soil nutrients (Peralta et al.,
330 2010) or extremophile presence. This is demonstrated in the boxplot (Fig. 2). The highest
331 proportion of Protoebacteria may have reduced the evenness of bacterial community distribution
332 under the same treatment. The absence of difference in richness due to the different management
333 practices or their interactions might be attributed to niche differentiation of the bacterial species
334 (Lennon et al., 2012).

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

342 *3.4. Bacterial community composition based on treatments*

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

353 *3.5. Topographic linkages to soil microbiota*

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

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

365 diversity was significantly negatively correlated with elevation (r = -0.59) and mid-slope position
366 (r = -0.47); while slope height, multi-resolution ridge top flatness index, and normalized height
367 showed a weak negative correlation with diversity (Table 4). The strong correlation between the
368 Shannon index and elevation agrees with a recent finding by Merino-Martín et al. (2020).
369 Elevation is among the most important terrain attributes and has been widely studied for its
370 effect on soil microbial composition and structure (Merino-Martín et al., 2020; Zhang et al.,
371 2019), although interacting effects from management and inherent soil properties and elevation
372 may occur (Xu et al., 2020; Zhang et al., 2019). Terrain attributes alone illustrated little
373 correlation with diversity; therefore, the TFU's which were developed based on combinations of
374 multiple terrain attributes, were evaluated.
375 The distribution of richness and diversity in different TFUs are shown in Fig. 4. Average
376 richness in the study area was 3,803 (±1764), where it was above average in TFU A, B, and C.
377 TFU A had the highest (5,804), and D had the lowest (2,626) richness. On the other hand, TFU
378 B, C, and D had diversity lower than its overall mean (231) in this silvopasture study. TFU A
379 had the greatest (373), and D (188) had the smallest diversity. Results showed that the richness
380 was influenced by TFUs. For example, richness in TFU A was different than from TFU B and D,
381 and that from D was different from TFU A, and C. Richness in TFU B and C were not different.
382 However, diversity among the TFU B, C, and D were not different, except for TFU A which was
383 different from the rest of the TFUs.
384 Unlike management practices, richness was influenced by TFU, suggesting topographic
385 effects may define soil microbial niches. The greatest soil bacterial richness was found in TFU
386 A (Fig. 4) and may be attributed to this topographic position remaining moist for long periods
387 (Adhikari et al., 2018). Moisture gradients along the landscape was also previously reported to

388 be a determinant for variations in soil bacterial composition and abundance (Lennon et al.,
389 2012). The effect of topography on soil microbial composition and diversity of bacterial
390 communities has also been reported by Liu et al. (2007), (2020).
391
392 **4. Conclusions**
393 This study evaluated the soil phylogenetic response to forage grass species, poultry litter
394 applications, and soil moisture regimes, as well as soil microbiota interactions with topography
395 using next generation sequencing to understand the interaction of management with terrain
396 attributes in a silvopasture system. Soil moisture regime x forage species interacted to effect
397 bacterial community diversity, with native grass species under dry (*udic*) soil moisture regime
398 resulting in the most diverse microbial assemblage relative to *udic* and wet (*aquic*) soil moisture
399 regimes for the introduced forage (orchardgrass). In addition, a strong correlation was observed
400 between soil bacterial diversity and terrain attributes (elevation and flow accumulation), thus
401 suggesting the significance of considering TFUs to understand soil microbial diversity dynamics.
402 Therefore, TFUs controlled water distribution, with the unequal distribution of water controlling
403 biologic responses. Hence, studies evaluating richness and diversity of soil microbes should
404 consider the sample location within the context of landscape position.
405
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639 **Figure Captions**

640 Figure 1. Mean relative proportion of bacteria population in phylum (A) and OTU (B) level.
641 Factors include soil moisture regimes [udic (D) and aquic (w)], and forage species orchardgrass
642 (OG) and native grass (NG).

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

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

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

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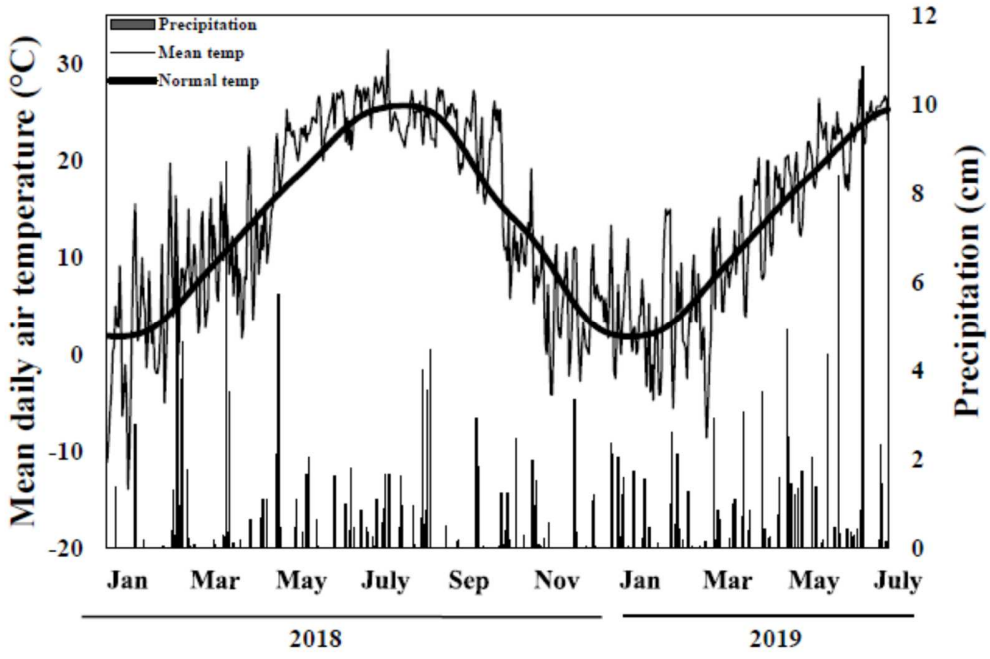
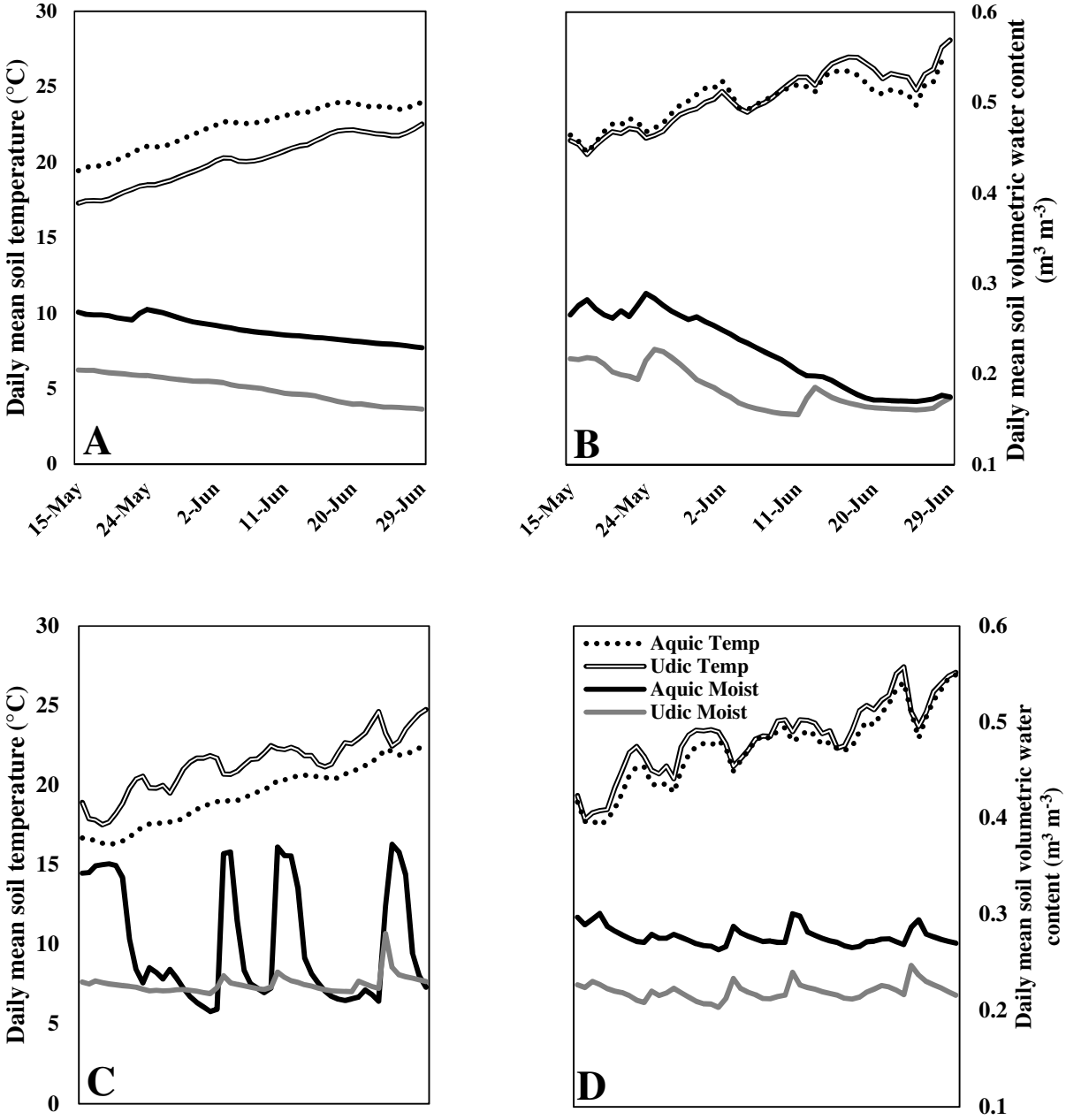
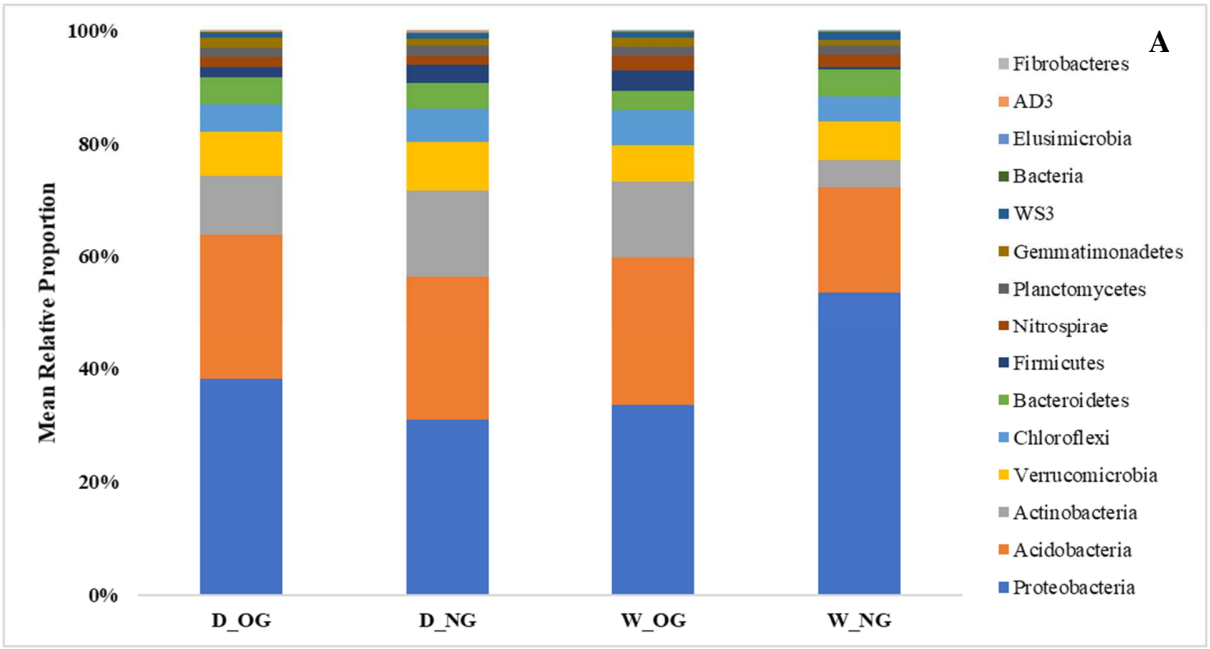
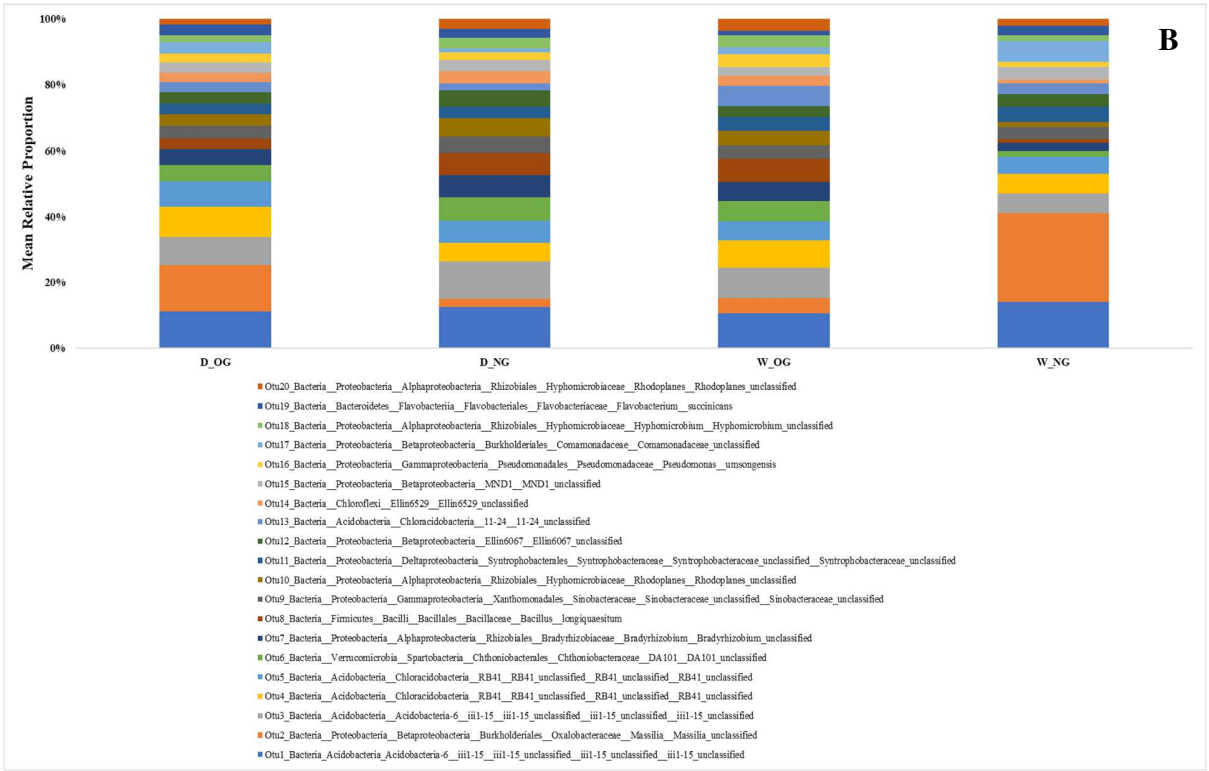

Precipitation
Mean temp
Normal temp
Mean daily air temperature (°C)
Precipitation (cm)
30
20
10
0
-10
-20
12
10
8
6
4
2
0
Jan
Mar
May
July
Sep
Nov
Jan
Mar
May
July
2018
2019


Daily mean soil temperature (°C)
Daily mean soil volumetric water content (m³ m⁻³)
30
25
20
15
10
5
0
0.6
0.5
0.4
0.3
0.2
0.1
15-May
24-May
2-Jun
11-Jun
20-Jun
29-Jun
A
B
C
D
Aquic Temp
Udic Temp
Aquic Moist
Udic Moist


A
100%
80%
60%
40%
20%
0%
Mean Relative Proportion
D_OG
D_NG
W_OG
W_NG
Fibrobacteres
AD3
Elusimicrobia
Bacteria
WS3
Gemmatimonadetes
Planctomycetes
Nitrospirae
Firmicutes
Bacteroidetes
Chloroflexi
Verrucomicrobia
Actinobacteria
Acidobacteria
Proteobacteria


B
100%
80%
60%
40%
20%
0%
Mean Relative Proportion
D_OG
D_NG
W_OG
W_NG
Otu20_Bacteria__Proteobacteria__Alphaproteobacteria__Rhizobiales__Hyphomicrobiaceae__Rhodoplanes__Rhodoplanes_unclassified
Otu19_Bacteria__Bacteroidetes__Flavobacteriia__Flavobacteriales__Flavobacteriaceae__Flavobacterium__succinicans
Otu18_Bacteria__Proteobacteria__Alphaproteobacteria__Rhizobiales__Hyphomicrobiaceae__Hyphomicrobium__Hyphomicrobium_unclassified
Otu17_Bacteria__Proteobacteria__Betaproteobacteria__Burkholderiales__Comamonadaceae__Comamonadaceae_unclassified
Otu16_Bacteria__Proteobacteria__Gammaproteobacteria__Pseudomonadales__Pseudomonadaceae__Pseudomonas__umsongensis
Otu15_Bacteria__Proteobacteria__Betaproteobacteria__MND1__MND1_unclassified
Otu14_Bacteria__Chloroflexi__Ellin6529__Ellin6529_unclassified
Otu13_Bacteria__Acidobacteria__Chloracidobacteria__11-24__11-24_unclassified
Otu12_Bacteria__Proteobacteria__Betaproteobacteria__Ellin6067__Ellin6067_unclassified
Otu11_Bacteria__Proteobacteria__Deltaproteobacteria__Syntrophobacterales__Syntrophobacteraceae__Syntrophobacteraceae_unclassified__Syntrophobacteraceae_unclassified
Otu10_Bacteria__Proteobacteria__Alphaproteobacteria__Rhizobiales__Hyphomicrobiaceae__Rhodoplanes__Rhodoplanes_unclassified
Otu9_Bacteria__Proteobacteria__Gammaproteobacteria__Xanthomonadales__Sinobacteraceae__Sinobacteraceae_unclassified__Sinobacteraceae_unclassified
Otu8_Bacteria__Firmicutes__Bacilli__Bacillales__Bacillaceae__Bacillus__longiquaesitum
Otu7_Bacteria__Proteobacteria__Alphaproteobacteria__Rhizobiales__Bradyrhizobiaceae__Bradyrhizobium__Bradyrhizobium_unclassified
Otu6_Bacteria__Verrucomicrobia__Spartobacteria__Chthoniobacterales__Chthoniobacteraceae__DA101__DA101_unclassified
Otu5_Bacteria__Acidobacteria__Chloracidobacteria__RB41__RB41_unclassified__RB41_unclassified__RB41_unclassified
Otu4_Bacteria__Acidobacteria__Chloracidobacteria__RB41__RB41_unclassified__RB41_unclassified__RB41_unclassified
Otu3_Bacteria__Acidobacteria__Acidobacteria-6__iii1-15__iii1-15_unclassified__iii1-15_unclassified__iii1-15_unclassified
Otu2_Bacteria__Proteobacteria__Betaproteobacteria__Burkholderiales__Oxalobacteraceae__Massilia__Massilia_unclassified
Otu1_Bacteria_Acidobacteria_Acidobacteria-6__iii1-15__iii1-15_unclassified__iii1-15_unclassified__iii1-15_unclassified

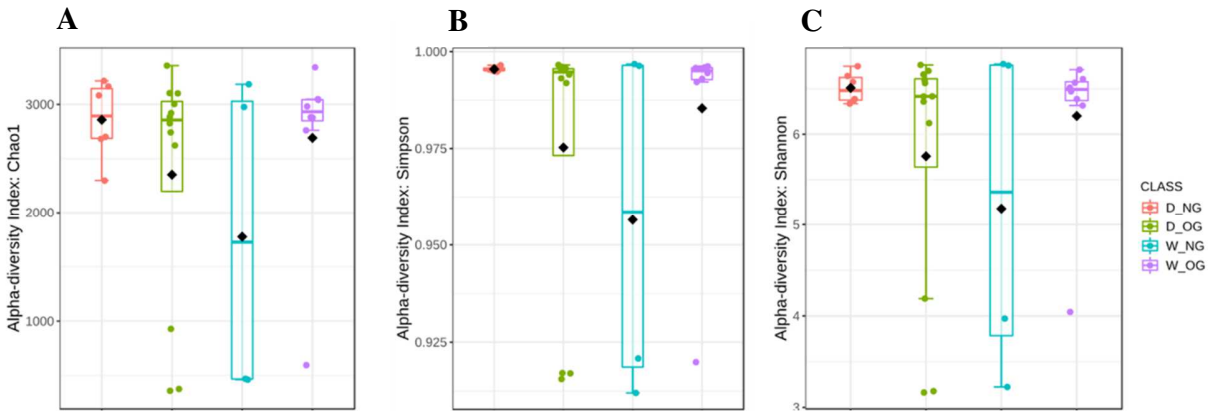

A
B
C
Alpha-diversity Index: Chao1
3000
2000
1000
Alpha-diversity Index: Simpson
1.000
0.975
0.950
0.925
Alpha-diversity Index: Shannon
6
5
4
3
CLASS
D_NG
D_OG
W_NG
W_OG

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 × forage), and three factors (moisture regime x fertility x forage).

Factor	**Pseudo-*F***	***P*-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 × 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 Richness (Chao1)	Diversity (Simpson)	2019 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 $P<0.01$.