Chapter

Spatio-Temporal Dynamics of Soil Microbial Communities in a Pasture: A Case Study of *Bromus inermis* Pasture in Eastern Nebraska

Taity Changa, Jane Asiyo Okalebo and Shaokun Wang

Abstract

Today's intensified agricultural production is characterized by crop and pasture monocultures, which have a significant impact on soil microbial diversity and abundance. This chapter provides a case study in which the relative importance of brome grass (*Bromus inermis*) monoculture pasture versus intra-site microhabitat diversity is explored using fatty acid methyl ester (FAMEs) assay to delineate the presence and abundance of several classes of soil microbes instrumental in soil nutrient cycling, plant health, plant organic matter decomposition, and soil stabilization. The chapter explores spatio-temporal variability of bacteria, actinomycetes, saprophytes, mycorrhizae, and micro-eukaryotes over two durations (summer and fall) collected using two distinct sampling methods. One of the methods is commonly employed, namely, transect-based, while the other is informed by soil electroconductivity measurements conducted over the entire pasture site from a previous survey.

Keywords: grassland, plant-soil feedback, soil electroconductivity, soil health, soil quality, *Bromus*

1. Introduction

The role of below-ground soil processes as well as plant-soil feedbacks, particularly those facilitated by the interaction between plants and soil microbes, are poorly understood despite having a key role in potentially regulating the productivity of ecosystems [1–4]. Soil microbial communities are critical for soil functioning and health as drivers for biogeochemical cycles [5, 6]. They have been shown to be shaped by the abiotic and biotic environments (e.g., plant and animal communities, edaphic characteristics, water, and climate), management practices (e.g., crop rotations and grazing) and are known to alter the amount, quality, and distribution of organic carbon (OC) within the soil profile—key for soil health [1, 7–10].

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Today's intensified agricultural production is characterized by crop and pasture monocultures, which have a significant impact on the soil microbial diversity and abundance. As an example, pastures in Eastern Nebraska grasslands have been shaped by Brome grass (*Bromus inermis*), a perennial grass species that was first introduced from Eurasia into North America in the 1880s as a forage grass and for soil stabilization [11]. The use of this species was promoted after the drought of the 1930s to control soil erosion and stabilize soil banks and ditches in Nebraska and elsewhere [11, 12]. The species has been successful at colonizing and spreading into pastures due its vigorous tillering and dense establishment of rhizomes and rootstocks [11, 13, 14]. In Eastern Nebraska, the species thrives well in clay loams forming even stands and monocultures in pastures [11, 13–15]. Deep roots (up to 2.7 m), debris, and exudates have been shown to influence both soil structure and texture positively through increased organic matter availability as well as binding properties which increase soil water holding capacity [11]. While there have been several studies that addressed the role of brome grass in soil processes and organic matter mineralization such as [13–15], some research has been conducting on its role in shaping the microbial community in pasture sites such as those conducted by Grigera et al.; Lauber et al.; Pereira e Silva et al.; and Segal et al. [16–19].

Understanding factors driving the spatio-temporal variability of soil microbial communities in monocultures can inform the selection of best management practices that can maintain and regulate soil biodiversity and enhance the microbial-soil ecosystem functions in these systems. However, quantifying microbial abundance and diversity have been a challenge over a range of spatial and temporal scales [20, 21]. This is in part attributed to the high heterogeneity in microbial populations found in two adjacent locations that are as close as 10 cm apart [21]. Sampling strategies have been proposed to capture the spatial heterogeneity in soil microbial biota [10]. One strategy is the randomized sampling method that is common among researchers [15, 22] and the other is the application of transects across the site of study guided by physical and biological variables including slope, water gradients, soil pH, or plant productivity [10, 23–25]. Each strategy has its strengths and shortcomings, especially where soils are spatially variable. It is agreed upon however, that besides vegetation, soil characteristics such as soil moisture, soil structure, organic matter, and temperature are some of the main drivers that govern spatio-temporal variability in microbial populations [21, 26].

Soil microbes can thrive under a wide range of temperatures. Nevertheless, microbial enzymatic catalytic functions perform optimally over a shorter range of temperatures. During spring and summer seasons (approximately 20–30°C), microbial abundance and activity are both at high levels compared to that measured during winter conditions (>10°C) characterized by reduced vegetation and less favorable edaphic conditions [17, 18]. Low temperatures affect the plant development resulting in the reduction of substrate (nutrients within the rhizosphere of plant roots) for soil microbes [17, 18]. Winter conditions characterized by both low temperatures and low nutrient availability trigger bacterial spore and fungal sclerotia formation. Soil microbial dormancy reduces the microbial abundance and ecosystem functions. Spores, sclerotia, and other soil microbial growths can remain dormant for long durations until the edaphic conditions become more favorable for reproduction and growth. During these periods of low temperatures, enzymatic activities and microbial functions are minimal [17, 18]. In order to understand temporal variation in soil microbial communities and their ecosystem functions, sampling over different temperatures and/or seasons is recommended and advisable.

Physical and chemical properties of soils in monocultures can potentially serve as a guide in delimiting zones of similar soil microbial abundance and diversity compared to sampling along a transect line. A property widely used to monitor soil

characteristics is the apparent electrical conductivity (ECa), which measures the amount of electric current that soil can conduct [27]. This valuable trait relates to soil texture, that is, soils with high clay content exhibit higher ECa values and vice versa [28]. The percentage of clay particles in soil influence its physical texture. Additional soil properties such as salinity, water, organic matter content, and cation exchange capacity (CEC) are also directly correlated with ECa. This soil characteristic is used for delineating homogeneous zones and is utilized by large commercial producers for guiding the fertilizer application and other land management practices [27].

This case study assesses the relative importance of brome grass monoculture versus intra-site microhabitat diversity in determining the microbial abundance and diversity. We hypothesize that soil microbial communities are in most part shaped by the brome monoculture rather than the intra-site variability in edaphic factors such as temperature, pH, N, ECa, and OC in a relatively homogeneous site. We tested two soil sampling techniques, ECa-guided sampling technique and transect-based sampling methodology, and used fatty acid methyl ester (FAMEs) assay [19, 29, 30] to determine and quantify the soil microbial diversity and abundance. The assay characterizes the composition of fatty acid species recovered from membranes of soil microbes which serve as unique markers for soil microbiota identification [30]. FAMEs assay is cost-effective and has been shown to give similar results to genomic analysis of bacterial and archaeal ammonia oxidizers [19].

2. Materials and methods

2.1 Site description

The experiment was conducted at a 15-ha pasture site, located at the Eastern Nebraska Research and Education Center (ENREC), University of Nebraska-Lincoln (latitude 41° 8′28.45″ and longitude N, 96°27′1.05”W) (**Figure 1**).

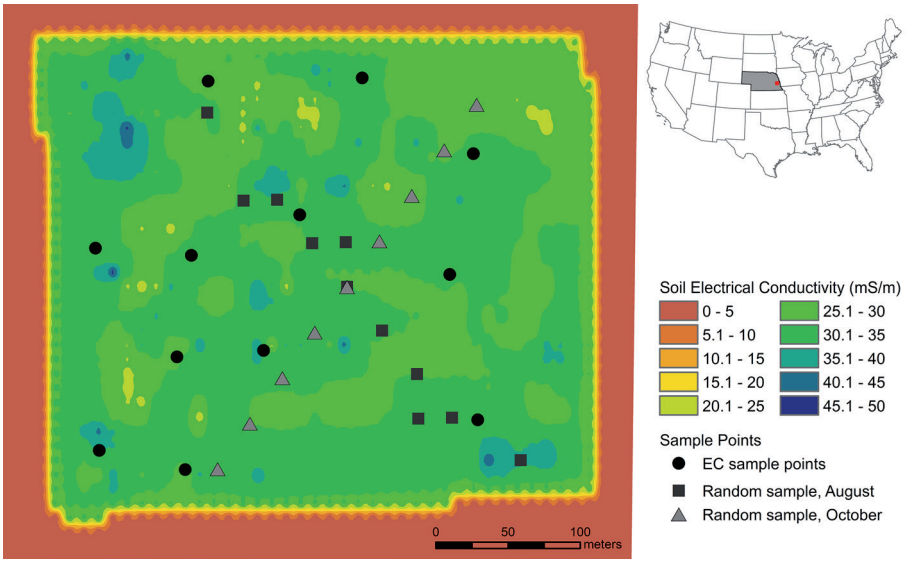


Figure 1.
Map showing distribution of soil apparent electrical conductivity (EC_a) mS m^{-1} in brome grass pasture study site. The points on the map indicate locations where soil sample were taken following EC (●) and random August (■) and October (▲)-based techniques. On the right corner is the site location in highlighted state of Nebraska and continental map of USA.

Annual average precipitation is 748 mm and the mean annual temperature is 9.9°C. January and July are the coldest and warmest months of the year with an average minimum of −11.3°C and maximum of 30.4°C, respectively (https://www.usclimatedata.com/climate/mead/nebraska/united-states/usne0316). The precipitation in 2017—when sampling took place—totaled 869 mm, with 112.3 mm in the month of August and approximately 24.4 mm falling 6 days prior to soil sampling on August 31. Minimal rainfall amounts of less than 2.54 mm had been recorded 11 days prior to sampling on 27th of October. The total amount of rainfall in October was 128.3 mm with most of it falling in the first half of the month.

The site is ~360 m above sea level with a topography that is relatively flat (0–2% slope). Under the older soil classification, the soil is characterized as sharpsburg silty clay loams of the fine, smectitic, mesic Typic Argiudoll series. Under the new classification, the pasture is comprised of yutan silty clay loam, tomek silt loam, and filbert silt loam with each occupying about one third of the pasture.

The soils are slightly acidic (pH 5.8) and are characterized by a high organic matter (OM 4.1%) (**Table 1**). The pasture was seeded over 20 years ago with smooth brome grass *Bromus inermis* Leyss which forms a monoculture. Brome grass is

Soil chemical properties	Value
1:1 Soil pH	5.80
WDRF Buffer pH	6.80
1:1 S Salts mmhocm^{-1}	0.09
Organic matter LOI %	4.14
Nitrate-N ppm N	4.42
Potassium ppm K	462.40
Sulfate-S ppm S	34.40
Zinc ppm Zn	2.81
Iron ppm Fe	189.38
Manganese ppm Mn	31.68
Copper ppm Cu	1.95
Calcium ppm Ca	2452.00
Magnesium ppm Mg	579.80
Sodium ppm Na	35.80
Boron ppm B	0.55
CEC/Sum of cations me/100 g	20.12
%H Sat	8.80
%K Sat	6.00
%Ca Sat	60.80
%Mg Sat	23.60
%Na Sat	0.80
Chloride ppm Cl	8.52
Mehlich P-III ppm P	30.40

Table 1.
Physical and chemical properties in the brome grass pasture site in eastern NE. (Ward Laboratories, Kearney, Nebraska, 2017).

characterized with low C:N ratio of about 60 [31]. In studies conducted by Vinton and Goergen [32], brome grass yielded a C:N ratio of 50.68 compared a high value (102.1) for *Panicum virgatus* (switchgrass) which makes it highly palatable for livestock [32]. It grows rapidly and aggressively at the start of the growing season and does well in soils that are rich in nitrogen. Its low C:N ratio results in relatively fast decomposition rates thereby increasing soil available N and rapid expansion and growth of the species in a positive plant-soil feedback system. Every year in spring, nitrogen fertilizer is applied at the rate of 14.6 kg ha^{-1}, and since 2014, animals (cattle) graze on this pasture between the end of April to end of September. N fertilization has been conducted as a management practice in the north central USA to improve beef animal daily gain (ADG) through increased forage biomass in pastures attributed to N availability through enhanced N cycling processes [33].

2.2 Apparent electrical conductivity (ECa)

A soil survey to characterize the soil ECa at the pasture site was conducted on April 4, 2017. The survey consisted of 22 transects running from north to south with an average spacing of 15 m between transects. A Geonics dual-pole EM38 (Geonics, Inc., Mississauga, Ontario, Canada) was mounted in a plastic sled and towed through the fields at about 5 km $h.^{-1}$ using a four-wheel all-terrain vehicle. Soil ECa sampling locations were determined using a Geode global positioning system (GPS) (Juniper Systems, Sunnyvale, CA, USA). The geographical coordinates as well as EM38 output were collected every 10 s and stored on a data logger. The raw data was processed using the ESAP-95 software [34] to identify 12 sampling points that best represented the spatial soil ECa variability within the field.

2.3 Soil physical and chemical properties

A set of samples used for the determination of the soil physical and chemical properties were collected in early June. These samples were collected at a depth of 20 cm and included soil cores taken from five randomly selected locations in the pasture to represent field scale soil properties. Samples were air-dried, sieved to remove large debris, and bulked into five composite samples and sent to Ward Laboratories Inc. (Kearney, NE.) for soil analysis (**Table 1**). The second set involved soil cores obtained from the designated 12 points that were chosen to represent the soil ECa gradient of the pasture as described above in the pasture ECa survey. Soil cores were collected from 10 to 15 cm depth increments and analyzed as follows: substrate water content was determined gravimetrically, samples were weighed, oven-dried at 105°C for 2 days, and weighed again. Laboratory EClab and pH were determined in 1:1 water:substrate slurries using a conductivity meter for EClab and a glass electrode for pH [35]. Extractable phosphorus (P) was determined using the method of [36] with P concentration determined spectrophotometrically at 882 nm using the phosphomolybdate blue method [37]. Inorganic nitrogen (N) in 1 M KCl extracts was measured colorimetrically using an AA3 flow injection ion analyzer (Seal Analytical, Inc. Mequon, WI). Nitrate-N was determined using the Cd reduction method [38]. Total C and N were measured by dry combustion (EA1112 Flash NC Elemental analyzer, Thermo Finnegan Scientific Inc., Waltham, MA) of air-dried ground soil.

2.4 Fatty acid methyl ester (FAMEs) analysis

Soil samples used for quantification of soil microbial community and abundance were collected in August at peak biomass and October when regrowth occurred. Measurement of ECa was conducted at the beginning of the season prior to

livestock being grazed on the pasture field. ECa is a product of dynamic soil factors (e.g., soil moisture) and static measurements (e.g., bulk density, clay type) [39] and is generally stable throughout the growing season. Sampling following the soil ECa gradient (described above) was repeated at the 12 selected points, while soil sampling following the transect method were taken at points about 15–25 m apart along a SE-NW and NE–SW transects during August and October sampling, respectively. The GPS coordinates of locations where soil samples were collected were obtained via the GPS app locator of a smartphone to identify transect sampling points on the pasture site map. Approximately, 50 g of soil core taken at a depth of 10–15 cm was transferred to plastic ziplock bag and kept in an icebox. Between samples, the soil corer was cleaned with alcohol (70%) to prevent cross contamination of samples. Soil samples were transported to the laboratory where they were processed using 2 mm sieves to remove pebbles and any plant material and immediately stored in the freezer (−20°C) until ready for assay.

For the assessment of microbial diversity and abundance, the total microbial fatty acids (FA) were extracted following the procedure described by Schutter and Dick [30]. Briefly, total microbial lipids in 5 g soil samples were extracted in 10 ml of 0.2 M methanolic potassium hydroxide and the mixture heated at 37°C for 1 h with intermittent shaking. The solution was then neutralized by adding 1 N acetic acid and the lipids dissolved in hexane. The mixture was centrifuged at 6000 rpm for 10 min and the supernatant was carefully recovered, filtered, and further processed before fatty acids were quantified using gas chromatography with 0.05 mg/ml nonadecanoic acid (C19:0) as an internal standard. A total of 19 FAMEs were retained and used to determine microbial community composition following FAMEs nomenclature of the IUPAC-IUB Commission on Biochemical Nomenclature (IUPAC-UIB, 1987). Specific fatty acids with 14–20 carbon composition were used to represent fungal, bacterial groups, and micro-eukaryotes. Bacterial biomass was represented by the sum of 10 FAMEs: iC14:0, iC15:0, aC15:0, C15:0, iC16:0, iC17:0, aC17:0, C17:0, cyC17:9, cyC19:9,10, and cyC19:11,12 [16]. Actinomycetes bacteria were quantified by 10Me fatty acids: 10MeC18:0 and 10MeC19 [22, 40], while saprophytic fungal biomass was represented by C18:2cis9,12 [41]. In addition, micro-eukaryotic biomass was represented by the sum of C20:3, C20:4 and C20:5 [19, 42]. Finally, the fatty acid C16:1cis11 was used as a biomarker for arbuscular mycorrhizal fungi (AMF) [43]. Total microbial biomass was estimated by summing up FAMEs representing bacteria, actinomycetes, saprophytic fungi, and AMF. In addition, total FAMEs for bacterial (bacteria and actinomycetes) and fungi (AMF and saprophytic fungi) were used to calculate the ratio of fungi to bacteria biomass in the soil.

3. Statistical analysis

The resulting datasets from FAMEs analysis on the microbial groups were analyzed using generalized linear mixed models procedure (GLIMMIX Procedure) in SAS® 9.4 software package and means separated at $p < 0.05$. This analysis involved testing sampling methods (ECa-directed vs. transect-based), the effect of sampling date/time as well as any interactions between sampling method and time. To further investigate any impacts of plant (brome grass monoculture) as well as the effect of soil physical and chemical parameters on microbial diversity and abundance, principal component analysis (PCA) was conducted. The PCA was performed to determine the contribution of soil characteristics (e.g., ECa, pH) in the variation and separation of both temporal and sampling method. Soil chemical, physical and biological attributes were used in the PCA to elucidate the effects of

sampling technique and timing of sampling. This was conducted using R programming software version 3.4.1, utilizing ade4 package [44] and factoextra package for visualization purposes [45].

In addition, the relationship between soil microbes and soil physicochemical characteristics was visualized using heatmap. The heatmap was generated using a combination of packages and their functions in the R programming language. The hclust function and scale both available in stats package were utilized, respectively, for hierarchical cluster analysis after applying the scale function to centralize the various data about the mean and to generate z-scores around each variable's standard deviation about the mean. Cluster analysis was performed using the default 'complete' agglomeration method. The melt function in the reshape2 package was used to organize the dataset before plotting the heatmap using ggplot function acquired from the ggplot2 package. The z-score legend was generated using scale_fill_gradient2 function in ggplot2 package and color breaks represented z-scores ranging from −3 to 3 over the entire dataset.

4. Results and discussion

4.1 Site characteristics

The physical and chemical characteristics of soil in the brome grass pasture are summarized in **Table 1**. Soil organic matter contents were relatively high, averaging 4.14%. High organic matter content in the top 20 cm of the pasture soil can be attributed to dense rhizomatous roots of brome grass root biomass and shoots [32]. Soil nutrient availability to crops is influenced by soil pH. The pasture site exhibited a slightly acidic soil pH (5.98) and was positively correlated (R^2 0.89, $p < 0.05$) with calcium but negatively correlated to percent H+ (R^2 0.95, $p < 0.05$). Available N (Nitrates-N ppm) of soils sampled from the pasture site averaged 4.42, which can be attributed to the cow dung manure, N fertilizer, as well as the high biomass from the brome grass shoots and roots. 1:1 soil soluble salts (mmho cm^{-1}) was strongly and positively correlated to measured available soil nitrates (R^2 0.79, $p < 0.05$). Available P in the pasture site was measured at 18.07 ppm P. Hydrogen (H^+) cations contributed significantly to the total sum of cations me 100 g^{-1} (CEC) of the pasture site.

Values for ECa ranged from 21 to about 44 mS m^{-1} (**Figure 1**), which indicated a low to moderate level of spatial site heterogeneity. The mean ECa value was 32 mS m^{-1} with ECa of 30.1–35 and 25.1–30 mS m^{-1} being more common (the two combined covered nearly the entire the pasture). Regions with lowest and highest ECa values mainly constituted small pockets that were randomly distributed across the pasture with no clear pattern that could be discerned (**Figure 1**). As a result, soil in this pasture was considered to be less variable and the ECa values fell within normal range of 0–150 mS m^{-1} for grass pasture [35] but lower than in a fertilized maize field [46] within Eastern Nebraska.

4.2 Soil microbial community

General composition of microbial communities, namely, total microbial biomass, diversity, and composition of soil microbes detected using FAMEs assay in soils sample collected over two seasons and methods is presented in **Figures 2** and **3**. Each sector of the pie chart (**Figure 3**) represents individual microbial composition as a percentage of total recovered microbial fatty acid. Microbial biomass was dominated by bacteria (55–65%), followed in declining order by arbuscular mycorrhizae (15–25%) saprophytic fungi (8–9%) actinomycetes (8%) micro-eukaryotes (4%).

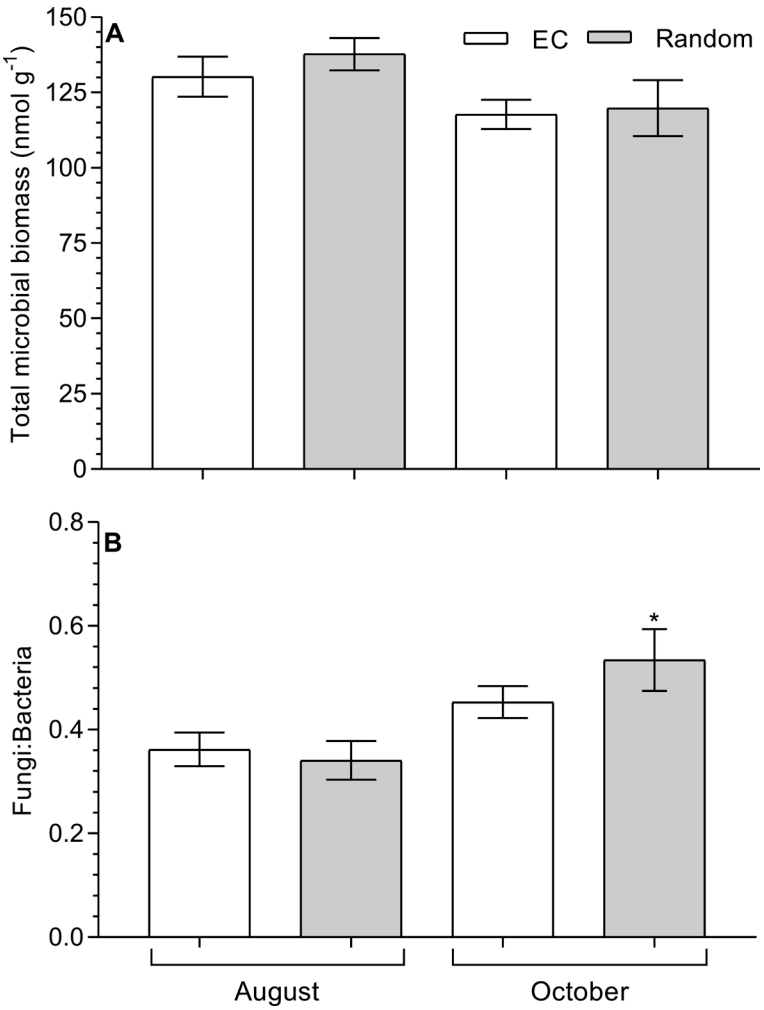


Figure 2.
Total microbial biomass (A) and the ratio of fungi to bacteria (B). Individual bars represent the mean and standard error collected following EC_a (clear bars) and random method (dark bars).

Bacteria was found to be highly correlated (R^2 0.84, $p < 0.05$) with actinomycetes. This was consistent across the two sampling durations. Bacteria correlation with saprophytic fungi (R^2 0.53–0.6, $p < 0.05$) and micro-eukaryotes (R^2 0.56–0.59, $p < 0.05$) was only significant during August and October sampling, respectively. The abundance of soil micro-eukaryotes showed significant correlation with AMF (R^2 0.74–0.77, $p < 0.05$) for both soil sampling methods while the correlation with saprophytic fungi (R^2 0.64, $p < 0.05$) and actinomycetes (R^2 0.72, $p < 0.05$) was observed only in August and October, respectively. Furthermore, the total recovered microbial FA was found to be strongly correlated with all microbial groups ranging between R^2 0.59 and 0.89 and was statistically significant across all microbial groups and sampling times except for AMF ($p = 0.59$) in October samples. The high correlation of bacteria with actinomycetes are similar to that of Grigera et al. [16] who established a high level of correlation (R^2 0.88, $p < 0.05$) in an agricultural field in Buffalo county, Nebraska, continuously cropped with corn. These high correlations highlight similarities in the edaphic conditions (e.g., pH and organic matter content) in which the microbes coexist in complementary biogeochemical functions in recycling both N and C [47].

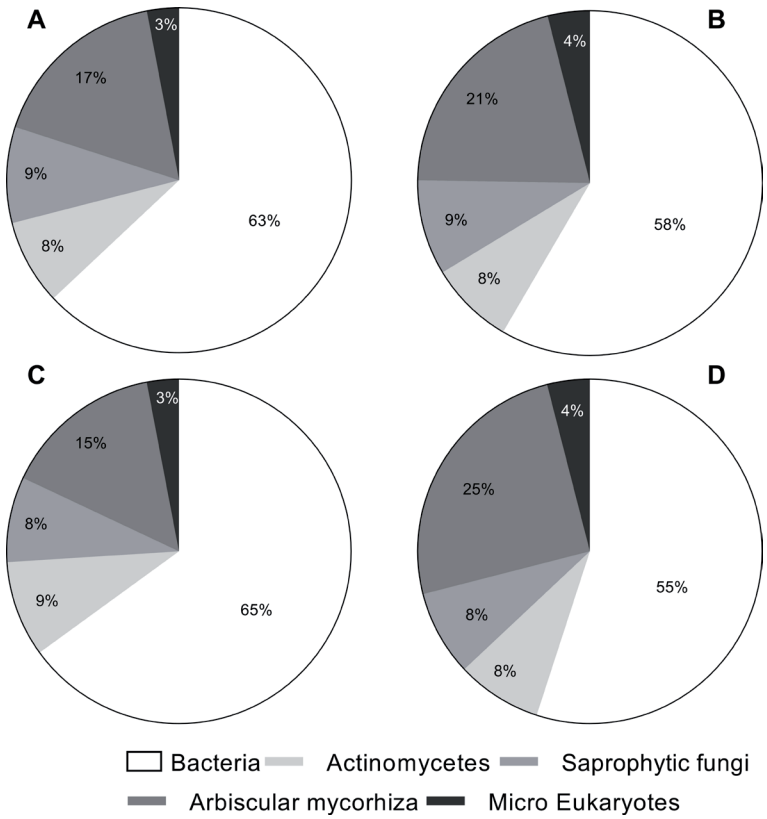


Figure 3.
Pie charts showing diversity and composition of soil microbes detected using FAMEs assay in soils sample collected in august (a and C) and October (B and D). Soil samples were collected following ECa-based method (A and B) and random method (C and D). Each sector represents individual microbial composition as a percentage of total recovered microbial fatty acid.

4.3 Comparison of sampling methods

Statistical analysis did not show any significant differences in soil microbial biomass of soil samples collected based on ECa stratification or transect sampling methods. The sampling method did not result in any significant differences (**Table 2.**) in the abundance of bacteria, actinomycetes, saprophytes, mycorrhizae, or micro-eukaryotes. Transect sampling technique has the same sensitivity and reliability as an ECa-based method in capturing the spatial and temporal dynamics of soil microbiota and can thus be used as a method of choice for sites with a relatively low range of ECa variability indicative of similar soil chemical,

		Type III tests of fixed effects			
Effect	**Bacteria**	**Actinomycetes**	**Saprophytes**	**Mycorrhizae**	**Micro-eukaryotes**
Date (D)	<.0001	0.002	0.239	0.076	0.740
Sampling (S)	0.583	0.650	0.660	0.413	0.589
DxS	0.257	0.274	0.656	0.318	0.951

Table 2.
Summary table of date (D) and sampling methodology (S) and their interactive effects of D and S on bacteria, actinomycetes, saprophytes, mycorrhizae, and micro-eukaryotes sampled at the PR-HPA site in Mead, Nebraska.

physical and microbial properties. The two soil sampling techniques used in this research (i.e., ECa- and transect-based) captured comparable soil microbial communities and abundance in both space and time highlighting the significant role of vegetation on soil microbial communities as highlighted by others like Grigera et al.; Lauber et al.; Pereira e Silva et al.; and Segal et al. [16–19].

4.4 Effects of temperature on soil microbes

There were statistically significant seasonal differences in the total soil microbial biomass irrespective of soil sampling technique with a considerably higher abundance in August compared to October. Total soil microbial mass in August and October soil samples had means of 130.2 and 137.7 nmol g^{-1} in August compared to 117.7 and 119.8 nmol g^{-1} in October for soil samples collected via ECa-directed and transect-based sampling methods. Despite the observed decline in October, which was cooler, no statistical difference was observed in space and time for microbial biomass.

When examining the effect of sampling date (i.e., August vs. October) on the abundance of individual microbial groups, results demonstrated a significant shift in soil biota with temporal changes affecting selective groups. Specifically, saprophytic fungi, actinomycetes, and micro-eukaryotes remained seasonally stable and constituted about 8–9 and 3–4% of the total microbial biomass, respectively (**Figure 2**). Bacteria and AMF abundance exhibited a significant temporal variability. In particular, a significant decline of bacterial biomass ranging from 4 to 10% observed in August and October, respectively, was observed irrespective of method of soil sampling. In contrast to bacteria, a significant increase (4–10%) in AMF abundance was noted in soil sampled in October compared to August (**Figure 4**). Bacterial abundance declined by up to 10% from August to October with a corresponding 10% increase in AMF observed during the same period.

Fungi to bacterial (F:B) ratio which is indicative of the changes in soil microbial communities [48] was calculated in August (0.30–0.39) and noted to have increased (0.45–0.53) in October, representing a 20 and 32% increase for ECa and transect soil samples, respectively. Although there was a general increase in F:B in October samples, statistical significance was only observed in soil sampled via the transect

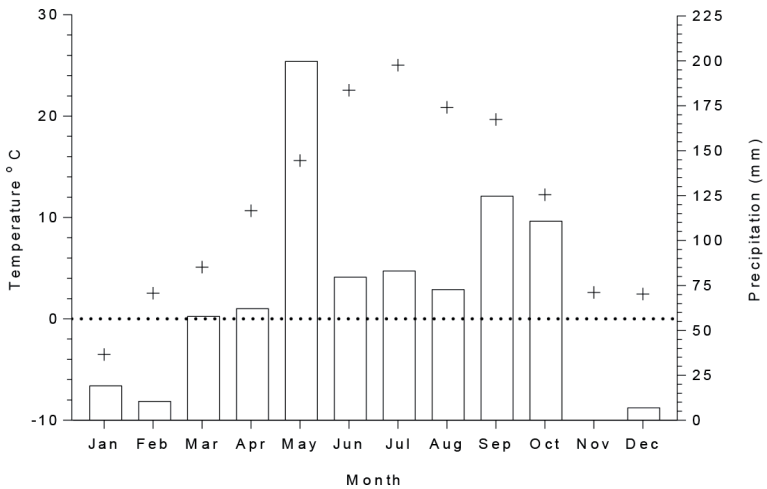


Figure 4.
Monthly average temperature and precipitation from Mead weather station (41.17° N, 96.47° W) closest to the pasture study site (4 km) at the East Nebraska research and Experimental Station (ENREC). Bars indicate total monthly precipitation while stars show mean monthly temperature.

method (**Figure 3B**). Increase in F:B ratio during the cooler month reflected the shift in compositional abundance of fungi and bacteria that was observed in October (**Figure 2**). Commonality in trend in total microbial biomass and the ratio of fungi to bacteria as observed along time illustrates the comparable sensitivity of the two soil sampling methods [16, 22, 49].

Shifts in soil microbial communities are affected by seasonality and specifically temperature and moisture. Temporal changes in soil microbial abundance observed in our work has also been demonstrated elsewhere in soil under controlled environment [3, 50] as well as in different ecosystems including forests [51–54], deserts [55], cultivated land, [17–19] as well as grasslands [17]. While fluctuation in microbial abundance across the soil types are common, and the key drivers tend to vary according to the location and soil type, with environmental factors such as temperature and precipitation being the most dominant factors [17, 50, 54, 55].

Our results concur with those of several researchers such as Papatheodorou, Argyropoulou, and Stamou [56] who conducted studies on soils from a grassland in Mediterranean Greece. They detected a linear decline in bacterial diversity, evenness, richness, and mean oxidation, especially of carbohydrate and carboxylic substrates over a 6-month (July to December) study. Substrate use efficiency and specifically carbon use efficiency have also been found to decrease with nutrient availability and increasing temperature [57]. Our study's August and October environmental conditions showed that the monthly mean temperature was higher (20.8°C) in August compared to October (12.2°C). Cumulative monthly precipitation measured 78.89 mm and 110.99 mm for August and October, respectively, which for this area is about average for August but double the average for the month of October (**Figure 2**). In addition, we examined in detail these two environmental parameters recorded 4 days preceding the soil sampling dates. The results summarized in **Table 3** showed that average temperature was 20.5°C in August and dropped to 10.7oC in October. With respect to precipitation, a cumulative 1.27 mm of rain was received 3 days prior to the August sampling and none prior to the October sampling. While there were differences in precipitation both monthly (August and October) and the days preceding the aforementioned sampling dates, this variation did not have a significant effect on soil moisture as revealed by computed soil gravimetric water content (**Table 3**). This implies that, changes observed in bacterial and fungal composition (**Figure 4**) may possibly have resulted from factor(s) other than soil moisture which are discussed in the next section below. These results concur with those of [58] who characterized soil microbial communities and their conditioning by varied plant species. They noted that soil bacterial communities are primarily influenced by abiotic conditions; namely temperature and ECa (**Figure 5**). Fungal communities on the other hand are determined by biotic conditions such, as plant species [58] as seen with the

Date	Sample type	Temperature (°C)	Precipitation (mm)	Gravimetric water content (%)
August 31	EC	20.5 ± 2.2	0.3 ± 0.4	18.7 ± 5.9
	Random			19.8 ± 6.2
October 27	EC	10.7 ± 2.5	0	17.84 ± 2.4
	Random			17.39 ± 2.1

Table 3.
Summary of weather data and soil water content. Values indicate means and standard deviation of temperature and precipitation recorded 5 days before soil samples were collected. Gravimetric soil water content was calculated as the difference between fresh and dry soil of a unit of soil and the values indicate mean and standard deviation.

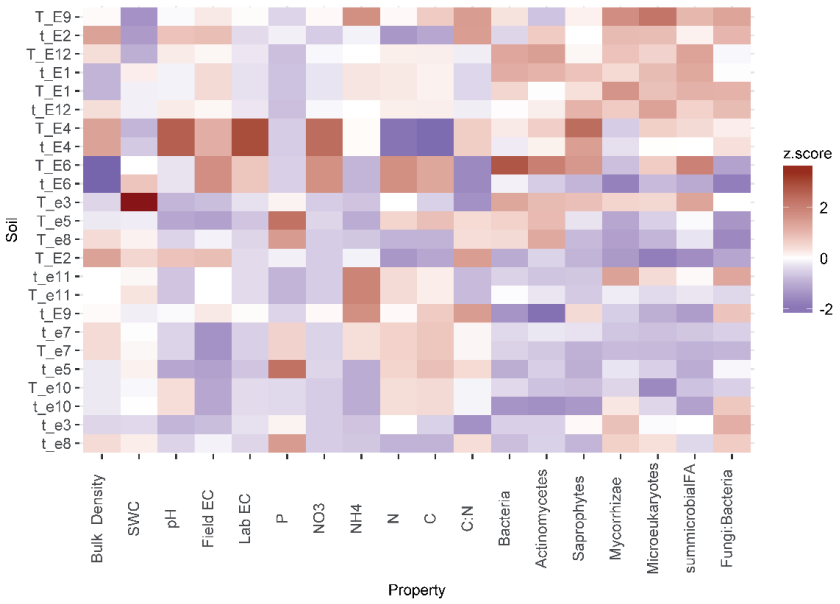


Figure 5.
Cluster heat map showing relationship between soil microbiota, soil physicochemical attributes, and environmental variables. Soil properties and microbes are indicated on the bottom, while soil EC_a and temperature are to the left of the image. The rows representing soil samples characteristics are clustered based on hierarchical cluster analysis of the values of the measured soil variables represented in the columns. These soil variables are sorted based on physical, chemical, and biological characteristics. Letters "E" and "e" represent high and low field EC_a, respectively, and classification was based on the median EC_a value of 32.5 mS m^{-1}. The EC_a values greater than 32.5 mS m^{-1} units are represented by "E," while those below the media are denoted by "e." Letters "T" and "t" represent the warmer and cooler month of August and October, respectively. The z values are represented by the blue color, while the color intensity shows the level of significance.

increased flush of Brome grass root growth during the late fall season. The brome plant-AMF microbial feedback elicits subsequent biases toward the development of brome grass monoculture.

4.5 Impacts of chemical, biological, and physical properties on soil microbial communities

The overall relationship between the soil microbes and soil physiochemical characteristics was computed and summarized in https://prhpa.unl.edu/supplementary-materials-ltar-pasture-soil-characteristics-0. The results show significant correlations between soil physical, chemical, and biological characteristics. In general, there were 30 and 24 statistically significant correlations among the measured soil parameters sampled in August and October, respectively. A total of 15 of these correlations were consistent across the two sampling time points (https://prhpa.unl.edu/supplementary-materials-ltar-pasture-soil-characteristics-0).

Concerning microbial groups and their abundance as impacted by soil physicochemical characteristics, bacteria was negatively correlated with bulk density (BD) and NO_3^- at –0.74 and – 0.66, respectively, while being positively correlated (R^2 0.73) with C:N. In addition, saprophytic fungi were strongly correlated with ECa, EClab, and NO_3^- at R^2 0.6, 0.84, and 0.88, respectively. On the other hand, in soil sampled in October, statistical significance was solely observed between AMF and soil NO_3^- (R^2–0.61). These results are in agreement with the direct effect of BD on soil drainage and its negative influence on bacteria populations similarly observed in crop-livestock studies [59]. Additionally, soils with high available NO_3^- demonstrate a lower population of bacteria necessary in nitrification processes

[47]. Soils with low N content, indicative of high C:N ratios, have higher bacterial populations. A highly positive association of ECa, EClab and NO_3^- with saprophytic fungi is attributable to the cationic byproducts as well as NO_3^- from extracellular breakdown of organic matter by the saprohytic fungi's enzymes. Seasonal variations that lead to lower soil temperatures influence bacteria populations and nitrification processes reducing NO_3^-. On the other hand, AMF colonize roots of regrowth brome grass roots resulting in their higher prevalence and abundance in October soil samples. Our findings concur with those of [58] who determined that plant species determined the relative abundance of AMF fungi in comparison to saprotrophs (e.g., saprophytic fungi and bacteria) which were influenced by soil abiotic factors such as pH.

The relationship between soil microbes and soil physicochemical characteristics as impacted by ECa variability of individual sampling point was examined using PCA (**Figure 6**). The first two PCA axes explained a total of 57.4% of the variability with PC1 and PC2 explaining 32.3 and 27.4%, respectively, of the total variation between several of the soil physicochemical characteristics, temperature, and microbial diversity (**Figure 5**). Specifically, PC1and PC2 largely explained variability in microbial groups and soil characteristics, respectively, (**Figure 6**). A consistent discrimination of soils was evident in the sum of microbial fatty acids which was noted as being negatively associated with relatively low ECa levels. High levels of ECfield, EClab, and NO_3^- where associated with soils of high microbial abundance which was similarly observed by [16] in a corn field in Buffalo county in Nebraska. This observation is also noted in this study as shown in the right hand corner of the heatmap which generally depicts soils of 32.5 mS m^{-1} exhibiting z-scores of above 0 across microbial types (**Figure 5**). High P levels on the other hand decreased AMF root colonization and spore density thereby decreasing microbial abundance and diversity in soils that are relatively high in P from sources such as fertilizers [60, 61]. Sources of P in this pasture site included effluents from the livestock in the form of manure and urine.

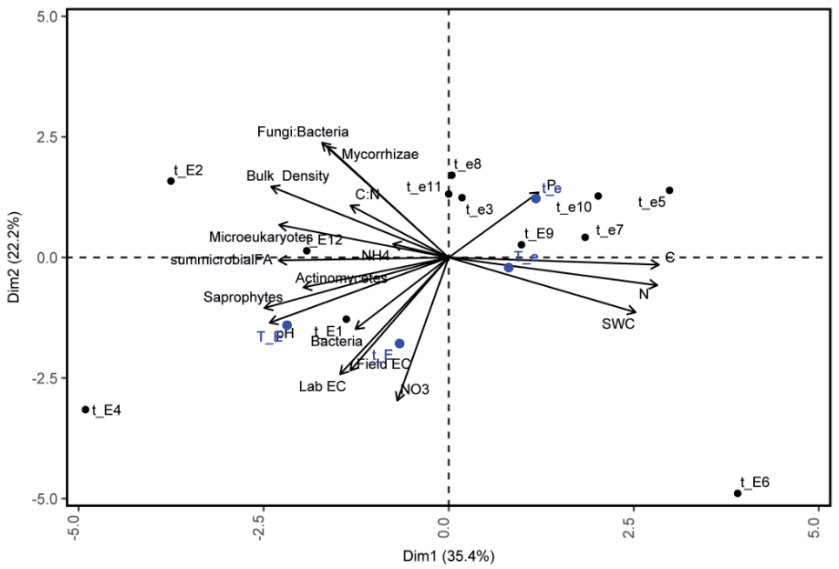


Figure 6.
Principal component analysis (PCA) of microbial groups in soil sampled via ECa method during the warm (August) and cooler (October) temperature. Abbreviations containing a combination of letters and numerals denote the seasons temperature (warm "T" and cool temperature "t") followed by soil ECa (high "E" or low "e" soil ECa values relative to the median value of 32.5 mS m^{-1}), while the numeral (1–12) indicates the point on the field where soil samples were collected following ECa gradient.

The pasture site comprised of a monoculture of brome grass, a cool season grass species with extensive below ground rhizomes and a unique a capacity to maintain active growth during cooler weather. Brome grass has been reported to yield high root biomass [62] with approximately 1014 g m^{-2} root mass measured in 0–10 cm depth of the soil. The seasonal shifts impacts in root biomass production impacted soil microbial communities specifically increasing AMF abundance [63] by influencing C availability [52, 64]. We speculate that as these plants were undergoing late season growth, facilitated by their inherent ability to withstand low soil temperatures (can withstand temperatures as low as –28°C); C allocation to the rhizomes (storage organs) increased as a survival strategy thereby affecting root exudate production. Exudates acting as cues coupled with changes in the production of these compounds have been shown to impact soil microbial community and composition [65]. Thus, elevated production of these signal molecules may have triggered a surge in species of AMF that preferentially associate or benefits from this grass species [66–68]. Bacteria in turn are critical for C nutrient cycling [69, 70]. Some bacteria species are also known to interact with plants symbiotically while fixing nitrogen and also externally in root zones, decomposing organic matter and releasing nutrients to plant roots [71]. Their abundance is largely influenced by the substrate quality of the roots and their exudates.

5. Conclusion

The underlying mechanisms that influence feedbacks and vegetation dynamics within a complex plant-microbial community interaction are largely unresolved [4, 56]. Soil ecosystems are dynamic and diverse, and their physicochemical characteristics vary spatially and temporally. In this study, we compared and contrasted the intra-microbial abundance and diversity of a pasture site in two sampling periods and sampling methods. Our results showed that several classes of soil microbes instrumental in soil nutrient cycling, plant health, plant organic matter decomposition, and soil stabilization were present. These included in order of abundance: bacteria (63%), AMF (17%), saprophytic fungi (9%), actinomycetes (8%), and micro-eukaryotes (3%). The composition of the soil communities changed with the falling temperature, with bacterial abundance diminishing by up to 10% from August to October with a similar magnitude of increase in AMF observed during the same period. Our results showed that the soil bacterial communities were primarily influenced by abiotic conditions, while fungal communities were shaped by the biotic environment such as the plant species such as seen during the flush of regrowth by brome grass (cool season grass) and reallocation of nutrients to root growth that contributed to AMF rapid proliferation and abundance. These findings may provide reasonable evidence that a prolonged positive feedback between brome grass plant-AMF microbial interactions elicits subsequent biases toward the continued dominance and development of brome grass monoculture in a site that was once natural grassland.

Our findings showed that the random sampling technique has the same sensitivity and reliability as an ECa-based method in capturing the spatial and temporal dynamics of soil microbiota and can thus be used as a method of choice for sites with a relatively low range of ECa variability, indicative of similar soil chemical, physical, and microbial properties, especially in locations with established legacy effects (in our case, more than 20 years of a brome grass monoculture). Our findings support and add to new information regarding temporal changes in plant-climate-soil interactions which have not been conducted previously for pasture sites dominated by cool-season grasses such as brome grass over several decades of development.

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Abbreviations

pH	potential of hydrogen
N	nitrogen
ECa	apparent electroconductivity
OC	organic carbon
WDRF BpH	Woodruff buffer pH

Author details

Taity Changa[1], Jane Asiyo Okalebo[1*] and Shaokun Wang[2]

1 School of Natural Resources, University of Nebraska, Lincoln, NE, USA

2 Urat Desert-Grassland Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

*Address all correspondence to: jane.okalebo@gmail.com

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