ISSN Online: 2349-7785

Relationship of Soil Microbial Community Structure and Soil Characteristics in Vertical Profiles in a Managed Meadow

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Received 11 November 2013; accepted in revised form 21 December 2013

Abstract: The relationship with depth of soil microbial communities to several soil characteristics was examined in two 25-cm deep trenches dug in a grass meadow covered with *Andropogon gerardii*, *Plantago lanceolata*, *Rudbeckia hirta,* and other grass species. Most measured soil characteristics tended to decrease in magnitude with distance from the surface to about 10-15 cm, below which the values were approximately constant. pH increased from 0 to 10 cm depth and did not change below that. Change in bacterial abundance with depth was similar to that observed for all soil characteristics except pH. Bacterial and fungal community structures were not highly correlated to one another in either trenches (P_B = 0.179 and P_D= 0.214). Only bacterial community structure of B trench was significantly correlated with soil characteristics by Mantel test (r_M = 0.618, P = 0.010). The lag distance at depth was not significantly associated with either bacterial or fungal community structures at either trench, while soil characteristics were significantly associated.Altogether, surface vegetation (*P. lanceolata* and *R. hirta* for B trench, and *A. gerardii* for D trench) as dominant factor differentiating microbial community structures between trenches with distinctive types of root exudates released from root systems.

Key words: Vertical profile, Vegetation effect, Mantel test, Non-metric multidimensional scaling, Canonical correspondence analysis.

Introduction

Soil presents a very diverse and heterogeneous environment for microbes in both its chemical and physical aspects. The structures of various soil microbial communities are shaped by and, in turn, influence their environment. The physical heterogeneity of the soil environment arises from the infinite combination of mineral and organic particles into aggregates of widely differing sizes, shapes, porosity, and pore size [39]. The chemical properties of soil are also spatially heterogeneous, and the combination of physical and chemical heterogeneity contributes to the diversity and heterogeneity of microbial communities by producing large numbers of different environments for microbial habitation. This physical and chemical diversity of soil accommodates a hyperdiverse assemblage of microbes [14,23,47], simply due to the bacteria's minute size within that heterogeneous landscape [16]. In addition, the relationship between soil microbes and the soil environment is fully interactive in that while soil properties influence the distribution of microbesidentity types [10,11] and activity, the soil

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microbes, in turn, affect soil structure by modifying the cohesiveness of soil particles through organic material secretion or entwinement in fungal or actinomycete hyphae [6,11].

The general relationship between plants and soil microbes generally involves the plants providing microbes a substrate ornutrient source [2,32,34]. However, mutualistic symbiosis between leguminous plants and nitrogen fixing bacteria is a good example of full bidirectional interaction, as is that of nutrient gathering and delivery to plants by mycorrhizal fungi.

The present study is part of larger project that investigates, among biotic and abiotic factors, those that exert control over the soil microbial community structure at a meadow undergoing secondary succession after revegetation with native grass species. The objective of the current study is quantitative understanding of the vertical profiles in which bacterial and fungal community structure co-varies with vertical profiles of soil characters, surface vegetation (through the root system) and lag distance in vertical sense. We wanted to know whether soil bacterial and fungal community structures vary along depth profile and which of measured candidate factors affect the changes most. We used denaturing gradient gel electrophoresis (DGGE) with universal primers for bacterial and fungal domains separately and series of multivariate statistical tools to investigate the latter objective.

Material and methods

Study site and sampling

A 14-ha meadow at the Blandy Experimental Farm (BEF), Boyce, VA, was burned and treated with herbicide then reseeded for re-vegetation with native grass species [24]. Three years after the treatment and reseeding, several shallow trenches were cut in the field for the examination of microbial distribution, and two of the trenches (B and D) located 5.66 m apart were selected for examination of depth profiles of microbial community structure. Two trenches were selected due to their distinct above ground vegetation: a nearly monospecific stand of *Andropogon gerardii* for B and a mixed 3 dominant species of *A. virginicus*, *Bouteloua curtipendula* and *Schzachyrium scoparium* for D. From each trench, approximately 30-40 g of samples was taken at 2-cm intervals from the surface down to 10 cm. Thus, the 4-6-cm sample was the entire interval from 4 cm to 6 cm below the surface. Samples were taken at 5-cm intervals from 10 to 25 cm depth. The samples were homogenized and separated into portions for soil characterization and microbial community structure analysis. Portions of soil samples for microbial analysis were stored and transferred to the lab in frozen with ample amount of ice, while portions for soil characterization were handled in fresh. The overlying plant species and depth of the root zone were recorded at each of the trenches.

Soil characterization

Approximately 15 g of fresh soil from each sample was dried for 24 hours at 105°C in a drying oven, and the percentage water (P_w) was determined as the weight loss divided by the dry weight of the soil. Total organic matter content (OM) was then measured on each sample as the weight loss from oven dried soils upon ignition at 450°C for 24 hours.

Soil pH was measured by placing approximately 5 cm^3 of fresh soil sample and about 10 ml of distilled water in asmall beaker. Suspensions were well mixed with a glass rod, and pH was measured in the overlying solution with a combination electrode after the soil had settled [46]. The actual reading was obtained when measurements were stable for at least 5 seconds. The electrode was rinsed with distilled water between each measurement, and care was taken not to touch the solids with the electrode while making the measurement.

Total carbon and nitrogen contents were measured using an elemental analyzer CE EA 1108 CHNS-O (Fisons Instruments, Italy) with accompanying Eager 200 software. Approximately 20 mg of sifted (approximately 700-µm), air-dried, soil sample was prepared in tin cups. Two different standards were included along with the soil samples: atropine ($C_{17}H_{23}NO_3$, 70.56 % carbon and 4.84 % nitrogen) and acetanilide (C_8H_9NO, 71.09 % carbon and 10.36 % nitrogen). Soil samples were assayed in duplicate. Due to

the amount of sample available, samples from only one trench (B) were analyzed for particle size distribution using the hydrometer method [18].

Bacterial abundance

The total abundance of soil bacteria was estimated by direct counting using epi-fluorescence microscopy with acrid ineorange (AO) staining of the bacterial cells [7]. Soil suspensions and AO solution were mixed and filtered to collect stained cells on a black-dyed, 0.2-µm pore diameter membrane filter (Osmonics, Livermore, CA). Cells were counted with an oil-immersion objective (1000 X total magnification) under epifluorescent illumination. Counting was done on a minimum of 5 fields and was continued until the total count reached 200 cells or until 10 complete fields were counted.

DNA preparation and microbial community structure analysis

DNA for soil microbial community analysis was extracted with the UltraClean™ Soil DNA isolation kit (MoBio Laboratories, Inc., Solana Beach, CA). The actual protocol incorporated an 'alternative lysis method' in which the mechanical lysis was replaced by multiple short incubations at 65°C resulting in less shearing of the extracted DNA to reduce the frequency of chimeric amplicons [35] which could artificially influence the results of further analysis [31]. To determine the DNA extraction yield and to determine the required volumes of suspended DNA for genetic finger printing techniques, the concentration of DNA in extracts was measured using the Pico Green®ds DNA Quantification kit (Molecular Probes, Eugene, OR) with a BioLumin 960 microassay reader (Molecular Dynamics, Piscataway, NJ). Absorbance was measured at 260 nm (A_{260}).

Soil microbial community structure was analyzed by using (DGGE) profiling of SSU rDNA within the community DNA. The primer sets for bacterial and fungal community and specific experimental protocols were described previously [25]. Briefly, universal primers sets for bacteria (P63f-P518r) and fungi (FF390-FR1) were used to amplify SSU rDNA fragments. The amplified SSU rDNA fragment mixtures were then separated on a polyacrylamide gel with urea-formamide denaturant, and the community structure was evaluated by analyzing the patterns of bands obtained on the gel.

Statistical analysis

The approach to analyzing the band patterns has been described and used previously [17,25]. In the present study, the binary data (presence or absence of bands obtained from the DGGE analysis) and the continuous variables, i.e.,soil characteristics, were analyzed by using two separate ordination methods, principal component analysis (PCA) and non-metric multi-dimensional scaling (NMDS) [33], to detect possible artifact of ordination [36]. Only NMDS results are presented, however, because the two ordination methods produced results that were virtually the same in their overall trends, and that led to the same conclusions. Analysis of variance (ANOVA) was performed on bacterial abundance data using SAS 8.2 (SAS Institute Inc., Cary, NC). Both partial Mantel tests [30] and Procrustes tests [37] of the NMDS ordinations were used for multivariate correlation analysis between microbial communities and soil characteristics. The auto-correlation in depth was removed by using distance matrix in partial Mantel test. Direct gradient analysis was done by first selecting the important soil characteristics using Bio-Env [12] and then canonical correspondence analysis [45]. Due to the confusing effect of noisy soil characteristics, Bio-Env procedure selected most significant subset of all soil characteristics by comparing all possible subsets of soil characteristics and microbial communities. Various functions of package 'vegan (version 2.0-6)' of R (R Development Core Team 2012) were used for all multivariate statistical analyses.

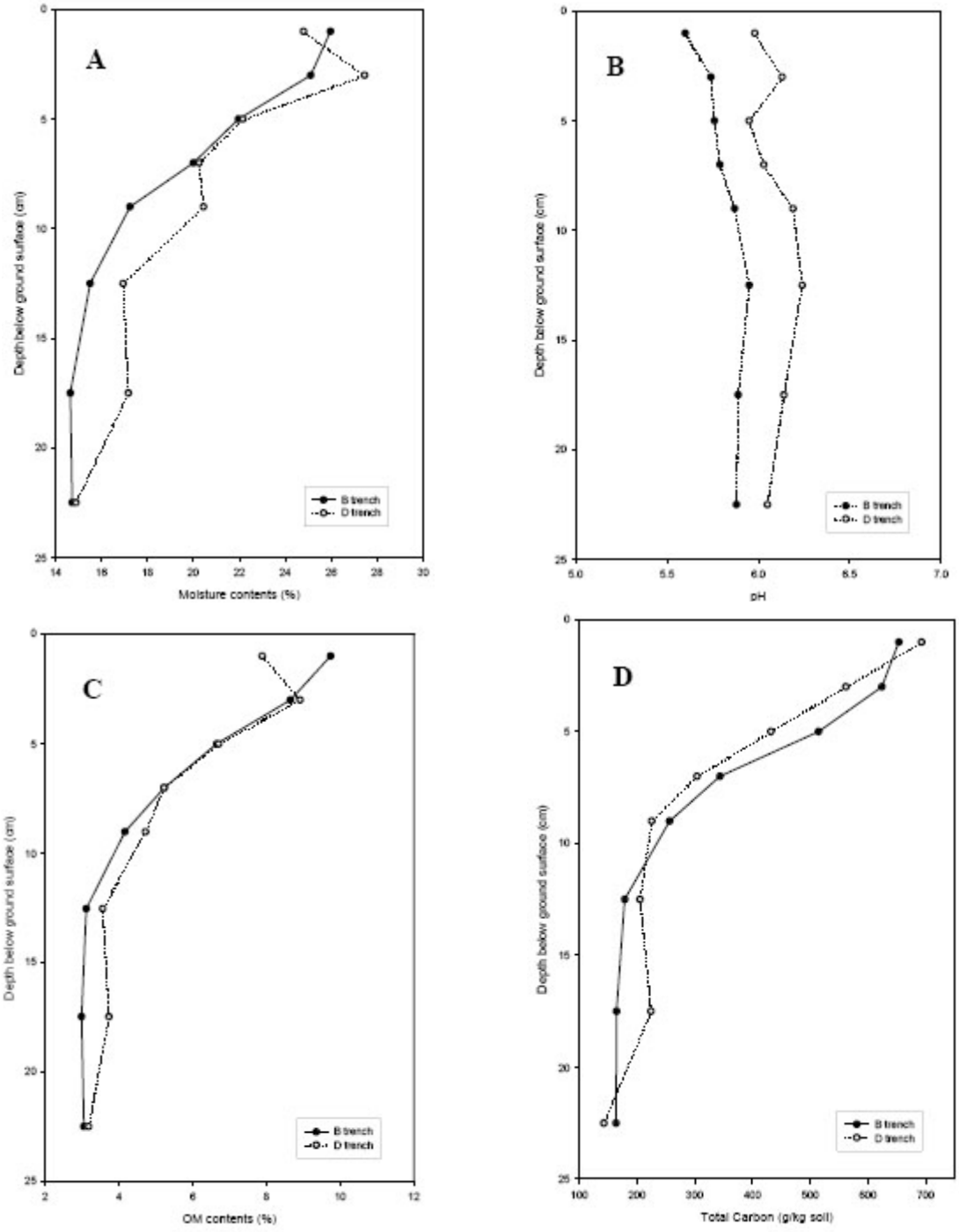
Results

Vertical profiles of soil characteristics

The vertical profile ofseveral soil chemical and physical properties was determined in each trench (Fig. 1). An overall trend of a decrease in the measured value with depth to a stable level at about 10-15 cm was observed for all the soil characteristics except for pH, the profile of which showed a slight increase with depth through the

first 10-15 cm. Moisture content of the soil was high at the surface (25.5 % in trench B and 26.1 % in trench D, average from 0 cm to 4 cm), and decreased continuously to around 15 % in each trench at 20-25 cm below the surface (Fig. 1A). pH increased down to 15 cm, then either wasconstant or decreased slightly thereafter (Fig.1B). Although differences in pH between the trenches are small, the B trench was slightly more acidic than the D trench at every measurement depth.

The general vertical profile of organic matter was very similar to that of moisture content in both trenches (Fig.1C). As expected, organic matter content was highest at the surface (9.2 % in B and 8.4 % in D, average from 0-4 cm) and



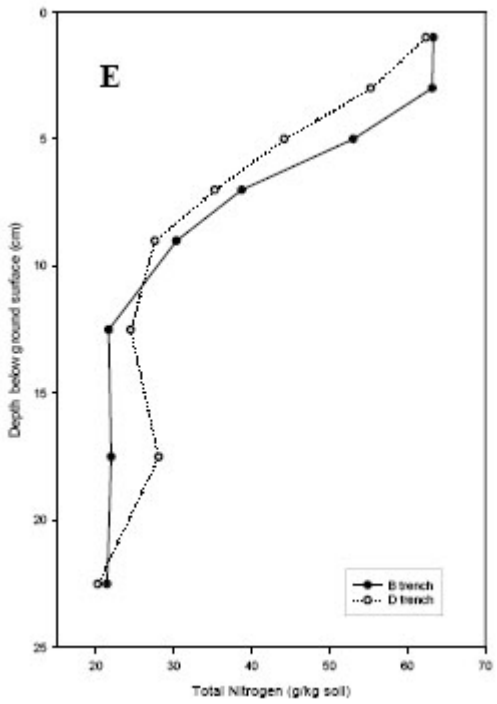


Fig. 1. Vertical profiles of soil characteristics measurements at both B and D trenches

gradually decreased to about 3 % in both trenches at 15-25 cm. As was seen for moisture content, organic matter content reached a stable minimum at around 10-15 cm below the surface in both trenches, and visible roots were observed mostly in the upper 10-15 cm in both trenches.Because of a limited amount of soil sample, data for % clay from the particle size analysis were not available for trench D. The vertical profile of % clay in trench B (data not shown) also followed the general profile trend; it was highest at the surface (44.0 %) and stabilized at around 30 % at the bottom of the trench. The only noticeable difference was significantly smaller % clay at 10-15 cm interval (25.3 %).

Total carbon content (Fig. 1D) was highest at the surface (30.11 g/kg soil in trench B and 31.25 g/kg soil in trench D) and lowest at the bottom (7.19 g/kg in B and 6.48 g/kg in D). Total nitrogen content followed that pattern closely (Fig. 1E). For both total carbon and nitrogen, minima were also reached around the 10-15 cm depth. The C/N ratio decreased slightly (11 to 7) and continually decreased from the surface to the bottom of the trenches (Fig.1.F).While the change is real, the small difference between the values at the top and the bottom create doubt that the change is meaningful.

All 16 depth samples from the two trenches were represented on the same ordination space, so that inter-relatedness between two trenches was revealed at qualitative level (Fig. 3.A). The overall trends between PCA and NMDS were very similar in that both trenches have nice monotonic trajectories from top to bottom of the sampling profile. Even if individual depths did not perfectly match between trenches, the overall match was quite good especially on dimension 1. The Mantel test showed very significant between-trench correlation soil characteristics as a whole (Table 1).

Vertical profiles of soil microbial communities

Since all measured soil characteristics were chosen based on the presumed close relationships with soil microorganisms, it was expected that the vertical profiles of the soil microbial community would be similar to those of the soil characteristics. Although the data were not congruent, the overall vertical profile of bacterial abundance followed the general soil characteristics pattern (Fig. 2). There was an overall

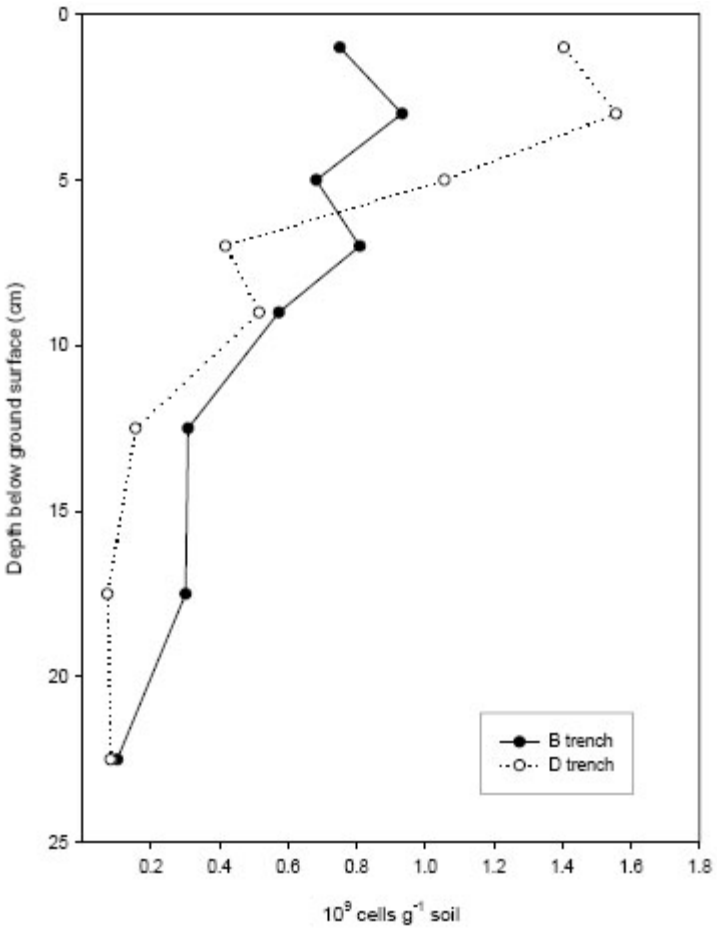


Fig. 2. Vertical profile of total bacterial abundance measured by epi-fluorescent microscope after Acridine Orange staining at both B and D trenches.

significant difference in the vertical profile of B trench (P = 0.001) and the total abundance data were grouped roughly 0 - 10 cm and 10 - 25cm by pairwise comparison using Ryan's Q test. Total abundance of D trench was also significantly different in overall profile (P< 0.001) and two groups (0-6 cm and 6-25 cm) were identified by pairwise comparison. ANOVA indicated no difference between two trenches (P = 0.550), and correlation analysis confirmed it with significant correlation (Table 1).

The ordinations between PCA and NMDS were very similar in their overall patterns for both bacterial and fungal community structures. However, the general trends were not as clear as it was on soil characteristics ordination (Fig. 3.B and C). In general B trench microbial community structures were relatively clear in their pattern in that the community structure shift were upper portion of clockwise direction in bacteria and lower portion of clockwise direction in fungi. Bacterial community structure was fairly well correlated between trenches by both Mantel test (Table 1) and Procrustes test (t_{bact} = 0.498, P = 0.100), while fungal community structure of two trenches were very different(t_{fung} = 0.290, P = 0.853).Comparison between bacterial and fungal community structures also observed as fairly weakly associated by the partial Mantel test with lag distance matrix (Table 2) and by the Procrustes test (t_B = 0.498, P = 0.340 and t_D = 0.417, P = 0.411).

Relationship between soil microbial community structure and soil characteristics in vertical gradient

The vertical profiles of bacterial and fungal community structures were not correlated

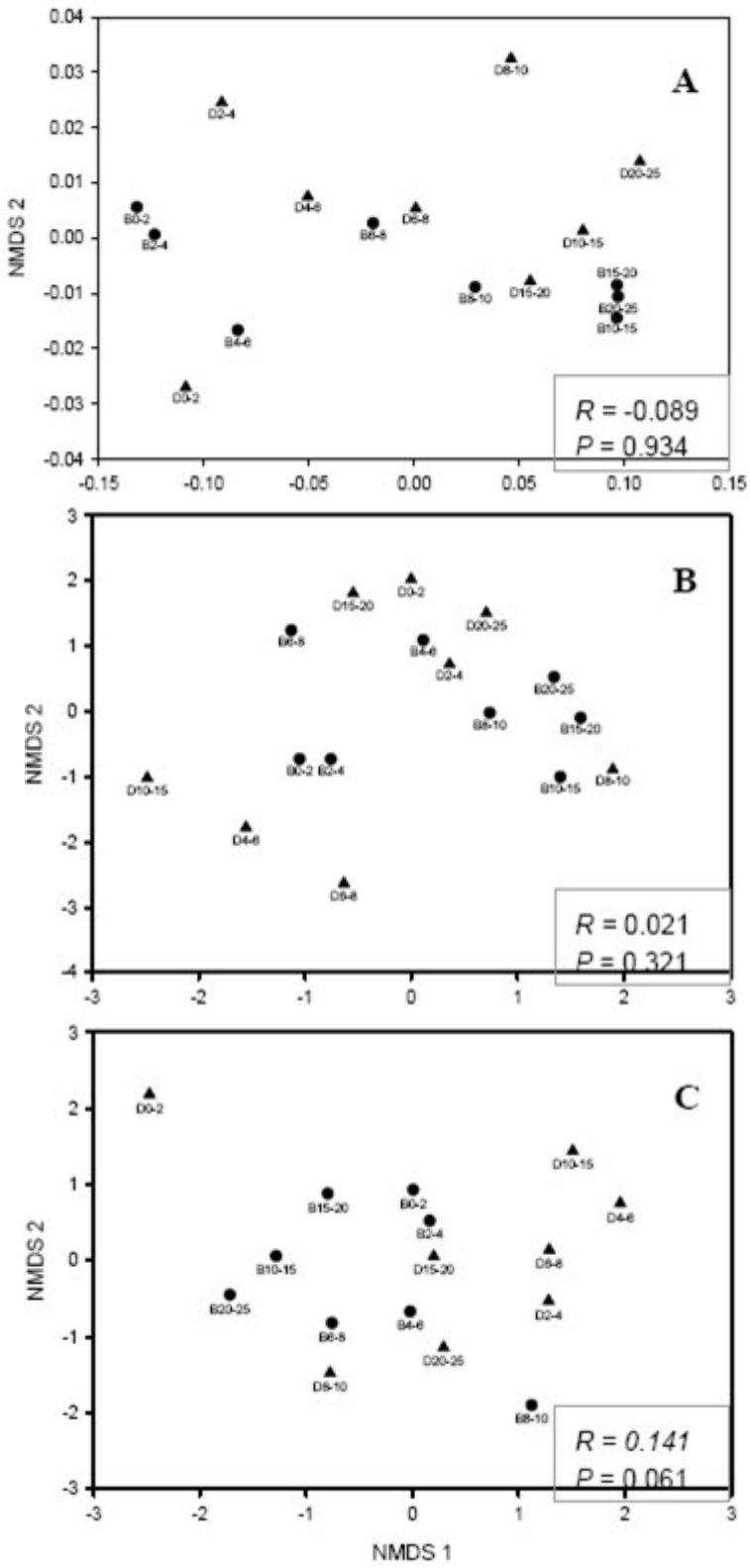


Fig. 3. Non-metric multidimensional scaling (NMDS) plot of soil characteristics (A), bacterial (B) and fungal (C) community structures. The plot of soil characteristics is based on Euclidean distance, and the plots of microbial communities were based on Jaccard coefficient (1 – *J*).

Table 1. Comparisons between B and D trenches by Pearson's product-moment correlation analysis and Mantel test

	Correlation analysis	Mantel test
Bacterial community		$r_M = 0.244$ $P = 0.051$
Fungal community		$r_M = -0.119$ $P = 0.296$
Bacterial abundance	$r_M = 0.822$ $P = 0.012$	$r_M = 0.507$ $P = 0.023$
Soil characteristics		$r_M = 0.897$ $P < 0.001$
Carbon	$r_M = 0.970$ $P < 0.001$	$r_M = 0.907$ $P < 0.001$
Nitrogen	$r_M = 0.972$ $P < 0.001$	$r_M = 0.909$ $P < 0.001$
C/N ratio	$r_M = 0.955$ $P = 0.051$	$r_M = 0.856$ $P < 0.001$
Moisture	$r_M = 0.945$ $P = 0.051$	$r_M = 0.827$ $P < 0.001$
Organic matter	$r_M = 0.966$ $P < 0.001$	$r_M = 0.912$ $P < 0.001$
pH	$r_M = 0.712$ $P = 0.048$	$r_M = 0.034$ $P = 0.381$

Table 2. Results of the partial Mantel tests (distance matrix holding) and Mantel test (with distance matrix) comparing the community structures of bacteria and fungi, soil characteristics and distance on vertical gradient. The significant test was based on 10,000 permutations

B trench	**Fungi**	**Soil characteristics**	**Distance**
Bacteria	$r_M = 0.165$ $P = 0.228$	$r_M = 0.581$ $P = 0.016$*	$r_M = 0.285$ $P = 0.072$
Fungi		$r_M = 0.310$ $P = 0.074$	$r_M = 0.250$ $P = 0.131$
Soil			$r_M = 0.610$ $P = 0.012$*
D trench	**Fungi**	**Soil characteristics**	**Distance**
Bacteria	$r_M = 0.147$ $P = 0.275$	$r_M = -0.072$ $P = 0.781$	$r_M = -0.014$ $P = 0.533$
Fungi		$r_M = 0.284$ $P = 0.152$	$r_M = -0.075$ $P = 0.600$
Soil			$r_M = 0.522$ $P = 0.029$*

*Mantel and partial Mantel test was significant at α level of 0.05

between trenches (Table 1). The high correlation of soil characteristics between trenches indicated that soil characteristics were not the major controlling factor on soil bacterial and fungal community structures. Only bacterial community structure in B trench was significantly associated with soil characteristics (Table 2).

The pairwise Pearson's correlation analysis was done among the total bacterial abundance data and all soil characteristic measurement (data not presented). As suggested from the graphic representations of vertical profiles, pH was the least correlated with other variables especially in the D trench (all negative Pearson's correlation coefficients). Moisture content was correlated with other variables better than any other variables ($r \geq |0.875|$ in B trench and $r \geq 0.892$ (except with pH, r = -0.529) in D trench). All measurement of soil characteristics were well correlated to each other including pH, which negative correlated. The only exception is pH in D trench, which did not have any significant correlation with other soil characteristics. % clay was well correlated with organic matter (C, N and C/N ratio) and moisture, but poorly correlated with total bacterial abundance (r = 0.570 and P = 0.1403). Bacterial abundance profile was very well matched with soil characteristics ($r \geq 818$), even relatively well with pH (r_B= -0.727, P = 0.041 and r_D = -0.425, P = 0.293) indicating importance prevalent changing trends at top 10 cm (root zone).

Associations among variables including microbial community structures, soil characteristics and vertical distance were analyzed using Mantel test. The overall pattern among soil characteristics was similar to that from correlation analysis, even if specific values of coefficients and probabilities were different in many cases. As summarized in Table 2, only bacterial community structures in B trench were significantly correlated with collective soil characteristics. The pairwise Mantel tests of individual soil characteristics confirmed it; bacterial community structures in B trenches were significantly correlated with all soil characteristics except pH (P< 0.05). All three other microbial community structures were not significantly correlated with any of soil characteristics, except total nitrogen content with fungal community in B trench (r = 0.370, P = 0.036).

The changes in soil microbial community structures in the soil profiles were more complex. The Mantel test results among bacterial and fungal community structures, soil characteristics and the average distance between the samples suggested complex relationships among them with inconsistency between the trenches. The overall assumption of close relationships between microbial community structure and soil characteristics (including physical characters and not just soil nutrients) was weakly confirmed (bacterial community in B trench). Some of the measurements of the soil characteristics might have had less influenceon soil microorganisms in the profile, for example, moisture content [43] that has been seen in more general settings [13,49].

Lag distances (vertical) between the samples were almost equal in influence on the vertical profiles of microbial community structure in the B trench, but the effect of distance was virtually negligible in D trench. This discrepancy between trenches is confirmed by the partial Mantel test that both bacterial and fungal community structures from two trenches were weakly associated (Table 2).

Rank correlation between dissimilarity matrices of all possible subgroups of soil characteristics and the similarity matrix of bacterial community structure in B trench by using Bio-Env selected moisture content and organic matter contents (r = 0.737). Canonical correspondence analysis (CCA) was performed to explain bacterial community structure by relating two most relevant soil characteristics measurements to it (gradient analysis). Two constraints used in the model were very significant (P< 0.001) and were able to explain 43.85 % of total variance. By looking at the loadings, it was determined that organic matter content was more correlated with bacterial communities closer to the surface (0-4 cm) and moisture content was at intermediate depth (4-8 cm) (Fig.4).All other microbial communities were not showed significant CCA model. CCA was also tried with all soil measurements, since the overall correlation was

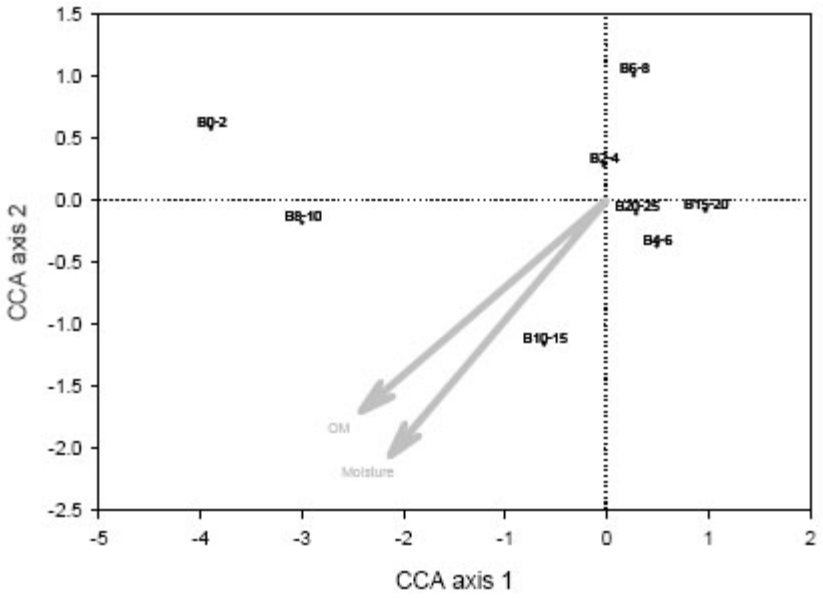


Fig. 4. Triplot of canonical correspondence analysis (CCA) between B trench bacterial community and 2 soil characteristics (moisture & organic matter content) selected by Bio-Env. Individual ribotypes are hidden for clearer description

significant by the Mantel test. The constraints were still fairly significant (P =0.077) but less than with 2 selected constraints, and the triplot is much less clear to be interpreted.

Discussion

Vertical profiles of soil characteristics

Since the experimental field was located in grassland vegetation, a strong O horizon was not expected to be found. O horizons require large accumulations of organic matter and most often are associated with soils developing in forests [9]. The 25 cm trenches included the A and upper portion of the B horizons. Although the root zone was 5 cm deeper in the D trenches, the dark, organic-rich A horizons were identified at about same depth (10 cm) in all the trenches. Note that in the vertical profile of organic matter (Fig. 1.C), organic matter content became almost identical below 10 cm in both trenches after a monotonic decrease in concentration from the surface to 10 cm. The 10-cm criterion was well matched with all other observations of soil characteristics and total bacterial abundance; all were relatively constant below the 10-cm depth. Vertical profiles were very similar among all measurements except pH. Organic matter content profile could be the key for explanation for all others in that moisture content, total carbon and nitrogen content, and total bacterial abundance have close relationships with organic matter content. The opposite pH trend is also explainable by the acidification effect of organic matter decomposition [9].

Moisture content was one of the properties showing a decrease with depth. In some studies, vertical profiles of moisture content were constant with depth [26,43], whereas others have reported mixed profiles (i.e., increasing or decreasing moisture with depth) dependent on the types of field cover [44]. Schulze *et al.*[44] measured volumetric water content (%) from five sites with distinctly different soil covers (from desert to forest) on a 140 km transect in Argentina. Except for the *Stipa* covered grassland site, which showed some downward increase in moisture over a 50-cm distance or constant overall profile, all of the sites examined showed downwardly decreasing moisture content, similar to that in the present study. In particular, sites with deciduous scrub and *Festuca*grass had profiles with a clear decrease in moisture content down to where the moisture increased again at about 2 m below the surface, which was roughly the extent of root zone.

This decreasing trend of soil moisture content is probably caused mainly by the existence of root zone, which extended 13 - 18 cm at the sampling site. The decreasing trend portion of the profile could be the result of the balance between precipitation and evapotranspiration. Almost constant moisture content profile below root zone inversely verifies. Another causal factor would be the higher relative concentration of clay and organic matter (i.e., higher field capacity) near surface and large amount of precipitation during the growing season of 2003 that larger amount of clay and organic matter could hold ample amount of moisture in the upper horizon minimizing downward infiltration and evaporation of the moisture. The average soil moisture content at the 5-cm depth in the same meadow in July 2002 (the last dry year), was about 8 % and it increased to about 22 % in 2003. Unique vertical profile of pH measurement is explainable by the fact of higher moisture content near surface and the organic matter content profile (Fig.1.C). Organic matter (C_2H_4ONS, generalized form) decomposition can produce carbonic acid, carboxylic acid and inorganic acids [9]:

$$C_2H_4ONS + 5O_2 + H_2O \rightarrow H_2CO_3 + RCOOH + H_2SO_4 + HNO_3$$

The ionization of those acids would have very strong acidification effect, especially from strong sulfuric acid ($pK_a \approx 0$) or nitric acid ($pK_a = -1.44$). The carbon dioxide availability profile could be another contribution. Soil carbon dioxide is generated mostly by soil respiration (microbial and plant root). The vertical profile of soil carbon dioxide must be similar to the general soil characteristics pattern, because of existence of root zone and soil bacterial abundance profile. Water and carbon dioxide form carbonic acid (H_2CO_3), and dissociation of carbonic acid produces bicarbonate (HCO_3^-) lowers pH.

Soil depth profiles from subtropical and semi-arid regions of Mexico reported by Rodriguez *et al*. [41] were relatively well matched with those of the present study. The soil depth profile reached 50 cm from the surface, and the depth profiles of pH, organic matter content and moisture content were of the same general trend as those of the present study. The only difference is in % clay that there was virtually no difference in 50-cm gradient. This is probably because of the climate regime difference. Typical soil in subtropical and semi-arid region is vertisol and it has higher relative concentration of swelling clay (smectite) to a depth of 1 m or more [9]. The trends of vertical profiles of soil characteristics might be extended even deeper. Blume *et al*. [5] measured the same soil properties as measured in the present study, along with the microbial community properties (biomass, diversity and activity) in a vertical profile in an agricultural field in Indiana. The soil pits were 1.7 m deep but the general trends of pH, moisture content, and total organic carbon were similar to those found in the present study in the 25 cm. For example, the pH of upper 20 cm was lowest regardless of the location of pits and seasons.

Vertical profiles of soil microbial communities

The vertical profile of total bacterial abundance was well matched with the expectation from the vertical profiles of soil nutrients (organic matter content, total carbon and nitrogen content), i.e., bacterial numbers were highest at the soil surface where organics and nutrients (including oxygen diffusing into the soil from above ground) were highest (Fig. 2). Although overall bacterial abundance patterns between trenches were not significantly different ($r = 0.822$, $P = 0.012$ in correlation analysis), larger between-trench difference in the upper 5 cm was identified (B trench was less abundant than D trench). Two trenches were located in two distinctive vegetation settings. Two species were identified at the B trench (*Plantago lanceolata* and *Rudbeckia hirta*), whereas only *Andropogon gerardii* was identified at the D trench. Different plants species are known to produce and release different types of organic carbon compounds [22,29,42], and those root exudates are the main carbon and energy source, and they are, therefore, a major controlling factor for the composition of soil microbial communities in the vicinity of plant roots [3,4,20,27]. It can be hypothesized that a more diverse suite of organic compounds from a more

diverse set of plant species would, in turn, support a more diverse microbial community. Such a diverse community might, under some circumstances, be highly internally competitive, resulting in lower overall abundance, for example through the antibiotic activities of genera such as *Streptomyces* [28].

The decreasing trend of bacterial abundance in the downward direction is commonly seen and is intuitively obvious given the fact the majority of soil microorganisms are heterotrophs and that the source of organic compounds is at or near the surface. A 10-cm deep profile from a pasture in Australia showed a very similar decreasing trend in total bacterial abundance along with a nearly constant moisture profile [43]. A similar trend was also found in a 15-cm deep profile of freshwater sediment in Illinois; here the cell number reached a nearly constant value at about 4-5 cm below the surface [19]. Almost constant profile in abundance was also seen in a 10 cm deep profile in the Arctic Ocean (Svalbard) sediment. The abundance of the an aerobic sulfate-reducing bacteria, however, increased down to 3 cm [40].

The rough trajectory of the vertical profiles of both bacterial and fungal community structure was only observed in B trench even with very liberal criterion. At another attempt, same data were grouped by cluster analysis results on soil characteristics measurements (0-6 cm, 6-10 cm and 10-25 cm). Three groups were then analyzed by using discriminant function analysis for ordination. The locations of centroids represented much clear monotonic trajectory of microbial community structure shift at B trench in counter-clockwise direction with opposite portions. The vertical profile of microbial community structures in a 200-cm soil column measured by PLFA followed by PCA in a California soil also showed a continuous trajectory in PC space from the surface to the bottom of the profile [15]. The 1.3 m vertical profiles of PLFA data obtained by Blume *et al*. [5] also showed smooth trajectories in both summer and winter measurements-their directions were opposite but the centroid locations were different in PC space, as was observed on the NMDS ordination space of the present study. Griffiths *et al*. [21] described bacterial communities in 20-cm vertical profile from a grassland in the UK. Community structures by DGGE and physiological trends by community-level physiological profiling (CLPP) both showed relatively smooth trajectories of centroids from top to bottom in the profile on the PC field. The trajectories of the profiles obtained by Griffiths *et al*. [21] showed a convergence at the bottom of the profile (i.e., 15-20 cm) compared with all the other groups.

The change in microbial community structures with depth is less intuitive than are decreasing vertical profiles of bacterial abundance. However, the simplest explanation might come from the close relationship with same soil variables that control overall abundance. Organic matter, including total carbon, content generally decreases from the surface to the bottom of the soil profile (Fig.1. A & C). In addition to the quantity of carbon, the carbon quality is also known to be lesser at greater soil depths [1,48]. Therefore, the composition of microbial communities might be expected to differ as total quantity and quality of major nutrients are changed. The microbial community near the soil surface probably is dominated by those heterotrophs that readily utilize labile carbon sources, and as profile goes deeper, micro-organisms adapted to more recalcitrant carbon sources would dominate in the community.

Conclusion

Vertical profiles of soil can be dynamics in its physico-chemical properties and residing microbial communities. Depth of plant root system and vertical distance was also measured for more comprehensive comparisons. The spatial autocorrelation on vertical direction was not noticeable. Plant root system extent and soil properties were well matched with soil microbial communities in vertical profile. However, plant cover was determined to be most influencing factor on the microbial community structures, because almost all the vertical profiles of soil characteristics were virtually identical between the experimental trenches while certain difference was found in microbial community and plant cover.

Acknowledgments

The authors thank David Carr of the Blandy Experimental Farm and Howard Epstein of the Department of Environmental Sciences at University of Virginia for helpful comments during the conduct of this work. SK was supported by fellowship from the Blandy Experimental Farm, and by start-up funds from the Office of Vice Provost of Research at Baylor University.

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