Irrigated Agriculture and Tillage Practices Impact Microbial Community Structure

DeEtta Mills[1], James A. Entry [2], Kalai Mathee[1], Giri Narasimhan[3], Krish Jayachandran[4], R. E. Sojka[2] and Warren J. Busscher[5]

[1]Department of Biological Sciences, Florida International University, University Park, Miami, FL, [2]USDA Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory, 3793 North, 3600 East, Kimberly, Idaho 83341. Department of Computer Sciences, Florida International University, [4]Department of Environmental Studies, Florida International University, University Park, Miami, FL. [5] USDA-ARS, Coastal Plains Soil, Water and Plant Research Center, 2611 W. Lucas Street, Florence, SC 29501
Email: busscher@florence.ars.usda.gov

Abstract: Irrigation increases carbon (C) input to soils via increased litter and root production. Intensively managed crop or pastureland has potential for C gain through the use of improved grazing regimes, fertilisation practices and irrigation management. Soil microbial diversity is important because it is often regarded as an index of soil health. Loss of biodiversity leads to loss of ecosystem resistance and resilience to anthropogenic as well as natural stresses. Organic C and microbial structural diversity present in Southern Idaho soils having long term cropping histories was measured. The sites sampled were native sagebrush vegetation (NSB); irrigated mouldboard ploughed crops (IMP), irrigated conservation-chisel-tilled crops (ICT) and irrigated pasture systems (IP). Organic C concentration in soils decreased in the order NSB 0-5 cm>IP 0-30 cm = ICT 0-15 cm>IMP 0-30 cm>NSB 5-15 cm = NSB15-30 cm. Amplicon length heterogeneity (ALH) LH-PCR, a DNA profiling method, was used to profile the eubacterial structural diversity in all soils sampled at the different depths. LH-PCR interrogates the variable domains of the ribosomal small subunit genes (SSU rRNA), and separates these variable domains on high-resolution genetic analysers. ALH assays are based on the natural variation in sequence lengths of the 16S rRNA genes and are independent of restriction enzyme recognition sites. The application of the ALH technique as a monitoring tool for microbial ecology has been shown to enhance and extend the current understanding of the structural dynamics of microbial communities in their specific environments.

Using the profiling data from four hypervariable regions of the 16S rRNA (V1, V1+V2, V3 and V9), it was shown that native sagebrush soil communities differed in bacterial richness (i.e. different phylotypes) within the top 30 cm when compared to the irrigated agricultural soils. Between the agricultural management systems (in the top 30 cm) the bacterial richness of conservation-tilled soils was greater than irrigated mouldboard ploughed soils but less than irrigated pastures. Soil C concentrations also correlated with eubacterial diversity indices for the four variable regions (r^2 = 0.91, 0.92, 0.68, 0.70, respectively), evenness indices (r^2 = 0.72, 0.68, 0.93, 0.80, respectively) and the active bacterial biomass (r^2 = 0.75, 0.75, 0.79, 0.79 respectively). Since ICT and IP increase C sequestration and appear to support higher eubacterial diversity in soils compared to IMP, producers can use these management practices on their lands to sequester organic C, improve soil microbial diversity and enhance soil biological processes.

INTRODUCTION

The carbon concentrations sequestered in soils is a balance between input (plant growth and litter) and output (microbial degradation and physical erosion). Irrigation increases C input to soils via increased litter and root production especially in semi arid or arid ecosystems (Entry, *et al.*, 2002). Intensively managed crop or pastureland has potential for C gain through the use of improved grazing regimes, fertilisation practices and irrigation management. Soil microbial diversity measurements are important because biodiversity is often regarded as an important index of soil health. Loss of biodiversity leads

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to loss of ecosystem resistance and resilience to anthropogenic as well as natural stresses (Bossio and Scow, 1995; Nyman, 1999; Yang *et al.*, 2000; Zhou *et al.*, 2002).

Ribosomal molecules have highly conserved sequence domains interspersed with hypervariable regions, and it is these variable regions that can be used distinguish one microbe from another and therefore, can be used as molecular markers to discriminate among taxa (Bowman and Sayler, 1996). The use of ribosomal DNA fingerprints as phylogenetic markers is able to provide rapid, economical methods to assess microbial diversity, although at lower resolution than nucleic acid sequencing. The application of the amplicon length heterogeneity-PCR (LH-PCR) technique as a profiling tool to assess microbial communities has been shown to extend the current knowledge of the dynamics of microbial communities in natural and disturbed environments (Litchfield and Gillevet, 2002; Mills *et al.*, 2003; Ritchie *et al.*, 2000; Suzuki, *et al.*,, 1998). Since increasing plant growth on arid and semiarid lands by conversion to irrigated agriculture increases C storage in soils (Entry, *et al.*,, 2002), we hypothesized that irrigated agriculture will also increase eubacterial structural diversity.

MATERIAL AND METHODS

Sites

The study area is located on the Snake River Plain, between 42° 30' 00" and 43° 30' 00" N. and 114° 20' 00" and 116° 30' 00" W and is classified as a temperate semi-desert ecosystem. The experiment was arranged in a completely randomized design. Soil samples were taken from native sagebrush (NSB) sites, irrigated pasture (IP), irrigated crop land managed with conservation tillage (ICT) and irrigated agricultural crop lands in mouldboard ploughing systems (IMP) as described previously (Entry, *et al.*,, 2002). All sites were located on fields managed by USDA Agricultural Research Service's Northwest Irrigation and Soils Research Lab, Kimberly, ID.

Sampling and carbon analysis

Separate 2.5 cm diameter cores were taken from each plot and partitioned into 0-5 cm, 5-15 cm, and 15-30 and 30-10 cm depths. Carbon in aboveground vegetation was estimated by measuring the amount of material in ten separate 1.0 m^2 plots at each site. Samples analysed for active microbial biomass and for microbial diversity using amplicon length heterogeneity (ALH) were collected and stored in water-tight plastic bags and prepared for microbial testing within 24 hrs or were frozen at –80°C until processed for molecular analyses. Concentration of organic C in each soil sample was determined by the Walkley-Black procedure and active bacteria numbers in soil were determined for each treatment using methods as described elsewhere (Entry, *et al.*, 2002).

LH-PCR: Whole community genomic DNA was extracted from the frozen soil samples using slight modifications to the FastDNA®SPIN Kit for Soil (QBiogene, Vista, CA). LH-PCR mixtures and run parameters are described elsewhere (Mills *et al.*, 2003). All samples were run on an ABI Prism™377 genetic analyser. LH-PCR products were loaded directly onto polyacrylamide gels without further purification. Community profiles were collected and analysed using the ABI Prism™ GeneScan®, ABI Prism™ Genotyper® software (PE Biosystems, Foster City, CA). Descriptive statistics were performed on the replicates and the mean relative ratios were used in subsequent analyses. The means of the peak heights were converted to binary data (presence/absence) and similarity indices were calculated using the Sørensøn's Index (pairwise similarity values) (Archer and Leung, 1998). The Shannon diversity index, phylotype richness, and evenness parameters were calculated as described elsewhere (Dunbar *et al.*, 1999).

RESULTS

Soil C, as measured by Walkley Black and loss on ignition, was higher in the NSB 0-5 cm soil depth than the 5-15, and 15-30 cm depths and all other soils. Soil C concentrations also correlated with eubacterial diversity indices for the four variable regions (r^2 = 0.91, 0.92, 0.68, 0.70, respectively), evenness indices (r^2 = 0.72, 0.68, 0.93, 0.80, respectively) and the active bacterial biomass (r^2 = 0.75, 0.75, 0.79, 0.79 respectively). Eubacterial community profiles were generated for four variable regions and all sites and depths but only the preliminary data from V1+V2 region are presented here. Figure 1 depicts the V1+V2 profiles from the four sites. While NSB, IP and ICT profiles all appear to have 23 amplicons (richness), careful scrutiny shows that not all amplicons are found at all depths (presence/absence) within the soil types.

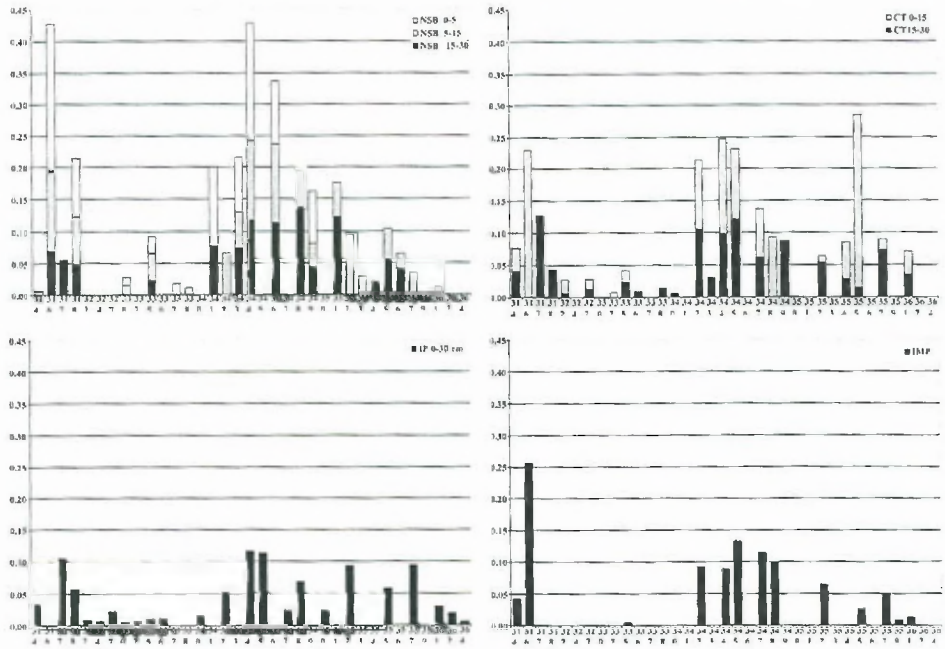

Figure 1. Comparison of amplicon lengths in the V1+V2 region of eubacterial 16SrRNA genes from native sagebrush soils (NSB) at 0-5 (light grey), 5-15(dark grey) and 15-30 (black) cm depths, irrigated conservation tilled soils at 0-15 (grey) and 15-30 (black) cm depths (ICT) , irrigated pasture soils at 0-30 cm (IP)

The dominant amplicons differ and the relative abundances vary. For example, the dominant amplicons in the NSB soils are 316, 344 and 346 bp long but the relative abundance of the amplicons varied with depth. For NSB 0-5 cm, the 316 bp amplicon is 23% of the relative abundance within that community while at 5-15 and 15-30 cm, the abundance is only 13% and 7% of the community, respectively. The 346 amplicon, on the other hand, represents approximately 10-12% of the community at all depths. In IP (0-30 cm), five peaks (317, 344, 345, 352, and 357 bp) seem to be equally represented across the profiles. The most dramatic decrease in amplicon richness can be seen in the IMP soils with only 13 amplicons and dominated by the 316 bp amplicon. However, one must proceed with caution when interpreting community profiles from a diversity standpoint. Keep in mind each amplicon most likely represents many taxonomically unrelated bacteria that coincidently produce the same length amplicon with the primer sets that were used. Cloning and sequencing of community libraries is currently being done in order to tease apart the true eubacterial diversity within each soil type.

However, given that the profiles represent the minimum community richness/diversity, each amplicon data point can be used to calculate commonly used ecological indices. The diversity, evenness and similarity indices (Table 1 & 2) for the V1+ V2 region were able to show community differences between sites. While the richness (S) and diversity (H) were highest for IP, evenness (E) was lower than in ICT (0-15 cm). The similarity *within* the NSB sites was higher than compared to the irrigated, managed sites (Table 2). One can speculate that the higher similarity indices for NSB 5-15 cm when compared to the irrigated sites may be correlated to the root zone and the associated rhizosphere populations. Cloning and sequencing of the DNA libraries will confirm this speculation.

Table 1. Richness, diversity and evenness indices for variable region (V1+V2) of the 16S rRNA genes for all soil samples at all depths.

Index	**NSB**	**NSB**	**NSB**	**IP**	**IMP**	**ICT**	**ICT**
(cm)	**(0-5)**	**(5-15)**	**(15-30)**	**(0-30)**	**(0-30)**	**(0-15)**	**(15-30)**
Richness (S)	11	19	14	**23**	14	16	21
Diversity (H)	2.13	2.71	2.51	**2.82**	2.36	2.71	2.74
Evenness (E)	0.89	0.92	0.95	0.90	0.89	**0.98**	0.90

Richness (S) = #of peaks in each sample. Diversity (H) = $-\Sigma(p_i)\ln(p_i)$ where p_i is the relative ratio of individual peak heights; value of 0 = no diversity; 4.6 = even distribution. Evenness (E) = H/H_{max} where $H_{max} = \ln(S)$; higher value = higher diversity and richness

Table 2. Similarity indices for variable region (V1+V2) for all samples and depths compared to natural sagebrush samples

Sample	**NSB**	**NSB**	**NSB**	**IP**	**IMP**	**ICT**	**ICT**
(cm)	**(0-5)**	**(5-15)**	**(15-30)**	**(0-30)**	**(0-30)**	**(0-15)**	**(15-30)**
NSB(0-5)	**1.00**	0.84	0.87	0.41	0.40	0.46	0.52
NSB (5-15)		**1.00**	0.96	0.75	0.82	0.76	0.79
NSB (15-30)			**1.00**	0.46	0.47	0.43	0.65

Table 3. Active bacterial biomass and whole community DNA concentration in native sagebrush and irrigated agricultural soils.

Sites (cm)	**Bacterial Biomass**	**[DNA] (ng/ml)**
NSB (0-5)	6.7b	41.8d
NSB (5-15)	6.1b	26.6e
NSB (15-30)	4.8b	31.6e
IP (0-30)	7.9b	75.3a
IMP (0-30)	12.0a	51.0bc
ICT (0-15)	7.9b	65.2b
ICT (15-30)	5.6b	40.0d

Values followed by the same letter are not significantly different as determined by the Least Square Means Test (P = 0.05; n = 9).

Irrigation increased plant biomass in summer months and the agricultural soils contain greater amounts of C in the top 30 cm of soil because of the increased soil density associated with tillage and fertilising operations. That resulted in greater amounts of soil and therefore C per m^2. Active bacterial biomass correlated with soil organic C in positive curvilinear relationships ($r^2 = 0.76$) (Entry, *et al.*, 2002). Active bacterial biomass measurements did not appear to correlate to the concentration of whole community DNA that was extracted from the soils (Table 3). However, the extraction procedure will extract all DNA that is present in the soil (i.e. nematode, plant, fungi, etc.) and all high

molecular weight DNA present in the sample was quantified not just bacterial DNA. Therefore, these concentrations may also reflect the amount of plant material and root density at each depth in the soils.

DISCUSSION

Soil microorganisms are of interest because of their universal presence in ecosystems and their importance in ecological function. Soil microbes are responsible for C degradation and the mineralisation of essential plant nutrients, such as nitrogen and are therefore a vital link in the function of the earth's ecosystem and any agricultural operation. Often, native sagebrush sites have far greater plant biodiversity than the irrigated agriculture because these field sites, including pastures, usually produce one major crop annually. The spatial distribution and community structural differences as seen in the LH-PCR profiles no doubt are a reflection of both the heterogeneity (NSB, IP) and homogeneity (ICT, IMP) associated with aboveground biomass as well as vertical distribution associated with root zones and moisture content. For example, when looking at the V1+ V2 ALH profiles, it appears that mouldboard ploughing dramatically impacts community structure; at the very least, it decreased the apparent community richness. Agricultural operations often decrease overall soil C concentration or, at a minimum, concentrate only certain types of carbon (i.e. monoculture crop residues) in the soils. This may be due to a single crop (i.e. single carbon source) being grown on these irrigated, fertilised soils. Therefore, by providing optimized inorganic nutrients via fertilisation practices, the microbial community, through selective exclusion, becomes dominated by bacterial populations that can most efficiently break down the carbon in the crop residues. On the other hand, native sagebrush soils had lower amounts of actively growing plant biomass (spatially dispersed), but greater plant biodiversity and that was reflected by greater eubacterial biodiversity. Add to this, a lower moisture content in NSB soils and the ALH profile probably depicts a community that may be more metabolically diverse (i.e. utilise multiple carbon sources) and trying to survive under nutrient-limiting conditions (low N, P, moisture content).

Within limits, as the biodiversity of an ecosystem increases, the resilience and stability of that system often increase. Loss of biodiversity can lead to loss of ecosystem resistance and resilience to anthropogenic as well as natural stresses (Brussaard *et al.*, 1997). Assessing the microbial community profiles associated with management practices (i.e. conservation tillage vs. mouldboard plough) is one step in determining the biological impact that is associated with tillage practices. Monitoring schemes, such as ALH, may be employed to link biogeochemical data from comparable ecosystems or agricultural management practices to the biological components and to serve as an early warning system to signal macro-ecological shifts. Based on the preliminary analyses of the microbial profiles from this study, comparisons between tillage practices would indicate that ICT supports higher 'biodiversity' than does IMP and may be a better approach for sustainable agriculture practices.

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