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Influence of irrigated agriculture on soil microbial diversity

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ABSTRACT

Organic carbon (C), bacterial biomass and structural community diversity were measured in Southern Idaho soils with long term cropping histories. The soils tested were native sagebrush vegetation (NSB), irrigated moldboard plowed 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 = NSB 15–30 cm. Active bacterial, fungal and microbial biomass correlated with soil C as measured by the Walkely Black method in positive curvilinear relationships (r^2 = 0.93, 0.80 and 0.76, respectively). Amplicon length heterogeneity (LH-PCR) DNA profiling was used to access the eubacterial diversity in all soils and at all depths. The Shannon–Weaver diversity index was used to measure the differences using the combined data from three hypervariable domains of the eubacterial 16S rRNA genes. Diversity was greatest in NSB 15–30 cm soil and lowest in the IMP soil. With the exception of IMP with the lowest diversity index, the samples highest in C (NSB 0–5 cm, IP 0–30 cm, ICT 0–15 cm) reflected lower diversity indices. However, these indices were not significantly different from each other. ICT and IP increase soil C and to some extent increase diversity relative to IMP. Since soil bacteria respond quickly to environmental changes, monitoring microbial communities may be one way to assess the impact of agricultural practices such as irrigation and tillage regimes.

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1. Introduction

Land use changes can impact the amount of organic carbon (C) stored in the soil by altering C inputs and losses. In forest, grassland and wetland ecosystems, conversion of native vegetation to agricultural cropping has resulted in substantial C transfer to the atmosphere as a result of loss of climax vegetation to the lower equilibrium C concentration in soil (Baker et al., 2007; VandenBygaart et al., 2003; Wang et al., 1999). Farm management practices, including conservation tillage and erosion control, have reduced the amount of CO_2 emitted to the atmosphere in both Canada and the United States (VandenBygaart et al., 2003; West and Marland, 2002; Paustian et al., 1997). Irrigation also increases C input to soils via increased litter and root production. In arid and semi-arid environments, plant survival and growth is limited by available water and irrigation is required to increase plant production to the point where crops become economically viable. Intensively managed crop or pastureland has potential for C gain through the use of improved grazing regimes, fertilization practices and irrigation management (Entry et al., 2002; Follett, 2001; Bruce et al., 1999).

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Soil microbial diversity is important because it is often regarded as an important index of soil ecosystem health. As the biodiversity of an ecosystem increases, the resilience and stability of the ecosystem should increase (Garbeva et al., 2004; Torsvik and Ovreas, 2002). Conversely, as ecosystems degrade, ecosystem biodiversity decreases (Garbeva et al., 2004; Sun et al., 2004). Loss of biodiversity leads to loss of ecosystem resistance and resilience to anthropogenic as well as natural stresses (Garbeva et al., 2004; van Elsas et al., 2002). Ecosystem resistance is the ability of the system to absorb the reduction of numbers of a species or set of species as a result of natural catastrophes such as hurricanes, periodic droughts, and insect or disease epidemics. Ecosystem resilience is the ability of ecosystem processes such as nutrient cycling, to survive and recover from these natural catastrophes.

Microorganisms exhibit an impressive diversity in their metabolic activities and in their interactions with other microbes, plants, and animals. Microbes have much shorter turnover rates than higher plants and therefore, respond more quickly to changes in land management than plants and may be the most sensitive indicator to anthropogenic activities. In the past it has been nearly impossible to discern underlying patterns within ecosystems because of the intrinsic structural and functional diversity of microbial communities. To date, only 1–5% of the world's microorganisms have been identified (Mills et al., 2007; Nannipieri et al., 2003; Hugenholtz et al., 1998) As a result, microbial processes, the microbes are often placed in a 'black box', inputs and outputs measured, but most of the processes that go on inside 'the box' are based on inference (Mills et al., 2007; Swift et al., 2004; Brussaard et al., 1997). Recently, there has been an explosion in the identification of microorganisms in the natural world using culture-independent techniques. The structure and function of these microbial communities using new molecular technologies can be determined (Mills et al., 2007; Thies, 2007; Nakatsu, 2007; Rogers et al., 2007; Suzuki et al., 1998).

Ribosomal molecules have highly conserved sequence domains interspersed with hypervariable regions (Kirk et al., 2004; Head et al., 1998), and it is these variable domains that can be used distinguish one microbe from another and therefore, can be used as molecular markers to discriminate among taxa. The use of ribosomal DNA profiles as phylogenetic markers has provided a rapid and economical method to assess microbial diversity, although at lower resolution than nucleic acid sequencing (Thies, 2007; Nakatsu, 2007; Mills et al., 2007, 2006). 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 their natural environment (Mills et al., 2006; Bernhard et al., 2005; Ritchie et al., 2000). For example, LH-PCR has been compared with fatty acid methyl ester (FAME) profiles in an effort to see which method could better assess the diversity present in the soil communities (Ritchie et al., 2000). LH-PCR has proven to be highly reproducible, robust and capable of monitoring microbial communities in a variety of soil, water and sediment ecosystems (Mills et al., 2003, 2006, 2007; Ritchie et al., 2000; Suzuki et al., 1998). Since increasing plant growth on arid and semi-arid lands by conversion to irrigated agriculture increases C storage in soils (Entry et al., 2002), we hypothesize that irrigated agriculture will also increase active bacterial biomass, and eubacterial structural diversity. Given that soil type and spatial distribution of resources have been found to be key drivers in the organization of soil communities (Johnson et al., 2003; Girvan et al., 2003; Zhou et al., 2002), organic carbon, microbial biomass and eubacterial structural diversity measures were used to assess the impact of ''disturbance'' or land management practices on Idaho soil under irrigation and different tillage regimes.

2. Materials and methods

2.1. Site descriptions

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. The sites occur across an elevation gradient ranging from 860 to 1300 m. The area is classified as a temperate semi-desert ecosystem (Bailey, 1998). The climate is typified by cool, moist winters and hot, dry summers with annual precipitation ranging from 175 to 305 mm, two-thirds of which occurs during October through March (Collett, 1982). Air temperatures range from 0 to 30 °C with annual average of 9–10 °C. Soils are typically well-drained loams and silt loams derived from loess deposits overlying basalt. Soil was classified as a fine, montmorillonitic, mesic Xerollic Haplargid on the Brown's Creek site, a coarse-loamy, mixed non-acid, mesic Xeric Torriorthents on the Simco site and a loamy, mixed, mesic lithic Xerollic Camborthids on the Kuna Butte site (Collett, 1982).

Vegetation throughout the general area was historically dominated by basin big sagebrush (*Artemisia tridentata* var. *tridentata* Nutt.), Wyoming big sagebrush (*Artemisia tridentata* var. *wyomingensis* Nutt.), and perennial bunch grasses, including Sandberg bluegrass (*Poa secunda* J. Presl), bottlebrush squirreltail (*Elymus elymoides* Raf. Swezy.), bluebunch wheatgrass (*Pseudoroegneria spicata* Pursh. A. Love), and Thurber's needlegrass (*Achnatherum thurberianum* (Piper) Barkworth).

2.2. Native vegetation sagebrush sites

Native sagebrush sites (NSB) were vegetated with native steppe vegetation and a low composition of exotic annual grasses. Sites were chosen for this study based on a history of no livestock grazing (BLM, Boise, Idaho, unpublished data). All study sites had 5–10% slope and were on areas that supported basin big sagebrush or Wyoming big sagebrush or mixed communities. Three geographically separated NSB locations, at least a minimum of 5 km apart, were sampled for this study.

2.3. Irrigated pasture (IP) sites

Three irrigated pastures were selected that were formerly crop land and converted to and maintained as irrigated pasture for the past 30 years. The Buhl site was vegetated with Kentucky bluegrass (*Poa pratensis* L.)—orchardgrass (*Dactylis glomerata* L.) on a Rakane-Blacknest soil complex, fine-loamy, mixed, mesic Xerollic Durargids soil. The Gooding site was vegetated with

smooth brome (*Bromus inermis* Leyss.)-orchardgrass on a Paulville-Idow soil complex, fine-loamy, mixed, mesic Xerollic Haplargid soil. The Kimberly site was vegetated with smooth brome-orchardgrass pasture on a Portneuf soil, coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid soil. Grazing rates on these pastures were 10–12 AUM year^{-1}. Slopes on fields sites ranges from 1.0 to 3.0%.

2.4. Irrigated conservation tillage (ICT) and crop sites

Three sites with fields rotating among alfalfa (*Medicago sativa* L.), wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.) and beans (*Phaseolus vulgaris* L.) were sampled. All sites were located on fields managed by USDA Agricultural Research Service's Northwest Irrigation and Soils Research Laboratory or the University of Idaho, Research and Extension Center. Soil on all sites was classified as a coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid, with 10–21% clay and 60–75% silt, and organic matter of approximately 13 g kg^{-1}. The soil has a pH of 7.6–8.0. Slopes on fields sites ranges from 1.0 to 3.0%.

2.5. Experimental design

The experiment was arranged in a completely randomized design (Kirk, 1982). Soil samples were taken from: (1) three sites supporting native sagebrush vegetation located near agricultural land in Southern Idaho (each site supported a basin big sage and a Wyoming big sage vegetation type); (2) three sites that were formerly crop land and converted to and maintained as irrigated pasture for the past 30 years; (3) three sites that were irrigated crop land and have been managed with conservation tillage (ICT) for the past 8 years growing potato and beans and (4) three irrigated agricultural crop lands in moldboard plowing systems (IMP) that were each growing (a) alfalfa, (b) wheat, (c) potato and (d) beans. Soil depths (0–5, 5–15 and 15–30 cm) for the IMP and IP and 0–15 cm layer of the ICT treatments were combined because the soil is tilled numerous times each before planting and during harvesting to promote even distribution of irrigation water to crops. The 15–30 cm layer of the ICT treatment was not plowed for the last 8 years.

2.6. Sampling procedures

Sampling locations were randomly chosen at each site or field. Separate 10 cm diameter cores were taken and partitioned into 0–5 cm, 5–15 cm, and 15–30 cm depths. Roots greater than 1.0 cm diameter were measured separately. A total of 210 soil cores were taken to measure total C in soil. Carbon in above-ground vegetation was estimated by measuring the amount of material in 10 separate 1.0 m^2 areas in each site or field (Embley and Stackebrandt, 1996). Samples analyzed for active bacterial and fungal biomass, and microbial diversity using amplicon length heterogeneity (LH-PCR) were collected and stored in water-tight plastic bags and prepared for microbial testing within 24 h to minimize the effects of storage on microbial activity (West et al., 1986). All soil for molecular analyses were subsequently stored at −80 °C until processed.

2.7. Carbon in soil and above-ground vegetation

Concentration of organic C in each sample of mineral soil was determined by the Walkley–Black procedure (Nelson and Sommers, 1996). The amount of C per ha^{-1} of the 0–100 cm of mineral soil was calculated assuming 0.44 g C g^{-1} organic matter with correction for soil bulk density. Above-ground vegetation was collected and separated into sage, grass, forbs, herbs and duff and then dried at 80 °C for 48 h, weighed and ground to pass a 1 mm opening. Carbon in above-ground vegetation was determined by loss on ignition (Nelson and Sommers, 1996). The amount of C in the above ground material was assumed to contain 0.44 kg C kg^{-1} organic matter on an ash free basis. Ten separate 10 cm diameter soil cores were taken to a 30 cm depth to determine bulk density and soil pH. Bulk density was measured by dividing the volume of the sample by the oven dry weight after drying at 105 °C for 48 h (Blake and Hartage, 1982).

2.8. Active bacterial and fungal biomass

Active bacteria and fungal numbers in soil were determined for each treatment using methods described by Ingham and Klein (1984). Active bacteria and fungi were estimated by taking a 1.0 ml water sample that was diluted in 9 ml of a phosphate buffer (pH 6.0) and shaken at approximately 120 rpm for 5 min. For fungal biomass, a 1 ml aliquot was removed and stained with 1 ml of a 20 fg ml^{-1} fluorescein diacetate (FDA) solution in a 0.2 M phosphate buffer for 3 min. One milliliter of 1.5% agar in a 0.1 M phosphate buffer (pH 9.5) was added to the FDA suspension. The sample was mixed and an aliquot placed on a microscope slide containing a cavity of known volume (Ingham and Klein, 1984). Immediately after preparation, slides were examined for FDA-stained hyphal length by epifluorescent microscopy. Total fungal biomass was estimated by measuring the length and diameter of hyphae in 3–6 fields with phase—contrast microscopy. Three slides were evaluated from each sample and ten fields per slide were evaluated with phase contrast microscopy for total hyphal length, and three transects were evaluated for FDA-stained (active) hyphal length at 160 total magnification.

Iodonitrotetrazolium (INT) stain was used for counting active bacteria (Stadmatiadis et al., 1990). A 1 ml sample of initial soil suspension was diluted to a final dilution in a 0.2 mg soil in 4 ml buffer. The suspension was incubated with 4 ml of filtered INT buffer for 60 min in the dark at 20 °C. Total bacteria per ml of water were estimated from the mean number of bacteria (fluorescent and non-fluorescent bacteria), their average diameter and length per field. Three slides were evaluated for each sample and ten fields per slide were evaluated using epifluorescent oil-immersion microscopy to determine numbers and size of fluorescent and total bacteria (Lodge and Ingham, 1991).

Bacterial biomass was computed from the numbers of active and total bacteria and active and total fungal biomass was determined from hyphal length. Bacterial biomass was computed from the number of soil bacteria per gram of soil by considering that the bacterial spheres were 1 μm in diameter. Active and total fungal biomass were computed by considering average hyphal diameter to be 1 μm in diameter and them multiplying

Table 1 – LH-PCR primer sequences

Primer name (hypervariable regions)	Sequence (5′-3′)	5′ modification
27 F (V1 + V2)	AGA GTT TGA TCM TGG CTC AG	6-FAM
355R (V1 + V2)	GCT GCC TCC CGT AGG AGT	None
P1F (V1)	GCG GCG TGC CTA ATA CAT GC	6-FAM
P1R (V1)	TTC CCC ACG CGT TAC TCA CC	none
338F (V3)	ACT CCT ACG GGA GGC AGC AG	HEX
518R (V3)	ATT ACC GCG GCT GCT GG	None
EC1055 F (V9)	ATG GCT GTC GTC AGC T	NED
EC1392R (V9)	ACG GGC GGT GTG TAC	None
536R[a] (V3 V1)	GWA TTA CCG CGG CKG CTG	

M = C:A, Y = C:T, K = G:T, W = A:T (possible nucleotide substitutions). Based on *E. coli* numbering of the 16S rRNA gene.
[a] Sequence primer.

by the length of observed hyphae (Jenkinson and Ladd, 1981). A carbon to volume conversion factor of 120 μg C mm^{-3} was used for both bacteria and fungi, assuming 1.1 g cm^{-3} wet density, 20% dry matter content, and a 0.41 carbon content in the bacterium or fungus (Jenkinson and Ladd, 1981).

2.9. Amplicon length heterogeneity

Whole community genomic DNA was extracted from replicate (3) frozen soil samples using slight modifications to the FastDNA® SPIN Kit for soil (QBiogene, Vista, CA) (Mills et al., 2003). After placing 500 mg of homogenized soil in the MULTIMIX 2 tubes with the appropriate buffers and reagents, samples were processed in the FastPrep® instrument (QBiogene, Vista, CA) for 20 s at setting 5.5. Samples were incubated for 45 min at 70 °C (to increase DNA yield and aid in cell lysis), placed back into the FastPrep® instrument and processed for an additional 10 s at 5.5. High molecular weight (HMW) DNA was quantified using a Bio-Rad fluorometer (Bio-Rad, Richmond, CA) and 10 ng of HMW DNA was used as the working stock in subsequent PCR reactions.

Only the forward primer of each primer set was fluorescently labeled (PE Biosystems, Foster City, CA) and paired with individual non-fluorescent reverse primers for each hypervariable region or domain examined (Table 1). Positive controls used DNA from pure laboratory cultured isolates. Negative controls used only diethylpyrocarbonate-treated (DEPC) water. Final concentrations of PCR reaction mixtures were: 1× PCR buffer, 2.5 mM $MgCl_2$, 0.250 mM dNTPs (Boehringer, Mannheim, GmbH), 0.5 μM forward and reverse primers, 0.25 U AmpliTaq Gold polymerase (PE Biosystems, Foster City, CA), 0.1% (w/v) bovine serum albumin (BSA), fraction V (ICN Biomedicals Inc., Aurora, OH), 10 ng HMW DNA, and DEPC-treated water to make up the final volume. The PTC-100 Programmable Thermal Cycler (M.J. Research Inc., Watertown, MA) was programmed for an initial denaturing step at 94 °C for 11 min followed by 25 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min with a final extension step at 72 °C for 10 min. LH-PCR products were loaded directly onto the polyacrylamide gels without further purification.

2.10. Electrophoresis

All samples were denatured in a 5:1:1 mixture of deionized formamide (98%, Sigma, St. Louis, MO), Blue Dextran-EDTA loading dye, and GeneScan 500 ROX internal standard (PE Biosystems, Foster City, CA), heated to 94 °C for 3 min, chilled on ice until loaded on a 36 cm WTR 5% Long Ranger polyacrylamide gel (Bio-Rad, Richmond, CA) and then run on a ABI® 377 DNA sequencing instrument. Gels were run for 3.5 h using standard electrophoresis run parameters.

2.11. Standardization and analyses of profiles

Microbial community profiles were collected and analyzed using the ABI Prism™ GeneScan®, ABI Prism™ Genotyper® software (PE Biosystems, Foster City, CA) and diversity, evenness and similarity indices were calculated using custom macros written for Microsoft Excel (Microsoft Corp., Seattle, WA). In GeneScan®, analysis parameters were set to the local Southern size calling, no peak correction and a minimum noise threshold of 50 fluorescent units. Standardized binning criteria were used to identify the reproducible peaks in each profile. The parameters were that each analyzed amplicon had to appear in at least three of the nine replicate profiles and, the relative intensity ratio was equal to or greater than 1%. The reason for setting these binning parameters was to eliminate the error introduced by the collection and analysis software (Dunbar et al., 1999). The GeneScan® software analyzes the amplicons to the nearest 0.01 base pair. By rounding data to the nearest integer, however, two peaks that may actually represent the same amplicon can erroneously be called as two separate fragments due to slight variations in migration during electrophoresis runs.

2.12. Standardization between sample profiles

In addition to using the same concentration of quantified HMW DNA in each PCR reaction, standardization of peaks between samples was performed in the following manner. Total fluorescence intensities (i.e., heights) were calculated for each profile as described in Dunbar et al. (2001). For example, if the total peak intensities for two profiles were 25,000 and 50,000, respectively, each peak in the latter profile would be standardized by a correction factor of 0.5. Relative intensity ratios were calculated using the corrected data by dividing each individual peak height by the total peak height of each electropherogram. This procedure was repeated for each comparison between sample profiles.

2.13. Cloning and sequencing

PCR products were obtained using non-fluorescent 27F and 1492R primers (Table 1) and the same PCR reaction mixture and amplification parameters as described previously. Cloning of the PCR products was performed using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Approximately 75 randomly selected positive white colonies were picked from the LB plates and grown up overnight using standard procedures. Qiagen Plasmid Prep kits were used to recover the plasmid. The plasmid preps were used for PCR using the 27F & 1492R primers and the PCR cycling parameters were: 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C for 35 cycles. The final extension step was for 10 min at 72 °C. PCR purification of the cloned products was performed using Exo-Sap (USB, Cleveland, OH) according to the manufacturer's protocol.

DNA sequencing reactions used 20 ng of the cloned PCR product, and 0.25 μM of the primer added to the standard mix of ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction mixture (PE Applied Biosystems, Foster City, CA). Cycle sequencing was performed using an initial heating step of 96 °C for 1 min and then 40 cycles at 96 °C for 30 s and 60 °C for 4 min. DNA sequencing reactions were purified by gel filtration using Sephadex G-50 (Sigma, St. Louis, MO) in the 96-well microtiter Millipore MultiScreen® Filtration System (Millipore, Bedford, MA) per the manufacturer's protocol. Samples were dried in a Speed-Vac for 45 min on medium heat, and then covered and stored at −20 °C until analyzed using standard parameters for the ABI® 3100 genetic analyzer. Sequence data were analyzed using Sequencher 4.2.2™ (GeneCodes,Corp, Ann Arbor, MI). Consensus sequences were compared to known sequences in the NCBI Ribosomal Database using the BLAST search option and the putative identity of each clone was assessed.

2.14. Statistical analysis

Carbon and bacterial biomass were subjected to a one-way analysis of variance (ANOVA) for a completely randomized design (Snedecor and Cochran, 1980; Kirk, 1982). Residuals were normally distributed with constant variance. Statistical Analysis Software programs (SAS, 1996) were used to conduct the analysis of variance. Significance of treatment means were determined at $P \leq 0.05$ with the Least Square Means test. Raw data from the 16S rRNA hypervariable domains were aligned by amplicon size for each sub-site, averaged and the averaged data was used for all other calculations. The Shannon–Weaver diversity index, phylotype richness, and evenness parameters were calculated as described elsewhere (Dunbar et al., 1999). The data matrices were converted to binary data (presence/absence) and similarity indices were calculated using the Sørensøn's Index (pairwise similarity values) (2). SPSS Version 13.0 for Windows (SPSS Inc., Chicago, IL) was used to compare indices. ANOVA with the Bonferonni post hoc test was used to assess the replicate sub-plots within the test samples. The mean indices for each site were subsequently tested for significance using independent sample *t*-tests. The *K*-means clustering algorithm was also used to evaluate the samples with $K = 3$ and $K = 4$.

3. Results

3.1. Carbon

Bulk density was less in the NSB 0–5 cm soil depth than the 5–15, and 15–30 cm depths and all other soils (Table 1). Soil organic C as was higher in the NSB 0–5 cm soil depth than the 5–15, and 15–30 cm depths and all other soils (Table 2).

3.2. Active bacterial and fungal biomass

In summer active bacterial biomass was higher in the IMP treatment than all other treatments (Table 3). In winter active fungal biomass was higher in the IP treatment than all other treatments. In both summer and winter microbial biomass was higher in the IP, and IMP than all other treatments except the ICT 0–15 cm treatment. Active bacterial, fungal and microbial biomass correlated with soil C in positive curvilinear relationships ($r^2 = 0.93$, 0.80 and 0.76, respectively).

3.3. Eubacterial structural diversity

Diversity indices for the various sites and depths were calculated based on a combined profile of three hypervari-

Table 2 – Bulk density, Walkley Black C in soils growing native sagebrush, irrigated moldboard plowed cropland, irrigated conservation tilled cropland and irrigated pastures in Southern Idaho

Treatment	Soil depth (cm)	Bulk density (Mg m^{-3})[a,b]	WBC (g C kg^{-1} soil)[a,b]
Native sagebrush	0–5	0.97 b	127 a
	5–15	1.28 a	47 c
	15–30	1.34 a	54 c
Irrigated moldboard plow	0–30	1.28 a	78 b
Irrigated conservation tillage	0–15	1.38 a	89 b
	15–30	1.38 a	69 bc
Irrigated pasture	0–30	1.33 a	85 b

[a] In each column, values followed by the same letter are not significantly different as determined by the Least Square Means test ($P \leq 0.05$), $n = 30$.
[b] Statistical comparisons in the ANOVA showed that soil bulk density with respect to soil depth were not significant at $P \leq 0.05$. Therefore, data were combined (Snedecor and Cochran, 1980; Kirk, 1982).

Table 3 – Active bacterial biomass, active fungal biomass, active microbial biomassa nd DNA in native sagebrush and irrigated agricultural soils

Treatment	Winter			Summer			
	Bacterial biomass (mg C g^{-1})	Fungal biomass (mg C g^{-1})	Microbial biomass (mg C g^{-1})	Bacterial biomass (mg C g^{-1})	Fungal biomass (mg C g^{-1})	Microbial biomass (mg C g^{-1})	DNA mg g^{-1}
Native sagebrush 0–5	4.0c	5.2b	9.2b	6.7b	6.1b	12.9b	41.8d
Native sagebrush 5–15	1.1c	0.8c	1.9d	6.1b	4.1bc	10.3b	26.6e
Native sagebrush 15–30	3.4c	3.7b	7.1c	4.8b	2.8c	7.7c	31.6e
Irrigated moldboard plow 0–30	7.8c	3.9b	11.2b	12.0a	5.0b	17.0a	51.0bc
Irrigated conservation tillage 0–15	1.4c	1.0c	2.4d	7.9b	6.5b	14.5ab	65.2b
Irrigated conservation tillage 15–30	0.2d	0.6c	3.0d	5.6b	3.5c	9.2bc	40.0d
Irrigated pasture 0–30	10.5a	9.5a	20.0a	7.9b	9.6a	17.4a	75.3a

In each column, values followed by the same letter are not significantly different as determined by the Least Square Means test ($P \leq 0.05$; $n = 9$).

Table 4 – Richness, diversity, and evenness indices for three concatenated hypervariable 16S rRNA domains: V1, V1 + V2, and V3 (±S.D.)

Sample	Richness[a,b]	Diversity[a,b]	Evenness[a,b]
NSB 0–5 cm	20 (±0.2) b	2.41 (±0.15) c	0.80 (±0.00) b,c
NSB 5–15 cm	26 (±3.8) a	2.63 (±0.18) a	0.81(±0.04) a
NSB 15–30 cm	25 (±0.6) a	2.68 (±0.13) a	0.84(±0.03) a
IMP 0–30 cm	19 (±1.5) b	2.26 (±0.11) b	0.76(±0.02) c
IP 0–30 cm	21 (± 1.0) b	2.50 (±0.03) b	0.82 (±0.01) a
ICT 0–15 cm	20 (±0.6) b	2.49 (±0.13) b	0.83 (±0.04) a
ICT 15–30 cm	21 (±2.0) b	2.36 (±0.13) b	0.78 (±0.02) b,c

[a] Richness (S) = #of peaks in each sample; diversity (H) = $-p_i \ln(p_i)$ where p_i is the relative ratio of individual peak heights; evenness (E) = H/H_{max} where $H_{max} = \ln(S)$.
[b] In each column, values followed by the same letter are not significantly different from each other ($P < 0.05$).

Table 5 – *K*-means clustering of soils under different management practices at the stated soil depths with *K* = 3 or 4

Cluster 1	Cluster 2	Cluster 3
IP 0–30 cm	IMP 0–30 cm	NSB 0–5 cm
CT 0–15 cm		NSB 5–15 cm
CT15–30 cm		NSB 15–30 cm

able domains of the 16S rRNA genes (Table 4). NSB 0–5 cm diversity was significantly less ($P \leq 0.05$) than NSB 15–30 cm but not significantly different than other sites. NSB 5–15 cm was significantly different than IMP while NSB 15–30 cm had the highest diversity index and was significantly different from IMP and ICT 15–30 cm. The overall trend of the diversity was: NSB 15–30 cm > NSB 5–15 cm > IP 0–30 cm > ICT 0–15 cm > NSB 0–5 cm > ICT 15–30 cm > IMP 0–30 cm. The evenness indices indicated significant differences between NSB samples and the other sites. IMP had the smallest value for all measured indices (richness = 19; diversity = 2.26; evenness = 0.76) while NSB 15–30 cm had the highest values.

K-Means cluster results: *K*-means clustering is an unsupervised classification method and therefore, the centroid is arbitrarily chosen. When $K = 3$ or $K = 4$ were chosen as a parameter, the samples were clustered in three groups (Table 5). The NSB samples formed one group, the IP and ICT samples formed a second group and the IMP samples fell into the third group.

Sequencing: clone libraries were constructed and a rapid screening of the clones showed many of the samples had common members. The gamma-proteobacteria group (55%) were the most abundant, then *Acidobacteria* (25%), bacteria (12.5%) and 5% *Firmicutes* and 2.5% *Pseudomonas* (Table 6).

4. Discussion

Agricultural treatments were implemented furrow irrigated fields. Farm managers plow the sampled fields 8–10 times each year to ensure that irrigation water flows evenly from the top of the field to the bottom. If irrigation water is not spread evenly the top of the filed will be flooded while the bottom of the field experience drought. It was not necessary to sample 0–5 cm, 5–15 cm, 15–30 cm soil layers in irrigated agricultural fields because the soil C is evenly distributed throughout the soil profile.

Our study surveyed the hypervariable regions in the 16S rDNA gene using LH-PCR. No one set of ''universal'' primers efficiently amplifies *all* members of the community. Therefore, by increasing the number of primer sets used, we would be more likely to amplify most members of the communities with at least one or more of the primer sets; thus, increasing our knowledge about the community as a whole. Amplicons produced most likely represent multiple genera/species/sequences that coincidently produce the same length amplicon. Virtual alignments for V1 + V2 region, showed 29 different genera (listed in Genbank) that could be represented by a

Table 6 – Primers, number of clones, Genbank accession number, species and affiliation that best matched sequences with ≥98% blast search for eubacterial DNA cloned and sequenced for each treatment and soil depth

Site (sample)	Primer	Clones	Accession #	Species	Affiliation
NSB 5–15; NSB 0–5	27F	3; 2	AY922152.1	Uncultured acidobacterium	Bacteria; acidobacteria; acidobacterium; environmental samples
NSB 0–5	27F	3	AY375058.1; AJ583184.1	Uncultured bacterium clone B182; uncultured gamma proteobacterium	Bacteria; environmental samples
IMP	27F	1	AF317372	Uncultured feedlot manure bacterium	Bacteria; firmicutes; environmental samples.
NSB 5–15	907R	2	AY921837.1; AJ252631	Uncultured acidobacteria; agricultural soil bacteria	Bacteria; acidobacteria; acidobacterium; environmental samples
NSB 0–5	907R	1	AY289367	Uncultured soil bacterium (sandy soil;0–7 cm)	Bacteria; environmental samples
ICT 0–15; ICT 15–30; NSB 5–15; IMP; NSB 15–30	907R	3; 1; 1; 1; 2	AY375058; AJ583184; Z83205.1	Uncultured bacterium clone B182; uncultured gamma-proteobacterium; *E. coli*	Gamma-proteobacteria; enterobactereticaeae
ICT 15–30	907R	1	AF529090	Uncultured pseudomonas sp. clone	Gamma-proteobacteria; pseudomonas; environmental samples
ICT 0–15; NSB 15–30; NSB 5–15	1492R	1; 1; 1	AY177759; AY921839; AB116463	Uncultured acidobacteria	Bacteria; acidobacteria; acidobacterium; environmental samples
IMP	1492R	1	AB034713	Uncultured compost bacterium	Bacteria; environmental sample
IP	1492R	1	AF317372	Uncultured feedlot manure bacterium	Bacteria; firmicutes; environmental samples

343 bp fragment. A profiling approach, however can provide a simple method by which communities can be monitored over time or compared to each other. This limits the more time-consuming and expensive cloning and sequencing steps to times when the communities change dramatically due to perturbations. More clone libraries and sequencing the full 16S rRNA genes would be able to provide a much better description of the soil communities. However, even with limited sequence information, similar organisms were found in each of the libraries, suggesting that many of the dominant members may be similar. These dominant bacterial populations apparently were not affected by disturbance.

Given the technique's limitation, rapid profiling methods, such as LH-PCR, can provide insight into the composition of the community organization by measuring the community richness, diversity, and evenness without the costly and labor-intensive construction of clone libraries and DNA sequencing analysis. However, most of these ecological indices are based on the correct identification to ''species'' level and their statistical application for microbial ecology studies has been questioned because the concept of species in bacteria is still being debated (Sun et al., 2004; Mills et al., 2003). Some recent studies have applied these indices to restriction fragment analyses using the individual peaks in DNA fingerprint profiles to represent discrete data points or operational taxonomic units (OTU) (Head et al., 1998; Embley and Stackebrandt, 1996). The evenness index considers both the richness and the diversity, and measures how similar the abundance of different phylotypes is in the profile. A low evenness value reflects a less diverse community with a frequency distribution of the diversity more skewed. These values are useful when assessing perturbations to the system (i.e., tillage practices, pollution, nutrient effects, etc.) and the response of the community to selective pressures (Dunbar et al., 2000).

In this study, diversity and evenness indices for three hypervariable regions of the 16S rDNA were calculated. However, the evenness indices seem to better reflect the differences in the soils and management practices based both in depth and the associated environmental disturbances or management practices (i.e., moldboard plow, conservation tillage, etc.). Unlike the results from the diversity indices where IP, ICT and NSB 0–5 were found not to be significantly different, the *K*-means results grouped all the NSB samples together. While diversity is a measure of differences in the richness and distribution of phylotypes, it reduces complex data sets that are produced to a single number. Univariate measures, such as diversity and evenness also assume a normal distribution within the community, a parameter that is impossible to measure for most soil microbial communities. Multivariate measures, however, take in consideration the complexities of the data sets. Measures such as *K*-means clustering treat each data point as an attribute and classify data based on similiarity (or dissimilarity) and distance measured from an arbitrary centroid, or *K* value. With this data set, $K = 3$ & $K = 4$ produced the same, thus the most stable, clusters. The undisturbed NSB samples grouped into one cluster while IP and ICT grouped together as the intermediate ''disturbance'' and IMP stood alone as the most disturbed system.

Soil microbial diversity is important because within limits, as the biodiversity of an ecosystem increases, the resilience and

stability of the ecosystem increases; conversely, as ecosystems degrade, ecosystem biodiversity decreases (Weis et al., 2007; Kardol et al., 2007; Balvanera et al., 2006). Loss of biodiversity can lead to loss of ecosystem resistance and resilience to anthropogenic as well as natural stresses (Waldrop et al., 2006; Fierer and Jackson, 2006; Hooper et al., 2005). Ecosystem resistance is the ability of the system to absorb the reduction of numbers of a species or set of species as a result of natural catastrophes such as hurricanes, periodic droughts, and insect and/or disease epidemics. Ecosystem resilience is the ability of the system's processes such as nutrient cycling, to survive and recover from these natural catastrophes. Conversion of NSB ecosystems to IP or ICT will decrease soil eubacterial diversity compared to native sagebrush soils at the 0–5 cm depth, but since irrigated agriculture will produce more crops per unit land converted to agriculture less land will be required to put into production relative to rainfed agriculture.

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