Final Report:

EFFECTS OF AGE AND DEPTH ON TOPSOIL PROPERTIES AND SEED BANK CHARACTERISTICS

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By
David B. Wester
Caesar Kleberg Wildlife Research Institute
Texas A&M University-Kingsville

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Executive Summary

The project studied seed bank and microbial community dynamics as affected by depth and age of stock-piled topsoils at two study sites in the western Texas Rio Grande Plains. Stock-piled topsoil and adjacent non-disturbed topsoil samples were collected on five occasions at 0-10, 10-20, 20-30 and 30-40 cm depths for seed bank analysis and 0-10, 10-20 and 20-30 cm depths for microbial analysis. Seed bank dynamics were quantified with the seedling emergence method. Microbial community dynamics were assessed with EL-FAME and chloroform fumigation extraction methods. Soil seed banks differed between study sites, which was expected given the differences in soils, past and present management, and surrounding vegetation. Soil seed banks also differed across sampling times, also expected given phonological changes in surrounding vegetation and weather influences. Stock-pile age did not affect seed bank characteristics and differences between stock-piled soil and intact soil and amongst depths varied between sites. Seed bank species diversity was generally unaffected by sampling depth in the stock-pile and species richness and number of emerged seedlings decreased with increasing sampling depth at both study sites. Mean seedling densities sites ranged from *ca* 1 to 6 (Hixon study site) and from less than1 to 2 (San Ysidro study site) seedlings per 1,288 cm^2 which meets standards for restoration success of 0.5 to 1 plant ft^2. In general, microbial community size (whether measured by MBC, MBN, TFA, or amounts of each community group) decreased with increasing sample depth in the undisturbed intact soil but did not change by depth in the stock-pile. Furthermore, microbial community size was generally smaller at a 0-10 cm depth in stock-piles compared to intact soil. Samples in winter months were also often different from summer months. At both study sites mean amount (nmol g^{-1} soil) Gram positive bacteria was highest in August 2013 while mean amount (nmol g^{-1} soil) Gram negative

bacteria was lowest. Microbial communities were uniform among depths in stock-piles, but not intact soil. Stock-piling affected seed banks and microbial communities. Protection of soil would increase the value of stock-piled soil for future restoration.

Table of Contents

Introduction and Project Background

A common recommendation following soil disturbance associated with pipeline and oil pad construction is topsoil stockpiling sites (Barkworth and Bateson 1964; Miller et al. 1985). In fact, US law requires removal, storage and replacement of topsoil in surface coal mining (Wick et al. 2009). Topsoil is set aside in piles during the construction of mining/drilling sites, and is then later replaced to facilitate site restoration (Rivera et al. 2012). Storage of topsoil can affect the value of the soil in the restoration process (Barkworth and Bateson 1964; Dickie et al. 1988). Topsoil stripping and storage may protect soil from hazards and disturbances associated with intended anthropogenic disturbances such as compaction, chemical contamination and complete loss (Stahl et al. 2002). However, effects of this practice depend on many factors including length of storage time, depth of pile, methods of stripping and environmental conditions during storage. Timing, method and duration of topsoil stripping and storage should be considered in light of the sensitivity of the biotic component of the soil to these factors (e.g., Rokich et al. 2000; Wick et al. 2009; Rivera et al. 2012).

Stockpiled topsoils are important for their seed banks. Knowledge of seed bank characteristics is essential in planning for successional changes and for determining the value of that seed bank for use in restoration projects (D'Souza and Barnes 2008). Because the soil seed bank resides in the topsoil, removal of the topsoil may equate to removal of the seed bank (Bakker et al. 2005). Soil microbial communities are also sensitive to soil disturbance (Mummey et al. 2002; Acosta-Martínez et al. 2004): disruptive management practices significantly affect microbiota (Blume et al. 2002; Gros et al. 2004). Soil management decisions are important and, ultimately, affect successful reuse of reserved topsoil.

Soil microbial communities are complex and dynamic (e.g., Wakelin 2009) and are fundamental indicators of ecosystem function and health (e.g., Acosta-Martínez et al. 2010b). Soil microbial communities are sensitive to and respond to soil disturbances (Mummey et al. 2002a; Acosta-Martínez et al. 2004; Wakelin et al. 2009), which may cause changes in microbial community structure, size and activity (Mummey et al. 2002b). Soil may take up to centuries to recover from severe disturbances (Tate III 2000) and disruption of soil processes affects ecosystem stability (Mummey et al. 2002a).

The challenge of effectively managing excavated soils has far-reaching consequences in the process of restoration. In south Texas, energy development industries are growing. In light of recent advancements in the oil and gas fields, ecologists have many opportunities to add to knowledge of soil and restoration ecology. Increases in economic activities and the human population make destructive extraction of resources likely to increase. The conditions both of the seed bank (D'Souza and Barnes 2008) and microbial communities (Gros et al. 2004) of the segregated topsoil are crucial for ecological restoration. The focus of this study is to provide some insights into the effects of current soil management practices on soil seed bank characteristics and microbial processes and offer information for future restoration endeavors

Materials and Methods

Study Site Descriptions

Study sites were located on two private ranches in western Rio Grande Plains. Stock-piles were less than 45 days old at each study site. The Hixon Ranch study site is a pad located near the center of La Salle County near Cotulla, TX. The stock-pile is approximately 15 meters long, 6 meters wide and 3 meters tall. Cotulla County receives an annual average of 53 to 66 cm of precipitation with the majority occurring between May and October (NOAA 2015). The

average temperature is 21 to 22 C. This study site includes both the Zavco soils series and the Dilley soil series. Samples were collected from the Dilley soil series (loamy, mixed, active, hyperthermic, shallow Ustic Haplargids). The A horizon of this series is a fine sandy loam to loamy fine sand that is approximately 20 cm deep (NRCS 2015). Ustic Haplargids are Aridsols that are typically found in arid environments and contain calcium carbonate with a clay accumulation. Mixed, active hyperthermic soils are those which have a mixed mineralogy class, a cation exchange capacity of 0.4 to 0.6 and are exposed to high temperatures (Brady and Weil 2008; UI-CALS 2013).

The historic plant community was a midgrass-dominated savannah with grasses including false rhodesgrass (*Chloris crinita*), little bluestem (*Schizachyrium scoparium*), feathery bluestem (*Andropogon ternarius*), sideoats grama (*Bouteloua curtipendula*), multiflowered false rhodegrass (*C. pluriflora*), Arizona cottontop (*Digitaria californica*), and pink pappusgrass (*Pappophorum bicolor*). Mesquite (*Prosopis glandulosa*), whitebrush (*Aloysia gratissima*), condalia (*Condalia* sp.) and wolfberry (*Lycium carolinianum*), along with diverse forbs would have been scattered throughout this area (NRCS 2015).

The San Ysidro Ranch study site is adjacent to a fracking pond located in the south central area of Dimmit County near Catarina, TX. The stock-pile is approximately 38 meters long, 12 meters wide and 5 meters tall. Dimmit County receives an average annual 43 to 61 cm of precipitation with the rainy season between May and October (NOAA 2015). The average temperature is 22 to 23 C. The Brundage soil series (fine-loamy, mixed, active, hyperthermic Aridic Natrustalfs) is the dominant soil series of this site. The A horizon of this series is a fine sandy loam that is about 20 cm deep. Aridic Natrustalfs are Alfisols (moderately leached with a subsurface accumulation of clay) which are found in arid and subhumid environments; their

natric horizon is similar to an argillic horizon, except for differences in structure and the addition of a subhorizon having greater than 15% saturation of exchangeable sodium. Mixed, active hyperthermic soils are those which have a mixed mineralogy class, a cation exchange capacity of 0.4 to 0.6 and are exposed to high temperatures (Brady and Weil 2008; UI-CALS 2013).

The historic plant community was a savannah habitat dominated by grasses including false rhodesgrass, tanglehead (*Heteropogon contortus*), plains bristlegrass (*Setaria macrostachya*), silver bluestem (*Bothriochloa laguroides*), Arizona cottontop, hooded windmill grass (*Chloris cucullata*), pink pappusgrass, lovegrass tridens (*Tridens eragrostoides*) and curly mesquite (*Hilaria belangeri*). Scattered among the grasses would have been various and woody species, for example, bush sunflower (*Simsia calva*), orange zexmenia (*Wedelia texana*), western ragweed (*Ambrosia psilostachya*), guajillo (*Acacia berlandieri*), blackbrush (*Acacia rigidula*.) and spiny hackberry (*Celtis ehrenbergiana*) (NRCS 2015).

Methods—Seed Bank Study

Six soil samples were collected from random locations from the stock-pile of topsoil and also from the nearby intact non-disturbed topsoil at both study sites on 5 sample occasions (June 2013, August 2013, April 2014 May 2014 and July 2014). Each 400 g sample (Eldridge and Lunt 2010) was collected with a sharpshooter and bucket auger and placed in marked, whirl-pak bags.

For seed bank studies, many small samples are generally more acceptable than a few large samples (Page et al. 2006). Therefore, six soil cores (i.e., bucket augers) were collected from each stock-pile. Cores were separated into 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm depths. It was not possible to collect deeper samples because of soil compaction. There were a total of 24 samples from each stock-pile for each trial. Six samples of intact soil were also

collected from the A horizon at each study site. Each core from the intact topsoil was mixed into a composite sample because this better represents the stripped topsoil in the stock-pile (Mason et al. 2011). After collection, soil samples were transported on ice to a Texas A&M University Kingsville soils laboratory.

Samples were sieved using a 5.6 mm sieve to smooth soil aggregates, rocks and large pieces of vegetative debris, spread out on covered tables to air dry at room temperature (D'Souza and Barnes 2008), and then transported in their same marked bags to the South Texas Natives greenhouse. The greenhouse was maintained at ~30°C during the day and ~20°C at night; there was no artificial lighting.

Seed banks were assessed with the seedling emergence method. Each sample was spread out in a prepared, 30.5 cm by 46 cm tray as evenly as possible. The tray had about 9 cm of clean, coarse sand in the bottom; the soil sample was about 0.5 cm deep across the top of the sand (Erenler et al. 2010).

Six negative control trays and six positive control trays were also prepared. The negative controls had only 9 cm of sand and no soil (Eldridge and Lunt 2010). The positive controls had 9 cm of sand and were inoculated with about 20 seeds each of green sprangletop (*Leptochloa dubia*), awnless bush sunflower and a mixture of redseed plantain (*Plantago rhodosperma*) and Hooker's plantain (*P. hookeriana*). Positive control trays were used to indicate that environmental conditions were appropriate for germination of some common south Texas species; negative control trays were used to detect contamination from the sand that was placed in each tray and from inside the greenhouse.

All trays were positioned on five benches in a completely random fashion (Erenler et al. 2010). Trays were watered daily to maintain soil water and checked daily to monitor germination. Trays were fertilized after three weeks with Miracle-Gro® Liquafeed® Bloom Booster® liquid fertilizer (12% nitrogen, 9% phosphorus and 6% potash) (Kinucan and Smeins 1992).

Seedlings were identified as soon as possible after emergence and then removed with a pair of scissors or tweezers by cutting or pinching the plant (rather than uprooting it) in order to avoid disturbing nearby seeds and seedlings (Thompson and Grime 1979). Each germination trial lasted six weeks, after which unidentified plants were transplanted into pots which were observed until they reached an identifiable stage (Eldridge and Lunt 2010). Each trial took five to eight months to complete.

Statistical Analysis—Seed Bank Study

The soil seed bank of each greenhouse tray was characterized by germinable seed bank size (total numbers of emerged and identified seedlings) and species composition based on seedling density. Species richness (S), diversity (exp(H′), where H′ is Shannon's index) and evenness (exp(H′)/S) were calculated from species composition. Neither seed bank size nor these diversity metrics are normally distributed; therefore permutational analyses were used to test hypotheses addressing effects of sampling time and sampling depth as well as comparisons between stock-piles and nearby intact (undisturbed) soil on seed bank size and diversity measures with Euclidean distance as a resemblance measure. Two analyses were performed to examine: (1) changes over sampling times and sampling depths on the stock-pile and (2) comparison between the stock-pile and intact soil. The first analysis used a linear mixed model with sample core as a random nuisance block effect and sampling depth as an effect of interest in

a randomized block design. Data from the five sampling times were analyzed together to test effects related to seed bank age; in this model, core was nested in time, and the interaction between core and depth was nested with sampling time. In the second analysis, seed bank characteristics were compared between intact soil and stock-piles, with each soil depth analyzed separately in a comparison with intact soil. Therefore, this analysis tested effects of location (intact soil or stock-pile) and sampling time, as well as their interaction using core nested within time and location as an error term. These two analyses were also performed on mean species composition with a permutational multivariate analysis of variance based on an unweighted Bray-Curtis similarity measure. Reported *P* values are based on permutation unless otherwise indicated. Past seed bank studies have shown high variability in the distribution of seeds in the soil (e.g., Gioria and Osbourne 2009); thus, a 0.10 alpha level was used to detect statistical differences.

Given the spatial variability in soil characteristics, even over short distances, and the confounding effects of variable machine operators, it is likely that no two stock-piles can reasonably be considered as two replications in the true sense. Therefore, in this research, each stock-pile is defined as the population of inference (Wester 1992) and soil samples from randomly selected locations on each stock-pile are samples from these populations.

Methods—Soil Microbial Study

Soil samples were collected from both the stock-pile of topsoil and also from the nearby intact topsoil at both study sites. Sample sites were randomly selected. Each sample, about 400 g of soil (Eldridge and Lunt 2010), was collected from the field, placed in marked, whirl-pak bags and then stored in coolers with ice. Samples were collected using a sharp-shooter shovel and a bucket auger. The samples were stored on ice until they were shipped (on ice), as soon as

was feasible, to the USDA Agricultural Research Service's Cropping Systems Research Laboratory (CSRL) in Lubbock, Texas.

Soil samples were collected in February 2013 and then every six months through February 2015 (a total of five trials for the soil analyses portion of the study). Five cores (i.e, bucket augers) each of nearby intact topsoils and the stock-pile were collected at three depths: 0-10 cm, 10-20 cm and 20-30 cm. Soil samples were not pooled because this method has the potential of not accurately representing the soil ecosystem due to variability of microbial distribution (Nunan et al. 2002).

There were a total of thirty samples taken from the San Ysidro Ranch for each trial. At the Hixon study site, half of the intact portion of the intact, undisturbed study area was sprayed with Sendero® herbicide in August 2013 in an effort to control mesquite in this area of the property. For samples collected in August 2013 and thereafter, ten samples were collected from the intact soil; five were collected from the Sendero® sprayed area and five from the unsprayed area.

FAME Analysis

Although there are several variations of FAME extraction and analysis (Zelles 1999), we are using the Ester Linked (EL) method as the best possible and most cost effective method.

Microbial Biomass Carbon and Nitrogen Analysis

Microbial biomass carbon and nitrogen were analyzed using the chloroform-fumigation extraction method (Acosta-Martínez et al. 2004, 2010b).

Statistical Analysis—Soil Microbial Study

For tests of hypotheses addressing effects of sampling depth, location and sampling time, soil cores from each treatment, intact and stock-piled soil, were the experimental units from

which three depths were separated and where core was treated as nuisance variable. Quantitative response variables, MBC, MBN, TFA, fungi:bacteria ratio and amounts of each individual microbial group, were analyzed with a mixed model repeated measures analysis in SAS, with depth as the repeated measure. Relative soil microbial community composition was analyzed with a similar model using permutational *F* tests. Non-metric multidimensional scaling was used to illustrate patterns in relative microbial size as affected by sampling date, location and depth.

Given the spatial variability in soil characteristics, even over short distances, and the confounding effects of variable machine operators, it is likely that no two stock-piles can reasonably be considered as two replications. Therefore, in this research, each stock-pile is defined as the population of inference (Wester 1992) and soil samples from randomly selected locations on each stock-pile are samples from these populations.

Results—Seed Bank Study

Hixon Study Site

Changes on the Stock-pile

Mean species richness, diversity and evenness changed over time on the stock-pile. Richness and diversity were highest in August 2013 and lower but similar at other sampling times; evenness was lowest in August 2013 and higher but similar at other sampling times. Although richness generally decreased with increasing sampling depth, neither diversity nor evenness changed with depth. Mean species composition also differed across sampling times; these differences, however, were consistent across sampling depths. Additionally, mean species composition did not differ across sampling depths.

Mean total seedling number was affected by a sampling time × depth interaction. Total numbers were similar across depths at each sampling time except the May 2014 sampling. Total seedling numbers declined as sampling depth increased at the May 2014 sampling time. At all depths, total number of seedlings were highest at the August 2013 sampling time and similar at all other times.

Although emerged seedling density of the stock-piled soil is of interest, dynamics of native and exotic species are also of interest in restoration projects. Mean number of exotic seedlings changed over time on the stock-pile, but was not affected by sampling depth. Mean total number of native seedlings was affected by both sampling time and sampling depth: the August 2013 sampling time had the highest mean number of native seedlings and mean number of native seedlings decreased as sampling depth increased.

Comparison of Stock-pile and Intact Soil

Seed bank species richness differed across sampling times at 0-10 cm, 10-20 cm, and 20-30 cm sampling depth strata. Mean species richness was highest at the August 2013 sampling time at each of these depths and in the intact soil. In contrast, location (stock-pile or intact soil) and sampling time interacted in their effects on seed bank species richness at the 30-40 cm depth.

Seed bank diversity was affected by sampling time at all sampling depths. Species diversity was highest in August at each depth. Although species diversity was higher in the intact soil seed bank than the pile at the 30-40 cm, diversity was unaffected by location at other depths on the pile.

Seed bank species evenness was affected by sampling time at 0-10 cm, 10-20 cm, and 20-30 cm sampling depths. Mean species evenness was lowest at the August 2013 sampling time at

each of these depths as well in the intact soil. Species evenness was higher in the stock-pile at the 0-10 cm depth and 20-30 cm depth than in intact soil. At the 30-40 cm depth, location (stock-pile or intact soil) and sampling time interacted in their effects on seed bank species evenness.

Mean species composition differed across sampling times at the 10-20 cm, 20-30 cm and 30-40 cm sampling depths. At the 10-20 cm depth, seed bank mean species composition of the intact soil was different from the stock-piled soil. Sampling time and location interacted at the 0-10 cm depth.

Location and sampling time interacted in their effects on mean total number of seedlings at all sampling depths. Total seedling numbers were higher in the intact soil than stock-piled soil from the 10-20 cm depth at the May 2014 sampling time and the 30-40 cm depth at the August 2013 and May 2014 sampling times. Conversely, stock-piled soil had higher mean total numbers of seedlings at the 0-10 cm depth at the August 2013 sampling time and the 20-30 cm depth at the June 2013 and April 2014 sampling times. Samples collected in August 2013 produced more seedlings than any other sampling time in both the stock-piled soil and the intact soil.

San Ysidro Study Site

Changes on the Stock-pile

Mean species richness and evenness were not affected by sampling time. However, mean species richness decreased as sampling depth increased and species evenness increased with increasing sampling depth. Seed bank diversity was not affected by sampling time, depth, or their interaction. Sampling time and depth interacted in their effects on seed bank mean species composition.

Sampling time and depth interacted in their effects on mean total number of emerged seedlings. Mean total numbers were reduced in the 0-10 cm depth at the June 2013 sampling time. Total numbers were similar across time at all other depths. At the June 2013 sampling time, total numbers did not different across depths. At all other depths, total number of seedlings decreased as sampling depth increased.

Only 5 exotic species seedlings emerged from San Ysidro samples which were too few to analyze. Therefore the dynamics of the native species seedlings are sufficiently described by the total number of seedlings analysis.

Comparison of Stock-pile and Intact Soil

Sampling location and time interacted in their effects on mean species richness at the 0-10 cm and 10-20 cm sampling depths. Richness of the stock-piled soil seed bank at the 0-10 cm depth was lower than the undisturbed soil at the June 2013 sampling time; species richness was similar between the undisturbed soil and the stock-pile at this depth at all other sampling times. Species richness was consistently higher in the undisturbed soil than the stock-piled soil at the 10-20 cm depth at each sampling time except at the April 2014 time.

Sampling time did not affect species richness at the 20-30 cm or 30-40 cm depths. Seed bank species richness was higher in the intact soil than the stock-pile at both the 20-30 cm and 30-40 cm depths. Mean species richness of the intact soil seed bank was not affected by time at these sampling depths.

Sampling time and location acted independently in their effects on diversity and evenness. In addition, neither diversity nor evenness was affected by sampling time. However, diversity was higher in the intact soil than the stock-piled soil at all sampling depths. Evenness

was similar between locations at all sampling depths except 20-30 cm, where evenness was higher in the stock-pile.

At the 0-10 cm depth, mean species composition differed across time and intact species composition was different from the pile. Sampling time and location interacted at the 10-20 cm and 30-40 cm depths. Mean species composition was not affected by either sampling time or location, nor did time and location interact at the 20-30 cm depth.

Mean total number of seedlings was not affected by sampling time at any depth. Total number of seedlings was identical between the intact soil and the 0-10 cm depth of the stock-piled topsoil. However, total seedling numbers were higher in the intact soil than the deeper depths of the stock-pile. Mean total number of seedlings was not different across sampling times.

Discussion—Seed Bank Study

Soil seed banks at Hixon study site were, in general, very different from the San Ysidro study site with respect to size, diversity and composition. Differences were expected because of differences in micro-climate, soil order, and past and present management. Study sites differ in other ways as well. For example, the stock-piles were made by different machine operators on different dates. Species diversity generally was unaffected by sampling depth in the stock-pile and species richness and numbers of emerged seedlings decreased with increased depth in the stock-pile at both study sites.

Changes Over Time

At the Hixon study site, sampling time strongly influenced nearly every aspect of the study. The August sampling time, in particular, was different from all other sampling times:

species diversity, richness and total number of seedlings was highest in August, and evenness was lowest in August. Mean species richness was more than 4 times higher and mean species diversity was more than 2 times higher in August 2013 than other times. Mean total numbers of seedlings was nearly 6 times higher at the August 2013 sampling time than any other sampling time. Composition also changed over time on the stock-pile and in comparisons of stock-pile depths to intact soil.

Precipitation events prior to the August sampling time may have contributed to increase in emerged seedlings. One possible explanation begins with precipitation events prior to sampling in June 2013 which resulted in a flush of germination of a large portion of resident seeds. This may explain the relatively low number of emerged seedlings in the June greenhouse trial. In addition, seedlings which resulted from this flush of germination flowered after being triggered by a period of low precipitation in July and August and consequently contributed a significant and immediate input of seeds into the soil seed bank of both intact soil and the stock-pile, which was subsequently recovered in the August greenhouse trial.

Sampling time had no effect on mean species diversity richness or evenness at the San Ysidro study site. However, species composition was affected by a time-by-depth interaction and total number of seedlings was affected by an interaction between time and depth. Total number of seedlings increased from 0 seedlings to an average of up to 5.5 seedlings at the 0-10 cm depth on the stock-pile after June 2013. This may be a result of dispersing seeds from nearby vegetation. Precipitation events may have influenced seed bank dynamics in a similar manner, though to a lesser extent than at the Hixon study site.

Duration of residence in a stock-pile had no discernible pattern of effects on seed bank characteristics at the Hixon study site after 18 months of storage. Results from the San Ysidro

study site also suggest that more than 18 months of storage do not impact seed bank characteristics. Original seed bank composition may determine the effects of storage time on seed bank characteristics. There have been no previous studies of the upland seed banks of the western Rio Grande plains and lack of conformity to previously mentioned studies may be due, in part, to differences in vegetation and climate. The environment in south Texas is extreme and resident vegetation is adapted to survive in spite of those challenges. Seed banks of these soils are evidently resilient to environments typical of stock-piles. Alternatively, the resident seed bank of this area may consist of mainly transient seeds which are easily missed in seedling emergence trials and would be expected to decline in stock-pile conditions. Transient seeds are often missed in seed bank analyses because they exist in the soil for only a short time before germinating or dying.

Changes by Depth

Species richness decreased with increasing depth at the Hixon study site. Mean species richness was twice as high in the 0-10 cm depth as the 30-40 cm depth. The decline in species richness may be an indication of death or depletion of some species which are transient or more sensitive to environmental conditions. Mean total number of seedlings also decreased with increasing depth, but only at the May 2014 sampling; mean total number of seedlings remained constant throughout other sampling times. Seed rain may have influenced these results. Contributions of seed from surrounding vegetation would have impacted only the soil exposed at the top of the stock-pile. The upper depths of the stock-pile would have also been more quickly exposed to changes in temperature than the deeper depths. This may have relieved dormancy of some warm season species in the upper depths of soil.

At the San Ysidro, total number of seedlings also decreased with increased sampling depth. This is consistent with findings of Rivera et al. (2012) who determined that germinability was reduced as depth of the sample in a stock-pile increased. Changes by depth interacted with sampling time at the San Ysidro. Total number of seedlings was not different between depths at the June 2013 sampling time. However, at subsequent sampling times, significant changes occurred. The top depth had 7.5 times higher mean number of emerged seedlings than lower depths in August 2013. Also, at the last sampling time (July 2014), the 10-20 cm depth had nearly 8 times higher mean total numbers of seedlings than 20-30 and 30-40 cm depths. This indicates the possibility that the duration of storage in the stock-pile is nearing an age at which seed viability and density have begun to reduce.

After 8 months of storage, the seed bank of the stock-pile at the San Ysidro study site developed a profile by depth. Deeper samples had fewer total emerged seedling and fewer species among those which did emerge. This is similar to what would be expected in the intact soil profile (Iverson and Wali 1982; Rosef 2008). Changes in seed bank dynamics by depth could be the result of dormancy triggered by darkness, lack of moisture or other factors. Stock-piling topsoil results in soil compaction and decimation of earthworm populations (Boyer et al. 2011) and these may be important factors in limiting contributions of dispersed seed to the vertical soil profile (Thompson 1987). Although some species may not survive well in stock-pile conditions, an alternative explanation of differences in seed bank characteristics by depth would be that the soil at the top of the stock-pile is exposed to seeds from nearby vegetation. This is a better explanation of these results because mean total numbers of seedlings remained fairly stable across times in each depth.

Mean seedling densities at the stock-pile at the Hixon study site ranged from less than 1 to 6 seedlings per 1,288 cm^2 tray depending on time of sampling. Mean seedling densities ranged from less than 1 to 2 seedlings per 1,288 cm^2 tray at the San Ysidro study site. This meets common standards for restoration success of an average 0.5 to 1 plant ft^{-2} (Dahl et al. 1988).

Potential of the seed bank of stock-piled soils for restoration efforts would be increased if native species dominated the soil seed bank, thereby reducing the need for seeding native species. In this study, both the mean number of exotic seedlings and mean number of native seedlings was affected by sampling time at the Hixon study site. However, depth had no effect on exotics species, whereas native species declined in the deeper depths of the stock-piled soil. Changes in mean numbers of exotic and native species over sampling time may be a function of precipitation or other seasonal factors such as temperature. Increases and decreases evidently were not affected by the age of the stock-pile. Decline of native seedling germination in deeper stock-pile depths suggests that native species may be less able to withstand these conditions than exotic species. At the San Ysidro study site, very few exotic species emerged which hindered analysis of differences in natives and exotics. Only 5 exotic seedlings emerged (from the 0-10 cm depth) from the May and July 2014 sampling dates.

Stock-piled Soil vs Intact Soil

Samples collected from the stock-pile at the Hixon study site, in general, did not exhibit different seed bank dynamics from the intact soil. On the other hand, samples from the stock-pile at the San Ysidro study were often different from intact soil samples. Species diversity, richness and total number of seedlings was generally higher in the intact soil than in the stock-piled soil at the San Ysidro. Mean total number of emerged seedlings was not different from the

intact soil at the 0-10 cm depth. At the 10-20, 20-30 and 30-40 cm depths the intact soil had more than 2, 6 and 12 times, respectively, higher mean total numbers of seedlings that the stock-pile. The observations at the San Ysidro site are compatible with those made by Iverson and Wali (1982) who found that both numbers and diversity of seeds decreased as depth in the stock-pile increased.

Many studies of seed banks in stored topsoil have found that duration and depth in the stock-pile affected seed bank characteristics (Iverson and Wali 1982; Dickie et al. 1988; Rokich et al. 2000; Rivera et al. 2012). Studies of mixed grass prairies in North Dakota (Iverson and Wali 1982), woodlands in Australia (Rockich et al. 2000), the Mojave Desert in Nevada (Scoles-Sciulla and DeFalco 2009) and annual dominated grassland in Spain (Rivera et al. 2012) each concluded that seedlings decreased as time in the stock-pile increased. A range of 6 to 13 months was considered sufficient to negatively impact seed bank dynamics.

The results of this study are different from many of these studies: seed bank studies are strongly influenced by site-specificity. There have been no published studies of seed banks in stock-piled topsoil in this eco-region. Further, no two studies of stock-pile seed banks have been designed the same way. For example, whereas Rivera et al. (2012) examined similar depths in the stock-pile, they examined effects of burial on a specific species which they buried in the stock-pile for only 6 months. And although Dickie et al. (1988) examined the seed bank of stock-piled soil using a seedling emergence method, they examined effects over a period of four years and three meters in depth.

Management Implications—Seed Bank Study

Averaged over soil depth, mean number of emerged seedlings at the Hixon study site ranged from 6 seedlings 1,277 cm^{-2} (4.3 ft^{-2}) in August to less than 1 seedling 1,277 cm^{-2} (0.5 ft^{-2}) at other sampling dates (5 native seedlings 1,277 cm^{-2} (3.6 ft^{-2}) in August to less than 1 native seedling 1,277 cm^{-2} (0.1 ft^{-2}) at other sampling dates). At the San Ysidro study site, emergence ranged from 3 seedlings 1,277 cm^{-2} (3.2 ft^{-2}) to less than 1 seedling 1,277cm^{-2} (0.2 ft^{-2}). Because these ranges generally fall within recommended plant establishment guidelines and it is reasonable that seed rain from adjacent vegetation will also contribute colonization of the re-spread soil, there would be little reason to re-seed these stockpiles.

However, there are many factors that affect the range of emerged seedlings that that were observed in this study. Although seedling emergence varied among my greenhouse trials, it is likely that the factors responsible for this variability are more closely related to the season when the stockpile was constructed and then sampled, as well as the immediately-preceding climatic conditions, rather than the age of the stock-pile per se. That is, there were no apparent patterns to changes in seed bank dynamics of the stored soil that can be attributed solely to storage time (up to the 18-month sampling period of this study). Furthermore, in areas where exotic species already dominate the plant community, managers should realize the possibility that these species will continue to dominate the stock-pile seed bank for at least this period. This should be taken into consideration if management goals include alteration of current seed bank characteristics. Therefore, chances for future restoration success likely would be improved if the stockpile (1) could be constructed after a period of ample seed rain (to enhance seed input into the stockpile); (2) spread soil out on the restoration site following an episode of seed rain (again, to enhance

seed input); and (3) time the restoration effort to the most appropriate season for adequate precipitation to enhance chances of success.

Results—Soil Microbial Study

Hixon Study Site

Changes in amounts of microbes (nmol g^{-1} soil) in the Intact Soil: Sendero® vs no Sendero®

As described above, a portion of the study area was sprayed with the herbicide *Sendero®* as part of its ongoing brush management protocol. However, effects of herbicide on microbial communities are not part of the objectives of this study and so the following results will be based on samples collected from intact soil that was not sprayed with Sendero® at the Hixon study site. Samples in February 2013 (which were collected from the area that was sprayed in summer 2013) were also excluded from subsequent analysis.

Changes in Amounts of Microbes (nmol g^{-1} soil)

Mean amount of Gram positive bacteria was affected by sampling time and also by an interaction between location and depth. Mean amounts of Gram positive bacteria were highest at the August 2013 sampling time and similar at all other sampling times. In the stock-pile, mean amounts of Gram positive bacteria were not affected by depth. However, mean amounts of Gram positive bacteria decreased with increasing depth in the intact soil. The intact soil had higher mean amounts of Gram positive bacteria than the stock-pile at the 0-10 cm depth. However, the stock-pile had higher mean amounts of Gram positive bacteria than the intact soil at the deeper sampling depths.

Mean amounts of Gram negative bacteria were affected by a three-way interaction between sampling time, sampling depth and location. Gram negative bacteria (nmol g^{-1} soil) in the August 2013 samples were not affected by location, depth or their interaction. However,

location and depth interacted in their effects on amounts of Gram negative bacteria in samples collected at the later sampling times.

Actinomycetes were also affected by a three-way interaction of sampling time, depth and location. Additionally, location and depth interacted at each sampling time. For all sampling times, depth was not significant on the stock-pile but was significant in the intact soil. At all sampling times mean amounts of actinomycetes decreased with increasing depth in the intact soil. During the summer sampling times, there was no difference between locations at any depth. During both winter sampling times, there was no difference between locations at the 10-20 cm depth.

Location and depth interacted in their effects on mean amount of fungi, as did location and sampling time. However, there was no three-way interaction between sampling time, depth and location. Depth had no effect on mean amount of fungi (nmol g^{-1} soil) in the stock-pile. However, amounts of fungi (nmol g^{-1} soil) decreased with increasing depth in the intact soil. Mean amount of fungi differed between the stock-pile and the intact soil at all depths. At the 0-10 cm depth, mean amounts of fungi were higher in the intact soil than the stock-pile. However, mean amounts of fungi were lower in the intact soil than the stock-pile at deeper depths. Although the mean amount of fungi did not change over time in the intact soil, mean amount of fungi was higher at the February 2014 sampling time than at other times which were similar to one another. Also, at the February 2014 sampling time, mean amounts of fungi were higher in the stock-pile than the intact soil whereas amounts were similar between locations at all other times.

Location interacted separately with both sampling time and depth in their effects on the fungi to bacteria ratio. Sampling time and depth also interacted in their effects on the fungi to

bacteria ratio. There was, however, no three-way interaction with sampling time, depth and location. The fungi to bacteria ratio did not change over time in the stock-pile. However, in the intact soil, fungi to bacteria ratios were lower in the winter sampling times than the summer sampling times. Further, fungi to bacteria ratios were lower in samples collected during 2014. At each sampling depth, fungi to bacteria ratios changed across time.

Changes in Relative Microbial Community Composition

Time interacted separately with location and with depth in its effects on relative microbial community composition at the Hixon study site. Depth also interacted with location in its effects on relative community composition. There was no three-way interaction with time, location and depth. Relative microbial community composition was different in the stock-pile than intact soil at all sampling times. However, in both the stock-pile and the intact soil, winter sampling months were similar to each other and different from the summer sampling times. Also, in both locations, August 2013 was different from July 2014. Relative microbial community composition was different in the stock-pile than intact soil at all depths. In both the intact soil and stock-piled soil, the 0-10 cm depth was different from the deeper depths and the deeper depths were similar to each other.

Changes in Microbial Biomass

Time interacted with location and with depth in their effects on MBC (mg kg^{-1} soil). Also, depth and location interacted in their effects on MBC. There was no three-way interaction. MBC was higher in the stock-pile than the intact soil during the winter months, lower in the stock-pile than the intact soil in July 2014, and not different between locations at the August 2013 sampling time. In the intact soil, all sampling times were similar except July 2014 which was higher than other sampling times. In the stock-pile, winter sampling times were similar to

each other and consistently higher than summer sampling times. Summer sampling times were similar to each other.

MBC changed by depth at all sampling times except August 2013. At all times in which depth was significant, MBC decreased with increasing depth. MBC did not change over sampling time at the deeper depths. MBC did not change by depth in the stock-pile. In the intact soil, however, all depths were different from each other and MBC decreased with increasing depth.

An interaction of sampling time and depth as well as an interaction of location and sampling depth affected MBN (mg kg^{-1} soil). There was no three-way interaction. MBN did not change by depth in the summer sampling times. During the winter sampling times, MBN decreased with increasing depth. The summer sampling times had lower MBN than winter and February 2015 was higher than February 2014. Both the intact and stock-piled soil changed by depth. Whereas in the intact soil the deeper depths were similar to each other but not the top depth, in the stock-pile the upper depths were similar to each other but not the deepest depth. Overall, MBN decreased with increasing depth.

Total fatty acids (TFA; nmol g^{-1} soil) were affected by sampling time and an interaction of location and depth. The August 2013 sampling time was higher in TFA than all other sampling times. TFA were different between the stock-pile and intact soil at all depths. TFA did not change by depth in the stock-piled soil. In the intact soil, TFA decreased with increasing depth.

San Ysidro Study Site

Changes in Amounts of Microbes (nmol g^{-1} soil)

Sampling location and sampling depth interacted in their effects on both the Gram negative and Gram positive bacteria. Sampling time also affected amount of Gram positive and Gram negative bacteria in the soil. For each group, amounts of bacteria were not affected by depth in the stock-pile. Amounts of each group, however, declined with depth in the intact soil. Amounts of both Gram positive and Gram negative bacteria (nmol g^{-1} soil) were higher in the intact soil at the 0-10 and 10-20 cm depths. Amounts of these groups were similar at the deepest depth (20-30 cm) between the intact soil and stock-pile. Amounts of the Gram positive community group were highest at the August 2013 and February 2014 sampling times and lowest at the July 2014 sampling time. Amounts of Gram negative bacteria, on the other hand, were consistently highest during the winter sampling months.

Actinomycetes were affected by a three-way interaction between sampling time, sampling depth and location. Sampling depth and location did not interact nor was location or depth significant at the February 2013 and July 2014 sampling times. At the August 2013 sampling time, although sampling depth and location interacted in their effects on mean amounts of actinomycetes, depth was not significant on either the stock-pile or the intact soil and location had no effect at any depth. There was no difference in mean amounts of actinomycetes in samples from the February 2014 and 2015 sampling times across depth in the stock-pile. Mean amounts of actinomycetes declined with increasing depth in the intact soil at both sampling times. Also, mean amounts of actinomycetes were higher in the intact soil at the 0-10 cm in samples from both February 2014 and 2015. In February 2014, it was also higher in the intact soil at the 10-20 cm depth. Samples from the 10-20 cm depth at the February 2014 sampling time and from the 20-30 cm depth at both February 2-14 and 2015 were similar between locations.

The amount of fungi in the soil was affected by sampling time and location. Amounts of fungi were generally higher during the winter months and lower during the summer months. Mean amounts of fungi were higher in the intact soil than the stock-piled soil. The fungi to bacteria ratio was unaffected by sampling time, depth, location or any of their interactions.

Changes in Relative Microbial Group Composition

Sampling time, location and sampling depth interacted in their effects on mean microbial community composition. Neither location nor depth affected mean microbial community composition during the winter sampling times. Mean microbial community composition was different between locations during the summer sampling times. Sampling depth and location interacted in their effects on microbial community composition during the August 2013 sampling time. Sampling depth did not affect microbial community composition in the intact soil. In the stock-pile, however, community composition was different at each depth. Mean microbial community composition was different between the stock-pile and intact soil at the 0-10 and 10-20 cm depths, but was not different at the 20-30 cm depth. Although depth did not affect microbial community composition at the July 2014 sampling time, mean microbial community composition of the intact soil was different from that of the stock-pile.

Changes in Microbial Biomass

MBC (mg kg^{-1} soil) at the San Ysidro was affected by each two-way interaction among the factors under study. Sampling time interacted separately with location and depth in its effects on MBC. And location and depth also interacted in their effects on MBC. On the stock-pile, the February 2014 sampling time was higher than all other sampling times. There were no differences detected in MBC between locations in 2013 or 2015. In 2014 however, there were

differences between the stock-piled and intact soil. MBC was higher in the stock-pile in February 2014 and higher in intact soil in July 2014. MBC in the intact soil did not change over time.

MBC (mg kg^{-1} soil) in the intact soil, however, changed by depth. The 0-10 cm depth was higher in MBC than the deeper depths which were similar to each other. MBC did not change by depth in the stock-piled soil. And at the deeper sampling depths there were no differences detected between locations. At the 0-10 cm depth MBC was higher in the intact soil than the stock-pile.

MBN (mg kg^{-1} soil) was affected by a three-way interaction of sampling time, depth and location. Sampling depth and location did not interact in samples from the first four sampling times. MBN in samples from the February 2013 and July 2014 sampling times were not different amongst depths, nor were they different between locations. Samples from the August 2013 sampling time were also similar between the stock-pile and intact soil. However, MBN increased with increasing depth in samples from August 2013. Conversely, MBN decreased with increasing depth in February 2015 during which time MBN was also higher in the stock-pile than the intact soil.

TFA (nmol g^{-1} soil) at the San Ysidro study site changed over time. Location and depth also interacted in their effects on TFA. TFA was similar during winter sampling times. Summer sampling times differed in TFA.; August 2013 had higher TFA than July 2014. In the stock-pile, depth did not affect TFA. In the intact soil, on the hand, all depths were different from each other and TFA decreased with increasing depth. TFA was higher in the intact soil at the top two depths; there was no difference between locations at the 20-30 cm depth.

Discussion—Soil Microbial Study

This study examined changes in amounts and relative amounts of identifiable FAME biomarkers, and fungi to bacteria ratios, MBC, MBN and TFA in stock-piled soil over time and by depth at two study sites. Because of differences of the two study sites in micro-climate, soil order, and past and present management, among other factors, it is to be expected that stock-piling would affect microbial communities differently between sites. However, microbial communities responded to stock-piling similarly in a number of respects. In general, microbial community size (whether measured by MBC, MBN, TFA, or amounts of each community group) decreased with increasing sample depth in the undisturbed intact soil but did not change by depth in the stock-pile. Furthermore, microbial community size was generally smaller at a 0-10 cm depth in stock-piles compared to intact soil. Samples in winter months were also often different from summer months. At both study sites mean amount (nmol g^{-1} soil) Gram positive bacteria was highest in August 2013 while mean amount (nmol g^{-1} soil) Gram negative bacteria was lowest.

Depth of Soil Sample Effects

One expectation of stripping and storing topsoil is that microbial biomass will decrease (Harris 2003) and that those impacts are affected by depth of storage (Visser et al. 1984). Dilution of the SOM rich upper layers of the topsoil with the predominantly mineral layers during construction of the stock-pile may be the reason for the changes in organic carbon (Visser et al. 1984). Stripping and stock-piling topsoil can homogenize soil nutrients (Strickland and Rousk 2010) which affects the distribution of soil microbial communities. Spatial arrangement and variability of soil biota is essential to understanding other facets of soil ecology and patterns of spatial distribution vary both horizontally and vertically through the soil profile (Nunan et al. 2002, 2003). Bacterial communities and activities are variable but not randomly distributed in

the soil. Visser et al. (1984) reported that organic carbon and microbial biomass was lower in the upper depths of the stock-pile than undisturbed soil and lower than or not different from the intact soil at deeper depths and suggested that nutrient dilution was likely a factor in their findings. Although this study did not analyze soil nutrients and chemicals, it would be expected that the process of stripping and stacking the soil would result in a relatively uniform distribution of soil organic matter and soil nutrients, thereby eradicating the natural depth gradient of soil nutrients and soil microbial communities.

This absence of depth gradient in the stock-piled soil with respect to microbial community size was apparent throughout this study, as expected. Microbial community composition and size were often homogenous throughout the soil profile in the stock-pile. Departures from this pattern were instances in which sampling depth interacted with sampling time in their effects on the soil microbial communities. Of the many factors which influence microbial biomass, climate plays a major role in causing variation, especially over time, but also sometimes by depth as well. The stock-pile lacks the depth gradient which is prevalent in the intact soil and is especially lacking in the high amounts of all microbial groups in the top 10 cm of soil that are present in the intact soil. Loss of concentrated soil organic matter due to dilution of soil horizons during the stripping and piling procedure and exposure to harsh environmental conditions are likely factors in this lack of depth gradient in the stock-pile.

Time Since Disturbance Effects

It has been well documented that soil microbial communities may take long time periods to recover from severe disturbances. Wick et al. (2009) suggested that recovery of microbial communities in instances of extreme soil disturbance may require at least three years. Mummey et al. (2002b) found, in a study of re-spread and seeded (previously stored) soil, a decrease in all

FAME biomarkers, MBC and SOM after 20 years of recovery time. As depth in the stock-pile increases, microbial activity decreases; overall, increase in time and depth of stock-piled topsoil negatively impacts microflora and microfauna (Harris et al. 1989). These effects on microbial communities are a long-term issue (Blume et al. 2002; Gros et al. 2004).

It is reasonable to expect that microbial biomass and/or community composition in the stock-pile would have become similar to the intact soil in the event that the soil microbial community had recovered during the 24 month period of this study. The differences between the disturbed and undisturbed soil, however, were still apparent at the close of the study, especially in regard to the lack of depth gradient in the stock-piled soil. Although there were differences in size and composition of the microbial community between sampling times, these differences were indicative of seasonal and environmental variation rather than changes since disturbance. The fact that microbial characteristics of stock-piles differed from those of intact soils throughout the duration of this study (e.g., a depth gradient did not become reestablished in the stock-pile) does not mean that these stock-piles are devoid of value or sources of microbes in soil after replacement even after 24 months of storage.

Seasonal and Climatic Effects

Season and climate are well known to affect ecological communities and microbial communities are no exception. The response of microbial communities to climatic variation may depend upon the origin of the microbial community. Some ecosystems have communities which are more resilient to fluctuating environmental conditions (Waldrop and Firestone 2006). This may also be important in consideration of changes in microbial communities in soils which have been stripped and stock-piled. Areas which have historically faced harsh environmental

conditions or soil disturbances may be better able to withstand the disturbances of stripping and the harsh environmental conditions which are expected during storage.

Soils in the western Rio Grande Plains are cyclically exposed to extreme heat and low moisture conditions and the microbial communities sampled in this study varied seasonally. These differences suggest that microbial community response to stock-piling is related to both seasonal and climatic changes between sampling times.

Disturbance Effects on Individual Community Groups

A common expectation is that bacterial species are more competitive in high disturbance events. Bacteria are able to more efficiently benefit from new nutrients sources made available from the carcasses of microbes and other soil organisms which succumbed to the perturbation (Strickland and Rousk 2010). Ingham et al. (1986) found in a study of soil microbial communities in a semi-arid grassland in Colorado that bacterial numbers, in general, declined during hot and dry summer months and Spedding et al. (2004) proposed that fungi can be more tolerant of water stress than bacteria and an increase in fungi should be expected during drier summer months in Quebec.

The results of this study suggest different processes. Mean amounts of fungi were similar in the intact and stock-piled soil at all times except in February 2014 at the Hixon study site, when it was higher in the pile than the intact soil. At the San Ysidro study site, mean amounts of fungi were higher in the winter sampling months than the summer sampling months and were higher in the intact soil than the stock-pile. Our results suggest that excessively high temperatures in the summer, combined with extremely low moisture conditions are less conducive to fungi, actinomycetes and Gram negative bacteria native to this region than Gram positive bacteria. Bacteria, Gram positive *Bacilius* sp. especially, are well known to be resilient

in harsh environments and able to persist through sporulation. For example, a viable *Bacilius* sp. endospore was discovered in New Mexico in a 250 million-year-old salt crystal (Nicholson 2002). In a study of tillage practices effects on soil microbes in the Great Plains, Frey et al. (1999) refuted the idea that fungi are better able to thrive in low moisture conditions. Their results show that fungi populations increased as soil moisture increased. Soil microbial communities in the western Rio Grande Plains experience extreme variations in temperature and moisture availability and some species of Gram positive bacteria native to the area may be more resilient to those extremes during the summer months than other members of the community such as fungi and Gram negative bacteria.

Another expectation is that the intact soil would have a higher fungal to bacterial ratio than that of stock-piled soils (Strickland and Rousk 2010). Fungi growth forms make them especially vulnerable to tissue damage during soil disturbance events and fungi are expected to be most severely impacted in the case of extreme soil disturbance (e.g., Mummey et al. 2002). Also, the filamentous form in which fungi often grow is advantageous when soil nutrients are distributed unevenly throughout the soil. This advantage is nullified when soil management practices homogenize the soil, e.g., stripping and stock-piling (Strickland and Rousk 2010). However, other factors may be involved in the effects of disturbance on fungi to bacteria ratios. In a review of literature which discusses fungal:bacterial dominance, Strickland and Rousk (2010) suggest that the implications of fungal:bacterial dominance depends on both the methods used and relationships with the environment. Several studies have indicated that, in some regions and soil series, seasonal variation and moisture have a greater impact on microbial communities and fungi to bacteria ratios and that disturbances had little, if any effect the fungi to bacteria ratio (e.g., Acosta-Martínez et al. 2010a).

At the Hixon study site, the fungi to bacteria ratio was higher in the intact soil than the stock-pile during the August 2013 sampling time when it increased with increasing sampling depth. However, the reverse was true for winter sampling months when the stock-pile has a higher mean ratio of fungi to bacteria and in February 2015 decreased with increasing depth. Fungi to bacteria ratios were not affected by sampling time, depth or location at the San Ysidro study site. So, although fungi amounts increased during the winter months, increases were similar to the increases seen in the bacterial groups during those times. Also contrary to much of the literature, fungi to bacteria ratios were not affected by the extreme disturbance associated with stripping and stock-piling of soil. Bell et al. (2008) examined microbial community responses to seasonal variations in moisture and temperature in the Chihuahuan Desert. They reported that fungal carbon use was more closely related to temperature than moisture. Their findings suggest that fungi are less able to tolerate high temperatures than bacteria and only responded to substantial rain events during the summertime. This more in line with the results from the San Ysidro study site where fungi may have been able to take advantage of both precipitation events and cooler temperatures. Fungi to bacteria ratios at both study sites in both disturbed and undisturbed soils are low, indicating bacterial dominance with or without disturbance. This suggests that, in these soils, fungi are simply less prevalent and therefore not present in sufficient amounts to demonstrate an apparent loss of dominance after a disturbance event. If fungi are not dominant prior to stripping and stock-piling, they could hardly lose dominance afterwards.

Results from this study are in general agreement with results from other studies which factor environmental conditions. Spatial arrangement and community size and composition are impacted by stripping and storage of topsoil. These effects are, however, dependent on depth and

time of sampling. Depth gradients are especially affected and those natural depth gradients are not reestablished in a short time period. Recovery time for the microbial communities of these soils will take more than 24 months.

Management Implications—Soil Microbial Study

Many factors affect the condition of soil microbial communities. Stock-piling soil may compound the negative influence of some of those factors such as soil compaction and climatic conditions. Environmental conditions on the surface of the stock-pile may deter the development of a microbe rich upper layer in the soil after the construction of the stock-pile. Dilution of soil nutrients during stripping and stock-piling is also a concern in consideration of the spatial distribution of soil microbial communities. Covering the stock-pile with an organic cover or establishment of vegetation following pile construction would alleviate some of the issues associated with high temperatures and low moisture typical of bare soils. Furthermore, establishment of vegetation or an organic covering on the stock-pile would provide organic matter for the uppermost layer of soil. This would provide environmental conditions which are more conducive to the growth, development and recovery of microbial communities.

The results of this study show a change of spatial organization of the microbial biomass as a consequence of stripping and stock-piling topsoil. Also, microbial community composition was affected by seasonal changes on the stock-pile. This was especially evident at the San Ysidro study site where stock-piled soil microbial community composition was different from intact soil microbial community composition only during the summer sampling times when environmental conditions are most likely to be extreme. Therefore, chances for future restoration success likely would be improved if the stockpile (1) were amended with an organic covering during storage;

(2) amended with organic matter after replacement; and (3) tested for amounts soil chemicals e.g., nitrogen, phosphorus and amended appropriately after replacement.

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