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Influence of Drying–Rewetting Frequency on Soil Bacterial Community Structure

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ABSTRACT

Soil drying and rewetting represents a common physiological stress for the microbial communities residing in surface soils. A drying–rewetting cycle may induce lysis in a significant proportion of the microbial biomass and, for a number of reasons, may directly or indirectly influence microbial community composition. Few studies have explicitly examined the role of drying–rewetting frequency in shaping soil microbial community structure. In this experiment, we manipulated soil water stress in the laboratory by exposing two different soil types to 0, 1, 2, 4, 6, 9, or 15 drying–rewetting cycles over a 2-month period. The two soils used for the experiment were both collected from the Sedgwick Ranch Natural Reserve in Santa Ynez, CA, one from an annual grassland, the other from underneath an oak canopy. The average soil moisture content over the course of the incubation was the same for all samples, compensating for the number of drying–rewetting cycles. At the end of the 2-month incubation we extracted DNA from soil samples and characterized the soil bacterial communities using the terminal restriction fragment length polymorphism (T-RFLP) method. We found that drying–rewetting regimes can influence bacterial community composition in oak but not in grass soils. The two soils have inherently different bacterial communities; only the bacteria residing in the oak soil, which are less frequently exposed to moisture stress in their natural environment, were significantly affected by drying–rewetting cycles. The community indices of taxonomic diversity and richness were relatively insensitive to drying–rewetting frequency. We hypothesize that drying–rewetting induced shifts in bacterial community composition may partly explain the changes in C mineralization rates that are commonly observed following exposure to numerous drying–rewetting cycles. Microbial community composition may influence soil processes, particularly in soils exposed to a significant level of environmental stress.

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Introduction

In most ecosystems, surface soils undergo periods of prolonged drying interspersed with relatively rapid rewetting events. Soils of central California, and other semiarid Mediterranean-type ecosystems, are particularly susceptible to drying–rewetting stresses due to the infrequency of rainfall events and the often warm, dry climate that favors rapid soil drying. If we define a stress as any disturbance which causes a community to shift resources from growth and production of biomass to physiological maintenance [33], then the rewetting of a dry soil may represent a significant stress for the soil microbiota. The rapid increase in total soil water potential associated with a rewetting event will cause microbes to undergo osmotic shock, possibly inducing microbial cell lysis and a release of intracellular solutes [21, 25, 30]. It has been estimated that 30–60% of the microbial biomass C contained in soil may be released during an individual rewetting event [5, 25].

Numerous studies have focused on the short-term effects of drying–rewetting cycles on soil processes. Most of these studies have shown that the rapid rewetting of a dry soil yields a 1–4 day increase in C and N mineralization rates when compared with an unstressed control [2, 11, 39, 43]. This short-term spike in C and N mineralization rates probably results from the release of labile substrates during microbial cell lysis or osmoregulation [28, 30, 49].

Previously, we showed that drying–rewetting cycles can also have longer term effects on microbial processes. After numerous drying–rewetting cycles, soil C mineralization rates were significantly lowered compared to the unstressed controls [16]. We hypothesized that this decrease in respiration rates was accompanied by changes in microbial functioning brought about by drying–rewetting-induced shifts in microbial community structure [16]. This study was partially designed to test the validity of this proposed hypothesis.

Although the effects of drying–rewetting on microbial biomass and activity have been well studied, few studies have examined the effects of drying and rewetting on microbial community composition and structure. Physiological stress, such as that imposed by drying and rewetting, could reduce total soil microbial diversity by favoring a portion of the microbial community best adapted to coping with the given stress [1, 42]. Alternatively, microbial diversity may increase in response to drying and rewetting by enhancing the spatial and temporal heterogeneity of the soil environment, promoting species coexistence [12, 32, 45].

Frequent drying–rewetting cycles may alter the specific composition of microbial communities by selecting for microbes that can survive rapid changes in water potential. Actively growing microbes have been found to be more susceptible to drying–rewetting stress than slower growing microbes, probably due to differences in cell wall characteristics [5, 47, 48]. Rapid changes in soil water potential may also select for gram-positive bacteria and fungi which have thicker, more rigid cell walls and compatible solutes that enhance osmoregulatory capabilities [22, 24, 41, 42]. Alternatively, other studies have suggested that frequent drying and rewetting may select for fast-growing (copiotrophic) microbes that are capable of rapid growth on the labile substrates released into the soil during a rewetting event [13, 23, 28, 39].

The objective of this study was to determine how the frequency of drying–rewetting cycles affects bacterial communities and how this response is mediated by soil type. Since drying and rewetting is common in surface soils and probably represents a significant source of stress for microbial communities, we would expect the composition of soil microbial communities to be partially controlled by the frequency of exposure to drying and rewetting cycles. We manipulated the frequency of soil rewetting cycles in the laboratory so two soil types received 0, 1, 2, 4, 6, 9, or 15 drying–rewetting cycles over a 2-month period. Soil moisture content was adjusted so the average moisture content over the course of the incubation was the same for all treatments, with only the drying–rewetting stress frequency differing between treatments. After the 2-month period, we assessed the effects of stress frequency and soil type on bacterial community structure by examining terminal restriction fragment length polymorphisms (T-RFLP) of PCR-amplified 16S rDNA extracted from soil samples. The T-RFLP method was chosen because it has been proven to be a reproducible method with sufficient resolution to detect differences in soil bacterial community structure [9, 15, 27, 34].

Methods

Soils

The two soils used for this experiment were collected at the University of California Sedgwick Reserve, a 2364 ha reserve

located in Santa Ynez, California, USA (N 34° 42′ 30″, W 120° 2′ 30″). The climate is Mediterranean, with an annual rainfall of 50 cm yr^{-1} (Cachuma Reservoir, Santa Barbara County Water District) with most of the rain occurring between December and March. The soils of the field site are haploxerolls [17]. Surface soils (0–10 cm) were collected from beneath isolated oak trees (*Quercus agrifolia*) and from an adjacent annual grassland (primarily *Bromus* spp.). These soils will be referred to as "oak" and "grass" soil, respectively. The two soils are found in close proximity to one another, yet are distinct with respect to composition and local microclimate. The oak soil has a higher total C and N content (3.9% and 0.3%, respectively) than the grass soils (2% and 0.2%, respectively) and a larger microbial biomass (N. Fierer, unpublished data, [16]). The oak soil is a loam with a pH in water of 6, the grass soil is a clay loam with a pH of 6.5. More details on the two soils can be found in Fierer and Schimel [16] and Parker and Muller [36].

Experimental Design

The T-RFLP analyses were conducted on the same soil samples used in the experiment described in Fierer and Schimel [16]. The soils were sieved to 4 mm, homogenized, and conditioned for 1 week at 35% of water holding capacity (WHC). Treatments were done in triplicate with 10 to 15 g of soil incubated at 20°C in sealed glass 2 L Mason jars with weights measured periodically to monitor soil moisture content. For the experiment, soils were incubated for 2 months and exposed to six different drying–rewetting stress regimes: 1, 2, 4, 6, 9, or 15 drying–rewetting cycles during the course of the experiment. The control treatment consisted of soils kept at a constant moisture content, 35% of water holding capacity, which corresponds to a water potential of approximately −60 kPa in both soils, as measured on a thermocouple psychrometer (Decagon Devices, Inc. Model SC-10a). The drying–rewetting cycles were evenly spaced throughout the 2-month incubation with all of the treatments receiving the final (and only, in the case of the soils that received only one) drying–rewetting treatment at the end of the incubation. Drying–rewetting cycles consisted of a 2-day drying period followed by a rewetting. Soil drying was accomplished by removing the jar lids and incubating in a 20°C room with substantial air flow. By the end of the drying period, the soils always dried down to approximately 5% gravimetric water content (approximately −15 MPa for both soils). Soils were rewet by adding a single aliquot of a predetermined amount of sterile deionized water to the middle of the soil sample. The water content after rewetting was adjusted to an average water content of 35% of water holding capacity during the 2-month incubation. To maintain all the treatments at the same average water content, the frequently dried and rewet soils were adjusted to a higher post-rewetting water content. For example, the oak soils that received six drying–rewetting cycles during the course of the incubation were adjusted to 42% gravimetric water content while the oak soils that received 15 drying–rewetting cycles were adjusted to 49% (approximately −40 kPa).

At the end of the 2-month incubation all samples were maintained at a constant moisture content (35% WHC). Triplicate samples of each soil from each treatment were collected for T-RFLP analyses at 1 day, 7 days, and 5 weeks following the 2-month incubation. The sampling times were chosen to assess the shorter and longer term impacts of the stress regimes on microbial community composition.

T-RFLP Analyses

Soil DNA was extracted from soil samples using the MoBio UltraClean DNA extraction kit (Solana Beach, CA) as recommended by the manufacturer. The amounts of extracted DNA were qualitatively assessed on a 0.8% agarose gel run at 70 V for 40 min and stained with ethidium bromide; any extractions that yielded insufficient DNA were repeated. Extracted DNA yields were quantified by PicoGreen fluorometry as per manufacturer's instructions (Molecular Probes, Eugene, OR).

16S rDNA from the extracted DNA samples was PCR-amplified using the universal eubacterial primers 8 F hex, a fluorescently labeled forward primer (5′ AGAGTTTGATCCTGGCTCAG, [26]) and 1389R (5′ACGGGCGGTGTGTACAAG; [34]). Primers were obtained from Operon Technologies (Alameda, CA). Each 100-μL PCR reaction contained 50 ng template DNA, 3 units *Taq* polymerase (Qiagen, Chatsworth, CA), 0.2 mM each dNTP, 50 pmol DNA primers, 1× PCR buffer (Qiagen), and 2.5 mM $MgCl_2$. Reaction mixtures were held at 94°C for 6 min and then held at 60°C while *Taq* and dNTPs were added. Reactions were cycled 28 times through three steps: 45 s of denaturation at 94°C, 45 s of annealing at 58°C, and 90 s of primer extension at 72°C. The final primer extension step was 7 min. Two separate PCR reactions were performed with each DNA sample; the PCR products were then combined and purified using the UltraClean PCR Clean-Up Kit (MoBio).

PCR products were digested with either *Rsa*I or *Msp*I restriction enzymes (New England Biolabs, Beverly MA). Approximately 500 ng of purified PCR product was digested in a 20 μL reaction volume with six units of restriction enzymes for 10 h at 37°C. Restriction enzymes were inactivated by heating (65°C, 10 min). The length and area of fluorescently labeled fragments was determined using an Applied Biosystems Instruments (Foster City, CA) model 373A automated sequencer [26]. Terminal restriction fragment sizes between 100 and 850 bp with peak heights >30 fluorescent units were measured using Genescan analytical software (Applied Biosystems Instruments).

Analysis of T-RFLP Profiles

The analysis of terminal restriction fragment (TRF) patterns was conducted in a manner similar to that described by Dunbar et al. [14]. First, the total TRF peak area per sample was calculated; any sample with a very low peak area, probably the result of a poor or incomplete restriction digest, was discarded. The peak area for each DNA fragment was recalculated as a proportional peak area (the quotient of the individual peak area/total peak area). This

normalization process is necessary because of the inherent variability in total DNA quantity between samples on the sequencing gel. Any peaks with peak areas less than 0.5% of the total or with peak heights less than 75 fluorescence units were not used in analyses.

Alignment of TRF profiles was necessary because the Genescan software calculates DNA fragment sizes to 1/100 of a base pair but the error associated with sequencing can be up to 0.5 bp [14]. Therefore, TRF profiles had to be manually aligned to prevent incorrectly identifying one TRF peak as two separate peaks. TRF peaks that differed by less than 0.5 bp in different profiles, but represented the same peak, were considered identical and clustered together. Any error introduced by this manual clustering of the peaks is likely to emphasize the peaks held in common by samples and deemphasize any unique peaks that would be found in only a few samples.

For comparisons between TRF profiles, we used both the proportional TRF area and the base pair length. Since TRFs of similar size can be produced from different organisms [26], TRFs of different lengths were assumed to represent distinct operational taxonomic units (OTUs), not necessarily unique bacterial species. The proportional TRF area was then used as an indicator of the proportional "abundance" of a particular OTU in a given sample. Although absolute TRF area can be subject to error associated with preferential primer annealing during the PCR amplification of extracted 16S rDNA [37, 44], proportional TRF areas do provide a reproducible measure of relative OTU abundances in a given sample [9, 14, 34].

Statistical Analyses

The effects of drying–rewetting regimes on bacterial communities were assessed using data on both TRF fragment length and proportional area to calculate community structure indices. OTU richness (as per [31]) is defined as the number of individual TRF fragments identified in a soil sample using the T-RFLP profile data analyzed using the methods described above. OTU diversity incorporates both OTU richness and evenness ("equitability") into a single parameter. OTU diversity was calculated using the Shannon index [31] using the number of distinct TRFs in a sample and the peak height associated with each TRF. Principal-component analysis (PCA) was conducted using Systat [46] to reduce the *n*-dimensional data of TRF lengths and heights obtained from each sample into a series of linear axes that explain the maximum amount of variance in the data. The relative position of each sample along the principal-component axes can then be used to describe the degree of community-level similarity between samples. PCA was first conducted with the entire dataset to ascertain the degree of similarity between the two soil types. Separate principal-component analyses were also conducted with each soil type independently to examine the effects of stress treatment on community differentiation.

The data from the two separate restriction digests, *Rsa*I and *Msp*I, were combined for all statistical analyses [14]. Multiple factor ANOVAs were conducted using Systat [46] to look for the effects of postincubation time or water stress frequency (treatment) on community richness and diversity.

In both soil types, there were no significant effects of postincubation sampling time on diversity and richness measurements; nor were there any significant interactions between sampling time and stress treatments ($P > 0.3$ in all cases, data not shown). Similarly, by PCA, we were unable to identify any substantial degree of community-level differentiation based on sampling time (1 day, 7 days, or 5 weeks after the last stress) with either of the soil types. The small number of replicates for each stress treatment/sampling time combination ($n = 3$) made it difficult to ascertain any influence of sampling time (after the last stress) on community structure. Therefore, for Fig. 1–4, we combined the data from each of the three sampling times, simplifying the graphical representation of the data and giving us a high enough sample number ($n = 9$ for each experimental treatment) to adequately examine the effects of stress frequency on community structure.

Results and Discussion

Differences between the Two Soils

While the primary objective of this experiment was to determine the effects of stress frequency on bacterial community structure, we also wanted to assess the degree of differentiation between the communities of the two soil types and how these differences may have influenced the stress responses. Among all the samples (both oak and grass soils), a total of 42 and 85 peaks were identified with the *Rsa*I and *Msp*I restriction enzymes, respectively. The total number of identified peaks, the OTU richness, was approximately the same for the two soil types (Fig. 1). The grass soil and oak soils were also very similar with respect to the overall OTU diversity (Fig. 2).

Principal-component analysis demonstrated that the bacterial communities inhabiting the two soil types, oak and grass, were distinct from one another (Fig. 3). When scores for the first two principal component axes were plotted, the samples clustered together by soil type with no overlap. Similar results were obtained with factor analysis of the data in binary form, ignoring OTU abundances and looking only at OTU presence or absence (results not shown). So, while the simple community-level parameters of richness and diversity show limited differences between the two soils, the T-RFLP technique had sufficient resolution to separate the bacterial communities of the two soils on the basis of OTU abundances.

Although the grass and the oak soils are found in close proximity to one another, they have very distinct bacterial

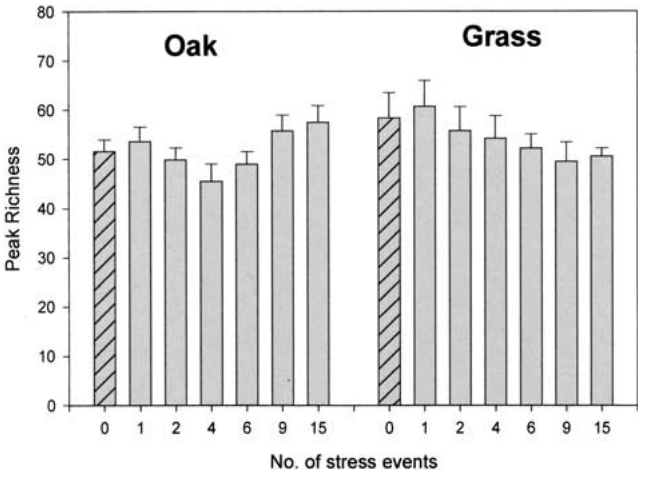

Fig. 1. Bacterial community richness, the number of identifiable OTUs, in oak and grass soils exposed to different numbers of drying–rewetting cycles. Hatched bars indicate soils not exposed to drying and rewetting. Error bars indicate 1 SE. n = 8 or 9 for each bar.

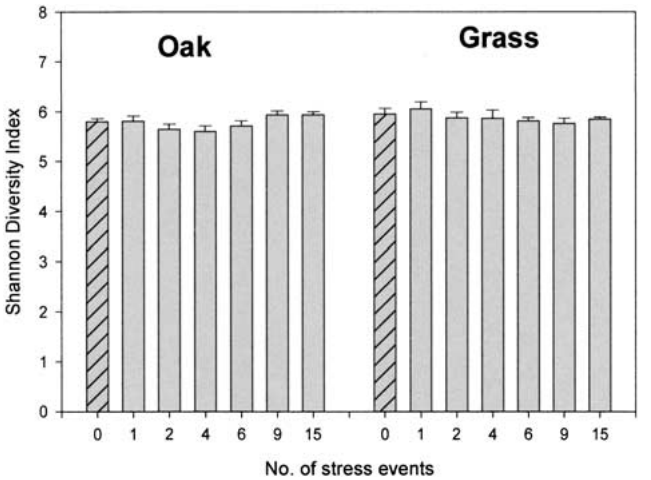

Fig. 2. Bacterial community diversity, as measured with the Shannon index, in oak and grass soils exposed to different numbers of drying–rewetting cycles. Hatched bars indicate soils not exposed to drying and rewetting. Error bars indicate 1 SE. n = 8 or 9 for each bar.

communities. There are a number of possible explanations why the two soils may harbor distinct bacterial communities. Differences in soil abiotic conditions, such as temperature [54] and soil moisture [3, 42, 52], have been shown to influence microbial community structure. The oak soils are, on average, cooler and more moist than the nearby grass soils because of canopy shading and a litter layer covering the mineral soil [36]. The grass soils do not have appreciable canopy shading during the summer and

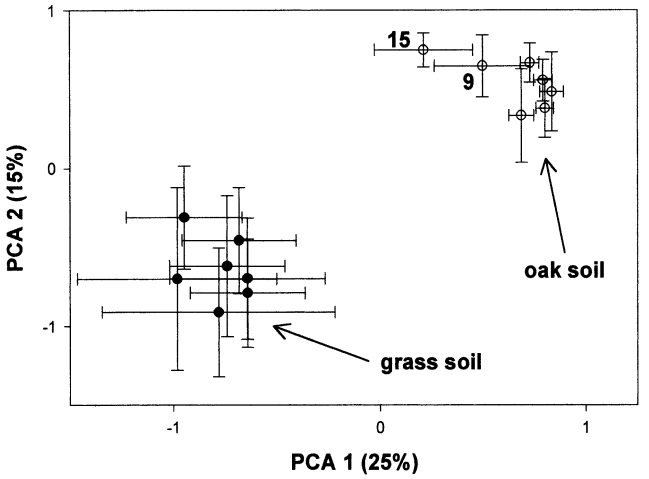

Fig. 3. Results from principal-component analyses combining data from both soils showing the degrees of similarity in the bacterial communities of the oak and grass soils. Error bars indicate ± 1 SE. Each symbol represents a different experimental treatment, n = 8 or 9. PCA 1 and PCA 2 represent 25% and 15% of the variance in the data, respectively. The oak soils exposed to frequent drying–rewetting cycles are indicated on the graph.

there is only a thin litter layer (0–2 cm thick). Microbial community structure could also be a function of organic matter availability, organic matter quality, and soil nutrient status [4, 19, 35]. The oak soil has significantly more organic C and N, higher rates of C and N mineralization, and higher nitrification rates than the grass soil [16]. The distinct above ground plant communities associated with the two soil types could also directly contribute to the differences in microbial community composition [6, 18, 45].

Effects of Stress Frequency

With the oak soil, there was a small, but statistically significant, effect of stress frequency on OTU richness (P = 0.02, Fig. 1). The soils that were exposed to frequent drying–rewetting cycles had slightly higher OTU richness than soils exposed to fewer drying–rewetting cycles. However, none of the individual treatments was statistically different from the unstressed control ($P > 0.3$, in all cases). With the grass soils, there was no significant relationship between the number of stress cycles and OTU richness (P = 0.42), but there was a trend of richness decreasing with an increase in the number of drying–rewetting cycles.

Diversity was not significantly affected by the drying–rewetting regimes in either soil types (P = 0.08 and 0.2 for oak and grass soils, respectively; Fig. 2). Oak soil exposed

to frequent drying–rewetting cycles had slightly higher levels of diversity compared to the unstressed control treatment, but these differences were small and not individually significant. These results contrast with those of McLean and Huhta [32], who found that fungal diversity increases under fluctuating moisture regimes. Similarly, Degens et al. [12] found that catabolic evenness (an index of functional diversity) increased after exposure to four successive drying–rewetting cycles, but decreased after exposure to eight drying–rewetting cycles in a 6-week period. Differences in the imposed moisture regimes, the levels of pre-adaptation of the soil communities to moisture stress, or the component of "microbial diversity" actually measured with any particular method may explain why we did not observe similarly large changes in bacterial diversity.

While the effects of drying–rewetting cycle number on OTU diversity and richness were limited in magnitude, the PCA data show drying–rewetting induced divergence in the bacterial communities of oak soil, but not grass soil (Fig. 4). Oak soils exposed to few drying–rewetting cycles have bacterial communities that cluster with the unstressed control along both principal-component axes (Fig. 4). The more drying–rewetting cycles to which the oak soil was exposed, the more the bacterial communities differed from the unstressed control. By PCA, the grass soil shows a high degree of community-level variability between samples and no apparent separation of bacterial communities on the basis of stress frequency (Fig. 4). Similar results were obtained for both soils with the data reduced to binary form, i.e., peak presence or absence (results not shown).

The effects of drying–rewetting stress frequency on oak soil bacterial communities were smaller than the differences between the two soil types (Fig. 3). These results are consistent with other studies which show that moisture regime can influence the structure of microbial communities, but the differences between litter or soil types are often greater in magnitude than any moisture effects [29, 32, 52].

In order to maintain all soils at the same average water content over the 2-month incubation, the frequently stressed soils were adjusted to higher water contents upon rewetting than the soils that received fewer stresses. One possible explanation for our results is that the bacterial communities inhabiting oak soil did not change in response to the frequency of drying–rewetting cycles, but rather to the higher water contents to which the frequently stressed soils were adjusted between dry-downs. The difference in water potential between the oak soil control (−60 kPa) and the "15 stress" treatment between dry-down (−40 kPa) is relatively small, so we do not believe that this is the most parsimonious explanation for the results. Furthermore, a previous study [16] has shown that at the absolute highest water contents used in this experiment (50% WHC), the oak soils did not show signs of anaerobiosis and the rates of most soil processes did not differ appreciably from those of the unstressed control (35% WHC).

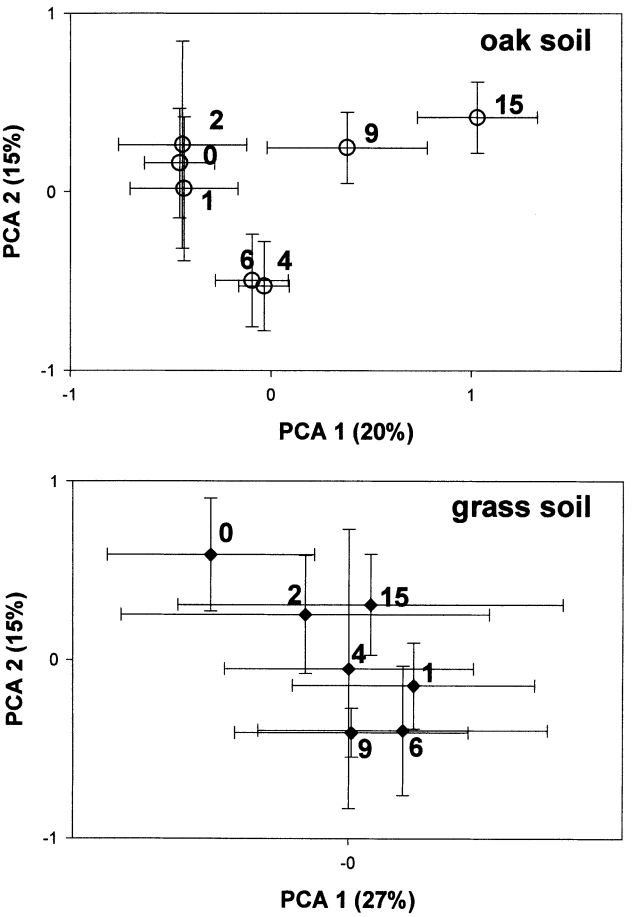

Fig. 4. Results from the principal components analyses of data from the oak and grass soils showing the influence of stress frequency on community differentiation. These principal-component analyses are distinct from those shown in Fig. 3 in that data from the two soil types were analyzed separately. Error bars indicate ± 1 SE. n = 8 or 9 for each symbol. For the oak soil, PCA 1 and PCA 2 represent 20% and 15% of the variance in the data, respectively. For the grass soil, PCA 1 and PCA 2 represent 27% and 11% of the variance in the data, respectively.

The oak soil bacterial communities changed in response to the stress regime while the grass soil communities were largely unaffected. One possible explanation

for this observation is that the grass soil bacterial communities are, in their natural state, already selected for by frequent drying–rewetting cycles, excluding the stress-intolerant microbes that are not excluded from the oak soils. The oak soils are covered by a thick litter layer and have perennial canopy shading so the natural variability in soil moisture is lower than in the more exposed grass soils [36]. Microbial processes (respiration and nitrification rates) in oak soils were also more strongly affected by moisture stress frequency than the processes in grass soils [16]. Other studies have reported that exposure to drying–rewetting cycles is often more stressful for microbial communities not preadapted to a high degree of variability in field soil moistures [25, 29, 48, 51].

Methodological Considerations

In this study, the indices of community structure, diversity and richness, did not differ appreciably between the two soil types or in response to stress treatments. Either bacterial diversity and richness are relatively unaffected by drying–rewetting stresses, or the T-RFLP method has limited utility in the calculation of these parameters. Dunbar et al. [15] suggest that the T-RFLP method, while effective at elucidating similarities between communities, is of limited utility in describing relative OTU richness and diversity. They suggest that the low degree of phylogenetic resolution and inherent variation between restriction enzymes obscures differences in OTU richness and diversity between samples. In addition, diversity estimates with the T-RFLP method may be subject to bias as a result of the preferential amplification of rare OTUs after a high number of PCR cycles [44].

The T-RFLP method coupled with PCA proved to be useful in determining the degrees of similarity between bacterial communities. The method had sufficient resolution to detect differences in bacterial communities between soil types and oak soil stress regimes. We may have been able to enhance the resolution of the T-RFLP technique by employing additional restriction enzymes since a greater number of OTUs may have increased our ability to detect differences between soils or stress treatments [15]. However, it should be noted that the T-RFLP method offers a quantitative view of the PCR product pool obtained from a community, not necessarily the native soil community itself. There is possible PCR bias during amplification [38, 44] and considerable variability in rRNA operon copy numbers within bacterial genomes [10, 53].

Microbial Community and Soil Function

In an earlier paper, we reported that soil process rates are affected by the frequency of drying–rewetting [16]. For up to 6 weeks after exposure to the final drying–rewetting cycle, the frequently stressed soils had substantially lower respiration rates than the unstressed controls. Furthermore, the efficiencies of carbon substrate use were altered by frequent exposure of soil to drying and rewetting. The microbial processes in oak soil were always more strongly affected by drying–rewetting frequency than the processes in grass soil.

A number of studies have suggested a causal link between microbial community structure and soil process rates [7, 20, 40, 50]. The stress-induced changes in C mineralization rates described above may be a direct result of alterations to bacterial community structure following exposure to drying and rewetting. If this explanation is valid, we would predict (correctly) that C cycling in oak soil should be more affected by drying–rewetting frequency than C cycling in grass soil since the oak soil bacterial community is more sensitive to drying–rewetting stress frequency. In a similar study, Schimel et al. [42] also found that exposure to multiple drying–rewetting cycles can change soil microbial communities, lowering functional diversity and decreasing decomposition rates. The decrease in C mineralization rates following drying and rewetting observed by Clein and Schimel [8] and Magid et al. [30] could possibly be explained by drying–rewetting-induced changes in microbial community composition.

Conclusions

Gross measures of community structure, bacterial diversity and richness, were largely unaffected by soil type or numbers of drying-rewetting cycles. Only with PCA could we ascertain the relative similarities and differences between bacterial communities. The oak and grass soils have distinct bacterial communities and these differences in community structure are greater in magnitude than any differences induced by drying–rewetting stress frequency. While the oak soil communities changed in response to frequent drying and rewetting events, the bacterial communities inhabiting the grass soil were largely unaffected. The different responses of the two soils may be a result of prior adaptations of the bacterial communities to the moisture conditions found in the field. Drying–rewetting-induced changes to oak community structure may affect microbial functioning, providing a possible mechanism

for the changes in soil processes observed following exposure to numerous drying–rewetting cycles.

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References

1. Atlas R (1984) Use of microbial diversity measurements to assess environmental stress. In: M Klug, C Reddy (eds) Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington, DC pp 540–545
2. Birch H (1958) The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10:9–31
3. Bossio DA, Scow KM (1998) Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278
4. Bossio DA, Scow KM, Gunapala N, Graham KJ (1998) Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb Ecol 36:1–12
5. Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14-C and 15-N labelled plant material. Soil Biol Biochem 17:329–337
6. Broughton LC, Gross KL (2000) Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia 125:420–427
7. Cavigelli MA, Robertson GP (2000) The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology 81:1402–1414
8. Clein J, Schimel J (1994) Reduction in microbial activity in birch litter due to drying and rewetting events. Soil Biol Biochem 26:403–406
9. Clement B, Kahl L, DeBord K, Kitts C (1998) Terminal restriction fragment patterns (TRFPs), a rapid, PCR-based method for the comparison of complex bacterial communities. J Microbiol Methods 31:135–142
10. Cole ST, Saint Girons I (1994) Bacterial genomics. FEMS Microbiol Rev 14:139–160
11. Cui M, Caldwell M (1997) A large ephemeral release of nitrogen upon wetting of dry soil and corresponding root responses in the field. Plant Soil 191:291–299
12. Degens BP, Schipper LA, Sparling GP, Duncan LC (2001) Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol Biochem 33:1143–1153
13. Denef K, Six J, Bossuyt H, Frey SD, Elliott ET, Merckx R, Paustian K (2001) Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol Biochem 33:1599–1611
14. Dunbar J, Ticknor L, Kuske C (2001) Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Appl Environ Microbiol 67:190–197
15. Dunbar J, Ticknor LO, Kuske CR (2000) Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Appl Environ Microbiol 66:2943–2950
16. Fierer N, Schimel J (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34:777–787
17. Gessler PE, Chadwick OA, Chamran F, Althouse L, Holmes K (2000) Modeling soil–landscape and ecosystem properties using terrain attributes. Soil Sci Soc Am J 64:2046–2056
18. Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378
19. Griffiths B, Ritz K, Ebblewhite N, Dobson G (1999) Soil microbial community structure: effects of substrate loading rates. Soil Biol Biochem 31:145–153
20. Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F, Sorensen SJ, Baath E, Bloem J, de Ruiter PC, Dolfing J, Nicolardot B (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: An examination of the biodiversity–ecosystem function relationship. Oikos 90:279–294
21. Halverson LJ, Jones TM, Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci Soc Am J 64:1630–1637
22. Harris R (1981) Effect of water potential on microbial growth and activity. In: J Parr, W Gardner (eds) Water Potential Relations in Soil Microbiology. Soil Science Society of America, Madison, WI pp 23–95
23. Jager G, Bruins E (1975) Effect of repeated drying at different temperatures on soil organic matter decomposition and characteristics, and on the soil microflora. Soil Biol Biochem 7:153–159
24. Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high osmolality environments. Arch Microbiol 170:319–330
25. Kieft T, Soroker E, Firestone M (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol Biochem 19:119–126
26. Liu W, Marsh T, Cheng H, Forney L (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16s rRNA. Appl Environ Microbiol 63:4516–4522

27. Lukow T, Dunfield P, Liesack W (2000) Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. FEMS Microbiol Ecol 32:241–247
28. Lund V, Goksoyr J (1980) Effects of water fluctuations on microbial mass and activity in soils. Microb Ecol 6:115–123
29. Lundquist E, Scow K, Jackson L, Uesugi S, Johnson C (1999) Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biol Biochem 31:1661–1675
30. Magid J, Kjaergaard C, Gorissen A, Kuikman P (1999) Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added 14-C labelled plant material. Soil Biol Biochem 31:595–602
31. Magurran A (1988) Ecological diversity and its measurement. Princeton University Press, Princeton, NJ
32. McLean MA, Huhta V (2000) Temporal and spatial fluctuations in moisture affect humus microfungal community structure in microcosms. Biol Fert Soils 32:114–119
33. Odum EP (1985) Trends expected in stressed ecosystems. Bioscience 35:419–422
34. Osborn A, Moore E, Timmis K (2000) An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ Microbiol 2:39–50
35. Ovreas L, Torsvik V (1998) Microbial diversity and community structure in two different agricultural soil communities. Microb Ecol 36:303–315
36. Parker V, Muller C (1982) Vegetational and environmental changes beneath isolated live oak trees (*Quercus agrifolia*) in a California annual grassland. Am Mid Nat 107:69–81
37. Polz M, Cavanaugh C (1998) Bias in template-to-product ratios in multitemplate PCR. Appl Environ Microbiol 64:3724–3730
38. Reysenbach AL, Giver LJ, Wickham GS, Pace NR (1992) Differential amplification of rRNA genes by polymerase chain reaction. Appl Environ Microbiol 58:3417–3418
39. Scheu S, Parkinson D (1994) Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. Soil Biol Biochem 26:1515–1525
40. Schimel J, Gulledge J (1998) Microbial community structure and global trace gases. Global Change Biol 4:745–758
41. Schimel J, Scott W, Killham K (1989) Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Appl Environ Microbiol 55:1635–1637
42. Schimel JP, Gulledge JM, Clein-Curley JS, Lindstrom JE, Braddock JF (1999) Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biol Biochem 31:831–838
43. Sorensen L (1974) Rate of decomposition of organic matter in soil as influenced by repeated air drying–rewetting and repeated additions of organic material. Soil Biol Biochem 6:287–292
44. Suzuki M, Giovannoni S (1996) Bias caused by template annealing in the amplification of mixtures of 16s rRNA genes by PCR. Appl Environ Microbiol 62:625–630
45. Swift M (1976) Species diversity and the structure of microbial communities in terrestrial habitats. In: M Anderson, A Macfadyen (eds) The Role of Terrestrial and Aquatic Organisms in Decomposition Processes. Halsted Press, New York pp 185–222
46. Systat (2000) Systat for Windows SPSS Inc, Evanston, IL
47. VanGestel M, Merckx R, Vlassak K (1993) Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56:617–626
48. VanGestel M, Merckx R, Vlassak K (1993) Microbial biomass responses to soil drying and rewetting: The fete of fast- and slow growing microorganisms in soils from different climates. Soil Biol Biochem 25:109–123
49. VanVeen J, Ladd J, Amato M (1985) Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with 14-C glucose and 15-N ammonium sulfate under different moisture regimes. Soil Biol Biochem 17:747–756
50. Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32:1837–1846
51. West A, Sparling G, Speir T, Wood J (1988) Dynamics of microbial C, N-flush and ATP, and enzyme activities of gradually dried soils rom a climosequence. Aust J Soil Res 26:519–530
52. Wilkinson S, Anderson J, Scardelis S, Tisiafouli M, Taylor A, Wolters V (2002) PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biol Biochem 34:189–200
53. Wintzingerode FV, Goebel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: Pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229
54. Zogg G, Zak D, Ringelberg D, MacDonald N, Pregitzer K, White D (1997) Compositional and functional shifts in microbial communities due to soil warming. Soil Sci Soc Am J 61:475–481