MICROBIAL COMMUNITIES AND C CYCLING UNDER DEFICIT-IRRIGATED MAIZE

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ABSTRACT

Growing urban population, declining groundwater levels, and drought are factors leading to reduced water quantities for irrigated land in the semiarid western U.S. Developing sustainable limited irrigation systems necessitates an understanding of how reduced water availability affects soil microbial processes and ecological interactions critical to crop productivity and soil conservation. The objective of this study was to determine the effects of deficit-irrigated maize cropping systems on rhizosphere microbial communities and activities. Maize rhizosphere soil samples were collected from furrow or sprinkler irrigated field plots in 2006, shortly after tasseling. Samples were collected from four replicate plots of the following cropping systems × furrow or sprinkler irrigated treatment: maize-alfalfa rotation, 740 mm anticipated annual consumptive maize water use (ET) (fully irrigated); maize-alfalfa rotation, 410 mm ET; and maize-sunflower-winter wheat rotation, 310 mm ET. Soils were analyzed for microbial biomass carbon (MBC), community fatty acid methyl ester (EL-FAME) structure, and C mineralization and β-glucosidase activities. MBC and C mineralization activity were not significantly affected by type or amount of irrigation. β-glucosidase activity was significantly lower in sprinkler-irrigated plots than in furrow-irrigated plots and lower in rhizosphere soil from the deficit-irrigated maize-sunflower-wheat rotation compared to other treatments. Principle components analysis of EL-FAMEs separated rhizosphere communities according to deficit irrigation-rotation systems, and deficit irrigation significantly reduced relative amounts of fungal EL-FAME but increased relative amounts of Gram-positive bacterial EL-FAMEs. Bacterial stress ratio 17:0cy:16:1ω7c was not affected by deficit irrigation but was greater in furrow than in sprinkler-irrigated soil. This study indicates that fungi and β-glucosidase activity responded negatively to deficit irrigation, perhaps due to reduced biomass inputs of maize residue in these systems, and that furrow irrigation, which floods soils, posed a greater stress to soil bacteria than did decreased water availability.

INTRODUCTION

There is increasing competition for a limited water supply throughout much of the western U.S. Increasing competitions from urban and municipal water users, declining groundwater levels, and drought are factors that are leading to reduced irrigation water quantities for large areas of agricultural land. As an example, Colorado's population is expected to grow about sixty-five percent in the next twenty-five years (Colorado Water Conservation Board 2004), causing water use to shift from agriculture to municipal and industrial uses. Indeed, it is expected that 428,000 acres of irrigated farmland will dry up to meet future needs (Colorado Water Conservation Board 2004), and these estimates may be quite conservative. Changes in water allocation will have important implications for the economic and environmental

sustainability of these agroecosystems. In some cases, irrigated production systems will be transitioned to dryland farming methods. However, the low productivity of dryland maize production in the semi-arid west will not be able to support the regions cattle feeding industry and the growing demands of the ethanol industry. As an alternative to drying up irrigated acres, many growers are interested in adopting cropping practices based on the concept of limited irrigation. Limited irrigation practices are a partial dewatering of irrigated lands and seek to maintain a profitable level of productivity while conserving water resources for other uses. This presents a challenge, for reduced water availability will affect all aspects of management, including cropping rotations, tillage, and modifying irrigation schedules to maximize water availability at critical stages of crop growth. All of these factors are likely to affect the soil environment and subsequently soil microbial communities and their processes.

Soil moisture content directly affects the physiological status of soil microorganisms (Harris 1981, Griffiths et al. 2003). Water availability directly affects microorganisms by controlling the osmotic status of cells and indirectly by regulating substrate availability, diffusion of gases, soil pH and temperature (Griffiths et al. 2003). Also, plants stressed by low water potentials may alter rhizodeposition or belowground nutrient allocations, which may alter rhizosphere communities (Lynch and Whipps 1990). Few studies, however, have examined the effects of reduced water availability and low water potentials on microbial community diversity. In a 2-month long laboratory study, Griffiths et al. (2003) examined the effects of soil drying and soil drying + rewetting on culturable and total (16S rRNA genes and transcripts) microbial community diversity. They found that although there were physiological and functional responses of microbial communities to soil drying, genetic diversity was unaffected by water stress. In another laboratory study, Uhlirova et al. (2005) detected significant changes to microbial community structure in response to extreme soil water contents when communities were analyzed according to their phospholipid fatty acid (PLFA) profiles. Specifically, communities of dry soils were dominated by Gram-positive bacteria and actinomycetes, whereas greater soil water contents favored Gram-negative bacteria. Others have correlated microbial community structure and diversity with soil moisture in various field studies, and when no correlation was found, concluded that microbial communities are resilient to water stress (Cookson et al. 2006) and wide ranges of soil moisture conditions, except for sudden flooding events (Hamel et al. 2006). However, field-level responses of soil microbial communities to limited irrigation practices (which includes extreme soil drying and wetting) remains uninvestigated. Thus, the objective of this study was to gather preliminary information on the response of soil microbial communities to deficit-irrigated maize agroecosystems.

MATERIALS AND METHODS

Study Site

A pilot research project was initiated in 2005 on two locations (sprinkler-irrigated and furrow-irrigated) at the Colorado State University Agricultural Research, Demonstration, and Education Center in Fort Collins, CO. The project evaluates crop productivity and crop water use for agroecosystems with a range in applied irrigation water. In addition to variable water inputs, the experimental agroecosystems (Table 1) also vary in crop selection, cultural practices, and irrigation methods with the goal of using available knowledge to maximize water use efficiency while reducing net water consumption at the systems level.

Table 1. Experimental agroecosystems and associated crop selection, cultural practices, and anticipated average annual consumptive water use (ET) at CSU-ARDEC in Fort Collins, Colorado.

Cropping System	Cropping Rotation	Tillage Practice (sprinkler/furrow)	Irrigation Scheduling	Average Annual ET (mm)
Dryland	Winter wheat-summer fallow	No-till/no-till	No irrigation	100
Limited irrigation, grain	Winter wheat-maize-sunflower	No-till/no-till	Growth stage timed irrigation	320
Limited irrigation, forage	Maize-alfalfa	No-till/no-till	Growth stage timed irrigation	410
Full Irrigation	Maize-alfalfa	Plow/plow	Full ET	740

Soil Sampling and Analyses

In August 2006, shortly after tasseling, rooting systems from three maize plants were excavated with a shovel from each maize-planted plot of the following treatments: fully irrigated maize (maize-alfalfa rotation, furrow and sprinkler irrigated), limited irrigation maize (maize-alfalfa forage rotation, furrow and sprinkler irrigated), and limited irrigated maize (winter wheat-maize-sunflower grain rotation, furrow and sprinkler irrigated).

In the laboratory, rhizosphere soil was collected, passed through a 4-mm mesh sieve, and analyzed for microbial biomass carbon (MBC) by the chloroform fumigation incubation method of Jenkinson and Powlson 1976, using a K_c factor of 0.41. Microbial C mineralization activity was determined from the amount of CO_2-C evolved from non-fumigated soil during a 10 day incubation period (25 °C). β-glucosidase activity was determined following the colorimetric assay described by Tabatabai (1994). Microbial community structure was characterized by ester-linked fatty acid methyl ester (EL-FAME) analysis as described by Jiménez Esquilín et al. (2007). Data were analyzed as a split plot-block design in SAS (version 9.1, SAS Institute, Cary, NC) with irrigation method as the main effect, and amount of irrigation as the split effect. When significant effects were detected ($\alpha = 0.05$), means were separated by the least significant difference (LSD) method. Microbial community EL-FAME data were analyzed by Principal Components Analysis (PCA) after normalizing the data as relative mol%, followed by arcsine-square root transformation, using the PC-ORD statistical package (MjM Software, Gleneden Beach, OR, 1999).

RESULTS AND DISCUSSION

Microbial biomass C tended to decrease as the amount of irrigation water decreased, but results were not statistically significant (Fig. 1). Carbon mineralization activity was unaffected by irrigation type or amount, and averaged 14.3 mg C kg^{-1} soil d^{-1} (± 1.03 standard error) across all 24 samples (data not shown). Activity of β-glucosidase, an enzyme involved in cellulose decomposition, was responsive to differences in irrigation method (lower in sprinkler-irrigated plots than in furrow-irrigated plots) as well as to the amount of irrigation water applied during the growing season (lower in limited-irrigated maize than in fully-irrigated maize) (Fig. 1). Patterns observed for β-glucosidase may reflect reduced C inputs to soil because of reduced crop biomass in limited-irrigated maize. In the limited irrigated systems studied here, researchers

measured reduced biomass production in response to irrigation amounts in 2005. For example, maize biomass production averaged 58, 47, and 40 Mg ha^{-1} yr^{-1} for the full irrigation, limited irrigation-forage, and limited irrigation-grain systems, respectively (unpublished data).

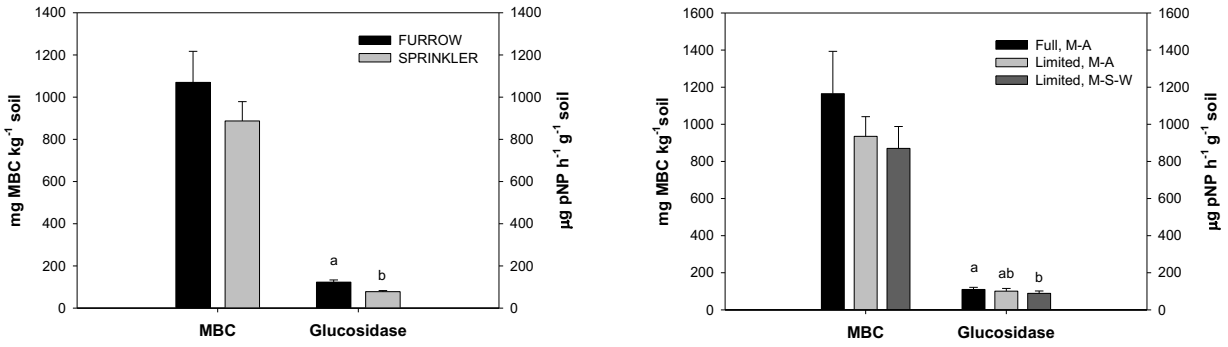

Fig. 1. Microbial biomass C (MBC) concentrations and β-glucosidase activity in rhizosphere soil under furrow- or sprinkler-irrigated maize (left) and with differing amounts of irrigation water (right; Full = fully irrigated maize, Limited = limited irrigated maize, M-A = maize-alfalfa rotation, M-S-W = maize-sunflower-winter wheat rotation). Standard error bars (± 1) are shown. Means labeled with different letters are statistically different at α = 0.05.

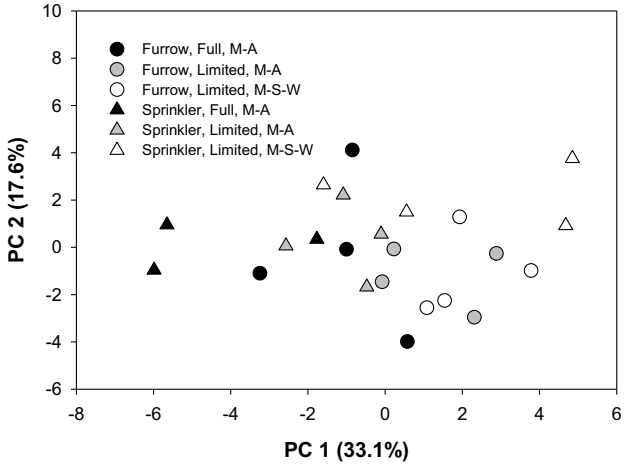

Fig. 2. Principal components (PC) analysis of rhizosphere microbial community EL-FAME patterns in maize with different furrow or sprinkler irrigation practices. Full = fully irrigated maize, Limited = limited irrigated maize, M-A = maize-alfalfa rotation, M-S-W = maize-sunflower-winter wheat rotation. The variance explained by each PC is shown in parentheses.

Principle components analysis of microbial community EL-FAMEs (expressed as relative percentages) demonstrated shifts in microbial community structure as soils transitioned from full to limited irrigation (Fig. 2). Specific EL-FAME biomarkers heavily weighted on PC 1 included fungal biomarkers 18:2ω6,9c, 18:1ω9c, AM fungal biomarker 16:1ω5c (all to the left of PC1), and Gram-positive bacterial markers i15:0, a15:0, a17:0, and i17:1 (all to the right of PC1). This weighted distribution indicates that microbial communities became more dominated by Gram-positive bacteria and less dominated by fungi under deficit irrigation.

This pattern was confirmed by ANOVA tests on relative amounts of several EL-FAMEs biomarkers (sum of 18:1ω9c and 18:2ω6,9c for fungi; sum of i15:0, a15:0, i16:0, i17:0, and a17:0 for Gram-positive bacteria; and sum of 16:1ω7c, 16:1 2OH, 17:0cy and 19:0 cy for Gram-negative bacteria) (Fig. 3). Thus, our preliminary results with Gram-positive bacteria agree with those of Uhlirova et al. (2005), but we found fungi rather than Gram-negative bacteria, to dominate

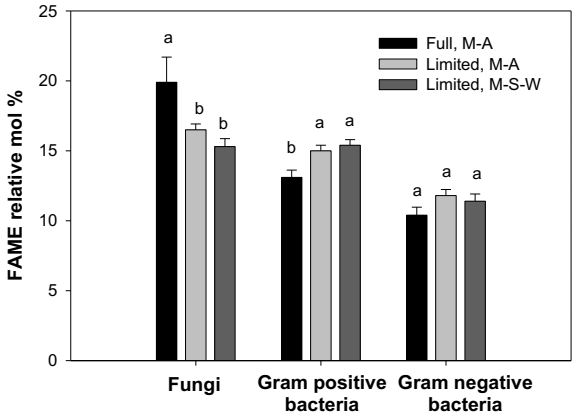

Fig. 3. Relative amounts of microbial EL-FAME biomarkers in maize rhizosphere soil under deficit irrigation. Full = fully irrigated maize, Limited = limited irrigated maize, M-A = maize-alfalfa rotation, M-S-W = maize-sunflower-winter wheat rotation. Standard error bars (± 1) are shown. Means labeled with different letters are statistically different at α = 0.05.

"wetter" (fully irrigated) soils. Within bacterial communities in general, Gram-positives are usually selected in drier soils because of their greater tolerance for low water potentials due to their thicker cell walls and higher cytoplasmic concentrations of compatible solutes compared to Gram-negative bacteria (Harris 1981). Physiologically, fungi are even better adapted for water stress, and are able to grow at water potentials as low as -0.33 MPa or less in soil (Griffin 1981). This is likely due to their thicker cell walls (compared to bacteria), the types of compatible solutes they produce (e.g., polyhydric alcohols), and their ability to spatially exploit soil for water and nutrient resources due to their hyphal nature. However, our preliminary study suggests that fungi preferred the fully irrigated soil and were not enhanced in the deficit-irrigated soil. Increased competition with bacteria due to lower C availability in deficit-irrigation systems could explain this pattern.

We also examined the ratio of 17:0cy-to-16:1ω7c concentrations as an indicator of bacterial stress (Stromberger et al. 2007). Interestingly, bacteria did not become stressed under deficit irrigation, at least not among bacteria containing 16:1ω7c (Fig. 4). Stromberger et al. (2007) also reported no increase in this stress indicator in warmer and drier soils across an evapotranspiration gradient in Colorado. Instead, bacterial stress was affected by irrigation method, with greater stress ratio in furrow than in sprinkler-irrigated soils (Fig. 4). Perhaps in soils of the semi-arid Great Plains, flooding (as would happen under furrow irrigation) poses a greater stress to microbial communities than does decreased water availability. Kieft et al. (1987) and Hamel et al. (2006) also reported that sudden re-wetting of dry soil may be particularly

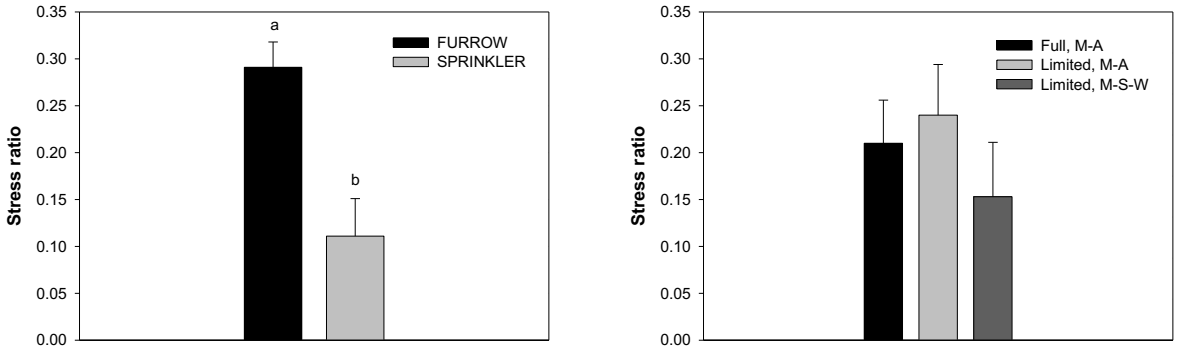

Fig. 4. Ratio of 17:0cy-to-16:1ω7c as an indicator of bacterial stress in rhizosphere soil under furrow- or sprinkler-irrigated maize (left) and with differing amounts of irrigation water (right; Full = fully irrigated maize, Limited = limited irrigated maize, M-A = maize-alfalfa rotation, M-S-W = maize-sunflower-winter wheat rotation). Standard error bars (± 1) are shown. Means labeled with different letters are statistically different at α = 0.05.

stressful to soil microorganisms, with lysis of a large number of cells likely to occur with a sudden change in soil water potential.

In conclusion, relative fungal biomass and β-glucosidase activity responded negatively to deficit irrigation, perhaps because they are sensitivity to water stress or because of reduced maize production in these systems. Furrow irrigation, which floods soils, posed a greater stress to soil bacteria than did reduced water availability. The negative response of fungi to deficit irrigation was surprising, and perhaps this microbial group is more sensitive to water stress than previously thought. Our results represent early responses of microorganisms to changes in agroecosystem management, as 2006 was only the second year of the study. We will continue to monitor soil biological properties to determine longer-term deficit irrigation effects.

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