Pedosphere **26**(2): 192–205, 2016
doi:10.1016/S1002-0160(15)60034-0
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Cover Crop and Irrigation Effects on Soil Microbial Communities and Enzymes in Semiarid Agroecosystems of the Central Great Plains of North America

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(Received July 2, 2015; revised December 14, 2015)

ABSTRACT

Cover crops can have beneficial effects on soil microbiology by increasing carbon (C) supply, but these beneficial effects can be modulated by precipitation conditions. The objective of this study was to compare a fallow-winter wheat (*Triticum aestivum* L.) rotation to several cover crop-winter wheat rotations under rainfed and irrigated conditions in the semiarid US High Plains. Experiments were carried out at two sites, Sidney in Nebraska, and Akron in Colorado, USA, with three times of soil sampling in 2012–2013 at cover crop termination, wheat planting, and wheat maturity. The experiments included four single-species cover crops, a 10-species mixture, and a fallow treatment. The variables measured were soil C and nitrogen (N), soil community structure by fatty acid methyl ester (FAME) profiles, and soil β-glucosidase, β-glucosaminidase, and phosphodiesterase activities. The fallow treatment, devoid of living plants, reduced the concentrations of most FAMEs at cover crop termination. The total FAME concentration was correlated with cover crop biomass ($R = 0.62$ at Sidney and 0.44 at Akron). By the time of wheat planting, there was a beneficial effect of irrigation, which caused an increase in mycorrhizal and protozoan markers. At wheat maturity, the cover crop and irrigation effects on soil FAMEs had subsided, but irrigation had a positive effect on the β-glucosidase and phosphodiesterase activities at Akron, which was the drier of the two sites. Cover crops and irrigation were slow to impact soil C concentration. Our results show that cover crops had a short-lived effect on soil microbial communities in semiarid wheat-based rotations and irrigation could enhance soil enzyme activity. In the semiarid environment, longer time spans may have been needed to see beneficial effects of cover crops on soil microbial community structure, soil enzyme activities, and soil C sequestration.

Key Words: crop rotation, enzyme activity, FAME profile, fatty acid methyl ester, winter wheat

Citation: Calderón F J, Nielsen D, Acosta-Martínez V, Vigil M F, Lyon D. 2016. Cover crop and irrigation effects on soil microbial communities and enzymes in semiarid agroecosystems of the Central Great Plains of North America. *Pedosphere*. **26**(2): 192–205.

INTRODUCTION

Cover crops have historically been defined as plantings between grain crops used to manage overly wet soils, prevent nitrate leaching, control soil erosion, suppress weeds, and/or enhance soil conservation without providing a direct economic benefit. Cover crops have been very effective in improving soil physical quality in relatively moist regions (Blanco-Canqui *et al.*, 2011; Liu *et al.*, 2005), but the benefit of cover cropping in semiarid regions is questionable because cover crops use water (Unger and Vigil, 1998). Cover cropping in different geographic areas has shown clear benefits to soil enzymes and soil microbiology.

Agricultural scientists have long recognized that increases in plant growth are not determined by total available resources, but by increases in the most scarce resource in a particular situation (van der Ploeg *et al.*, 1999). In the Central Great Plains of USA, the most scarce resource for crop growth is water, followed secondarily by nitrogen (N). In rainfed semiarid systems, the cover crop can reduce the water recharge of the soil profile relative to a fallow period, which can lead to a yield reduction of the following grain crop (Lyon *et al.*, 1995; Nielsen *et al.*, 2015). In the Central Great Plains, wheat yields decrease by 141 kg ha^{-1} for every cm of available soil water deficit found at wheat planting (Nielsen and Vigil, 2005). Because of this, the cover crop water demand has to be balanced against the possible conservation and management benefits resulting from the cover crop.

The composition and biomass of soil microbial co-

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mmunities are markedly influenced by soil moisture regimes (Williams and Rice, 2007; Clark *et al.*, 2009; Cregger *et al.*, 2012) and by changes in vegetation cover (Buyer *et al.*, 2002) because different plant species can favor distinct soil microbial taxa (Zak *et al.*, 2003; Kardol *et al.*, 2009, 2010; Mitchell *et al.*, 2010). Soil microbial communities have a profound effect on soil function through their enzymes, which catalyze the cycling of several nutrients (*e.g.*, C, N, P, and S) and regulate soil organic matter (SOM) dynamics. Measurement of several enzyme activities can provide early signs of changes in soil metabolic capacity due to soil disturbances such as tillage (Acosta-Martínez and Tabatabai, 2001), land management (Acosta-Martínez *et al.*, 2003), and crop rotations (Acosta-Martínez *et al.*, 2007b).

Fatty acid profiling followed by multivariate analysis has been used to study soil microbial community structure under different agronomic regimes (Calderón *et al.*, 2000; Acosta-Martínez *et al.*, 2007b; Bell *et al.*, 2009; Bowles *et al.*, 2014) because different microbial taxa contain fatty acids that vary in their chain length, number of unsaturations, and position of double bonds. The ester-linked total fatty acid (FAME) method has been used successfully to study tillage and crop rotation effects in rainfed wheat systems in agricultural plots (Drijber *et al.*, 2000; Acosta-Martínez *et al.*, 2007b).

The objectives of this study were to determine the effect of the presence or absence of different cover crop species or a 10-species mixture on soil microbial communities throughout a complete cycle of a cover crop-wheat (grain crop) system in the Central Great Plains of USA. Our hypothesis is that in a semiarid environment, soil microbial community structure and microbial enzyme activities will be enhanced by supplemental irrigation and will be negatively affected by fallowing the soil. The effect of cover crop diversity on soil microbial community structure and microbial enzyme activities will be secondary to that of irrigation and fallow. The effect of cover crop diversity was ascertained by comparing 10-species cover crop mixture with individual cover crop species. Soil water effect on soil microbial communities and enzyme activities was also assessed by comparing rainfed and irrigated conditions.

MATERIALS AND METHODS

Study sites

This study was conducted from 2012 through 2013 at two separate sites 135 km apart: the USDA-ARS Central Great Plains Research Station (40°09′ N, 103°09′ W, 1 383 m elevation) near Akron in Colorado of USA and the University of Nebraska-Lincoln High Plains Agricultural Laboratory (41°12′ N, 103°0′ W, 1 315 m elevation) near Sidney in Nebraska of USA. The soil at the Sidney site was a Keith silt loam (fine-silty, mixed, superactive, mesic Aridic Argiustolls in the USDA soil classification system), while the soil at Akron was a Weld silt loam (fine, smectitic, mesic Aridic Argiustolls in the USDA soil classification system). The 0–15 cm soils at Sidney and Akron have a pH of 7.0 as reported by Lyon *et al.* (2007).

Average temperatures during April–December 2012 were 15.1 and 13.7 °C for Akron and Sidney, respectively. These temperatures were higher than the long-term (1946–2013) average at both the sites, which are 12.6 °C for Akron and 12.1 °C for Sidney, respectively. The average temperatures during January–July of 2013 were 8.9 °C for Akron and 8.1 °C for Sidney. The long-term averages for this period were 9.0 and 8.5 °C for Akron and Sidney, respectively.

Precipitation during April–December 2012 was 306 and 284 mm, below the long-term averages of 378 and 388 mm at Akron and Sidney, respectively. Precipitation during January–June 2013 amounted to 214 mm at Akron and 241 mm at Sidney. Long-term average precipitation for this period was 215 and 233 mm at Akron and Sidney, respectively.

Experiments

Experimental design. At both the sites, the experiment was a split-plot design with four replications. The main plot factor was irrigation and the split-plot factor was cover crop species. The spring-planted cover crop treatments were established on no-till proso millet (*Panicum miliaceum* L.) residues under two water availability conditions at both study sites: rainfed (no irrigation) and irrigated to nearly non-water-stressed at Akron and to simulate average precipitation at Sidney. The irrigated treatment at Akron received a total of 588 mm of irrigation during the experiment, divided into 374 mm for the cover crop and 214 mm for the wheat. The irrigated treatment at Sidney received 255 mm, divided into 197 mm for the cover crop and 58 mm for the wheat. Prior to the experiment, the plots had been managed with no-till production practices in excess of 10 years. The cover crop treatments were deployed once in 2012. They consisted of a fallow treatment, a 10-species cover crop mixture of oat (*Avena sativa*), pea (*Pisum sativum*), flax (*Linum usitatissimum*), rapeseed (*Brassica napus*), lentil (*Lens culinaris*), vetch (*Vicia sativa*), clover (*Trifolium repens*), barley (*Hordeum vulgare*), safflower (*Carthamus tinc-*

torius), and phacelia (*Phacelia tanacetifolia*), and single species plantings of flax, oat, pea, and rapeseed.

Cultural practices at Akron. All cover crops were no-till seeded into proso millet residues on March 27, 2012. Row spacing was 20 cm and plot size for each replicate was 9.1 m by 6.1 m. Seeding rates were as follows: rapeseed, 7.4 kg ha^{-1}; flax, 39.2 kg ha^{-1}; oat, 94.0 kg ha^{-1}; pea, 114.5 kg ha^{-1}; mixture, 59.7 kg ha^{-1}. Emergence was mostly complete on April 9, 2012. The plots were sprayed with glyphosate (*N*-(phosphonomethyl)glycine) prior to planting and fertilized with 34 kg N ha^{-1}. Hand weeding was performed periodically during the growing season, with most of that performed during the last week of April. Required irrigation amounts were applied every two weeks with a linear-move irrigation system. The original plan called for the cover crops to be terminated by chemical spraying on July 1, 2012, but the termination date was moved up to June 16 because of the warm conditions during 2012 and the resulting faster plant development. Termination was accomplished with a spray application of paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride (3.51 L ha^{-1}). Rapeseed was not effectively terminated and required a second herbicide application on July 11, 2012. Biomass measurements (1 m of one row) were taken just prior to cover crop termination. Soil samples of 0–5 and 5–15 cm depths were taken on June 27, 2012 from each plot following cover crop termination and again at wheat planting and wheat harvest. Two soil samples from each depth were composited into one sample for each sampling time per plot. Soil samples were kept at 5 °C until microbiological analyses (described below) were performed. Winter wheat ('Settler CL') was no-till seeded at 67 kg ha^{-1} on September 21, 2012. Row spacing was 20 cm. Fertilizers were applied at planting at 67 kg N ha^{-1} and 17 kg P_2O_5 ha^{-1}. Wheat grain was harvested on July 8, 2013 (rainfed plots) and July 9, 2013 (irrigated plots).

Cultural practices at Sidney. All cover crops were no-till seeded into proso millet residues on April 4, 2012. Row spacing was 25 cm and plot size was 9.1 m by 4.6 m. Seeding rates were as follows: rapeseed, 6.7 kg ha^{-1}; flax, 39.2 kg ha^{-1}; oat, 100.8 kg ha^{-1}; pea, 112.0 kg ha^{-1}; mixture, 57.1 kg ha^{-1}. Emergence was mostly complete on April 16, 2012. The plots were sprayed with glyphosate prior to planting and fertilized with 34 kg N ha^{-1}. Hand weeding was performed periodically during the growing season. Required irrigation amounts were applied every two weeks with a linear-move irrigation system. Similar to the plots at Akron, the weather conditions required the plots at Sidney to be terminated early (June 15) with a spray application of glyphosate (4.68 L ha^{-1}). Biomass measurements (1 m of one row) were taken on June 1, 2012 and at cover crop termination. Soil samples (0–5 and 5–15 cm) were taken on June 28, 2012 from each plot following cover crop termination and again at wheat planting and wheat harvest. A hydraulic probe (diameter of 3.8 cm) was used to take one core near the center of each plot. Soil samples were placed at 5 °C upon collection in the field, and then frozen until microbiological analyses were performed. Visible plant material was removed from the soils prior to analysis. Winter wheat ('Pronghorn') was no-till seeded at 62 kg ha^{-1} on September 20, 2012. Row spacing was 25 cm. Fertilizers were applied at planting at 44 kg N ha^{-1} and 17 kg P_2O_5 ha^{-1}. Wheat grains were harvested on July 13, 2013 from each plot.

Soil sample analysis

Soil water content at Akron was measured with neutron probes (Model 503 Hydroprobe, CPN International, Inc., Martinez, USA) to a depth of 165 cm at Akron and a depth of 135 cm at Sidney in 2012. The 0–30 cm soil water content at Akron was also measured by time-domain reflectometry (Trase System I, Soil Moisture Equipment Corp., Santa Barbara, USA) during the cover crop and wheat growing seasons and during the intervening fallow period.

We followed the FAME method of Schutter and Dick (2000), which involves the following steps: 1) incubation at 37 °C for 1 h of 3 g of soil in 15 mL of 0.2 mol L^{-1} KOH in methanol for the saponification and methylation of the fatty acids, with the pH of the mixture neutralized with 1.0 mol L^{-1} acetic acid at the end of incubation; 2) adding 10 mL of hexane to allow for the separation of the FAMEs into the organic phase, followed by centrifugation at 480 × *g* for 10 min; 3) evaporation of the hexane using N_2; and 4) dissolution of the FAMEs in 100 μL of 1:1 methyl tert-butyl ether and hexane with methyl nonadecanoate (19:0) as an internal standard (0.5 mg mL^{-1}) to allow for quantification of the individual FAME using gas chromatography (GC). The GC analysis was performed using an Agilent 6890 N gas chromatograph with an Agilent HP-5 fused silica column (Agilent, Santa Clara, USA) and a flame ionization detector (Hewlett Packard, Palo Alto, USA) with hydrogen carrier gas. The temperature program ramped at 5 °C min^{-1} from 170 °C to 270 °C and then to 300 °C for 2 min. The FAME identification peak areas were determined using the TSBA6 aerobe program (Microbial ID, Inc., Newark, USA). Fatty acids were

attributed to microbial taxa as follows: *i*15:0, *a*15:0, *i*17:0, *a*17:0, from eubacteria in general, to anaerobic sulfate-reducing gram-positive bacteria (Zelles, 1997; Zelles, 1999); cy 17:0 and cy 19:0, *i*13:0 3OH, and *i*17:0 3OH to anaerobic gram-negative bacteria (O'Leary and Wilkinson, 1988); 10Me16:0, 10Me17:0, and 10Me18:0 to actinomycetes (Kroppenstedt, 1985); 16:1ω5*c* to arbuscular mycorrhizal fungi (Graham *et al.*, 1995; Calderón *et al.*, 2009); 18:1ω9*c*, 18:2ω6*c*, and 18:3ω6*c* to saprophytic fungi (Harwood and Russell, 1984; O'Leary and Wilkinson, 1988; Vestal and White, 1989); and 20:4ω6 to protozoa (Zelles, 1997).

The activities of three soil enzymes indicative of P cycling (phosphodiesterase), C cycling (β-glucosidase), and C/N cycling (β-glucosaminidase) were determined for the sampling at wheat maturity in 2013. One g of air-dried soil was mixed with the respective substrate and incubated for 1 h at 37 °C at their optimal pH as detailed in Tabatabai (1994) and Parham and Deng (2000). Assays included a control (no soil added) that received substrate, and control values were subtracted from the soil sample values.

At wheat maturity, the total C and N in dried and ground soil samples from the 0–5 cm depth were measured with an LECO Truspec analyzer (LECO Corporation, St. Joseph, USA).

Statistical analysis

The analysis of variance (ANOVA) for the total C, total N, FAME, and enzyme activity data were carried out using the PROC MIXED analysis of SAS Release 8.02 (SAS institute, Cary, USA). We tested for site, depth, irrigation, and vegetation cover main effects. Mean separations were done with Duncan's multiple range test. The redundancy analysis (RDA) of the individual FAMEs as a percentage of the total FAME concentration was carried out with the CANOCO software Version 5.02 (Microcomputer Power, Ithaca, USA), which allowed us to relate the FAME profiles to the cover crop treatments for selected depths and sampling times.

RESULTS

Cover crop biomass

Irrigation had a significant effect on the cover crop biomass at both sites, with a 58% increase at the Akron site and a 68% increase at the Sidney sites in the irrigated plots over the rainfed plots (Table I). The increases in biomass of most cover crops ranged between 35% and 75% with irrigation. Exceptions were an increase of 125% in rapeseed biomass with irrigation at Sidney and the subdued response to irrigation of the mixture with increases in biomass of only 35%–48%. Rapeseed had the least biomass regardless of the irrigation treatment and the site. The oat, pea, and mixture treatments consistently had the greatest biomass across the sites and irrigation treatments (Table I). The biomass at the Akron site was on average 56% greater than that at the Sidney site.

TABLE I

Cover crop biomass at cover crop termination of different cover crop and irrigation treatments at the two study sites (Sidney in Nebraska and Akron in Colorado, USA)

Water condition	Crop	Akron		Sidney	
		Mean	Standard error	Mean	Standard error
		kg ha^{-1}			
Rainfed	Flax	2 716	237	1 731	154
	Oat	3 090	274	2 282	234
	Pea	2 945	194	2 242	244
	Rapeseed	2 604	237	1 220	147
	Mixture[a)]	3 740	433	2 270	235
	Average	3 019	146	1 949	127
Irrigated	Flax	4 656	135	2 557	270
	Oat	5 250	428	3 777	372
	Pea	4 839	436	3 924	215
	Rapeseed	4 099	251	2 740	248
	Mixture	5 060	318	3 366	325
	Average	4 781	161	3 269	170
Both water conditions	Flax	3 686	388	2 144	212
	Oat	4 170	471	3 029	348
	Pea	3 892	421	3 083	352
	Rapeseed	3 352	325	1 971	317
	Mixture	4 400	352	2 818	278

[a)] A 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia.

Total soil C and N

The Sidney soil had 1.5 and 13.9 mg kg^{-1} of total soil N and C, respectively, while the Akron soils had 1.3 and 11.1 mg kg^{-1} of total soil N and C, respectively. There were no cover crop or irrigation main effects on total soil C or total N at wheat termination. There was a site main effect due to the higher total C and N of the soils from Sidney relative to the soils from Akron.

Effects of study site, soil depth, crop and irrigation on FAME results

The ANOVA results show that for all three sampling times there were significant ($P < 0.05$) soil depth and site main effects on soil microbial community composition (Table II). At wheat maturity, the total FAME concentration was 122% higher at the 0–5 cm depth than at the 5–15 cm depth for both sites. Likewise, at wheat maturity, the Sidney soils had 64% higher FAME concentration than the Akron soils (data not shown). Site and depth differences are not pertinent to the main objectives of this study so they will not be discussed further. For the total FAMEs, the interactions between irrigation and crop were not significant for any of the three sampling times (Table II). This prompted us to use the averaged site data to better illustrate the crop and irrigation main effects, which are the main focus of this study. The significant interaction between site and irrigation observed at wheat maturity will be explained below.

FAME results at cover crop termination in 2012

Irrigation did not affect FAME concentrations at cover crop termination (Table III). The ANOVA indicated a significant cover crop main effect, which was explained by the reduced concentration of total FAMEs and most FAMEs in the fallow treatment (Table III). The fallow soil had low total FAME concentrations at both the 0–5 and 5–15 cm depths. Compared to other cover crops, flax and rapeseed had a tendency for low FAME concentrations, but the rapeseed and flax values were always statistically higher than the fallow treatment. The pea treatment had higher Gram-negative and protozoan markers than the flax treatment at the 0–5 cm depth (Table III). The cover crop mixture treatment was not associated with increased total or individual FAMEs relative to the individual cover crops at either soil depth (Table III).

The separation of cover crop treatments according to FAME composition in the Sidney 0–5 cm soil layer at cover crop termination is illustrated Fig. 1a. It sho-

TABLE II

Results of analysis of variance (ANOVA) on the fatty acid methyl ester data for three soil sampling times (0–5 and 5–15 cm depths) of different cover crop and irrigation treatments at the two study sites (Sidney in Nebraska and Akron in Colorado, USA)

Sampling time	Source	Degree of freedom	Type I sum of squares	Mean square	F value	P value
Cover crop termination in 2012	Irrigation	1	852.627 1	852.627 1	0.36	0.549 7
	Soil depth	1	199 988.293 8	199 988.293 8	168.05	< 0.000 1
	Crop	5	60 278.870 2	12 055.774 0	5.08	0.000 2
	Site	1	33 379.040 5	33 379.040 5	14.06	0.000 2
	Irrigation × crop	5	2 226.003 0	445.200 6	0.19	0.967 0
	Irrigation × site	1	3 036.757 7	3 036.757 7	1.28	0.259 6
	Crop × site	5	20 505.612 6	4 101.122 5	1.73	0.130 8
	Irrigation × crop × site	5	6 830.152 4	1 366.030 5	0.58	0.718 7
Wheat planting in 2012	Irrigation	1	9 773.894 9	9 773.894 9	3.24	0.073 7
	Soil depth	1	262 242.105 9	262 242.105 9	179.60	< 0.000 1
	Crop	5	15 146.778 8	3 029.355 8	1.00	0.417 0
	Site	1	100 506.776 5	100 506.776 5	33.32	< 0.000 1
	Irrigation × crop	5	6 195.114 1	1 239.022 8	0.41	0.840 9
	Irrigation × site	1	88.361 1	88.361 1	0.03	0.864 3
	Crop × site	5	3 193.212 7	638.642 5	0.21	0.957 2
	Irrigation × crop site	5	4 163.619 0	832.723 8	0.28	0.925 8
Wheat maturity in 2013	Irrigation	1	114.877 1	114.877 1	0.02	0.892 7
	Soil depth	1	650 945.718 7	650 945.718 7	267.88	< 0.000 1
	Crop	5	14 397.104 3	2 879.420 9	0.46	0.807 2
	Site	1	219 485.323 7	219 485.323 7	34.89	< 0.000 1
	Irrigation × crop	5	8 921.173 0	1 784.234 6	0.28	0.921 6
	Irrigation × site	1	27 321.227 3	27 321.227 3	4.34	0.038 7
	Crop × site	5	20 640.158 2	4 128.031 6	0.66	0.657 1
	Irrigation × crop site	5	16 480.557 1	3 296.111 4	0.52	0.757 9

TABLE III

Fatty acid methyl ester (FAME) data[a)] at cover crop termination in 2012 for different cover crop and irrigation treatments at the two study sites (Sidney in Nebraska and Akron in Colorado, USA)

Soil depth	Treatment	Total FAMEs	Gram-positive bacteria[b)]	Gram-negative bacteria[c)]	Actinomycetes[d)]	Arbuscular mycorrhizal (AMF) fungi[e)]	Saprophytic fungi[f)]	Protozoa[g)]
cm		nmol g^{-1} soil						
0–5	Fallow	73.9b[h)]	10.4b	4.0c	7.7b	1.7b	13.3b	0.4c
	Flax	129.3a	17.4a	6.0b	11.8a	2.8a	25.5a	0.7b
	Mixture[i)]	144.4a	19.6a	6.7ab	13.3a	3.7a	26.4a	0.8ab
	Oat	152.2a	20.3a	6.7ab	13.3a	3.8a	27.9a	1.0ab
	Pea	152.4a	20.3a	7.5a	13.6a	3.6a	28.3a	1.1a
	Rapeseed	129.8a	17.3a	6.2ab	11.8a	2.9a	24.5a	0.7b
5–15	Fallow	46.1b	7.0b	3.1b	5.4b	1.6c	7.1b	0.3a
	Flax	80.9a	12.1a	4.4a	8.5a	2.5abc	13.8a	0.5a
	Mixture	64.7ab	10.2a	4.0ab	7.1ab	3.2ab	9.8ab	0.4a
	Oat	71.7a	10.9a	4.1ab	7.4ab	3.3a	12.4a	0.5a
	Pea	68.1ab	10.5a	4.1ab	7.3ab	2.6abc	11.2ab	0.4a
	Rapeseed	63.2ab	9.6ab	3.8ab	6.7ab	2.2bc	10.6ab	0.4a
Both depths	Irrigated	100.2a	13.9a	5.1a	9.5a	2.9a	18.3a	0.6a
	Rainfed	96.0a	13.7a	5.0a	9.4a	2.8a	16.8a	0.6a

[a)] Data are means across the two study sites ($n = 16$ for the cover crop averages and $n = 96$ for the irrigation averages).
[b)] Sum of *a*15:0, *i*15:0, *a*17:0, and *i*17:0.
[c)] Sum of cy 17:0, cy 19:0, *i*13:0 3OH, and *i*17:0 3OH.
[d)] Sum of 10Me16:0, 10Me17:0, and 10Me18:0.
[e)] 16:1ω5*c*.
[f)] Sum of 18:1ω9*c*, 18:2ω6*c* and 18:3ω6*c*.
[g)] 20:4ω6.
[h)] Means within a column followed by the same letter(s) are not significantly different according to Duncan's test ($P < 0.05$).
[i)] A 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia.

uld be noted that the RDA was carried out with individual FAMEs as a percentage of the total fatty acids, rather than the concentration of FAME used in the ANOVA. Thus, the RDA illustrated the differences in the relative amounts of FAMEs determined by the cover crop treatments. The distance between the fallow and the different cover crop treatment symbols in the RDA biplots is indicative of the dissimilarity (Euclidean distance) of FAME composition. The vectors point in the direction of increase for the corresponding FAME. The angle between arrows indicates the sign of the correlation between the FAMEs such that there is a positive correlation in sharp angles but negative if the angle is $> 90°$. It could be seen that the microbial community composition was similar between the flax, mixture, oat, and pea treatments, which formed a relatively tight cluster, different from the fallow treatment. The FAMEs of the rapeseed treatment were different from those of the rest of the cover crop treatments as well as the fallow treatment.

At the Akron site, the differences in microbial community composition between the fallow and the flax treatments were less pronounced than those at the Sidney site (Fig. 1b). The rapeseed treatment was also separated from the rest of the treatments. The oat, mixture, and pea treatments had similar microbial community composition. The correlation vectors in Fig. 1 show that the relative amounts of *i*17:0, *a*17:0, 10Me18:0, and cy19:0 were higher in the fallow treatment relative to the cover crop treatments regardless of site. In contrast, *a*15:0, 10Me17:0, *i*17:0 3OH, 18:1ω9, 18:2ω6, and 20:4ω6 tended to be negatively correlated with the fallow treatment (Fig. 1).

We carried out correlation analyses between the cover crop biomass and total and individual FAME concentrations at cover crop termination. The fallow plots, with zero plant biomass, were excluded from the correlation analysis. When both irrigation treatments were included, the Pearson correlation coefficient between crop biomass and total FAMEs of the 0–5 cm depth was 0.62 for the Sidney samples and 0.44 for the Akron samples. The correlation coefficient of the Sidney samples improved to 0.70 when only the irrigated total FAMEs and cover crop biomass were included in the analysis.

FAME results at wheat planting in 2012

Between the cover crop termination and the wheat planting, there was an interim of nearly 14 weeks in which all the plots were devoid of living vegetation. There was a 9% reduction in total FAMEs between the cover crop termination and wheat planting samplings

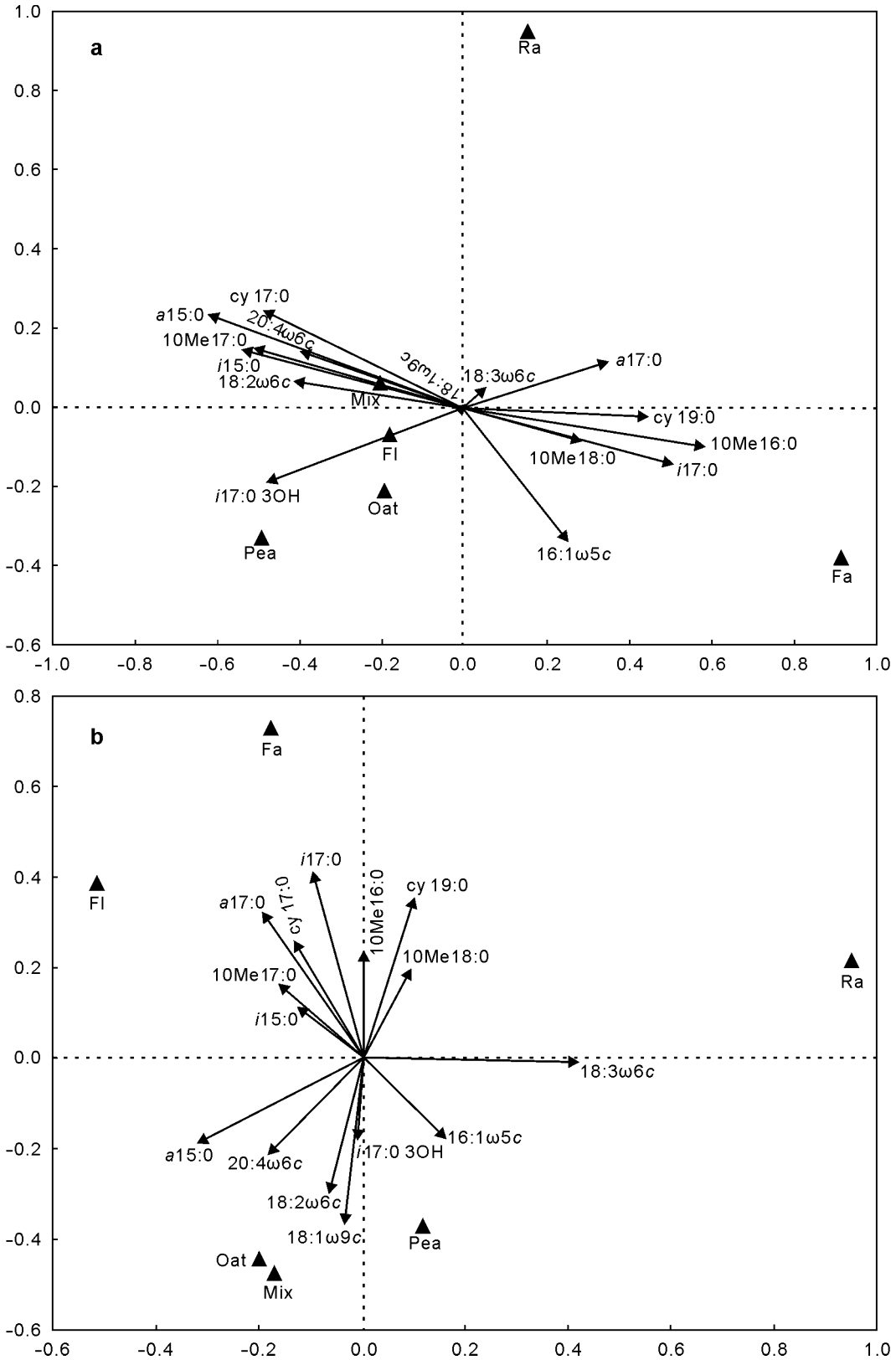

Fig. 1 Redundancy analysis of the individual fatty acid methyl esters (FAMEs) as a percentage of the total FAMEs for the soil samples of 0–5 cm depth from Sidney in Nebraska, USA (a) and Akron in Colorado, USA (b) at cover crop termination of different cover crop treatments. Fa = fallow; Fl = flax; Mix = mixture, a 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia; Ra = rapeseed.

TABLE IV

Fatty acid methyl ester (FAME) data[a)] at wheat planting in 2012 for different cover crop and irrigation treatments at the two study sites (Sidney in Nebraska and Akron in Colorado, USA)

Soil depth	Treatment	Total FAMEs	Gram-positive bacteria[b)]	Gram-negative bacteria[c)]	Actinomycetes[d)]	Arbuscular mycorrhizal (AMF) fungi[e)]	Saprophytic fungi[f)]	Protozoa[g)]
cm		nmol g^{-1} soil						
0–5	Fallow	105.7a[h)]	14.7a	5.0a	9.5a	2.1b	19.2a	0.6a
	Flax	119.1a	15.9a	4.9a	10.2a	2.8ab	23.5a	0.6a
	Mixture[i)]	146.5a	19.6a	6.0a	12.3a	3.3ab	28.5a	0.7a
	Oat	136.1a	18.4a	5.5a	11.7a	3.4a	26.3a	0.9a
	Pea	135.5a	18.6a	5.6a	11.6a	3.3ab	26.2a	0.6a
	Rapeseed	117.8a	16.1a	4.9a	10.1a	2.8ab	23.1a	0.6a
5–15	Fallow	46.5a	7.7a	2.8a	5.3a	1.6b	6.9a	0.3a
	Flax	49.5a	7.8a	2.8a	5.7a	1.7ab	8.0a	0.3a
	Mixture	58.5a	8.9a	3.3a	6.8a	2.5a	9.1a	0.3a
	Oat	56.5a	8.7a	3.0a	6.0a	2.5a	9.0a	0.3a
	Pea	54.8a	8.4a	3.0a	6.0a	2.5a	8.5a	0.3a
	Rapeseed	49.3a	7.6a	2.9a	5.7a	1.7ab	7.9a	0.3a
Both depths	Irrigated	96.5a	13.6a	4.3a	8.8a	2.8a	18.0a	0.6a
	Rainfed	82.2a	11.7a	3.9a	8.0a	2.3b	14.6a	0.4b

[a)] Data are means across the two study sites ($n = 16$ for the cover crop averages and $n = 96$ for the irrigation averages).
[b)] Sum of a15:0, i15:0, a17:0, and i17:0.
[c)] Sum of cy 17:0, cy 19:0, i13:0 3OH, and i17:0 3OH.
[d)] Sum of 10Me16:0, 10Me17:0, and 10Me18:0.
[e)] 16:1ω5c.
[f)] Sum of 18:1ω9c, 18:2ω6c and 18:3ω6c.
[g)] 20:4ω6.
[h)] Means within a column followed by the same letter(s) are not significantly different according to Duncan's test ($P < 0.05$).
[i)] A 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia.

when depths and cover crop treatments were averaged (Tables III and IV). The differences in the FAME markers between the fallow treatment and the cover crop treatments were reduced compared to the previous sampling at cover crop termination (Table IV). As with the previous planting, the total FAME concentration was less in the fallow treatment compared with the mixture treatment, but the difference was not significant. The mycorrhizal marker 16:1ω5c was higher in the oat treatment than in the fallow treatment at both depths (Table IV). Arbuscular mycorrhizal and protozoan markers were significantly higher in the irrigated relative to the rainfed soils (Table IV).

Redundant analysis illustrated the relative amounts of FAME associated with the cover crops as well as the irrigation treatments at wheat planting (Fig. 2a, b). No consistent clustering between the cover crop and fallow treatments was observed at the two sites, except that the fallow and rapeseed treatments tended to be close to each other (Fig. 2b). At the Akron site, the mixture treatment was close to the center of the RDA biplot, indicating that it did not foster a distinct microbial community structure relative to the rest of the cover crop treatments.

To determine if plant biomass from a cover crop affected soil microbial communities later when the main crop was planted on the cover crop residues, we carried out Pearson correlation analysis between the cover crop biomass at termination and the FAME concentration at wheat planting. We found a positive correlation coefficient of 0.43 between cover crop biomass and total FAME concentration in the Akron rainfed plots at the 0–5 cm depth. Correlation coefficients for 10Me18:0 and 16:1ω5c were both above 0.45. However, the correlation coefficients between FAMEs and cover crop biomass were reduced for the deeper soil samples and when the irrigated plots were included in the correlations. The correlation coefficient between cover crop biomass and total FAME concentration in the Sidney rainfed plots at the 0–5 cm depth was 0.31.

FAME results at wheat maturity in 2013

By the time of wheat maturity, 10.5 months had elapsed from wheat planting. The total FAME concentrations at wheat maturity were the highest among the three samplings. By this time, all statistical differences in total FAME concentration between the cover crop treatments had subsided (Table V). For all individual FAME markers, the mean values were statistically equal between the fallow and the cover crop treatments. However, differences in fungal markers 18:1ω9 and 20:4ω6 between the pea and fallow treatments per-

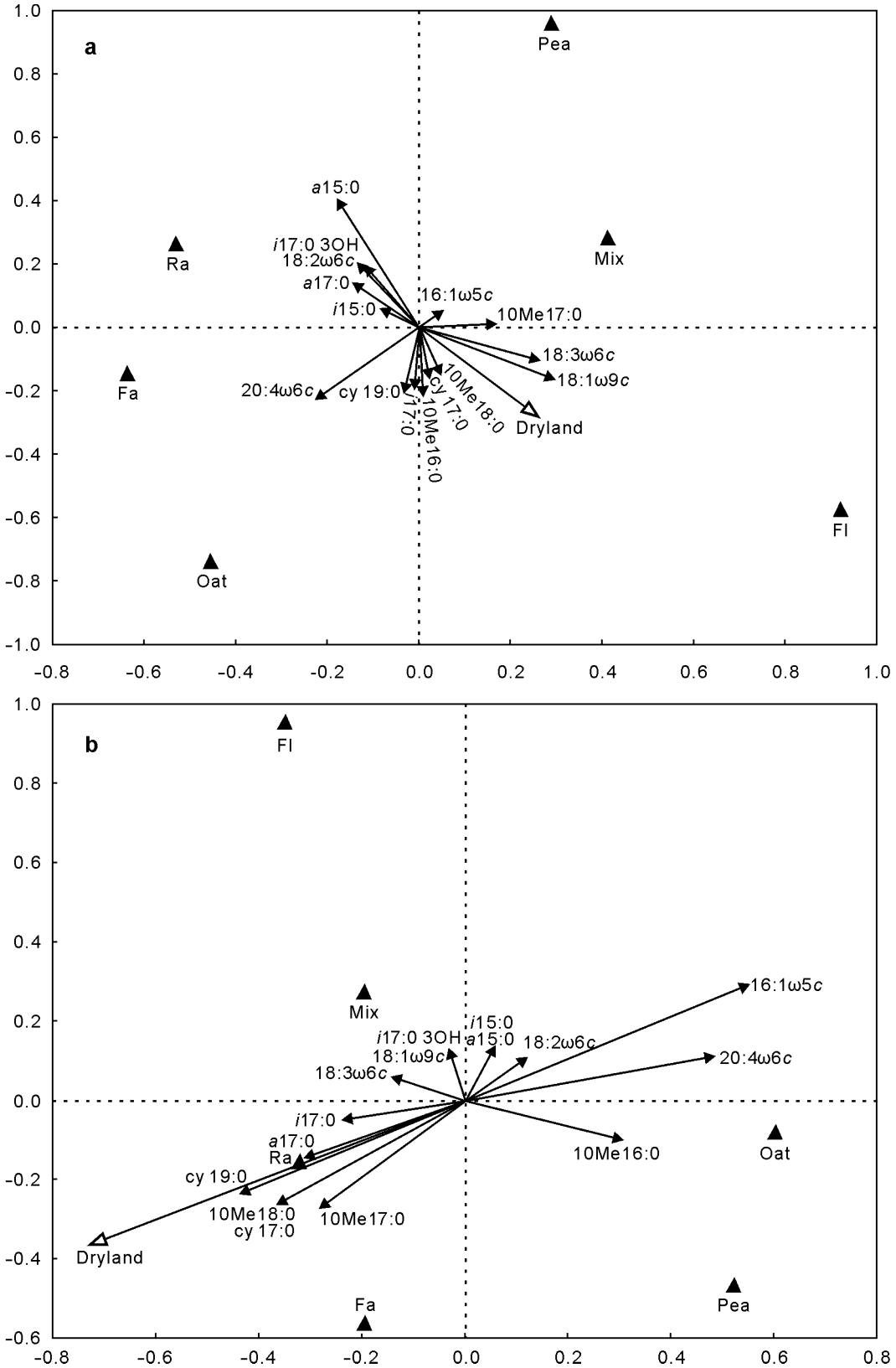

Fig. 2 Redundancy analysis of the individual fatty acid methyl esters (FAMEs) as a percentage of the total FAMEs for the soil samples of 0–5 cm depth from Sidney in Nebraska, USA (a) and Akron in Colorado, USA (b) at wheat planting of different cover crop treatments. Fa = fallow; Fl = flax; Mix = mixture, a 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia; Ra = rapeseed.

TABLE V

Fatty acid methyl ester (FAME) data[a)] at wheat maturity in 2013 for different cover crop and irrigation treatments at the two study sites (Sidney in Nebraska and Akron in Colorado, USA)

Soil depth	Treatment	Total FAMEs	Gram-positive bacteria[b)]	Gram-negative bacteria[c)]	Actinomycetes[d)]	Arbuscular mycorrhizal (AMF) fungi[e)]	Saprophytic fungi[f)]	Protozoa[g)]
cm		nmol g^{-1} soil						
0–5	Fallow	191.8a[h)]	27.0a	7.6a	15.6a	4.4a	31.5a	1.0a
	Flax	202.3a	26.3a	7.8a	15.6a	5.1a	37.4a	1.1a
	Mixture[i)]	210.0a	29.1a	8.0a	16.3a	5.6a	35.3a	1.2a
	Oat	205.1a	27.8a	7.6a	15.7a	6.5a	35.8a	1.2a
	Pea	236.9a	29.5a	8.1a	16.4a	5.4a	54.9a	1.4a
	Rapeseed	222.8a	31.1a	8.4a	16.7a	6.3a	37.5a	1.3a
5–15	Fallow	87.7a	13.2a	4.4a	8.4a	3.1a	12.6a	0.5a
	Flax	100.8a	14.7a	4.6a	9.3a	5.1a	15.6a	0.5a
	Mixture	88.3a	13.2a	4.4a	8.7a	3.5a	12.6a	0.5a
	Oat	96.4a	13.6a	4.7a	8.8a	5.0a	15.2a	0.6a
	Pea	94.0a	14.2a	4.7a	8.8a	3.3a	14.2a	0.5a
	Rapeseed	102.9a	15.1a	4.7a	9.3a	3.7a	16.8a	0.5a
Both depths	Irrigated	152.5a	21.1a	6.2a	12.2a	5.7a	25.2a	1.0a
	Rainfed	154.0a	21.3a	6.3a	12.8a	3.8b	28.0a	0.7b

[a)] Data are means across the two study sites ($n = 16$ for the cover crop averages and $n = 96$ for the irrigation averages).
[b)] Sum of *a*15:0, *i*15:0, *a*17:0, and *i*17:0.
[c)] Sum of cy 17:0, cy 19:0, *i*13:0 3OH, and *i*17:0 3OH.
[d)] Sum of 10Me16:0, 10Me17:0, and 10Me18:0.
[e)] 16:1ω5c.
[f)] Sum of 18:1ω9*c*, 18:2ω6*c* and 18:3ω6*c*.
[g)] 20:4ω6.
[h)] Means within a column followed by the same letter(s) are not significantly different according to Duncan's test ($P < 0.05$).
[i)] A 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia.

sisted at the 0–5 cm depth (data not shown).

At wheat maturity, the total FAME concentrations in the irrigated and rainfed soils were equal (Table V). However, the mycorrhizal marker 16:1ω5*c* and the protozoan marker 20:4ω6 were greater with irrigation. The significant interaction between site and irrigation was due to the overall positive response of the FAME to irrigation at the Sidney site, but an overall negative response to irrigation at the Akron site. For example, the total FAME concentrations at Sidney increased by 15% with irrigation, while the total FAME concentrations at Akron decreased by 17% with irrigation. Individual FAME concentrations followed a similar trend to the total FAME concentration in this interaction.

Soil enzyme activities

We chose three enzymes for our study, β-glucosidase, β-glucosaminidase and phosphodiesterase, which offer insights into the soil C, C/N, and P cycling, respectively. The enzyme activities were measured once at wheat maturity at the end of the crop cycle. There was no cover crop main effect on the activities of the three enzymes (Table VI). There was an irrigation main effect at the Akron site due to the higher β-glucosidase and phosphodiesterase activities in the irrigated soil (Table VI). The greatest increase was with the phosphodiesterase, with an 83% increase due to irrigation. The activity of β-glucosidase increased by 37%. There was a significant site main effect due to the reduced β-glucosidase and β-glucosaminidase activities and greater phosphodiesterase activity at the Akron site compared to the Sidney site.

DISCUSSION

Recently, there have been arguments in favor of widespread adoption of cover crops across North America, as well as suggestions that diverse cover crop mixtures offer advantages relative to single cover crops because of enhanced soil microbial activity (USDA-NRCS, 2013). Previous work indicates that a crop-free period is beneficial to winter wheat in the Central Great Plains because it allows for water recharge of the soil profile (Nielsen *et al.*, 2015). This study was designed to find the facts about the effects of the presence or absence of cover crops in a winter wheat rotation under the stated conditions, and whether the diversity of cover crops has an effect on microbial community structure and soil functioning. In addition, the role of the water regime in the context of cover crops was tested.

TABLE VI

Soil enzyme activities[a)] of different cover crop and irrigation treatments at the two study sites (Sidney in Nebraska and Akron in Colorado, USA)

Site	Treatment	β-glucosidase activity	β-glucosaminidase activity	Phosphodiesterase activity
		mg PNP[b)] g^{-1} soil h^{-1}		
Sidney	Fallow	147.6a[c)]	35.4a	43.4a
	Flax	168.0a	32.5a	56.9a
	Mixture[d)]	152.3a	34.4a	42.8a
	Oat	164.8a	37.3a	46.5a
	Pea	171.0a	39.5a	48.2a
	Rapeseed	182.0a	33.8a	63.5a
	Irrigated	157.8a	38.6a	51.2a
	Rainfed	170.7a	32.4a	49.2a
Akron	Fallow	104.3a	27.1a	57.6a
	Flax	98.7a	25.8a	56.3a
	Mixture	119.3a	31.3a	71.9a
	Oat	81.7a	21.4a	48.7a
	Pea	114.9a	29.0a	68.1a
	Rapeseed	93.0a	23.5a	60.6a
	Irrigated	118.0a	29.1a	78.4a
	Rainfed	86.0b	23.6a	42.7b

[a)] Data are means ($n = 8$ for the cover crop treatments and $n = 24$ for the irrigation treatments).
[b)] *p*-nitrophenol.
[c)] Means within a column followed by the same letter are not significantly different according to Duncan's test ($P < 0.05$).
[d)] A 10-species cover crop mixture of oat, pea, flax, rapeseed, lentil, vetch, clover, barley, safflower, and phacelia

Our results show that cover crops and irrigation were slow to impact soil C concentration, given that we did not observe soil C and N effects during the time span of this study. Previous studies in the US Corn Belt have shown that cover cropping and no-till can take up to 9 years to have an effect on soil C (Olson *et al.*, 2010). Bowman *et al.* (1999) reported that measurable increases in soil C and N due to changes in soil and crop rotation management can take 7 years or more to develop.

The lack of irrigation effects on FAME composition at cover crop termination indicates that irrigation took longer to affect microbial community composition, with the exception of fungi, which were favored by the added water. The biggest impact on the soil microbial communities at cover crop termination comes from the presence of roots, as shown by the detrimental effect of the fallow treatment on FAMEs. Individual cover crops and the mixture had equally positive effects on FAME at the 0–5 cm depth. However, the flax treatment had high levels of total FAMEs at the 5–15 cm depth. *Brassica* spp. are known to produce single tap roots (Ennos and Fitter, 1992), and our results suggest that root exudation from a deeper root system allowed rapeseed to enhance microbial growth at depth relative to other crop species. The positive correlations between cover crop biomass and total FAMEs indicate that microbial biomass at cover crop termination was strongly affected by plant primary productivity and transfer of photosynthates to the rhizosphere and soil.

At wheat planting, the total FAME concentrations suffered a 9% decline relative to the previous sampling, suggesting that the time between the cover crop termination and wheat planting was overall not favorable to the soil microbes, which may be due to the lack of growing roots and photosynthates moving into the soil, as well as the temperature and moisture effects. This indicates that root exudation from live roots was a more effective supply of energy to soil microbes than decomposing roots and crop residues in fallow soil.

Irrigation has been shown to increase soil microbial biomass, given that dry soil conditions limit residue decomposition and microbial growth processes. However, the effects on soil microbial communities have varied. Entry *et al.* (2008) observed that an irrigated pasture in southern Idaho, northwestern USA had significantly greater soil DNA content, fungal biomass, and microbial biomass than adjacent tilled or rainfed soils. However, 16S RNA sequences showed greater microbial diversity differences in the rainfed soils under native vegetation. Larkin *et al.* (2011) analyzed soil fatty acids in different cropping systems in northeastern Maine, northeastern USA with and without irrigation in the spring before planting a summer crop, and found that irrigation had little effect on soil microbial communities, which were mainly affected by the cropping system. In this study, we found that irrigation had a marked effect on soil mycorrhizal and

protozoan communities. The effect was most evident at wheat planting when the cover crop residues had some time to decompose and serve as substrate for the soil microbes. Plant-available water (0–180 cm depth) at wheat planting at the Akron site was 153 mm in the rainfed plots and 212 mm in the irrigated plots. At the Sidney site, plant-available water was 195 and 247 mm in the rainfed and irrigated plots, respectively. The added water would have favored residue decomposition, root proliferation, and microbial growth, and we found that fungi and gram-positive bacteria were most favored by this effect.

The differences in FAME composition between the fallow and cover crop plots at wheat planting were not as marked as those at cover crop termination. The late summer 2012 was extremely dry and warm at both sites, so the rainfed plots had drought conditions that were challenging for plants and soil microbes. Bacterial, fungal, and actinomycete markers were all reduced in the rainfed plots, though not significantly. Several actinomycete genera have been shown to be more resistant to soil desiccation relative to other bacteria (Zenova *et al.*, 2001). Correlation coefficients between cover crop biomass and total FAMEs at wheat planting indicate that the effect of cover crops can last through the late summer plant-free period, especially under drier soil conditions.

At wheat maturity, the total FAMEs had the greatest concentrations indicative of the greatest microbial biomass. By this time, the full growth cycle of the wheat had occurred before the last sampling, and it appears that the long period of plant and root growth favored the soil microbes. Irrigation effects were limited to greater mycorrhizal and eukaryotic protozoan markers with irrigation. The pea cover crop had the most long-lasting positive effect relative to the fallow, with high fungal and eukaryotic markers at wheat maturity although the differences were not statistically significant.

Our results indicate that the effects of the cover crop or fallow treatments did not last beyond the subsequent grain crop in this semiarid environment. Crop management effects due to intensifying rainfed rotations (reducing fallow frequency) have been shown to have a positive effect on enzyme activities at the Akron site (Acosta-Martínez *et al.*, 2007b). The β-glucosidase and phosphodiesterase enzyme activities increased with cropping intensity 15 years after the establishment of different crop rotations relative to the wheat-fallow rotation. This indicates that our experiment, spanning less than two years, might not have been long enough to detect differences in soil enzymes due to cover crops. On the High Plains of Texas, USA, winter cover crops were associated with increased soil enzyme activity within three years (Acosta-Martínez *et al.*, 2011), showing that at other sites with longer growing seasons, cover crops can take a relatively short time to influence soil function and can be used as an effective strategy to influence soil quality.

The Akron site received more irrigation than the Sidney site, which might explain the greater response in soil enzyme activities at the Akron site. Of the three enzymes studied, β-glucosidase had the greatest activity at both sites. β-glucosidase activity has been regarded as a rate-limiting step in cellulose degradation (Turner *et al.*, 2002) and the dynamics of this enzyme are a sensitive indicator of changes in SOM (Monreal and Bergstrom, 2000). Phosphodiesterase hydrolyzes ester bonds in phospholipids and nucleic acids, which are important sources of soil P to plant roots (Nannipieri and Giagnoni, 2011). Our results show that irrigation in a rainfed semiarid setting had a rapid and marked influence on soil functions related to C and P mineralization due to not only the direct effect of water on microbial activity, but also the indirect effect *via* increased primary productivity and possibly root exudation. β-glucosaminidase decomposes chitin, one of the most abundant biopolymers in soil (Parham and Deng, 2000), but we did not observe a significant change in this enzyme during our experiment. β-glucosaminidase activity is affected by land use and management changes (Acosta-Martínez *et al.*, 2007a; Sotomayor-Ramirez *et al.*, 2009). In this study, the lack of irrigation or cover crop effects on β-glucosaminidase could be explained by the addition of N fertilizer and thus the lack of N limitation to soil microbes and plants.

CONCLUSIONS

This study, carried out during a drier-than-average period, allowed comparison of the effects of cover crops under severe water-limited conditions of rainfed environment in the Central Great Plains. The soil enzymes at wheat maturity were more responsive to irrigation than to cover crops. This indicates that in semiarid systems, water was the main factor driving microbial function and community structure in the short term (months and years) through its direct effect on microbes, and also *via* indirect effects due to increased primary productivity. The use of cover crop mixtures did not offer an additional benefit to microbial community composition and microbial activity beyond that of individual cover crops. Cover crops and irrigation were

slow to impact soil C concentration. Our results suggest that in this semiarid environment, longer time spans may have been needed to see beneficial effects of cover crops on soil microbial community structure, soil enzyme activities, and soil C sequestration.

ACKNOWLEDGEMENT

We would like to thank Mr. Jon Cotton, USDA-ARS, USA, Mr. Rob Higgins, University of Nebraska, USA, Mr. Brandon Peterson, USDA-ARS, USA, Mr. Jamie Sauer, Colorado State University, USA, and Mr. Tyler Schumacher, University of Nebraska, USA, for their help with field sampling.

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