

Soil Organic Carbon and Nitrogen Fractions and Sugar Beet Sucrose Yield in Furrow-Irrigated Agroecosystems

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Soil organic matter (SOM) fractions were determined using extraction-, incubation-, and density-based fractionation techniques on samples collected from a range of furrow-irrigated sugar beet (*Beta vulgaris* L.) based rotations on the same soil series on farmers' fields in Wyoming. We hypothesized that extending the period of time between sugar beet crops in rotations beyond the 2-yr sugar beet–barley (*Hordeum vulgare* L.) (SB-BA) rotation by adding perennial or annual legumes would lead to higher levels of surface-soil (0–15-cm) organic C and N. Four rotations were compared: SB-BA, sugar beet–dry bean (*Phaseolus vulgaris* L.) (SB-DB), sugar beet–barley–dry bean (SB-BA-DB), and sugar beet–sugar beet–alfalfa (*Medicago sativa* L.)–alfalfa (SB-SB-Alf-Alf). Soils under SB-BA and SB-DB rotations on average contained 607 g soil organic C (SOC) m⁻² in the upper 15 cm, or 46% of the SOC found within SB-BA-DB and SB-SB-Alf-Alf soils. Potentially mineralizable C and N and microbial biomass C (MBC) were lower in SB-BA and SB-DB soils than SB-BA-DB and SB-SB-Alf-Alf soils, but, when normalized by SOC and total soil N (TSN), these labile C and N fractions were >1.5 times higher in SB-BA and SB-DB soils, suggesting greater SOM mineralization. Moreover, light-fraction C in SB-BA and SB-DB soils was about half that of SB-SB-Alf-Alf soils. Sugar beet sucrose yield was also higher in the SB-SB-Alf-Alf than any other rotation. There were strong linear relationships ($r^2 = 0.50–0.84$) between sugar beet sucrose yield and TSN, SOC, and MBC across all four rotations. To conserve high surface-soil organic C and N fractions on furrow-irrigated farm fields without sacrificing sugar beet sucrose yield, extending the 2-yr SB-BA rotation by adding 2 yr of alfalfa is recommended.

Abbreviations: AMF, arbuscular mycorrhizal fungi; BHB, Big Horn Basin; DOC, dissolved organic carbon; LFC, light-fraction carbon; MBC, microbial biomass carbon; PLFA, phospholipid fatty acid; PMC, potentially mineralizable carbon; PMN, potentially mineralizable N; SB-BA, sugar beet–barley; SB-DB, sugar beet–dry bean; SB-BA-DB, sugar beet–barley–dry bean; SB-SB-Alf-Alf, sugar beet–sugar beet–alfalfa–alfalfa; SOC, soil organic C; TSN, total soil N.

In a semiarid climate, soil disturbance caused by tillage, in conjunction with a lack of diversification of crop rotations, can lead to SOM depletion with time (Hurisso et al., 2013, 2014), underscoring the need for conservation-oriented approaches. Labile SOM fractions such as potentially mineralizable C (PMC), potentially mineralizable N (PMN), and MBC have different turnover times, ranging from days to a few years (Wander, 2004). These biologically active organic matter fractions have been shown to be more responsive to soil management and, consequently, have been put forth as sensitive indicators of disturbance-driven changes in SOM (Biederbeck et al., 1994; Franzluebbers et al.,

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1994, 1995; Wander, 2004). Moreover, light-fraction organic matter, a mineral-free or non-aggregate-protected organic material that is comprised largely of partially decomposed organic residues with a wide C/N ratio (Janzen et al., 1992), has been shown to be a sensitive indicator of SOM dynamics, especially in temperate soils (Alvarez and Alvarez, 2000; Tan et al., 2007). In a dryland wheat (*Triticum aestivum* L.) study in Wyoming, Norton et al. (2012) used ratios of MBC/SOC, PMC/SOC, and PMN/TSN to assess trends in SOM status under a range of management systems differing in disturbance intensity. They interpreted low ratios in soils under reduced tillage practices as indicative of SOC conservation via slowing the turnover of newly added C. Consequently, this study examined the effects that conservation-oriented, irrigated farming practices can have on soils that are low in SOM content and experience frequent mechanical disturbance associated with tillage.

In diversified crop rotations, rhizodeposited C during the course of a growing season and post-harvest crop residues, along with reduced tillage and reduced fallow frequency, generally increase total microbial biomass and shift the community structure toward a more fungal-dominated community (Liebig et al., 2006; Six et al., 2006). Frey et al. (1999) found the proportion of soil (0–5-cm depth) microbial biomass composed of fungi to be higher under reduced than conventional tillage at five sites in the US Great Plains. In a study by Acosta-Martinez et al. (2007) in Colorado, a greater proportion of fungi relative to bacteria at the 0- to 5-cm depth was associated with reduced tillage, diverse crop rotations, and a reduction in fallow frequency. In a Canadian study, Lupwayi et al. (1998) found microbial diversity to be significantly higher under wheat following legume than under continuous wheat or wheat–fallow cropping sequences. Collectively, these studies all discussed the combined effects of crop rotation and tillage-induced disturbance and suggested that changes in management toward increased crop diversity and reduced tillage are vital for SOM conservation and microbial diversity.

Sugar beet accounts for about 35% of global sugar production (FAO, 2013) and is a high-value crop because it provides economic benefits for farmers in temperate climatic regions (Tzilivakis et al., 2005; Stevens et al., 2007). In semiarid climates such as in the Big Horn Basin (BHB) in Wyoming, sugar beet is regularly irrigated and grown in rotation with other crops, primarily barley (Stevens et al., 2007). While cropping systems of the BHB are currently dominated by SB-BA, many farmers use rotation combinations including dry bean and sunflower (*Helianthus annuus* L.), and some include alfalfa crop and other legumes that are commonly grown for seed production. Soils under SB-BA are generally intensively tilled and managed with additional inputs of fertilizers and herbicides as needed. In arid or semiarid regions, the use of irrigation, along with adequate fertilizers and herbicides, can stimulate plant growth, greatly increasing the quantity of organic inputs via crop roots and residues. However, increased mechanical disturbance associated with tillage in recurring SB-BA production can accelerate microbial decomposition of crop residues,

leading to eventual depletion of SOM. While the importance of SOM management for soil fertility and productivity is well documented (Carter, 2002; Wander, 2004), only a few studies have examined the relationship between SOM and crop yields (Bauer and Black, 1994; Diaz-Zorita et al., 1999; Lucas and Weil, 2012; de Moraes Sá et al., 2014); however, all of these studies were conducted in dryland settings.

This on-farm study was designed to evaluate SOC in labile and density-fraction pools as well as sugar beet sucrose yield across a range of furrow-irrigated cropping systems, from a frequently tilled SB-BA rotation to a perennial-based SB-SB-Alf-Alf rotation. We hypothesized that extending the period of time between sugar beet crops in rotations beyond the 2-yr SB-BA rotation cycle by adding perennial or annual legumes would lead to higher levels of surface-soil organic C and N. Better understanding of the long-term effects that conservation-oriented production systems can have on nutrient-poor, semiarid soils will provide a basis for designing management strategies that support the buildup of SOM and soil fertility improvement. While this study offers some insight into how changes in soil and crop management affect SOC in labile and density-fraction pools on furrow-irrigated farm fields, it places more emphasis on overall system-induced differences rather than those of tillage-caused disturbance or inclusion of a legume crop alone.

MATERIALS AND METHODS

Study Site Description

The study was conducted on farmers' fields in the BHB near Powell in northwestern Wyoming (44°45' N, 108°45' W, elevation 1326 m asl), a region characterized by a semiarid climate with a mean 30-yr (1981–2010) annual precipitation of 193 mm and average annual maximum and minimum air temperatures of 15 and –0.9°C, respectively (Western Regional Climate Center, 2014). During this study (2008 and 2009), precipitation and temperature data were collected from a weather station located within 27 km of the study area and maintained by the University of Wyoming Powell Research and Extension Center. The study site is underlain by deep (0–1.0 m), well-drained soils formed in alluvial material from mixed sources and classified as fine-loamy and fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Haplargids belonging to the Garland series (Mukhwana, 2011; Soil Survey Staff, 2012). The site is known to have supported crops such as sugar beet, barley, dry bean, alfalfa, red clover (*Trifolium pratense* L.), and sunflower under furrow irrigation for many years.

Establishment of the Study

At the study site, four crop rotations (SB-BA, SB-DB, SB-BA-DB, and SB-SB-Alf-Alf) with similar landscape settings and slope characteristics were identified using soil surveys (Mukhwana, 2011; Soil Survey Staff, 2012). Soil similarity was confirmed in a 2008 sampling, in which soil properties to a depth of 30 cm were found to be very similar across all four sampling locations (Table 1). For comparative purposes,

Table 1. Site characteristics and selected soil properties (0–30 cm) of farm fields sampled during 2008 and 2009 in the Big Horn Basin, Wyoming.

Rotation	Description	Sand	Silt	Clay	Bulk density	pH	EC†
		g kg ⁻¹ soil			g cm ⁻³		dS m ⁻¹
SB-BA	2-yr, conventional sugar beet–barley rotation managed with furrow irrigation for >50 yr	250	230	520	1.20	7.36	0.19
SB-DB	2-yr, alternative sugar beet–dry bean rotation managed with furrow irrigation for ≥10 yr	120	290	590	0.97	8.07	0.11
SB-DB-BA	3-yr, alternative sugar beet–dry bean–barley rotation managed with furrow irrigation for ≥10 yr	130	290	580	1.27	7.82	0.15
SB-SB-Alf-Alf	4-yr, alternative sugar beet–sugar beet–alfalfa–alfalfa rotation managed with furrow irrigation for ≥10 yr	210	210	580	1.22	8.12	0.14

† Electrical conductivity.

the latter three rotations, each of which contained a legume component and had been implemented more recently (at least 10 yr before this study), were designated “alternative” practices, whereas the SB-BA rotation, which had been practiced for >50 yr by the growers in the BHB, was designated “typical” practice. All of the rotations were managed under furrow irrigation, with all phases of each rotation being present in both 2008 and 2009. Each rotation was implemented in two adjacent strips (~0.22 ha each) with the exception of SB-BA-DB, which was implemented in three adjacent strips alternating among sugar beet, barley, and dry bean crops. In this study, the typical SB-BA rotation was used as a reference by which to evaluate changes in soil C and N fractions as a result of the implementation of alternative cropping practices.

Crop Management

Across all rotations, cultural practices (type of tillage, herbicide and fertilizer use, crop cultivar, seeding rate, residue management, etc.) were very similar for the same crop both years. For example, the sugar beet crop was planted and treated identically in all four rotations. Primary tillage with a moldboard plow and roller-harrow was performed in the fall and was followed by two passes with a roller-harrow and two passes with a leveling blade in the spring. Because alfalfa was grown for two consecutive years in the same field out of every 4 yr, farm fields under the SB-SB-Alf-Alf rotation experienced less mechanical disturbance associated with tillage relative to the shorter 2-yr SB-BA rotation. In the spring of each year, sugar beet received 112 kg N ha⁻¹ as urea and 49 kg P ha⁻¹ as monoammonium phosphate before planting. Sugar beet received additional N and P fertilizers at planting (~10 kg N ha⁻¹ and 34 kg P ha⁻¹). Barley received a preplant application of 90 kg N ha⁻¹ and 34 kg P ha⁻¹. Alfalfa and dry bean crops received 54 kg N ha⁻¹ and 49 kg P ha⁻¹ each.

During the 2 yr of this study, the sugar beet cultivar planted was glyphosate-resistant Hilleshog 9036. The barley cultivars planted were Conrad 5057 in 2008 and Moravian 69 in 2009 based on seed availability. Sugar beet was planted in 56-cm rows on elevated beds, whereas barley was planted in 15-cm rows. The seeding rates were 2 kg ha⁻¹ for sugar beet, 126 kg ha⁻¹ for barley, 84 kg ha⁻¹ for dry bean, and 17 kg ha⁻¹ for alfalfa in both

2008 and 2009. Sugar beet was irrigated as many as 10 times throughout the growing season (mid-April–end of September), with the first irrigation occurring soon after planting to initiate germination. Barley was irrigated five to six times as needed during the growing season (late May–mid-July). Weeds were adequately controlled with conventional practices. Grain from barley and dry bean was harvested each year, and the aboveground residue from each crop was incorporated into the corresponding fields. After harvesting seeds from an alfalfa crop at the end of the second year, the crop was terminated with glyphosate [isopropylamine *N*-(phosphonomethyl) glycine] herbicide before seedbed preparation and tillage for sugar beet.

Sugar Beet Harvesting and Determination of Sucrose Yield

Sugar beet roots were harvested on 7 Oct. 2009 by hand digging 3.0 m of three center rows at each sampling area. The aboveground biomass was cut at the soil surface, weighed, and dried at 65°C for 48 h. Harvested roots were analyzed for sucrose content and impurities by Western Sugar Cooperative. Sugar beet sucrose yield was estimated by multiplying the sucrose content by the fresh weight of the harvested roots and corrected for impurities.

Soil Sampling and Analysis

We used the same experimental approach as Norton et al. (2012) for soil sampling. Briefly, the soil was sampled in 2008 and 2009 in the sugar beet phase of each rotation at three time points (spring, summer, and fall). Three rectangular plots, which were 225 m² in size, were established in the center of each sugar beet field and considered as replicates for statistical purposes ($n = 3$). It should be noted that these replicates within a specific rotation were within the same experimental unit (i.e., a sugar beet field within that rotation) and, as such, these are not statistically independent, true replicates. In on-farm studies, however, it is a common practice to use pseudo-replication to overcome a lack of replication (e.g., Blanco-Canqui and Lal, 2008; Christopher et al., 2009; Lucas and Weil, 2012). In July 2008, three soil cores (3.2-cm diam. by 30-cm depth) were collected per plot and used for bulk density measurements (Blake and Hartge, 1986). In each plot, five additional soil cores were collected to a 30-cm depth

at about 2-m intervals, divided into 0- to 15- and 15- to 30-cm increments, and sealed in separate plastic bags. All soil samples were placed in a cooler with frozen ice packs immediately following collection and returned to the laboratory for further sample processing. Once in the laboratory, soil moisture was determined gravimetrically (105°C for 24 h). The remaining soil was stored at 4°C until subsequent analyses.

Labile C and N fractions (dissolved organic C [DOC], MBC, PMC, and PMN) were measured in soils collected on the three sampling dates (spring, summer, and fall) in 2008 and 2009. Microbial biomass was measured using the chloroform fumigation–extraction method (Horwath and Paul, 1994). Briefly, two 10-g subsamples of field-moist soil that had been stored at 4°C were removed from each sample: one set was fumigated with ethanol-free chloroform for 48 h and the other was used as an unfumigated control. Both fumigated and unfumigated samples were extracted with 50 mL of 0.5 mol L⁻¹ K₂SO₄ by shaking them for 1 h on a reciprocal shaker and filtering through Fisherbrand Q5 filter paper (Thermo Fisher Scientific). Extracts were stored in the freezer at -20°C until they were analyzed on a UV persulfate TOC analyzer (Phoenix 8000, Tekmar Dohrmann). Microbial biomass C was calculated by dividing the difference in C contents of the fumigated and unfumigated samples by a correction factor of 0.35 (Voroney et al., 1991). Dissolved organic C (i.e., SOM that is <45 μm in solution and is extractable in dilute salt solutions [Marschner and Bredow, 2002; Wander, 2004]) was estimated from the quantity of soluble C measured in extracts from the unfumigated samples. These same extracts from the unfumigated samples were also used for the determination of initial inorganic soil N (NO₃-N and NH₄-N) before the incubations. Concentrations of NO₃-N (Doane and Horwath, 2003) and NH₄-N (Weatherburn, 1967) were determined on a microplate spectrophotometer (BioTek, Inc.).

Potentially mineralizable C and N were estimated from the quantities of CO₂-C and NO₃-N plus NH₄-N, respectively, mineralized from the soil during a short-term aerobic incubation (Zibilske, 1994). Briefly, the amount of water needed to bring soils to 50% of their water-filled pore space was determined gravimetrically before the incubation. Subsamples (22 g) of the field-moist soil were weighed into 50-mL specimen cups and placed inside 1-L Mason jars, deionized (DI) water was added to the soil, and the jars were capped tightly and incubated at 25°C in the dark for 2 wk. The CO₂-C concentration was determined by sampling 30 mL of air from the headspace at Days 1, 7, and 14 and injecting it into a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences). Potentially mineralizable C during the 2-wk incubation period was determined by summing the CO₂-C concentrations at the three samplings. At the end of the incubation, each soil sample was divided into two sets: one set was used for determination of gravimetric soil moisture content and the other was extracted with 0.5 mol L⁻¹ K₂SO₄, using the same extraction method as for the fumigated and unfumigated samples described above. Extracts were stored in the freezer at

-20°C until they were analyzed for NO₃-N and NH₄-N as described above. Potentially mineralizable N was calculated as the difference between the incubated and initial soil inorganic N (NO₃-N + NH₄-N) (Hart et al., 1994). Results for labile C and N pools were expressed on an oven-dry basis (105°C for 24 h).

The remaining soil was air dried, passed through a 2-mm sieve, and stored at room temperature for later analysis of the other parameters described below. Analysis of soil texture, SOC, light-fraction C (LFC), TSN, pH, and electrical conductivity (EC) was performed on the summer 2008 samples only. Subsamples of air-dried soil were finely ground and analyzed for total C and N by combustion using a Carlo Erba EA1100 CN elemental analyzer (Carlo Erba). Inorganic C was determined by a modified pressure-calculator (Sherrod et al., 2002) and subtracted from total C to calculate SOC. The light fraction was separated from the soil using a density-based method (Sohi et al., 2001). Briefly, subsamples of 6 g of rewetted soil were weighed into 50-mL polycarbonate centrifuge bottles. To each bottle, 35 mL of NaI solution (density = 1.8 g cm⁻³) was added and the bottles were shaken gently by hand for 30 s and then centrifuged at 2000 rpm for 15 min, after which floating light-fraction material was removed using a pipette and rinsed through a 5-μm filter with DI water into petri dishes. The light-fraction material was dried at 65°C for 24 h, finely ground, and analyzed for total C concentration as described above. Soil texture was determined by the hydrometer method (Gee and Bauder, 1986), and pH and EC were measured in a 1:1 soil/water slurry by electrode (Thomas, 1996). None of the C and N pools was corrected by the equivalent soil mass because no evidence for differences in soil bulk density was found among the four crop rotations we sampled.

Phospholipid Fatty Acid Analysis

In July 2009, three additional soil cores were collected (to the 5-cm depth) per plot for phospholipid fatty acid (PLFA) analysis, and soil samples were stored in the freezer at -20°C before extraction. Phospholipid fatty acids were extracted using a single-phase chloroform, methanol, phosphate buffer solution (1:2:0.8, pH 7.4) according to the modified Bligh and Dyer (1959) procedure described by Frostegård et al. (1991) and Buyer et al. (2002). In brief, fatty acids were separated on a solid-phase extraction column (Agilent Technologies), phospholipids were then methylated, and the resulting fatty acid methyl esters were separated on an Agilent 6890 gas chromatograph (Agilent Technologies) equipped with a flame ionization detector. The peaks of individual PLFAs were identified using MIDI identification software (MIDI Inc.). The PLFAs a15:0, i15:0, 15:0, i16:0, 16:1ω7c, 16:1ω9, i17:0, 17:0, cy17:0, 18:1ω9c, and cy19:0 were used as bacterial biomarkers (Frostegård and Bååth, 1996). The PLFAs i15:0, a15:0, i16:0, and i17:0 were used as biomarkers for Gram-positive bacteria, whereas PLFAs cy17:0, 18:1ω9c, and cy19:0 were used as biomarkers for Gram-negative bacteria (Zak et al., 1996). The

PLFA 18:2 ω 6 was used as a biomarker for saprotrophic fungi (Frostegård and Bååth, 1996), whereas the PLFA 16:1 ω 5c was used as a biomarker for arbuscular mycorrhizal fungi (AMF) (Olsson, 1999). The PLFA 20:4 ω 6 was used as a biomarker for protozoa (Stout, 1980).

Statistical Analysis

Differences in soil variables among rotations were compared separately for each sampling depth using a one-way analysis of variance approach to a completely randomized design with the PROC GLM procedure. Likewise, differences in PLFA concentration and sugar beet sucrose yield among rotations were tested using one-way analysis of variance using the PROC GLM procedure. For labile C and N data measured multiple times during the growing season, the date of sampling (spring, summer, or fall) was treated as a repeated measures factor, and analysis of variance was performed using the REPEATED option of PROC MIXED. None of the labile C and N fractions was significantly different between years (2008 vs. 2009), nor was an interaction between year and rotation regardless of sampling depth. Thus, data for each labile C and N fraction were averaged across the 2 yr, and the statistical analysis was rerun without the effect of year. When a significant rotation \times sampling date interaction was observed, the data are presented to reflect a rotation \times sampling date interaction only. Means were separated using a least square means test, with significant differences determined at $\alpha = 0.05$; however, differences at $\alpha = 0.10$ are also acknowledged. The relationships between sugar beet sucrose yield and labile C and N fractions, LFC, SOC, and TSN were assessed with correlation and stepwise multiple regression analyses using the PROC CORR and PROC REG procedures, respectively. For stepwise multiple linear regression, the significance level for each soil variable to enter the model and stay in the model was set at $\alpha = 0.05$. All analyses were conducted using SAS Version 9.3 (SAS Institute).

RESULTS

Climate and Sugar Beet Sucrose Yield

Annual average temperatures in 2008 and 2009 (5.7–6.0°C) were close to the 30-yr average (6.8°C; data not shown). Annual precipitation in 2008 and 2009 was within 70 to 79% of the 30-yr historical average (408 mm; Fig. 1). Sugar beet sucrose yield measured in 2009 was significantly higher (by 28–42%) in the SB-SB-Alf-Alf rotation than the other rotations (SB-BA, $P = 0.041$; SB-DB, $P = 0.034$; and SB-BA-DB, $P < 0.017$; Fig. 2). Likewise, sugar beet root dry matter yield was greatest in the SB-SB-Alf-Alf rotation (73.1 Mg ha⁻¹), lowest in the typical SB-BA rotation (50.8 Mg ha⁻¹), and intermedi-

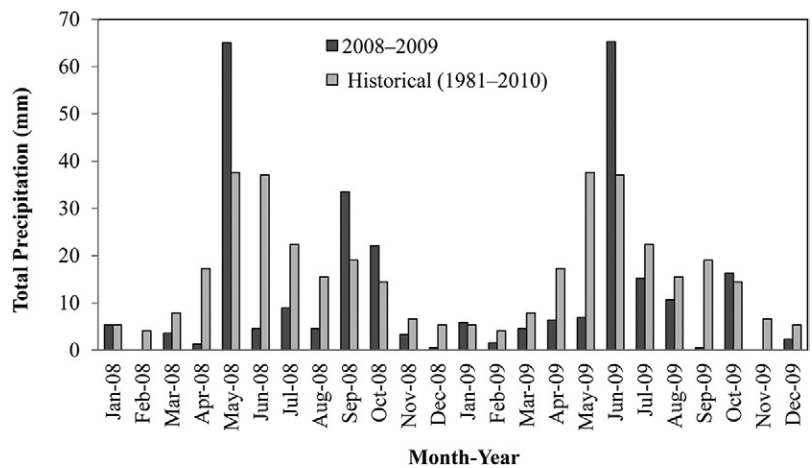


Fig. 1. Monthly total precipitation during the study period (2008–2009) and 30-yr (1981–2010) historical averages recorded at the weather station located within 27 km of the study near Powell, WY.

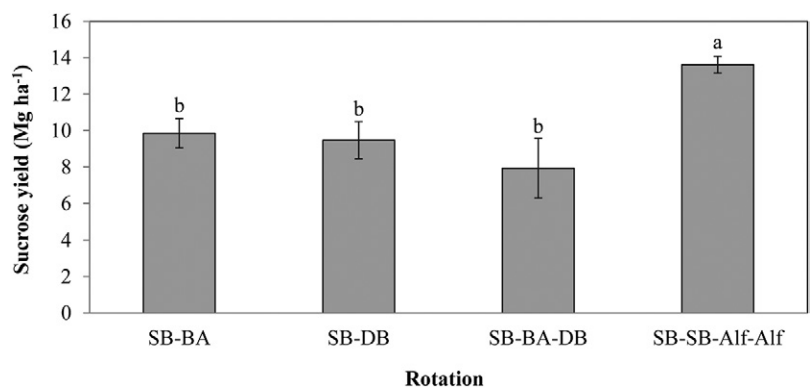


Fig. 2. Sugar beet sucrose yields (adjusted based on the amount of impurities in harvested roots) measured in 2009 under four furrow-irrigated sugar beet based rotations: sugar beet–barley (SB-BA), sugar beet–dry bean (SB-DB), sugar beet–barley–dry bean (SB-BA-DB), sugar beet–sugar beet–alfalfa–alfalfa (SB-SB-Alf-Alf). Mean values with different letters are significantly different at the $P < 0.05$ level. Error bars represent the standard error of the mean ($n = 3$).

ate in SB-DB and SB-BA-DB rotations (64.8–67.4 Mg ha⁻¹; data not shown).

Soil Organic Carbon, Light Fraction Carbon, and Total Soil Nitrogen

Soil organic C was significantly impacted by crop rotation in the 0- to 15-cm depth ($P = 0.004$; Fig. 3a), where SOC contents in soils under the SB-SB-Alf-Alf rotation were higher than those under the SB-BA and SB-DB rotations by 2.2 to 2.3 times but statistically similar to soils under the SB-BA-DB rotation. A similar trend was observed for LFC at the 0- to 15-cm depth, where soils under the SB-SB-Alf-Alf rotation had the largest LFC values, the SB-BA-DB rotation had intermediate values, and the SB-BA and SB-DB rotations had the lowest values (Fig. 4). There were no significant differences in SOC contents among crop rotations in the 15- to 30-cm depth ($P = 0.205$). Trends in TSN contents in the 0- to 15-cm depth ($P < 0.007$; Fig. 3b) were similar to those observed for SOC contents, with TSN in soils under the SB-SB-Alf-Alf rotation

being higher by 1.5 to 3.5 times that found in soils of the other three rotations.

Labile Carbon and Nitrogen Fractions

At the 0- to 15-cm depth, DOC contents were not significantly influenced by rotation, sampling date, or rotation

× sampling date interaction (Table 2). When normalized by SOC, however, DOC contents in soils under SB-BA and SB-DB rotations were 1.6 to 1.8 times higher than those under SB-BA-DB and SB-SB-Alf-Alf rotations (Fig. 5a; Table 2). A similar trend was seen for MBC when normalized by SOC, with soils under SB-BA and SB-DB rotations having twice as

much MBC as soils under SB-BA-DB and SB-SB-Alf-Alf rotations (Fig. 5b; Table 2). Regardless of sampling depth, MBC as well as MBC as a proportion of SOC also varied significantly by sampling date, with the greatest values occurring in summer compared with spring or fall when averaged across rotations (data shown only for the 0–15-cm depth in Fig. 6a and 6b; Table 2). In the 15- to 30-cm depth, MBC and PMC values followed the order SB-SB-Alf-Alf > SB-BA-DB ≥ SB-BA > SB-DB (data not shown).

In the 0- to 15-cm depth, there was a significant rotation × sampling date interaction for PMC as well as PMC as a proportion of SOC (Table 2). Soils under the SB-SB-Alf-Alf rotation contained 1.3 to 1.9 times higher PMC in spring and fall compared with those under the other rotations (Fig. 7a). Soils under the three alternative rotations did not differ in PMC contents in summer, but all of them had significantly higher PMC values than soils under the SB-BA rotation. When normalized by SOC, however, soils under SB-BA and SB-DB rotations had 1.3 to 1.6 times higher PMC contents in spring and fall than soils under SB-SB-Alf-Alf and SB-BA-DB rotations (Fig. 7b). For the summer sampling date, PMC contents when normalized by SOC decreased across the four rotations in the order SB-DB > SB-BA ≥ SB-BA-DB ≥ SB-SB-Alf-Alf.

As was the case for PMC, the rotation × sampling date interaction was significant for PMN as well as PMN as a proportion of TSN regardless of sampling depth (Table 2). Potentially mineralizable N values in spring and summer decreased across the four rotations in the order SB-DB ≥ SB-SB-Alf-Alf > SB-BA-DB ≥ SB-BA, with no significant differences among any of the rotations in the fall sampling date (data shown only for the 0–15-cm depth in Fig. 7c). Overall, an opposite trend was noted when PMN was normalized by TSN, where soils under the SB-SB-Alf-Alf and SB-BA-DB rotations had lower PMN contents in spring and summer (1.5–3.2%) than soils under SB-DB and SB-BA rotations (4.4–6.7%; Fig. 7d). There were no significant differences in PMN values across rotations for the fall sampling date, but when normalized by TSN, soils under SB-SB-Alf-Alf rotations had lower PMN content (1.2%) than soils under SB-DB and SB-BA

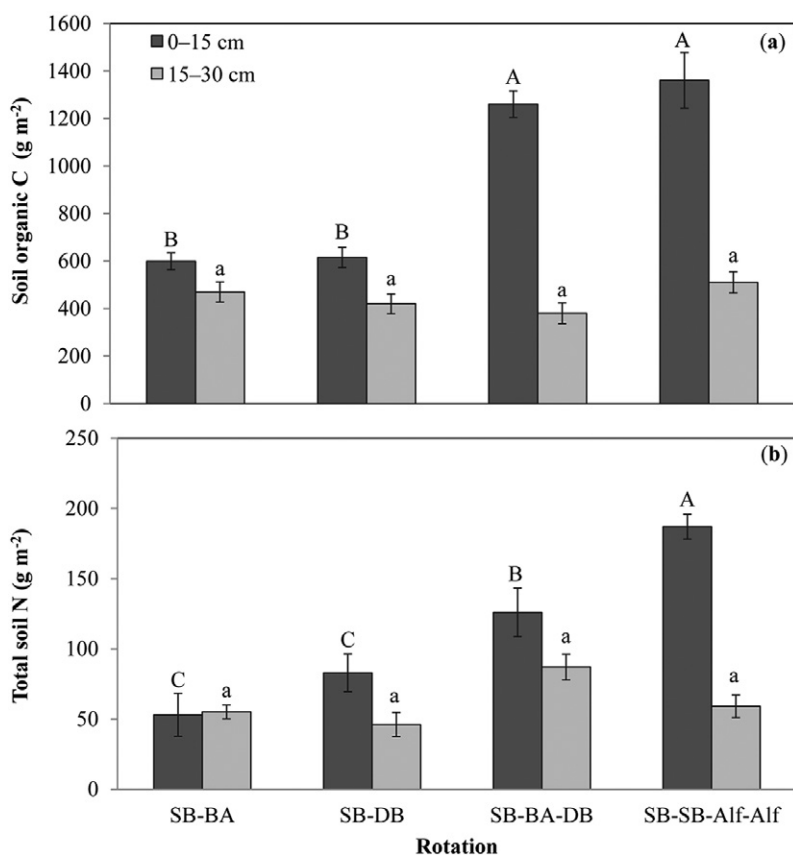


Fig. 3. (a) Soil organic C and (b) total soil N for the two sampling depths under four furrow-irrigated sugar beet based rotations: sugar beet–barley (SB-BA), sugar beet–dry bean (SB-DB), sugar beet–barley–dry bean (SB-BA-DB), sugar beet–sugar beet–alfalfa–alfalfa (SB-SB-Alf-Alf). Values with different uppercase (0–15 cm) and lowercase (15–30 cm) letters are significantly different at the $P < 0.05$ level. Error bars represent the standard error of the mean ($n = 3$).

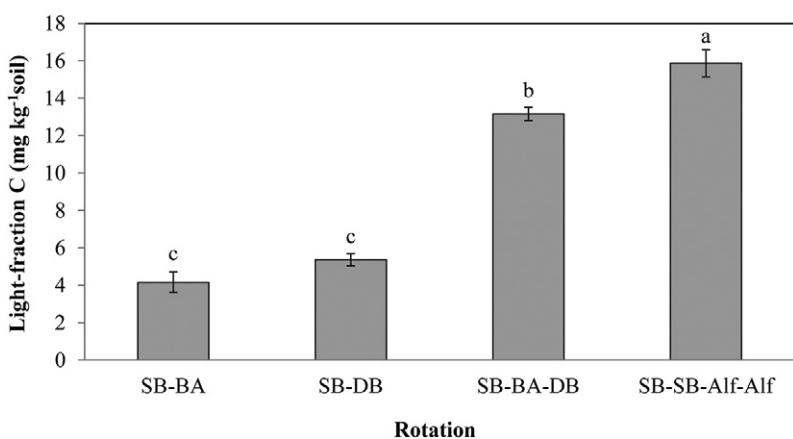


Fig. 4. Carbon contents in the light fraction of soils (0–15 cm) under four furrow-irrigated sugar beet based rotations: sugar beet–barley (SB-BA), sugar beet–dry bean (SB-DB), sugar beet–barley–dry bean (SB-BA-DB), sugar beet–sugar beet–alfalfa–alfalfa (SB-SB-Alf-Alf). Values with different letters are significantly different at the $P < 0.05$ level. Error bars represent the standard error of the mean ($n = 3$).

rotations (2.9–3.3%) and were similar to soils under the SB-BA-DB rotation (2.2%; Fig. 7d).

Microbial Community Structure

Soil (0–5 cm) sampled in 2009 showed minimal differences among microbial communities across the four crop rotations (Table 3). The concentration of the AMF biomarker PLFA (i.e., 16:1 ω 5c) was 1.6 to 1.9 times higher in soils under SB-BA-DB and SB-SB-Alf-Alf rotations than that measured in soils under SB-BA and SB-DB rotations ($P < 0.07$). The concentration of Gram-positive bacteria PLFAs was 1.4 to 1.7 times higher in soils under the SB-SB-Alf-Alf rotation than that measured in soils under the other three rotations ($P < 0.09$). Soils under SB-BA and SB-DB rotations had 1.3 to 1.7 times higher concentrations of the protozoa PLFA (i.e., 20:4 ω 6) than those under SB-BA-DB and SB-SB-Alf-Alf rotations ($P < 0.09$).

Correlations of Soil Parameters with Sugar Beet Sucrose Yield

Correlation analysis revealed that many of the measured soil properties were related to each other and to sucrose yield (Table 4). Soil organic C was strongly and positively related to LFC, with TSN, PMC, and DOC ranking second, third, and fourth behind LFC in terms of the strength of the relationship. Of all the measured soil properties, TSN and LFC were more strongly and positively related to sucrose yield than any other variable. Potentially mineralizable N as a proportion of TSN was negatively related to SOC but weakly related to sucrose yield. Stepwise multiple regression, which was used to determine the ability of a combination of variables to predict sucrose yield, selected TSN, MBC, and SOC as the best predictors in the final model (Fig. 8). None of the microbial biomarker PLFAs was related to soil measures or sucrose yield except for the AMF biomarker PLFA, which was weakly related to some of the labile SOM fractions.

DISCUSSION

This study was designed to assess whether extended sugar beet based irrigated rotations that include perennials such as alfalfa could increase SOM within soils that had a long history of sugar beet production in a shorter, 2-yr rotation with annuals such as barley and experience frequent mechanical disturbance associated with tillage. The results presented here support this hypothesis and demonstrate that the production of sugar beet in extended rotations resulted in the preservation of organic C and N fractions and a buildup of SOM without a decline in sucrose yield. Overall, our findings agree with a number of studies that have documented increased SOC contents in agroecosystems with rotational diversity and reduced till-

Table 2. Analysis of variance tests of significance for the response of labile C and N fractions to crop rotation and sampling date main effects and rotation \times sampling date interaction term for the two sampling depths.

Dependent variable†	P value		
	Rotation	Sampling date	Rotation \times sampling date
	0–15 cm		
DOC	0.276	0.408	0.739
(DOC/SOC)100	0.027	0.502	0.614
MBC	0.343	0.001	0.827
(MBC/SOC)100	0.002	0.001	0.743
PMC	0.043	0.226	0.019
(PMC/SOC)100	0.007	0.166	0.002
PMN	0.091	0.081	0.025
(PMN/TSN)100	0.053	0.126	0.084
	15–30 cm		
DOC	0.488	0.321	0.997
(DOC/SOC)100	0.414	0.304	0.994
MBC	0.002	0.006	0.853
(MBC/SOC)100	0.264	0.005	0.743
PMC	0.006	0.583	0.072
(PMC/SOC)100	0.309	0.641	0.067
PMN	0.150	0.305	0.026
(PMN/TSN)100	0.122	0.233	0.023

† DOC, dissolved organic C; MBC, microbial biomass C; PMC, potentially mineralizable C; PMN, potentially mineralizable N; SOC, soil organic C; TSN, total soil N.

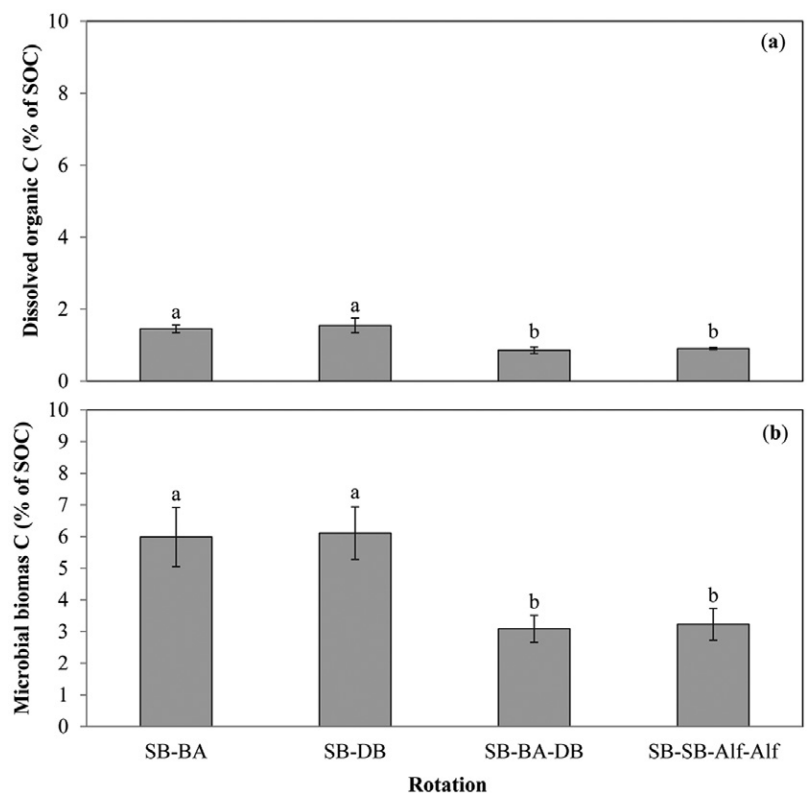


Fig. 5. (a) Dissolved organic C and (b) microbial biomass C expressed as a proportion of soil organic C (SOC) in soils (0–15 cm) under four furrow-irrigated sugar beet based rotations averaged across sampling dates: sugar beet–barley (SB-BA), sugar beet–dry bean (SB-DB), sugar beet–barley–dry bean (SB-BA-DB), sugar beet–sugar beet–alfalfa–alfalfa (SB-SB-Alf-Alf). Values with different letters are significantly different at the $P < 0.05$ level. Error bars represent the standard error of the mean ($n = 6$).

age practices (Huggins et al., 2007; Dou et al., 2008; Norton et al., 2012; Ghimire et al., 2014). Previous studies have suggested that changes in SOC continue to occur for several decades, the most dramatic of which occur during the first 10 yr following changes in management (Davidson and Ackerman, 1993; Norton et al., 2012; Hurisso et al., 2013). The four rotations evaluated in this study reflect the progression of furrow-irrigated farming practices from shorter 2-yr SB-BA production, which had been practiced for >50 yr in the BHB, to the relatively recent adoption of perennial or annual legume-based extended rotations for 10 yr or more. Therefore, evaluation of these farming practices after at least 10 yr of practice adoption is perhaps more relevant for better understanding of their potential impacts on long-term soil fertility and crop productivity in the region.

Below we briefly discuss SOM loss due to more frequent production of sugar beet, as is represented by the 2-yr SB-BA and SB-DB rotations, followed by the potential positive impacts of extended rotations and the relationships between soil measures and sugar beet sucrose yield. It should be noted that significant differences in SOC among rotations were observed only in the upper 15 cm (Fig. 3). While significant differences in some labile C and N fractions among rotations existed be-

low 15 cm (Table 2), they essentially mirrored those observed in the upper 15 cm. Thus to simplify our discussion, we have focused on the 0- to 15-cm sampling depth.

Organic Carbon and Nitrogen Fractions under Sugar Beet Based Rotations Shorter (Two-Year) Rotations

In this study, soils under SB-BA and SB-DB rotations on average contained 703 g m⁻², or 54%, less SOC than did soils under SB-BA-DB and SB-SB-Alf-Alf rotations (Fig. 3), indicating a loss of SOM associated with frequent production of sugar beet. Interestingly, despite notably having lower mean DOC and MBC values (data not shown), soils under SB-BA and SB-DB rotations had higher DOC/SOC and MBC/SOC ratios averaged across all sampling dates (Fig. 5; Table 2). These higher ratios in soils under SB-BA and SB-DB rotations may be due in part to disturbance-driven organic matter mineralization (Dou et al., 2008; Norton et al., 2012), especially given the intensive tillage associated with frequent production of sugar beet in shorter 2-yr rotations with annuals such as barley or dry bean compared with sugar beet produced in extended rotations that include an alfalfa crop. The lower concentration of the AMF biomarker PLFA (16:1 ω 5c) in soils under SB-

BA and SB-DB rotations relative to the perennial-based SB-SB-Alf-Alf rotation (Table 3) further supports this suggestion. This is because fungi, and AMF in particular, appear to be extremely sensitive to management practices (Allison et al., 2005) such as mechanical disturbance associated with tillage, which can directly affect AMF by disrupting hyphal networks (Wortmann et al., 2008; Helgason et al., 2010).

Larger MBC values and higher MBC/SOC ratios for the summer sampling date averaged across rotations (Fig. 6) demonstrate the large influence that soil temperatures have on mineralizing labile organic inputs. When the time period of practice adoption is factored in (50 vs. 10 yr for SB-BA and SB-DB rotations, respectively; Table 1), the lack of differences in SOC between these two rotations suggests that the production of sugar beet in a shorter 2-yr rotation with DB appeared to substantially accelerate the loss of SOM compared to sugar beet produced with barley. This generally agrees with observations of Huggins et al. (2007), who found a corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] sequence to have lower SOC contents (0–7.5 cm)

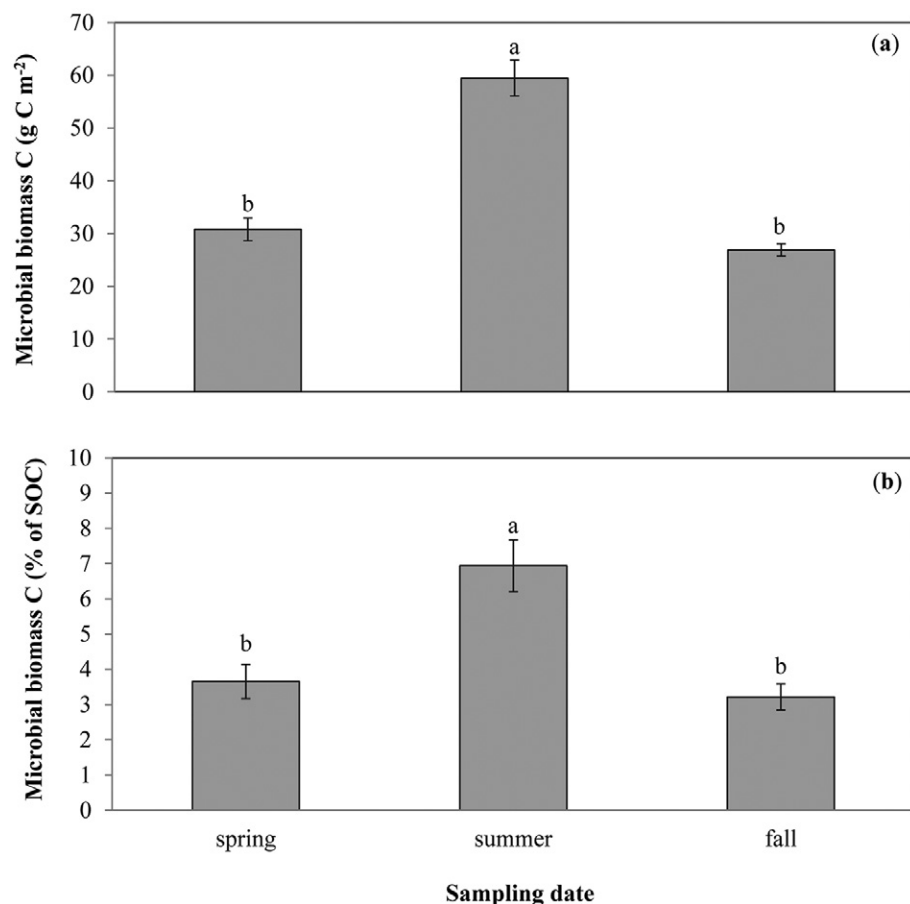


Fig. 6. (a) Microbial biomass C and (b) microbial biomass C expressed as a proportion of soil organic C (SOC) in the 0- to 15-cm depth at three sampling dates averaged across rotations. Values with different letters are significantly different ($P < 0.05$) among sampling dates. Error bars represent the standard error of the mean ($n = 6$).

than continuous corn. They attributed the lower SOC levels in the corn–soybean sequence to both tillage and lower residue differences with continuous corn.

Extended (Three- and Four-Year) Rotations

Soils under SB-BA-DB and SB-SB-Alf-Alf rotations on average contained 1310 g SOC m⁻² (Fig. 3), which was 54% more SOC than that found in soils under SB-BA and SB-DB rotations, thus highlighting the potential for diverse and perennial-based rotations to significantly and positively impact long-term SOM storage (Huggins et al., 2007; Sainju and Lenssen, 2011). The PMN/TSN ratio averaged across sampling dates followed the order SB-DB (5.4%) ≥ SB-BA (4.7%) > SB-BA-DB (2.3%) ≥ SB-SB-Alf-Alf (2.1%; Fig. 7d). This indicates that rates of N mineralization and hence N availability became progressively lower, most notably in soils under SB-BA-DB and SB-SB-Alf-Alf rotations. This further suggests that soils under extended sugar beet based rotations appeared to have “tightly coupled” or conservative nutrient cycling similar to grassland soils, where diverse and growing microbial communities immobilize N as fast as it mineralizes from decomposing SOM (Norton et al., 2004). A higher fungal/bacterial ratio, while not statistically significant, along with higher concentrations of AMF and Gram-positive bacteria biomarker PLEAs in soils under SB-BA-DB and SB-SB-Alf-Alf rotations (Table 3), might therefore indicate that a trend toward increasing C inputs was beginning to appear under more diverse and extended rotations (Frostegård and Bååth, 1996; Lupwayi et al., 1998; Frey et al., 1999; Liebig et al., 2006; Six et al., 2006; Acosta-Martínez et al., 2007). Findings by Drinkwater et al. (1998) suggested that greater temporal diversity in cropping sequences can substantially increase the retention of soil C and N in legume-based systems, as indicated by our findings (Fig. 3 and 4).

It should be noted that soils under the SB-SB-Alf-Alf rotation in this study experienced only minimum mechanical disturbance associated with tillage relative to those under SB-BA and SB-DB rotations because farm fields under an SB-SB-Alf-Alf rotation are typically not tilled in 2 out of every 4 yr, when the alfalfa crop is allowed

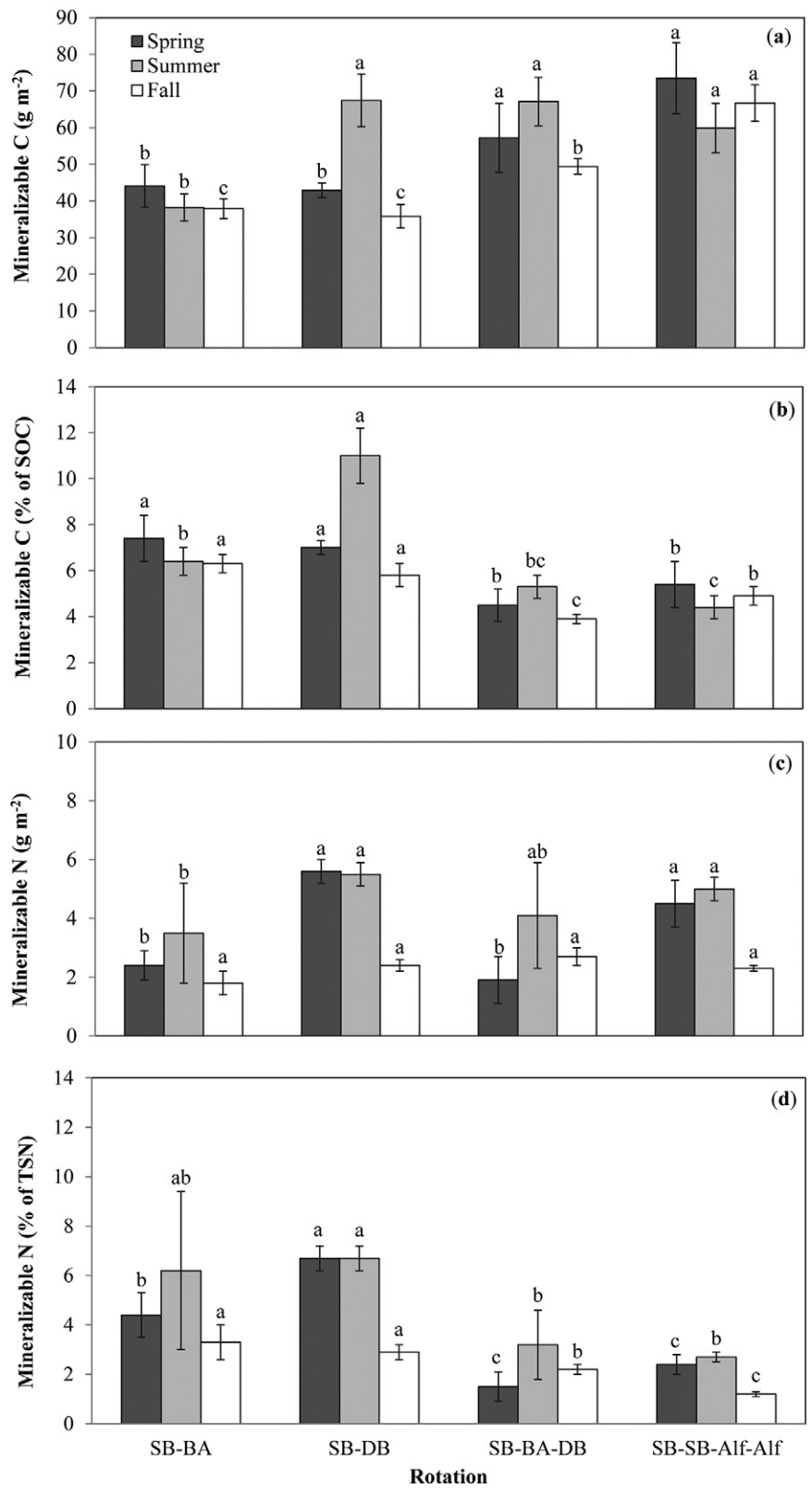


Fig. 7. (a) Potentially mineralizable C (PMC), (b) PMC expressed as a proportion of soil organic C (SOC), (c), potentially mineralizable N (PMN), and (d) PMN expressed as a proportion of total soil N (TSN) in soils (0–15 cm) under four furrow-irrigated sugar beet based rotations by sampling date: sugar beet–barley (SB-BA), sugar beet–dry bean (SB-DB), sugar beet–barley–dry bean (SB-BA-DB), sugar beet–sugar beet–alfalfa–alfalfa (SB-SB-Alf-Alf). Values with different letters in the same sampling date are significantly different ($P < 0.05$) among rotations. Error bars represent the standard error of the mean ($n = 6$).

Table 3. Microbial community structure based on phospholipid fatty acids analysis from surface soil (0–5 cm) collected in 2009 under four furrow-irrigated sugar beet based rotations. Values in parentheses represent the standard error of the mean ($n = 3$).

Rotation†	Phospholipid fatty acids						
	Total bacteria	Gram-positive bacteria	Gram-negative bacteria	AMF‡	Saprotrophic fungi	Protozoa	Fungal/bacterial ratio
	nmol g ⁻¹ soil						
SB-BA	23.0 (1.5) a§	13.2 (1.3) b	9.82 (0.9) a	2.33 (0.4) b	3.45 (0.8) a	3.23 (0.4) a	0.25 (0.02) a
SB-DB	21.6 (1.3) a	14.4 (1.4) b	7.23 (1.3) a	2.65 (0.4) b	3.28 (0.5) a	3.67 (0.6) a	0.27 (0.01) a
SB-DB-BA	23.6 (1.2) a	15.2 (1.1) b	8.43 (1.0) a	4.21 (0.5) a	4.58 (0.8) a	2.21 (0.4) b	0.37 (0.02) a
SB-SB-Alf-Alf	32.1 (2.2) a	21.8 (1.4) a	10.3 (1.7) a	4.38 (0.7) a	5.71 (1.4) a	2.58 (0.6) b	0.31 (0.02) a
LSD	11.1	6.02	3.42	1.83	2.47	1.06	0.18

† SB-BA, sugar beet–barley; SB-DB, sugar beet–dry bean; SB-BA-DB, sugar beet–barley–dry bean; SB-SB-Alf-Alf, sugar beet–sugar beet–alfalfa–alfalfa.

‡ AMF, arbuscular mycorrhizal fungi.

§ Means with standard errors in parentheses. Means followed by the same letter within each column are not significantly different ($P < 0.05$).

Table 4. Correlation coefficients (r) between selected soil measures and sugar beet sucrose yield across four furrow-irrigated sugar beet based rotations ($n = 12$).

Dependent variable†	SOC	Sucrose yield	AMF	(PMN/TSN)100	PMC	MBC	DOC	LFC
TSN	0.90**	0.92**	0.55‡	–0.66*	0.86**	0.54‡	0.65*	0.93**
LFC	0.94**	0.81**	0.51	–0.65*	0.76**	0.51‡	0.66*	
DOC	0.60*	0.71*	0.36	–0.14	0.65*	0.73**		
MBC	0.43	0.72*	0.54‡	–0.28	0.48			
PMC	0.78**	0.77**	0.50	–0.62*				
(PMN/TSN)100	–0.73**	–0.51‡	–0.51‡					
AMF	0.44	0.43						
Sucrose yield	0.71*							

* Significant at the α value of 0.05.

** Significant at the α value of 0.001.

† AMF, arbuscular mycorrhizal fungi; DOC, dissolved organic C; LFC, light fraction C; MBC, microbial biomass C; PMC, potentially mineralizable C; PMN, potentially mineralizable N; SOC, soil organic C; TSN, total soil N.

‡ Significant at the α value of 0.10

to grow for seed production. Thus, the higher levels of SOC we observed in soils under the SB-SB-Alf-Alf rotation (Fig. 3 and 4) appeared to be due to a combined effects of reduced tillage and the relatively large quantity of organic inputs (from both aboveground residues and roots) associated with alfalfa relative to soils under SB-BA and SB-DB rotations, in which frequent tillage accelerates rates of decomposition of the vast majority of labile organic C and N fractions. Previous studies have shown plant roots to be the main drivers of SOC accumulation (Rasse et al., 2005; Kramer et al., 2010). Although our study did not document root biomass, findings by Sainju and Lenssen (2011) showed that continuous alfalfa had larger root biomass and SOC values (0–15 cm) than durum (*Triticum turgidum* L.)–barley sequences. In minimally disturbed soils such as those under reduced tillage practices, SOC accumulation is primarily attributed to an increase in C concentration in the light fraction (Tan et al., 2007). For example, findings by Janzen et al. (1992) indicated that the light fraction accounted for 2 to 17.5% of the total SOC (0–7.5 cm), which was greater in rotations that included perennial forages than wheat–fallow. The larger LFC values that we observed in soils under the SB-SB-Alf-Alf rotation (Fig. 4), therefore, suggest increasing input of organic residues relative to those under SB-BA and SB-DB

rotations. Finally, because PMC is a laboratory incubation mediated by microbial biomass and activity, it reflects both the amount and availability of C. Therefore, high levels of PMC measured within soils under the SB-SB-Alf-Alf rotation on all three sampling dates (Fig. 7a) might indicate continued litter inputs from the alfalfa crop.

Sugar Beet Sucrose Yield and Soil Properties

The differences in sugar beet sucrose yield among rotations (Fig. 2) roughly resembled the differences we observed for SOC in the upper 15 cm of the soil. The findings of Wilson et al. (2001) indicated a positive effect on sucrose yields of extending the period of time between sugar beet crops in rotations by adding perennial forage legumes. Specifically, they found sugar beet grown following 5 yr of alfalfa to have a higher sucrose yield than sugar beet grown following other annual crops in Nebraska, supporting our findings (Fig. 2). Despite clear similarities between our findings and those of Wilson et al. (2001), the factors responsible for increased sucrose yield are difficult to predict. Previous studies have shown that excess mineral N toward the end of the growing season reduces sucrose content by stimulating vegetative growth (Malnou et al., 2008; Stevanato et al., 2010). Agroecosystems with increased SOC stocks tend to create a more

immobilizing, N-limited microbial environment (Norton et al., 2012; Drinkwater et al., 1998). Nitrogen immobilization, in particular, has been found to be driven largely by the proportion of light-fraction organic matter, an active SOM fraction with a wide C/N ratio (Janzen et al., 1992; Whalen et al., 2000). Thus, by facilitating C accumulation in the light fraction (see Fig. 4), the SB-SB-Alf-Alf rotation probably reduced N availability, which is evident from the lower PMN/TSN ratio on the final sampling date (see Fig. 7d), when late-season mineral N tends to reduce sucrose content (Malnou et al., 2008; Stevanato et al., 2010). This idea is supported by the negative correlation between sucrose yield and the PMN/TSN ratio, although marginally significant ($P < 0.09$; Table 4). Soil organic C was one of the three variables selected in the final stepwise model as the best predictor of sucrose yield (Fig. 8), and it was negatively related to the PMN/TSN ratio (Table 4). This further supports the idea that SOC-induced N immobilization might have been responsible for the higher sucrose yield we observed under the SB-SB-Alf-Alf rotation. Although not documented in our study, improved soil aggregation and water-holding capacity could account for some of the observed increase in sucrose yield under the SB-SB-Alf-Alf rotation (Diaz-Zorita et al., 1999; Stine and Weil, 2002; de Moraes Sá et al., 2014).

CONCLUSIONS

This on-farm study provides data on soil organic C and N fractions as well as sucrose yield from long-term irrigated sugar beet based cropping systems—widespread forms of land management throughout the BHB in Wyoming. The results presented here contribute to the literature documenting SOM dynamics and crop productivity in furrow-irrigated agroecosystems by: (i) indicating that production of sugar beet in shorter rotation cycles, such as the 2-yr SB-BA or SB-DB systems reported here, leads to loss of SOC from the upper 15 cm of the soil, and (ii) highlighting the importance of extending the period of time between SB crops in rotations beyond the recurring 2-yr SB-BA or SB-DB rotation cycle by adding perennial legumes, like the 2-yr alfalfa crop in this study, for preservation of surface-soil (0–15-cm) organic C and N fractions, thus enhancing SOM buildup without compromising sucrose yield. Despite the negative correlation we observed between sucrose yield and the PMN/TSN ratio, mechanisms leading to higher sucrose yield under the SB-SB-Alf-Alf rotation that had greater SOC values are not fully understood, which stresses a need for further research in this area.

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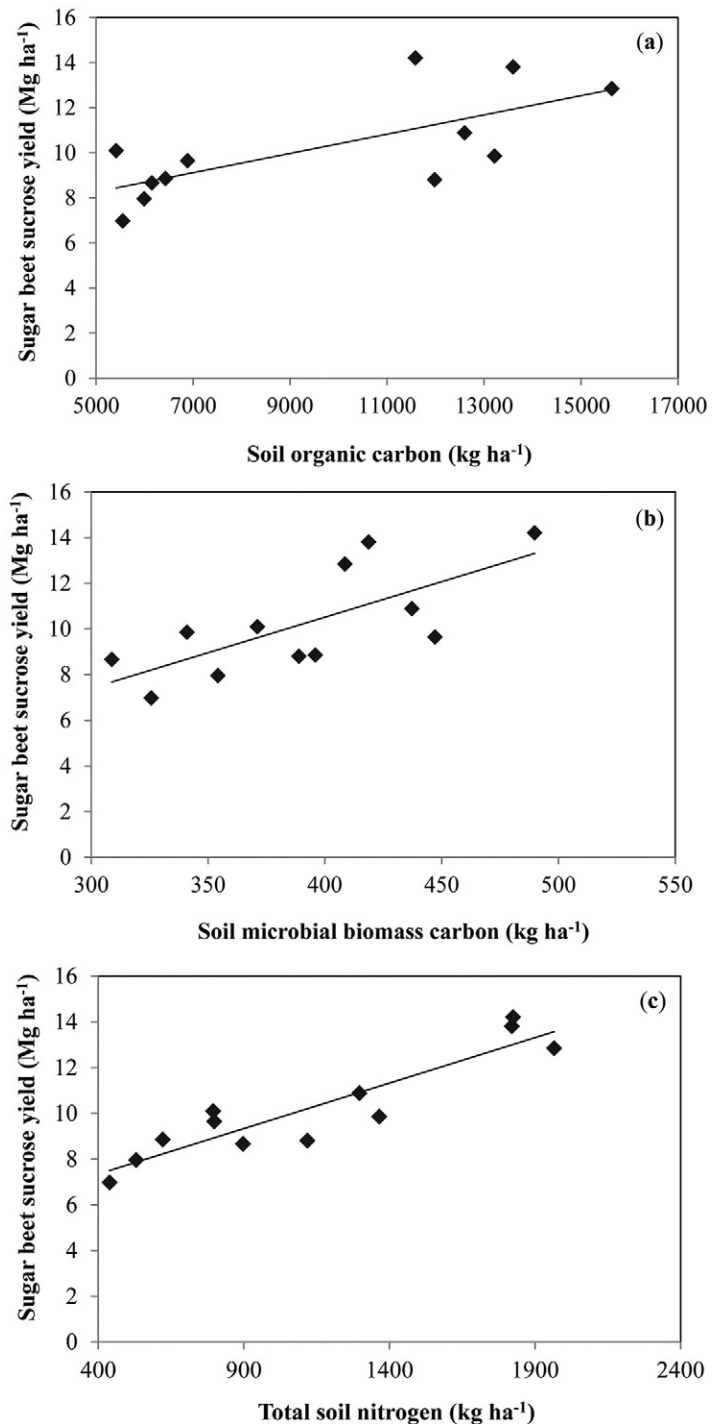


Fig. 8. Relationship between sugar beet sucrose yield and (a) soil organic C (SOC), (b) soil microbial biomass C (MBC), and (c) total soil N (TSN) across all four sugar beet based rotations ($n = 12$) averaged across all sampling dates. Coefficients of determination (r^2) between sugar beet sucrose yield and SOC, MBC, and TSN were 0.50 ($P = 0.015$), 0.52 ($P = 0.009$), and 0.84 ($P < 0.001$), respectively. Note that the x axis does not begin at zero.

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