Organic and Biodynamic Management: Effects on Soil Biology

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ABSTRACT

Biodynamic agriculture is a unique organic farming system that utilizes, in addition to the common tools of organic agriculture, specific fermented herbal preparations as compost additives and field sprays. The objective of this work was to determine whether biodynamic compost or field spray preparations affect the soil biological community in the short term, beyond the effects of organic management. Four fertilizer options: (i) composted dairy manure and bedding (organic fertilization), (ii) the same material composted with biodynamic compost preparations, (iii) mineral fertilizers, and (iv) no fertilizer were investigated with and without the biodynamic field spray preparations. Both biodynamic and nonbiodynamic composts increased soil microbial biomass, respiration, dehydrogenase activity, soil C mineralized in 10 d (MinC), earthworm (*Lumbricus terrestris*) population and biomass, and metabolic quotient of respiration per unit biomass (qCO_2) by the second year of study. No significant differences were found between soils fertilized with biodynamic vs. nonbiodynamic compost. Use of biodynamic field sprays was associated with more MinC and minor differences in soil microbial fatty acid profiles in the first year of study. There were no other observed effects of the biodynamic preparations. Organically and biodynamically managed soils had similar microbial status and were more biotically active than soils that did not receive organic fertilization. Organic management enhanced soil biological activity, but additional use of the biodynamic preparations did not significantly affect the soil biotic parameters tested.

Organic agriculture disallows the use of synthetic pesticides and fertilizers, relying instead on cultural, biological, or natural methods of pest control and fertility. A growing number of studies show that organic farming leads to higher quality soil and more soil biological activity than conventional farming. Drinkwater et al. (1995) documented higher pH, organic C and N, N mineralization potential, and actinomycete abundance and diversity in organic fields as compared with conventionally managed fields. Reganold (1988) found greater pH, organic C and N, cation-exchange capacity, microbial biomass, and several microbial enzymes in organic than in conventionally managed soils. Other studies have found similar benefits of organic soil management (Fraser et al., 1988; Wander et al., 1994; Workneh and van Bruggen, 1994; Gunapala and Scow, 1998).

Biodynamic farming is a type of organic farming and has many similarities to other organic farming systems, including a reliance on organic fertilizers. Biodynamic agriculture differs from traditional organic systems primarily in the use of fermented preparations in compost and as field sprays. These unique preparations (Table 1) consist of specific minerals or plants treated or fermented with animal organs, water, and/or soil (Steiner, 1974). The preparations were developed to improve soil and crop quality and hasten composting (Koepf et al., 1976). Six preparations (numbered 502–507) were applied to compost piles and three more preparations (500, 501, and 508) were applied directly to soil or crops as field sprays. Their primary purpose was not to add nutrients, but to stimulate the processes of nutrient and energy cycling (Koepf et al., 1976). If the preparations affect nutrient cycling, they may have their effect via soil microorganisms that mediate many nutrient transformations. The aim of this work was to test biodynamically prepared compost and the field spray preparations for effects on soil biotic parameters and to compare the effects of biodynamic soil management with the effects of organic fertilization with dairy compost.

Soil quality is often described as the soil's natural ability to produce good yields of high-quality crops and protect human and animal health without harming the natural resource base (Parr et al., 1992; Doran and Parkin, 1994). The meaning and quantification of soil quality depend on chemical, physical, and biological parameters. Of these, the biological measurements are least understood (Kennedy and Papendick, 1995). Because of its claimed reliance on beneficial microbial activity and enhanced soil quality, biodynamic agriculture is a potential case study of biological soil quality.

Generally studies have found that biodynamically farmed soils have better soil quality than conventionally farmed soils (Reganold, 1995). Fertilization with biodynamic compost can result in more organic C and N, dehydrogenase activity, biomass, and a higher dehydrogenase/biomass ratio than fertilization with chemical fertilizer or nonbiodynamic compost (Abele, 1976). Goldstein (1986) found that biodynamically managed plots had more organic matter, microbial biomass, and respiration than conventional or organic systems. In the DOC (bio-Dynamic, Organic, Conventional) plots maintained in Therwil, Switzerland, since 1978, biodynamically managed plots had greater microbial biomass even than the organic plots, while biodynamic and organic plots both had greater microbial activity (basal respiration and dehydrogenase activity) than conventional, mineral-fertilized, or unfertilized plots (Mäder et al., 1995). In an ongoing study, Penfold et al. (1995) found that biodynamic management resulted in lower extractable P than conventional or organic management

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Abbreviations: FAME, fatty acid methyl ester; HSD, Tukey's honestly significant difference; PC, principal component; PCA, principal component analysis; MinC, mineralized C; qCO_2, metabolic quotient of respiration per unit biomass; SIR, substrate induced respiration; TBME, tert-butyl methyl ether.

Table 1. Preparations used in biodynamic agriculture (Steiner, 1974), their main ingredients and agricultural uses.

Preparation	Main ingredient	Use
500	Cow (*Bos taurus*) manure	Field spray
501	Ground silica from quartz or feldspar	Field spray
502	Yarrow (*Achillea millefolium* L.) blossoms	Compost additive
503	Chamomile (*Matricaria recucitata* L.) blossoms	Compost additive
504	Stinging nettle (*Urtica dioeca* L.) shoot	Compost additive
505	Oak (*Quercus robur* L.) bark	Compost additive
506	Dandelion (*Taraxacum officinale* Weber) flowers	Compost additive
507	Valerian (*Valeriana officinalis* L.) extract	Compost additive
508	Horsetail (*Equisetum arvense* L.)	Field spray

systems. However, in this and many other studies the level of fertilization differed among systems, which probably affected the results.

In on-farm studies, biodynamically managed fields had more soil organic matter, protozoans, and nematodes, and greater respiration and enzyme activities than neighboring conventionally managed mineral-fertilized fields (Foissner, 1987). Commercial biodynamic farms were found as financially viable as neighboring conventional farms, while arguably maintaining greater soil quality (Reganold et al., 1993). Specifically, soils in biodynamically managed fields had lower bulk density, more total C, more respiration, more mineralizable N, a higher ratio of mineralizable N to C, and thicker topsoil than neighboring conventional field soils (Reganold, 1994). Droogers and Bouma (1996) found no significant differences between biodynamic and conventionally managed arable fields in directly measured soil physical parameters, but simulated crop yields were significantly greater from biodynamic fields because of improved soil structure and better water relations.

In companion studies, we found that Biodynamic Preparations 502 to 507 altered the microbial community phospholipid fatty acid makeup of compost and raised the temperature of composting dairy manure and bedding by an average of 3.4°C during an 8-wk development period (Carpenter-Boggs, 2000b). In short-term field trials, there were no differences in crop yield, crop quality, or soil fertility between plots fertilized with biodynamic or nonbiodynamic compost. Use of Biodynamic Sprays 500, 501, and 508 was correlated with higher yield of lentil (*Lens culinaris* Medikus) per unit plant biomass, lower C and crude protein contents in lentil, higher NO_3^- content in soft white spring wheat (*Triticum aestivum* L.), and greater NH_4^+ concentration in soil (Carpenter-Boggs et al., 2000a). On established biodynamic farms and neighboring conventional farms, 10-d soil respiration (MinC) and dehydrogenase enzyme activity were greater in biodynamically managed soils (Carpenter-Boggs, 1997).

The objective of this study was to determine whether biodynamic soil management affected soil biotic biomass, activity, or community fatty acid methyl ester (FAME) profiles. In studying biodynamics, the effects of the unique biodynamic preparations themselves must be separated from other management factors, such as additions of organic matter to the soil, that biodynamic management has in common with organic management. Therefore, a second objective of this study was to determine whether effects of biodynamic management differed from the effects of organic soil management. This study was unique because it differentiated the effects of biodynamic field sprays and compost preparations from the nutritional or biological effects of mineral or organic fertilization. The hypothesis to be tested was that the biodynamic preparations affect soil biotic parameters in the Palouse region of eastern Washington.

Table 2. Initial surface soil (0–15 cm) conditions at study sites in eastern Washington where organic, biodynamic, conventional, and mixed management treatments were imposed in April 1995.

	Study site	
Parameter	Spillman farm	Palouse farm
Soil series	Palouse	Palouse
pH	6.2	5.4
Organic matter, g kg^{-1}	35	35
Available P, μg g^{-1}	9	10
Available K, μg g^{-1}	292	310
Spring soil NO_3, μg g^{-1}	13	4
Spring soil NH_4, μg g^{-1}	7	2

MATERIALS AND METHODS

Experimental Site and Management

Farm sites were the Palouse Conservation Farm maintained by USDA-ARS and the Spillman Research Farm maintained by the Department of Crop and Soil Science, Washington State University. Mean annual precipitation is 55 cm and annual mean temperature is 8.3°C. Soil on both farms is Palouse silt loam (fine-silty, mixed, mesic Pachic Ultic Haploxeroll). Initial soil conditions are shown in Table 2. Initial determinations of soil available P (Morgan phosphorus test) (Murphy and Riley, 1962), available K (Teech and English, 1944), and organic matter were made by the Holm Laboratory, University of Idaho. A barley (*Hordeum vulgare* L.)–lentil–wheat rotation is common in the Palouse region and was present on study sites prior to and during this study. The eight treatments applied were a factorial of four fertilizer and two spray treatments (Table 3). Biodynamic compost, nonbiodynamic compost, mineral N-P-K fertilizers, and no fertilizer were used as fertility treatments, with and without the Biodynamic Field Sprays 500, 501, and 508. Each of the eight treatments was replicated four times in randomized complete blocks at each of the two farms.

Dairy manure mixed with bedding (pine shavings) was composted each autumn, beginning in October of 1994 and 1995. Material had been pressed to reduce moisture to 600 to 650 g kg^{-1}. Biodynamically active compost was prepared by inoculation with Preparations 502 through 507 (Table 1). The preparations used in this study were purchased from the Josephine Porter Institute (JPI, Woolwine, VA) immediately prior to their use.[1] The preparations were applied to compost piles according to JPI instructions, described here. Six holes were bored into the compost pile using a rod. Each packet of Biodynamic Preparation 502 to 506 was poured into a separate hole in the compost pile. Preparation 507 is a liquid, and must be stirred into 8 L of water before application to compost. One-

[1] Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the product by the USDA.

Table 3. Factorial design of fertilizers and field sprays used in eastern Washington in 1995 and 1996 to test effects of biodynamic (BD) compost and field sprays on soil biological parameters.

Treatment	Description	Fertilization	Field sprays
1	Negative control	None	Water
2	Mixed	None	BD 500, 501, 508
3	Conventional	Mineral N,P,K	Water
4	Mixed	Mineral N,P,K	BD 500, 501, 508
5	Organic	Nonbiodynamic compost	Water
6	Mixed	Nonbiodynamic compost	BD 500, 501, 508
7	Mixed	Biodynamic compost	Water
8	Biodynamic	Biodynamic compost	BD 500, 501, 508

half of the 507 solution was poured into the sixth hole, and one-half was sprinkled over the entire compost pile. The holes were then filled with soil from the surrounding field, requiring ≈1 L soil for each hole. In total, ≈19 g of moist weight of preparations were added to ≈3.5 Mg of compost material. In order to keep nonpreparation factors constant among treatments, control composts also received additions of ≈6 L of Palouse series field soil and 8 L of well water, applied similarly as in biodynamic piles but without the biodynamic preparations. Six-month-old composts were applied to field plots in April of 1995 and 1996 prior to tillage. Final compost C/N ratio was 30:1 in 1995 and 31:1 in 1996. Biodynamic Field Sprays 500, 501, and 508 were each applied once during each field season to the appropriate plots using JPI product instructions. One packaged unit of Preparations 500, 501, and 508 (Table 1), containing 38, 1.8, and 40 g moist weight, respectively, were applied as fine aqueous sprays in 11, 11, and 8 L of water, respectively, to the total biodynamic-sprayed plot area of 595 m^2. Final application rates were 64, 3, and 67 mg preparation m^{-2}, respectively, and 18, 18, and 13 mL water m^{-2}, respectively. Plots not receiving the biodynamic sprays were sprayed with a similar amount of well water.

Fertilizers were applied to plots each spring before rototilling and planting. Nutrient application levels were designed to meet the N needs of each year's crop (1995, 'Brewer' lentil; 1996, 'Penewawa' spring wheat) and meet or exceed needs of P and K, according to soil tests and university fertilization bulletins (Morrison et al., 1982; Murray et al., 1987). Estimated N availability from composts was used as the primary determinant of fertilization level. Because compost C/N ratio was 30:1 in 1995 and 31:1 in 1996, and because of the relatively high lignin content of pine shavings still visible in the compost, net N mineralization during the first growing season after application was expected to be low. Using measurements of total and soluble N, C/N ratio, and previous findings (Castellanos and Pratt, 1981; Vigil and Kissel, 1991; Hadas and Portnoy, 1994) relating these factors and material origin to N mineralization, it was estimated that all soluble N (≈7% of total N) was available to crops in the first growing season after application, with an additional 5% of total compost N available to the second crop after application. Sodium carbonate-extractable P and K in compost was assumed fully available (Rynk, 1992). Dry commercial NH_4NO_3, $NH_4H_2PO_4$, and KCl were applied at rates of 38, 22, and 48 kg ha^{-1}, respectively, in 1995 and 82, 18.5, and 126 kg ha^{-1}, respectively, in 1996 to equal N, P, and K available in compost treatments (Table 4).

Tillage, weed control, and all other management factors not part of the experimental design were similar among all plots (Table 4). Plots were disked once each fall and rototilled once each spring to incorporate fertilizers and compost. Weeds were controlled manually during the growing season. No synthetic insecticides or herbicides were applied, but lentils were sprayed once in 1995 with a tobacco (*Nicotiana tabacum* L.) infusion to control aphids (David and Gardiner, 1953; Ware, 1980, p. 24–25; Grainge and Ahmed, 1988).

Soil Analyses

Soils were sampled just prior to harvest and stored at 4°C. Six subsamples per plot were collected to a depth of 15 cm and pooled. All samples were brought to 0.225 kg kg^{-1} moisture (approximately −45 kPa) prior to storage or laboratory testing, and materials >2 mm were removed. Dehydrogenase was measured using a colorimetric procedure involving reduction of triphenyl tetrazolium chloride to triphenyl formazan (Tabatabai, 1994). Reduced products were extracted with methanol, centrifuged, and the supernatant's absorbance was read at 492 nm with a Bio-Rad Model 2550 EIA Reader (Bio-Rad, Hercules, CA). For soil respiration tests, moist soil (10-g dry weight equivalent) was placed into vials, capped with septa, and incubated 10 d at 22°C in darkness. After 10 d, the total CO_2 was measured using a Hewlett-Packard 5730A gas chromatograph (Zibilske, 1994) to give the 10-d MinC (Davidson et al., 1987). Then 0.5 mL of distilled water was added, vials were capped and incubated at 22°C for 3 h, and CO_2 was measured again to determine basal respiration. Substrate induced respiration (SIR) was subsequently measured using the same soil samples. After samples rested overnight, 0.5 mL of 30 g L^{-1} aqueous solution of glucose was added, for a concentration of 1.5 mg glucose g^{-1} soil. Vials were capped for 3 h, and CO_2 measured again. Substrate induced respiration in microliters CO_2 per gram soil per hour × 40.4 + 0.37 estimates micrograms microbial biomass C per gram soil (Anderson and Domsch, 1978). Earthworm populations were esti-

Table 4. Fertilizers applied, tillage, weed and pest control, and dates of management of field plots in eastern Washington.

Management descriptor	1995	1996
Available N-P-K, g kg^{-1} dry compost	0.8-0.3-1.3†	1.3-0.2-2.8†
Application rate, Mg dry compost ha^{-1}	19.0	24.0
Available N-P-K, compost or mineral, kg ha^{-1}	16-6-25†	31-5-66†
Organic C applied in compost, Mg ha^{-1}	4.6	8.4
Rototilling date	April 27	April 22
Planting date	May 4	May 2
Weed control	Wheel hoe, hand-pick	Wheel hoe, hand-pick
Weed control dates	June 12–16	June 10–14, July 8–12
Pest control	Tobacco (*Nicotiana tabacum* L.) infusion	None

† Expressed as elemental N-P-K.

Table 5. Soil microbial biomass, activity, and substrate measurements in plot soils (0–15 cm depth) in eastern Washington in 1995 after one cropping season under management varying by fertilizer type and application of biodynamic (BD) field sprays.

Treatment	Dehydrogenase activity	Microbial biomass (SIR)	Basal soil respiration	10-d mineralized C (MinC)	qCO_2	SIR/MinC
	μg TF g^{-1} soil h^{-1}	μg C_{mic} g^{-1} soil	μL CO_2 g^{-1} soil h^{-1}	μg C_{avail} g^{-1} soil	mg CO_2–C g^{-1} C_{mic} h^{-1}	μg C_{mic} μg^{-1} C_{avail}
Fertilizer						
BD compost	3.54a*	418	19.0	89.62a*	22.4	4.66
Non-BD compost	3.65a	418	16.2	95.74a	19.1	4.37
Mineral fertilizers	3.06b	368	10.1	55.51b	13.5	6.63
None	3.07b	351	10.3	65.08b	14.4	5.39
BD sprays						
Yes	3.27	402	13.9	79.32a*	17.0	5.04
No	3.39	375	13.9	73.66b	17.7	5.49
			P value			
Statistical contrasts†						
Compost vs. no compost	0.001	NS	0.06	0.001	NS	NS
Compost vs. mineral	0.001	NS	NS	0.001	NS	0.06
Organic vs. biodynamic	NS	NS	NS	NS	NS	NS

* Means with different letters are significantly different $\alpha = 0.05$.

† Compost vs. no compost, all plots receiving BD or non-BD compost vs. all plots not receiving compost; compost vs. mineral, all plots receiving BD or non-BD compost vs. all plots receiving mineral fertilizers; organic vs. biodynamic, plots receiving non-BD compost and no BD sprays vs. plots receiving BD compost and BD sprays.

mated by collecting and hand-sifting four 15-cm-deep, 15-cm-diam. soil cores (at ≈0.20 kg kg^{-1} field moisture content, or approximately −50 kPa) per plot in May 1996. After collection, earthworms were weighed to determine biomass.

Fatty acids were extracted from whole 1-g soil samples taken just prior to harvest in 1996, using the procedure of Microbial ID, Inc. (1992). Samples were saponified with 150 g L^{-1} NaOH in 1:1 methanol/water, methylated in acidified methanol, extracted with 1:1 hexane/tert-butyl methyl ether (TBME), and washed with dilute NaOH. The organic phase was removed, evaporated, and redissolved in a known volume (usually 200 ≈L) of hexane and TBME. Fatty acid methyl esters in samples were separated using a gas chromatograph (5890 GC Series II, Hewlett Packard, Wilmington, DE) equipped with a 25 by 0.2 mm fused silica capillary column and flame ionization detector. Seventy-six FAMEs with chain lengths of 10 to 20 C atoms were identified and quantified using the Eukary software and standard solutions from Microbial Identification Inc. (MIDI, Newark, DE).

Statistical Analyses

Statistical comparisons were made using the General Linear Model in SAS (SAS Institute, 1988). Means were separated using Tukey's honestly significant difference (HSD) test. Linear contrasts were used to compare groups of treatments. The contrast "compost vs. no compost" compares the average of Treatments 5 through 8 with the average of Treatments 1 through 4; "compost vs. mineral" compares Treatments 5 through 8 with Treatments 3 and 4; "organic vs. biodynamic" compares Treatment 5 through 8; "any fertilizer vs. no fertilizer" compares Treatments 3 through 8 with Treatments 1 and 2 (Table 2). Data set for total earthworm biomass did not fulfill the assumptions of parametric statistics and was transformed using $(x + 1)^{0.5}$ (Snedecor and Cochran, 1989).

Soil FAME profiles were subjected to principal component analysis (PCA), to combine the information of many FAMEs into a few principal components (PCs) (Pielou, 1984). Area percentage of all gas chromatographic peaks identified as FAMEs with chain lengths of 10 to 20 carbons were used as initial data. Each year, a subset of FAMEs was chosen for PCA using a preliminary round of PCA to determine which FAMEs significantly affected total profile variability at that sampling. Only FAMEs with a loading value $>|0.5|$ (17 fatty acids in 1995, 18 in 1996) were used in the final PCA. A separate PCA was performed on all FAME data each year. Resulting PC values were then used in GLM procedures, HSD tests, and contrasts as described above.

Treatment differences were considered significant at *P* values ≤0.10. All *P* values ≥0.05 and ≤0.10 are explicitly stated for the reader's consideration. Interactions between fertilizer and spray treatments are not discussed because none was statistically significant. In both years of the study, significant interaction between treatment and farm site in FAME PC1 led to subsequent analysis of variance of those values within each farm site.

RESULTS AND DISCUSSION

Soil Microbial Biomass and Activity

In both years of study, the values of many biological parameters were greater in soils fertilized with either of the composts than in soils not amended with compost. In 1995, compost-fertilized soils supported greater dehydrogenase activity, more soil respiration ($P = 0.06$), and had more MinC than noncompost plot soils (Table 5). In 1996, dehydrogenase activity, SIR, respiration, and MinC were all greater in plots receiving compost (Table 6). Compost may supply an additional source of labile C and other nutrients to the soil for microbial growth and activity. None of these parameters was different when comparing biodynamic and nonbiodynamic compost, nor when comparing organic and biodynamic managements (Treatments 5 and 8, respectively) (Table 3).

Soil MinC is a good indicator of microbially available C (Davidson et al., 1987). In 1995, plots receiving the biodynamic field sprays had more MinC than water-sprayed soils (Table 5). This difference was not present the following year. The supply of readily decomposable C is usually a good indicator of a soil's capacity for enzymatic activity (Burford and Bremner, 1975), but no other parameters indicated enhanced activity in biodynamically sprayed plots.

Maximum respiration response upon addition of substrate (SIR) is proportional to the size of the living microbial biomass (Anderson and Domsch, 1978). How-

Table 6. Soil microbial biomass, activity, and substrate measurements in plot soils (0–15 cm depth) in eastern Washington in 1996 after two cropping seasons under management varying by fertilizer type and application of biodynamic (BD) field sprays.

Treatment	Dehydrogenase activity	Microbial biomass (SIR)	Basal soil respiration	10-d Mineralized C (MinC)	qCO_2	SIR/MinC
	μg TF g^{-1} soil h^{-1}	μg C_{mic} g^{-1} soil	μL CO_2 g^{-1} soil h^{-1}	μg C_{avail} g^{-1} soil	mg CO_2–C g^{-1} C_{mic} h^{-1}	μg C_{mic} μg^{-1} C_{avail}
Fertilizer						
BD compost	3.83a*	558a*	30.9a*	155.11a*	27.2a†	3.60b*
Non-BD compost	3.55a	536a	28.4a	140.76a	26.1a	3.81b
Mineral fertilizers	2.07b	372b	10.3b	60.91b	13.6b	6.11a
None	2.09b	363b	9.8b	64.96b	13.3b	5.59a
BD sprays						
Yes	2.93	459	21.6	106.3	21.5	4.61
No	2.84	456	18.1	104.6	18.6	4.95
			P value			
Statistical contrasts†						
Compost vs. no compost	0.001	0.001	0.001	0.001	0.004	0.004
Compost vs. mineral	0.001	0.001	0.002	0.001	0.01	0.007
Organic vs. biodynamic	NS	NS	NS	NS	NS	NS

* Means with different letters are significantly different $\alpha = 0.05$.
† Contrasts as described in Table 5.

ever, the respiration response can also be affected by growth phase (Anderson and Domsch, 1978) and soil mineral nutrition (Sparling et al., 1981; Smith et al., 1985). The SIR method may also be used as a measure of soil community metabolic response to added substrate, without inference to soil microbial biomass.

Data gained by SIR may be more informative when viewed in relation to other factors such as basal respiration (qCO_2) (Anderson, 1994). The metabolic quotient of respiration per unit biomass (qCO_2) represents C flow through microbial biomass, that is, the energy needed to support a given biomass. A high qCO_2 is common in communities in initial stages of development and in communities with a large ratio of active to dormant biomass (Anderson, 1994). Less favorable soil conditions such as acidic pH can also increase qCO_2 by increasing metabolic stress (Anderson and Domsch, 1993). Compost-fertilized plots had high qCO_2 in 1996 (Table 6), suggesting a microbial biomass with high energy requirement. This could reflect the presence of a growing microbial community, a community under metabolic stress, or a greater proportion of active to dormant microbial biomass in compost-amended soils.

In 1995 compost-fertilized plots had a lower SIR/MinC than mineral-fertilized plots ($P = 0.06$, Table 5), and in 1996 compost-amended plots had lower SIR/MinC than either noncompost treatment (Table 6). This lower ratio of microbial biomass to available C may indicate lower efficiency of substrate utilization or that microbial biomass in compost-fertilized soils was limited by something other than available C. It should be noted that increased qCO_2 and decreased SIR/MinC could have resulted from an underestimation of the microbial biomass (SIR) in compost-amended soils relative to noncompost soils. The microbial decomposition of glucose leading to maximal respiration in SIR depends on many factors, including the balance of available C with available N and other nutrients (Chahal and Wagner, 1965). Addition of glucose alone will cause maximal respiration from a given biomass only when available C alone limits microbial activity (Smith et al., 1985). A high MinC combined with low SIR/MinC ratio suggests that readily available C is relatively abundant in compost-fertilized soils, and addition of more labile C as glucose does not induce a proportionate increase in respiration compared with noncompost soils. A lower

Table 7. Earthworm count in plot soils (0–15 cm depth) in eastern Washington in 1996 after two cropping seasons under management varying by fertilizer type and application of biodynamic (BD) field sprays.

Treatment	Earthworm population	Earthworm biomass	Biomass per earthworm
	no. m^{-2} topsoil	g m^{-2} topsoil	g
Fertilizer			
BD compost	95.6ab*	51.2ab*	0.45
Non-BD compost	145.1a	88.0a	0.56
Mineral fertilizers	46.0b	26.9ab	0.52
None	49.6b	22.8b	0.37
BD sprays			
Yes	92.0	48.7	0.44
No	76.1	45.8	0.52
		P value	
Statistical contrasts†			
Compost vs. no compost	0.01	0.01	NS
Compost vs. mineral	0.01	0.04	NS
Organic vs. biodynamic	NS	NS	NS
Any fertilizer vs. no fertilizer	0.10	0.05	0.07

* Means with different letters are significantly different $\alpha = 0.05$.
† Contrasts as described in Table 5 plus: any fertilizer vs. no fertilizer, all plots receiving either compost or mineral fertilizer vs. plots receiving no fertilizer.

Table 8. Fatty acids extracted from plot soils on two farms in eastern Washington under management varying by fertilizer type and application of biodynamic field sprays. Only those fatty acids contributing to Principal Components 1, 2, and 3 in 1995 (Fig. 1) and 1996 (Fig. 2) are listed.

1995				1996			
	Correlation				Correlation		
Fatty acid	PC1	PC2	PC3	Fatty acid	PC1	PC2	PC3
10:0 3OH	−0.9	0.1	−0.2	10:0	0.6	0.6	0.2
12:0	−0.9	0.2	−0.3	11:0 iso	0.6	0.6	0.1
14:1 ω7c DMA	0.4	0.6	0.1	11:0 iso 3OH	−0.1	−0.1	0.8
15:0 iso 3OH	−0.3	0.1	0.7	12:0	−0.1	0.9	0.2
16:0 2OH	0.4	0.8	−0.2	12:0 3OH	−0.7	0.2	0.1
16:0 iso	0.6	−0.7	−0.1	13:0 iso 3OH	0.2	−0.1	0.8
16:1 ω5c	0.6	−0.5	−0.4	14:0	0.1	0.8	0.1
17:0 iso	0.4	−0.5	0.5	15:0 anteiso	0.8	0.2	−0.2
17:0 iso 3OH	0.6	0.7	0.3	15:0 iso	0.9	0.1	0.2
17:0 cyclo	−0.1	0.0	0.9	16:0 iso	0.9	−0.2	−0.2
17:1 iso ω?	−0.1	−0.8	0.1	16:1 2OH	0.7	−0.2	−0.3
17:1 iso ω5c	0.5	0.8	0.0	16:1 ω7c OH	0.1	−0.3	0.7
17:1 ω8c	0.8	−0.4	−0.2	16:1 ω5c	0.8	0.0	−0.1
18:1 ω9t	0.7	−0.5	−0.1	16:1 ω9c	0.8	−0.2	0.1
18:1 ω9t OH	−0.1	−0.3	0.8	17:0 iso	0.6	−0.4	0.4
19:1 ω8t	−0.9	0.0	−0.2	17:1 ω7c	−0.7	0.2	−0.1
19:2 ω6c	0.6	0.8	0.1	17:1 ω8c	0.8	0.0	−0.3
				19:0 cyclo	0.0	−0.7	−0.1

SIR/MinC in compost-amended plots may suggest that available C limited microbial activity more so in mineral-fertilized and unfertilized plots than in compost-fertilized plots.

Earthworms were more abundant in compost-fertilized plots, especially in plots receiving nonbiodynamic compost (Table 7). Earthworm population and biomass were greater in compost-fertilized plots than non-compost plots. The weight of individual earthworms was similar among mineral- and compost-fertilized plots, but lower in unfertilized plots ($P = 0.07$). Pfiffner et al. (1995) found more earthworms under organic than biodynamic management, and fewest in mineral-fertilized or unfertilized plots. Earthworm populations and development are affected by both the quantity (Satchell, 1967) and quality (Lofs-Holmin, 1983) of food source. Partially decomposed material such as compost supports rapid growth of earthworms (Bostrom, 1987). Compost probably supplied an additional food source via the compost material itself and the microbial community within the compost.

These data support previous findings of many others that application of compost or manure can rapidly improve biological aspects of soil quality (Fraser et al., 1988; Parr and Hornick, 1992; Angers et al., 1995). Only MinC measurement in 1995 suggested any added benefit of using biodynamic spray preparations. There were no indications in these biomass and activity measures that compost prepared with biodynamic preparations affected soil biota differently than normally prepared compost.

Fatty Acids

Fatty acid profiles can be used as a fingerprint of microbial community structure (Turco et al., 1994).

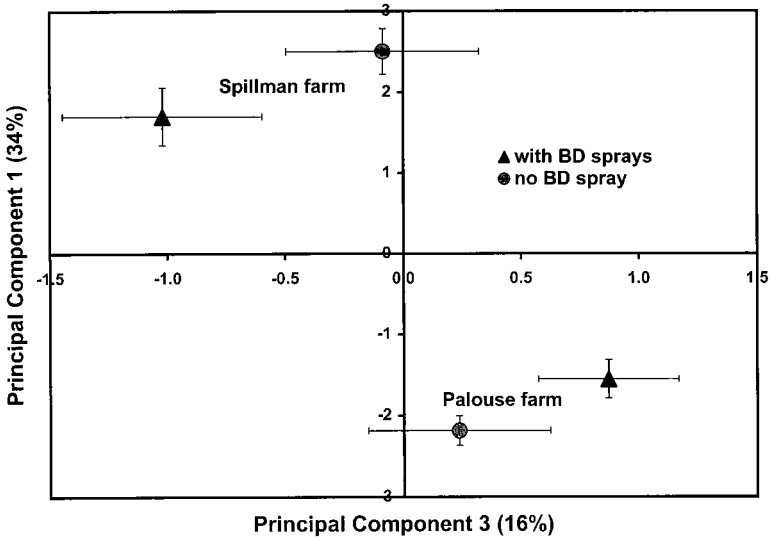

Fig. 1. Principal Components 1 and 3 (mean ± SE) of total soil fatty acid analysis for the fall 1995 plot samples by farm site and use of biodynamic (BD) field sprays.

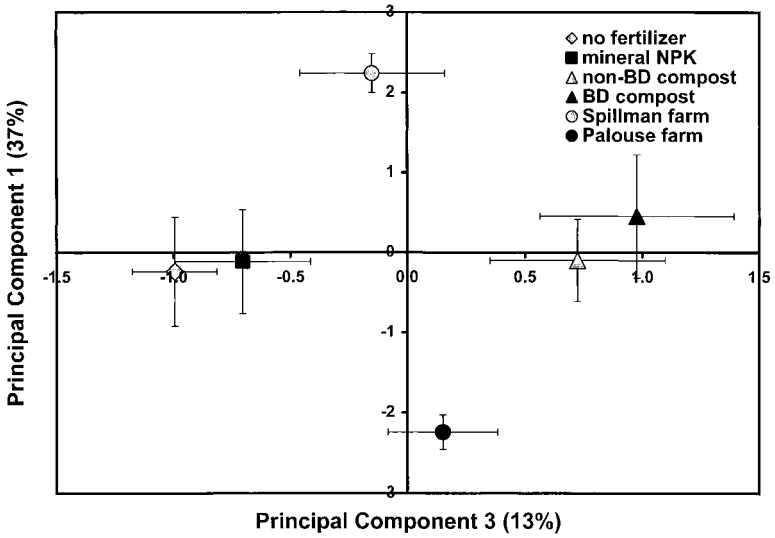

Fig. 2. Principal Components 1 and 3 (mean ± SE) of total soil fatty acid analysis for the fall 1996 plot samples by farm site and type of fertilizer. BD is biodynamic.

Whole soil FAME profiles have been used to identify differences in microbial community due to taxonomic makeup (Haack et al., 1994), management system, and sampling date (Buyer and Drinkwater, 1997).

Three PCs described 78% of the variance among the 1995 soil samples and 68% of the variance among the 1996 soil samples. Fatty acids contributing the most to total FAME variability were used in PCA each year (Table 8), so that these FAMEs are not the same from year to year, and PCs cannot be directly compared across years. A sample's rank in any PC only describes differences among samples and does not imply more or less microbial biomass or other general merit.

Fatty acids differentiated samples primarily by farm site, but also indicated smaller differences in soil microbial community between treatments. In 1995, soils of the two farms were separated in PC1 ($P = 0.001$) and PC3 ($P = 0.05$) (Fig. 1). Principal Component 2 was related only to replicate blocks, which separated landscape positions ($P = 0.001$) (data not shown). Biodynamically sprayed plots ranked differently than unsprayed plots in PC1 on both the Spillman farm ($P = 0.06$) and on the Palouse farm ($P = 0.03$) (Fig. 1). Soil FAME profiles did not differentiate fertilizer treatments in 1995 (data not shown).

In 1996, FAME profiles differentiated samples by fertilization as well as by farm. Principal Component 1 differentiated between the two farm sites ($P = 0.001$) (Fig. 2). Principal Component 3 differentiated among soil samples under different fertilization ($P = 0.01$). Plots fertilized with biodynamic compost ranked highest in PC3, followed by plots receiving nonbiodynamic compost, mineral fertilizer, and no fertilizer. Statistically, noncompost plots were similar and compost plots were similar, but plots receiving either type of compost ranked higher in PC3 than plots receiving no compost ($P = 0.001$). The biodynamic field sprays affected PC1 only on the Palouse farm in 1996 ($P = 0.07$) (data not shown).

Fatty acid analysis thus indicated that soil communities were affected more by sample location (farm site and landscape position) than by the applied treatments. Effects of fertilization on the soil community FAMEs were apparent by the second year of the study. Compost-fertilized plots were distinguishable from mineral-fertilized and unfertilized plots, but FAMEs did not differentiate biodynamic and nonbiodynamic compost-fertilized plots and gave only weak indication of an effect of the biodynamic sprays.

CONCLUSIONS

The soil biological parameters tested indicated many differences between soils that had received compost additions and those that had not. Both biodynamic and nonbiodynamic composts increased soil microbial biomass, respiration, dehydrogenase activity, MinC, earthworm population and biomass, and qCO_2. No differences were found between soils fertilized with biodynamic vs. nonbiodynamic compost. Use of biodynamic field sprays was associated with more MinC and slightly different FAME profiles in the first year of study. These effects were transient, and P values generally were near 0.05; therefore the indications of biodynamic spray effects on the soil are still questionable.

These data support earlier findings that organic fertilization rapidly benefits soil microbial biomass and activity, but provide few indications that the biodynamic compost and field sprays further affect soil microbial biomass, community structure, or activity in the short term. Although it is beyond the scope of this study to address possible effects of long-term use of the biodynamic preparations, in the short term it appears that benefits to soil quality from the biodynamic farming system are primarily due to the use of organic fertilization.

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Comparison of Fatty Acid Methyl Ester (FAME) Methods for Characterizing Microbial Communities

Mary E. Schutter and Richard P. Dick*

ABSTRACT

Fatty acid profiling is a popular method for characterizing microbial communities of natural systems. Direct extraction of microbial fatty acids in situ would be useful compared with methods that extract lipids first and subsequently release fatty acids from lipids. In this study, two methods for the direct extraction of fatty acids from soils were compared for three cultivated silt loams and one forested sandy clay loam. Fresh soils were analyzed for their fatty acid methyl ester (FAME) profiles by an ester-linked (EL) method and the method of MIDI (Microbial ID, Inc., Newark, DE). Soils were stored four different ways (moist at 4°C, moist at −20°C, air-dried at 25°C, and partially dry at 4°C) and analyzed for FAME profile changes after 30 and 90 d of storage. Eleven and 17 FAMEs were unique to the EL and MIDI method, respectively, but unique FAMEs generally were found in only minute amounts. Soils extracted with the MIDI method yielded more hydroxy FAMEs and short-chain saturated and branched FAMEs. Conversely, EL-extracted soils generally produced more long-chain saturated and branched FAMEs, unsaturated FAMEs, and FAMEs with cyclopropane and methyl groups. Both extraction methods were able to differentiate among communities of different soil types, regardless if soils were fresh or stored. Changes in FAME profiles did occur in stored soils, but the effectiveness of each storage protocol for preserving FAME patterns over time was different among the four soils. While community analyses should be conducted on fresh soil, overall effects of storage were slight compared with those of extraction method and soil type.

The use of microbial lipids to identify microorganisms and characterize microbial communities in natural systems has become increasingly popular. Several methods for the analysis of microbial phospholipid fatty acids (PLFAs) exist and have been in use for over 20 yr to estimate microbial biomass and community structure in sediments (White et al., 1979; Guckert et al., 1985; Rajendran et al., 1992). Since its introduction, PLFA methods have been applied to determine the effects of stress on bacterial isolates (Kieft et al., 1994, 1997), of root exudates on rhizosphere microorganisms (Griffiths et al., 1999), and P on arbuscular mycorrhizal fungi (Olsson et al., 1997). The methods also allowed for the characterization of microbial communities from agricultural soils (Zelles et al., 1992; Wander et al., 1995; Reichardt et al., 1997; Bossio et al., 1998; Ibekwe and Kennedy, 1998b), from soils contaminated with heavy metals, alkaline dust, and acid rain (Pennanen et al., 1996; Bååth et al., 1992; Pennanen et al., 1998), and from other diverse habitats (Sundh et al., 1997; Klamer and Bååth, 1998; Steinberger et al., 1999).

Although analysis of microbial PLFA profiles has proven extremely useful, the methods involved are time consuming. Microbial lipids are extracted from environmental samples in a phase-mixture of chloroform, methanol (MeOH), and water (Bligh and Dyer, 1959). Lipids associated with the organic phase are then fractionated into neutral, glyco-, and phospholipids on silicic acid columns, while the residue at the organic:aqueous interphase can be separated into lipopolysaccharides, teichoic acids, and muramic acid (Vestal and White, 1989). Finally, the phospholipids are subjected to alkaline methanolysis to produce fatty acid methyl esters (FAMEs) for analysis by gas chromatography (GC).

Recently, a simpler method has been developed to extract microbial fatty acids directly from soils. The MIDI protocol was designed to extract fatty acids from pure cultures of bacterial isolates for identification purposes, but it also has been applied to whole-soils. With this method, microbial cells in soil are saponified by heat and the addition of a strong base. Once fatty acids are cleaved from lipids, they are methylated to form FAMEs. The FAMEs are extracted in an organic solvent and analyzed by gas chromatography (Sasser,

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Abbreviations: EL, ester-linked; FAME, fatty acid methyl ester; GC, gas chromatography; MANOVA, multivariate analysis of variance; MIDI, Microbial ID, Inc.; NMS, non-metric multidimensional scaling; PCA, principal components analyses; PLFA, phospholipid fatty acid; TOC, total organic carbon.