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Rapid changes in microbial biomass and aggregate size distribution in response to changes in organic matter management in grass pasture

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ABSTRACT

Adding high quantities of organic matter can increase carbon (C) inputs to soil and help maintain soil structure. This study investigated short-term effects of application of different levels of composted dairy manure (CDM) versus interseeding a legume into grass pasture on aggregate stability and soil C and nitrogen (N) contents. CDM was added to a mixture of perennial grasses at 22.4, 33.6 or 44.8 Mg ha^{-1}. A grass–legume treatment was established by interseeding alfalfa (*Medicago sativa*) into the grass mixture. A no-input control was sampled as a reference. Soils (0–5 and 5–15 cm) were sampled approximately 1.5 years after study implementation and wet sieved to obtain four aggregate size classes: large macroaggregates (>2000 μm), small macroaggregates (250–2000 μm), microaggregates (53–250 μm) and silt and clay fraction (<53 μm). Significant CDM influences were found in the 5–15 cm depth. The addition of 44.8 Mg CDM ha^{-1} and alfalfa resulted in higher proportions of macroaggregates (>250 μm) and mean weight diameter (MWD) than CDM added at 22.4 or 33.6 Mg ha^{-1}. Addition of CDM at low dose rate and alfalfa did not affect total soil or aggregate-associated organic C or N. However, addition of CDM at 44.8 Mg ha^{-1} and alfalfa resulted in higher total soil microbial biomass C and N compared to CDM added at 22.4 and 33.6 Mg ha^{-1}. Large macroaggregates were found to be positively correlated with total soil microbial biomass C ($R=0.81$, $p=0.002$). In conclusion, compared to a low application rate of CDM, addition of a high application rate of CDM or alfalfa interseeding resulted in higher total soil microbial biomass C and N and macroaggregates, and these changes in microbial biomass and aggregation occurred very rapidly.

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1. Introduction

The addition of high quantities of organic soil amendments such as animal manure, compost and green manure, as well as soil-building leguminous crops increases soil organic matter (SOM) levels and the formation and stability of aggregates (Aoyama et al., 2000; Janzen, 2006; Tisdall and Oades, 1982). The added organic amendments provide active organic compounds such as particulate organic matter (POM) that act as nucleation sites for the formation of macroaggregates (Chivenge et al., 2011). The dynamics of aggregate turnover in time (i.e. aggregate formation and breakdown) influence short-term nutrient cycling and the stabilization of soil organic carbon (SOC) in the long-term (Plante and McGill, 2002; Six et al., 2002a,b).

The addition of animal manure has been noted to influence aggregation, i.e. high manure application results in higher aggregate mean weight diameter (MWD) than no addition (Gulde et al., 2008; Min et al., 2003; Whalen et al., 2003). For example, Grandy et al. (2002) and Wortmann and Shapiro (2008) observed higher macroaggregate formation with composted manure application than unamended control. Similarly, Min et al. (2003) observed that dairy manure added at 32.7 Mg C ha^{-1} resulted in 30% higher aggregate stability than an unamended control. In contrast, however, Whalen and Chang (2002) observed a lower macroaggregate proportion at 0–5 cm depth compared to 5–10 cm depth when manure was added at 60 Mg ha^{-1} year^{-1}. They attributed the decrease to the breakdown of larger macroaggregates due to dispersion of soil colloids caused by monovalent cations present in manure, suggesting that high levels of Na^{+} and K^{+} negate the positive effects of animal manure on aggregation. Manure has also been shown to have greater influence on aggregate stability than chemical fertilizers. Min et al. (2003), for example, observed an 18% increase in aggregate stability with dairy manure slurry compared to NH_4NO_3 fertilizer. Similarly, Mikha and Rice (2004) observed greater macroaggregate (>2000 μm) proportions (74%) with cattle manure compared to NH_4NO_3 fertilizer.

The growth of perennial grasses has also been shown to enhance aggregate formation due to the production of large quantities of polysaccharide and phenolic binding agents by the large microbial biomass in the pasture rhizosphere (Haynes and Beare, 1997; Milne

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and Haynes, 2004; Tisdall and Oades, 1982). Additionally, the fine grass roots and associated fungal hyphae physically enmesh fine soil particles into aggregates (Haynes and Beare, 1997; Milne and Haynes, 2004; Tisdall, 1994; Tisdall and Oades, 1982). For example, microbial biomass C has been shown to be positively correlated with aggregate stability, indicating the important role of microbial biomass C in aggregation (Milne and Haynes, 2004). Similar to grasses, legumes have also been shown to positively impact aggregation. Haynes and Beare (1997), for example, observed higher aggregate stability and microbial biomass C (63 and 7%, respectively) under lupin compared to wheat. In the U.S., the majority of irrigated grass pasture production is based on a mixture of different cool-season perennial grasses such as orchardgrass, bromegrasses or fescues (McBride and Greene, 2009; Miller, 2010). In organic farming systems with only limited options for certified fertilizers, the use of animal manure or its compost has become a viable way to address soil fertility challenges (ERS, 2008). Often, many livestock producers also improve the productivity of grass-based pastures by interseeding legumes.

The aim of this study was to evaluate aggregate stability, aggregate-associated organic C and N, including microbial biomass C and N, as affected by the addition of composted dairy manure (CDM) and alfalfa interseeding to perennial grass pasture. The hypotheses of this study were: (i) short-term microbial biomass and aggregate stability will be greatest with the addition of CDM at the highest dose rate as compared to lower CDM rates, and (ii) the interseeding of legume into grass-based pasture will induce higher microbial biomass and aggregate stability compared to the addition of CDM at lower rates.

2. Materials and methods

2.1. Site information

The study was conducted at the Agricultural Research Development and Education Center (ARDEC), Colorado State University, Fort Collins, Colorado (40°39′ N, 104°59′ W; 1554 m above sea level). The field site was on transitional organic land in the process of certification. Average annual precipitation for the area is 330 mm. Of this, about 88% occurs between April and October. The mean monthly temperature ranges from 0 °C in January to 22 °C in July. The soil is classified as a mixed, superactive, mesic, Aridic Haplustalf and has a loamy texture (NRCS, 1980).

2.2. Experimental design, trial management, and sampling

The site was in alfalfa (*Medicago sativa*) for 4 years before being converted to perennial grasses in the fall of 2007. The alfalfa was killed using tillage in the summer of 2007, and the field was clean-tilled and received a blanket basal application of 22.4 Mg CDM ha^{-1} by broadcasting and incorporating into the soil by disking. The experimental site consisted of 3 field blocks, each containing four plots measuring 3 m × 12 m. In September 2007, all plots were planted with a seed mixture containing orchardgrass (*Dactylis glomerata*), meadow brome (*Bromus biebersteinii*), and smooth brome (*B. inermis*). The experiment was set up as a randomized complete block design with three replicates.

In April 2008, out of the 4 plots in each block, three received 22.4 Mg CDM ha^{-1}, and one was interseeded with alfalfa to establish a grass–legume treatment. However, visual inspection and quantification of alfalfa revealed that establishment of alfalfa was inadequate due to competition from grasses. Thus, alfalfa was seeded again in March 2009. For the 2009 growing season, CDM was applied in fall 2008. CDM plots that received 22.4 Mg ha^{-1} in spring 2008 were split into three and received 0, 11.2 or 22.4 Mg ha^{-1} CDM on October 23, 2008. This approach resulted in cumulative additions of 22.4, 33.6 and 44.8 Mg CDM ha^{-1}. Fig. 1 summarizes the timeline of different interventions in the preparation of the trial. Since all CDM-amended plots received equal amounts of manure in spring 2008, any significant differences in microbial biomass or aggregate stability can, therefore, be attributed mainly to the effect of CDM treatments established in fall 2009. The CDM application rates in this study were in keeping with those commonly used in this area. Selected characteristics of CDM are listed in Table 1. The border area surrounding the research plots was also seeded to a mixture of orchardgrass, meadow and smooth bromegrass was not fertilized with CDM and thus was sampled as a reference no-input control. The field site, including border plots, was irrigated 1 to 2 times per week using a linear move sprinkler system, and plots were harvested five to six times per year to simulate rotational grazing. In July 2009, soil samples were collected in two depth increments (0–5 and 5–15 cm), including from six locations in the border area to provide a reference of soil properties. Baseline soil data from the trial before the establishment of the treatments indicated SOM by loss on ignition of 2.4%, KCl extractable nitrate–N (NO_3–N) of 15.4 mg kg^{-1}, Olsen P of 29 mg kg^{-1}, pH (1:1 w/v) of 8.3, and EC (1:1 w/v) of 0.4 dS m^{-1}.

2.3. Determination of soil microbial biomass

Microbial biomass was analyzed on field moist soil samples within 72 h of collection by the fumigation–extraction method (Horwath and Paul, 1994). Briefly, a 15-g subsample was immediately extracted with 75 ml of 0.5 *M* K_2SO_4 (non-fumigated control). Another 15-g subsample was fumigated with chloroform for 5 days and extracted as described above following fumigation. Total dissolved N (TDN) and dissolved organic C (DOC) were analyzed with a Shimadzu TOC Analyzer (TOC-V Series, Shimadzu, Kyoto, Japan). Microbial biomass N (MBN) was determined by subtracting K_2SO_4 extractable TDN in non-fumigated samples from that of fumigated samples and dividing the result by K_N (the proportion of biomass N released following chloroform fumigation and extracted by K_2SO_4). K_N was estimated from the equation $K_N = -0.014 (C_F/N_F) + 0.39$ (Voroney and Paul, 1984), where C_F and N_F were DOC and TDN, respectively, extracted by 0.5 *M* K_2SO_4 from fumigated samples. Microbial biomass C (MBC) was calculated as $MBC = E_C/K_C$, where E_C is the difference between DOC extracted in K_2SO_4 from fumigated and non-fumigated soils. K_C (the proportion of biomass C released following chloroform fumigation and extracted by K_2SO_4) was derived experimentally (Section 2.4).

2.4. Determination of K_C for calculating MBC

Substrate addition was performed on soil samples collected from the no-input reference plots. We used cellobiose (a glucose disaccharide derived from the partial hydrolysis of cellulose) as our substrate because it is a natural C compound that can be easily broken down extracellularly by many microorganisms prior to incorporation into the cell (Steinweg et al., 2008). According to Steinweg et al. (2008), the degradation process facilitates measurements of respiration due to cellobiose addition and of the remaining cellobiose concentration over time. A 1.5 ml aliquot of cellobiose solution (20 mg ml^{-1}) was added to 30-g sample (field moist soil). This resulted in an addition rate of 421 μg C g^{-1} soil. Control samples received 1.5 ml deionized (DI) water to maintain soil moisture content similar to that in cellobiose amended soils.

Each sample was split into three: one 5- and two 10-g subsamples and placed into separate jars. One 10-g subsample was immediately extracted with 0.5 *M* K_2SO_4, while the second 10-g subsample was fumigated with chloroform for 5 days to determine MBC. The 5-g subsample was used to monitor soil respiration and substrate remaining at three time points (0, 4 and 20 h after addition). Soil respiration was monitored by analyzing CO_2 concentration in gas samples taken from the headspace of jars using a LI-6252 CO_2 Analyzer (LI-COR Bioscience, Lincoln, NE). Cellobiose-derived CO_2 was determined by subtracting CO_2 concentration in the control samples from that of the amended samples. Cellobiose remaining (i.e., non-respired cellobiose-C) was analyzed using the sulfuric acid–anthrone method for water-soluble carbohydrates (Brink et al.,

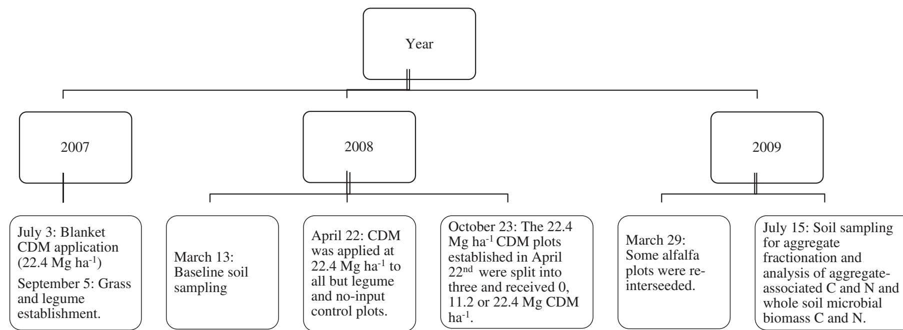

Fig. 1. Description of timeline from trial preparation to sampling for this study.

1960). Briefly, following headspace gas sampling, the 5-g sample was extracted with 50 ml DI water on a shaker for 30 min. The suspension was poured into 50 ml centrifuge tubes, balanced, and centrifuged for 10 min at 10,000 rpm. A 25 ml extract supernatant was transferred to a 100 ml volumetric flask, diluted to volume with DI water, and stored at 4 °C until analysis. Standards, blanks (DI water), and soil-extracts (5 ml each) were transferred to glass test tubes, and 10 ml anthrone–sulfuric acid reagent [0.2% anthrone in 95% (v/v) sulfuric acid] was rapidly added. A 10 ml solution was then transferred to glass cuvettes, and absorbance was read at 625 nm using a spectrophotometer. The absorbance was converted to cellobiose concentration using a standard calibration curve. The concentration of cellobiose remaining in the soil samples was determined as:

$$\text{Cellobiose}\left(\mu g\ g^{-1}\text{soil}\right) = Axsx\ [(100\ \text{ml})\ (50\ \text{ml extract})/(25\ \text{ml extract})\ (5-\text{g soil})]$$

where A is the solution absorbance at 625 nm and s is the slope (μg ml^{-1} nm^{-1}) of the standard curve.

Non-biomass C, which was not assimilated by microbes, was estimated from cellobiose-derived CO_2 and cellobiose remaining (i.e., anthrone-positive) in the soil samples. The amount of cellobiose-C assimilated by microbes was determined by difference as total cellobiose added to soil samples minus non-biomass C. Then, K_C was determined by dividing measured MBC (i.e., MBC from cellobiose-amended samples minus unamended control samples) by cellobiose-C assimilated, which is assumed to be lysed and released following chloroform fumigation.

2.5. Aggregate fractionation

Field-moist soil was passed through an 8-mm sieve by gently breaking soil clods along natural planes of fracture and air-dried for subsequent analyses. Water stable aggregate size distribution was determined by wet-sieving the air dried soil through a series of three sieves according to Elliott (1986). Briefly, a subsample of 100-g air dried soil was spread evenly on a 2000 μm sieve and slaked for 5 min in DI water. The soil was then sieved by manually moving the sieve up and down (approximately 3-cm amplitude) 50 times during a period of 2 min. The >2000-μm fractions (i.e., large macroaggregates) remaining on the 2000 μm sieve were backwashed into a beaker for drying. Soil plus water passing through the 2000 μm sieve was transferred to the next smaller-sized sieve (250 μm), and the above process was repeated to obtain the small macroaggregate fraction (250–2000 μm). The sieving process was repeated once more, and soil that passed through the 250 μm sieve was transferred to a 53-μm sieve to separate microaggregates (53–250 μm) from the silt and clay fraction (<53 μm). The <53-μm fraction was determined upon drying and weighing a subsample of 300 ml from the suspension that passed through the 53-μm sieve and correcting the weight for a total volume of the suspension. *All aggregate fractions* were oven-dried at 105 °C and weighed. The recovery of soil mass after fractionation was on average 98%. Aggregate mean weight diameter (MWD) for each treatment was calculated as:

$$\text{MWD} = \left(f_{>2000-\mu m} x\ 5\right) + \left(f_{250-2000-\mu m} x\ 1.125\right) + \left(f_{53-250-\mu m} x\ 0.1515\right) + \left(f_{b53-\mu m} x\ 0.0265\right)$$

where f is the weight of aggregates recovered after sieving as a fraction of the total dry weight of soil used for any particular size range, with size given in the subscript. The numbers, i.e. 5, 1.125, 0.1515 and 0.0265, are mean diameter values (mm) of aggregates in that size range.

2.6. Carbon and nitrogen analysis

Subsamples of oven-dried aggregate fractions, along with whole soil were finely ground and analyzed for total C and N concentrations using a *LECO CN-2000 analyzer (Leco Corp., St. Joseph, MI). Carbon and N contents of the aggregates were corrected for total sand in the aggregate fraction. Sand-free C and N concentrations (g kg^{-1} sand-free aggregates) were calculated according to* Six et al. (1998) *as:*

$$Sand-free\ (C\ or\ N)_{fraction} = (C\ or\ N)_{fraction} / \left[1-(sand\ proportion)_{fraction}\right]$$

where sand content (>53 μm) of the aggregates was determined on a subsample of 2- to 5-g aggregates that were dispersed in 5 g L^{-1} sodium hexametaphosphate by shaking for 18 h (Six et al., 2000). *Following shaking, dispersed samples were sieved using a 53 μm sieve and the >53 μm material remaining on the sieve was oven-dried at 105 °C and weighed.* Quality check was performed by comparing the total soil C and N data with the calculated soil C and N content based on the sum of the fractions and questionable values considered to be outliers were excluded from the dataset prior to statistical analyses.

Table 1
Physical and chemical characteristics of composted dairy manure (CDM) used in this study.

Parameter	Summer 2007	Spring 2008	Fall 2008
Moisture, (%)	25.2	28.6	20.4
Total solids, (%)	74.8	71.4	79.6
Carbon, (%)	7.4	9.1	3.6
Ash, (%)	60.7	54.2	72.7
Total N, g kg^{-1}	6.2	10.7	2.9
Organic N, g kg^{-1}	5.7	10.1	2.6
NH_3–N, g kg^{-1}	0.02	0.13	0.33
NO_3–N, g kg^{-1}	0.6	0.5	0.01
Total P, g kg^{-1}	2.4	5.1	1.1
Total K, g kg^{-1}	6.4	13.2	4.7
EC[a], dS m^{-1}	2.1	5.1	2.7
pH-H_2O[a]	7.9	8.6	9.1

[a] EC and pH were determined on 1:5 CDM to water ratio.

Table 2
Microbial biomass C and MBN as affected by different levels of composted dairy manure (CDM) and grass–legume treatment at two soil depths. Values in parenthesis are SD (n = 3).

Treatment	MBC		MBN	
	0–5 cm	5–15 cm	0–5 cm	5–15 cm
	———————μg g^{-1} soil———————			
Control[a]	146.1 (36.2)	168.7 (43.3)	21.5 (3.8)	37.0 (5.2)
Grass–legume[b]	346.1 (62.7) a[c]	208.7 (36.9) a	109.8 (8.3) a	72.0 (8.4) a
22.4	136.5 (37.9) b	152.1 (28.9) b	32.2 (17.0) b	43.3 (22.8) b
33.6	170.5 (36.9) b	145.5 (28.4) b	30.2 (8.0) b	46.0 (15.0) b
44.8	264.6 (13.3) a	214.2 (47.8) a	94.2 (2.4) a	67.3 (12.8) a
Mean	229.4 (41.9) A[d]	180.1 (35.5) B	66.6 (8.9) A	57.2 (14.4) A
	ANOVA		ANOVA	
Treatment	***		***	
Depth	**		ns	
Treatment × depth	**		**	

Level of significance: * $P<0.1$; ** $P<0.05$; *** $P<0.001$; ns, not significant.
[a] The no-input control was not included in the statistical analyses because it was not replicated within the experimental design.
[b] The grass–legume treatment had alfalfa (*Medicago sativa*) interseeded into a mixture of perennial grasses.
[c] Values within the column followed by common lowercase letter were not significantly different ($p<0.05$).
[d] Values followed by common uppercase letter were not significantly different ($P<0.05$) between the two sampling depths.

2.7. Statistical analyses

The field experiment was arranged in a randomized complete block (split plot) design with three replicates, where the different levels of CDM plus grass–legume treatment were considered as main plots, and sampling depth was considered as split plots. Data were analyzed using a mixed model (PROC MIXED) analysis of variance (ANOVA) for a randomized split-plot design using SAS software version 9.2 (SAS Institute, 2002). The model included CDM application rates plus grass–legume treatment, sampling depth, their interaction as fixed effects, and block as a random effect. Separation of means was achieved using the PDIFF option of the LSMEANS statement. Significance was evaluated at $P \leq 0.05$. No-input control was not included in the statistical analysis because it was not replicated within the experimental plots.

Simple Pearson's correlation analysis was also performed to determine relationship between microbial biomass and aggregate-size fractions. Significant predictors (MBC and/or MBN) were selected using a stepwise model that used a $P \leq 0.05$ to enter or remove predictors from the model.

3. Results

3.1. Microbial biomass

At 0–5 cm depth, MBC followed the trend 22.4 = 33.6 < 44.8 Mg CDM ha^{-1} = grass–legume treatment (Table 2). There was a similar trend in the 5–15 cm depth, where MBC was statistically similar under grass–legume and 44.8 Mg ha^{-1} CDM application rate, but was significantly higher as compared to 22.4 and 33.6 Mg ha^{-1} CDM application rates (Table 2). Microbial biomass N showed a similar pattern as MBC in relation to treatments, with grass–legume and 44.8 Mg ha^{-1} CDM application rate resulting in higher MBN than CDM added at 22.4 and 33.6 Mg ha^{-1}(Table 2). Since there was a similar trend in both MBC and MBN across treatments at 0–5 and 5–15 cm depth, further comparisons were made only for the 0–5 cm depth. Under 44.Mg ha^{-1} CDM application rate and grass–legume treatment, MBC and MBN respectively ranged between 265 and 346 μg C g^{-1} soil and between 94 and 110 μg N g^{-1} soil in the 0–5 cm depth, significantly higher than MBC and MBN under 22.4 and 33.6 Mg ha^{-1} CDM application rates (Table 2).

3.2. Determination of K_C for calculating biomass C

Four hours after substrate addition, there were still very high levels of anthrone-positive cellobiose (i.e., cellobiose remaining in the soil samples), but concentrations decreased significantly thereafter as observed at 20 h after addition (Table 3). Thus, we used soil respiration and cellobiose remaining at 20 h to determine the K_C. At 20 h, cellobiose-derived CO_2 was 103.7 μg C g^{-1} soil and anthrone-positive cellobiose was 9.9 μg C g^{-1} soil (Table 3), resulting in non-microbial C of 113.6 μg C g^{-1} soil. This indicates that the amount of cellobiose-C assimilated was 307.4 μg C g^{-1} soil (i.e. 421 μg C g^{-1}–113.6 μg C g^{-1}). However, measured MBC was 136.1 μg C g^{-1}, which was determined by subtracting MBC

Table 3
Soil respiration, cellobiose remaining and microbial biomass C with and without cellobiose addition determined at 4 and 20 h after the addition of cellobiose. Values in parenthesis are SD (n = 3).

Sample[a]	Time (h)[b]	Total CO_2 respiration (μg C g^{-1} soil)		Cellobiose-derived CO_2 (μg C g^{-1} soil)	Remaining cellobiose-C (Anthrone positive) (μg C g^{-1} soil)		Remaining non-respired cellobiose-C (μg C g^{-1} soil)	Microbial biomass C (Total μg C g^{-1} soil)		Microbial biomass due to Cellobiose (μg C g^{-1} soil)
		With cellobiose	Without cellobiose		With cellobiose	Without cellobiose		With cellobiose	Without cellobiose	
1	4	9.47(3.42)	2.95(0.87)	6.52	237.97(38.87)	11.23(3.30)	226.74	239.58(56.06)	163.93(21.44)	75.65
2	4	17.32(6.23)	5.51(1.01)	11.81	188.46(16.32)	9.25(1.28)	179.21	388.13(33.90)	324.03(19.64)	64.10
3	4	13.49(2.14)	4.76(1.01)	8.73	240.94(6.79)	14.20(3.76)	226.74	247.83(42.32)	256.18(11.33)	−8.35
Ave.				9.02			210.90			43.80
1	20	112.85(24.43)	7.02 (2.77)	105.83	30.98(3.60)	19.15(12.06)	11.83	314.43(143.80)	151.03(52.99)	134.40
2	20	125.30 (34.23)	21.61(10.26)	103.69	23.11(6.20)	13.21(5.02)	9.90	411.08(87.71)	275.33(87.02)	135.75
3	20	117.90 (17.53)	16.28(8.55)	101.62	24.86(6.55)	16.98(6.24)	7.88	508.78(34.48)	399.73(47.17)	136.05
Ave.				103.71			9.87			136.06

[a] Sample numbers refer to replicates, and each sample was performed in triplicate.
[b] Time at which measurements were taken after cellobiose addition.

in the control samples (275.3 μg C g^{-1} soil) from that of the cellobiose-amended samples (411.1 μg C g^{-1} soil). Thus K_C, determined by dividing measured MBC (136.1 μg C g^{-1}) by the amount of cellobiose-C assimilated (307.4 μg C g^{-1}), was 0.44 implying that only 44% of the biomass C released following chloroform fumigation was actually extracted by 0.5 *M* K_2SO_4.Thus E_C, the difference between DOC extracted in K_2SO_4 from fumigated and non-fumigated samples, was divided by 0.44 to calculate MBC (Table 2).

3.3. Aggregate size distribution

Although no significant difference among treatments was observed for aggregate size distribution at 0–5 cm depth (Table 4), the percentage of total soil accounted for by large (>2000 μm) and small (250–2000 μm) macroaggregates was lower in the 0–5 cm depth compared to 5–15 cm, when averaged across all treatments (Table 4). Significant influence of treatment on aggregate size distribution was found in the 5–15 cm depth, where soils under 44.8 Mg ha^{-1} CDM application rate and grass–legume each had just over 20% small macroaggregates and between 18 and 21% large macroaggregates, significantly greater than soils under 22.4 and 33.6 Mg ha^{-1} CDM application rates (Table 4). This trend was further reflected in aggregates MWD, with the 44.8 Mg ha^{-1} CDM application rate and grass–legume respectively resulting in on average 1.6- and 1.8-times higher MWD compared to 22.4 and 33.6 Mg ha^{-1} CDM application rates (Table 4).

Table 4
Water-stable aggregate size distribution and mean weight diameter (MWD) as affected by different levels of composted dairy manure (CDM) and grass–legume treatment at two soil depths. >2000 μm = large macroaggregates; 250–2000 μm = small macroaggregates; 53–250 μm = microaggregates; <53 μm = silt and clay fraction. Values in parenthesis are SD (n = 3).

Treatment	Depth	Aggregate-size distribution (g 100 g^{-1} soil)				
		>2000 μm	250–2000 μm	53–250 μm	<53 μm	MWD
Control[a]	0–5 cm	11.2 (2.1)	12.3 (1.8)	68.8 (2.6)	7.7 (1.5)	0.81 (0.31)
Grass–legume[b]		9.6 (1.1) a[c]	13.9 (1.1) a	69.0 (2.9) a	7.5 (1.4) a	0.74 (0.06) a
22.4		11.8 (1.3) a	15.2 (2.2) a	66.3 (0.4) a	6.7 (1.2) a	0.86 (0.05) a
33.6		11.1 (4.7) a	14.5 (1.8) a	66.1 (3.7) a	8.3 (0.5) a	0.82 (0.22) a
44.8		12.6 (1.1) a	14.8 (2.0) a	65.0 (1.5) a	7.6 (0.6) a	0.90 (0.05) a
Mean		11.3 (2.5) B[d]	14.6 (1.6) B	66.6 (2.6) A	7.5 (1.0) A	0.83 (0.12) A
Control	5–15 cm	12.1 (2.3)	15.3 (4.0)	63.3 (2.1)	9.3 (1.1)	0.88(0.06)
Grass–legume		20.9 (1.8) a	20.8 (5.3) a	50.0 (6.0) a	8.3 (1.0) a	1.36 (0.12) a
22.4		9.1 (2.9) b	13.7 (3.1) b	68.0 (1.1) b	9.2 (2.5) a	0.71 (0.14) b
33.6		10.5 (0.8) b	16.0 (1.3) b	65.0 (1.7) b	8.5 (1.5) a	0.80 (0.03) b
44.8		18.0 (0.9) a	20.5 (1.1) a	53.7 (2.6) a	7.8 (1.0) a	1.22 (0.05) a
Mean		14.6 (5.4) A	17.7 (4.1) A	59.2 (8.4) B	8.5 (1.5) A	1.02 (0.29) B
ANOVA						
Treatment		**	ns	**	ns	*
Depth		**	**	***	ns	***
Treatment × depth		***	*	***	ns	***

Level of significance: * $P<0.1$; ** $P<0.05$; *** $P<0.001$; ns. Not significant.
[a] The no-input control was not included in the statistical analysis because it was not replicated within the experimental plots.
[b] The grass–legume treatment had alfalfa (*Medicago sativa*) interseeded into a mixture of perennial grasses.
[c] Values within a column followed by common lowercase letter were not significantly different ($P<0.05$).
[d] Values followed by common uppercase letter were not significantly different ($P<0.05$) between the sampling depths.

3.4. Whole soil and aggregate-associated organic C and N

No significant difference among treatments was observed for total SOC and N of the whole soil at either of the sampling depths (data not shown). Although no significant difference between the two sampling depths was observed for aggregate-associated SOC except for clay and silt fraction, there was clear effect of treatment on SOC content of both small and large macroaggregates at 5–15 cm depth (Table 5). The addition of CDM at 44.8 Mg ha^{-1} increased SOC content of the small macroaggregates at 5–15 cm depth compared to other treatments (21% higher compared to 22.4 Mg CDM ha^{-1}, 20% higher compared to grass–legume treatment, and 16% higher compared to 33.6 Mg CDM ha^{-1}; Table 5). Similarly, large macroaggregates of 5–15 cm depth had higher SOC content under 44.8 Mg ha^{-1} CDM application rate than other treatments (34% higher compared to grass–legume treatment, 20% higher compared to 22.4 Mg CDM ha^{-1}, and 16% higher compared to 33.6 Mg CDM ha^{-1}; Table 5).

3.5. Relationships between microbial biomass and aggregation

Of the microbial parameters measured in this study, both MBC and MBN were found to be positively correlated with large macroaggregates (Table 6). Small macroaggregates showed positive but weak correlation with MBN. Microaggregates, on the other hand, showed strong negative correlation with both MBC and MBN (Table 6). According to the results of our stepwise regression analysis, MBC and MBN were selected as better predictors of the amounts of large macroaggregates ($r^2 = 0.65$, $P = 0.002$; data not shown) and microaggreagtes ($r^2 = 0.72$, $P = 0.001$; data not shown), respectively.

4. Discussion

4.1. Effects of CDM and alfalfa interseeding on soil C and N dynamics

The results presented here suggest that addition of CDM or alfalfa interseeding to grass-based pasture had no significant effect on total soil C and N. This may be because of the short duration of the trial (approximately 1.5 years) as total soil C and N tend to change slowly (Haynes, 1999). We suggest that cumulative impact of the addition of CDM or alfalfa interseeding on total soil C and N may only be obvious in longer term studies. We hypothesized that higher CDM addition rates would result in greater microbial biomass in the short term compared to lower CDM rates (Haynes and Francis, 1993). In general, our results suggest that addition of CDM had notable effects on microbial biomass. This is evidenced by a significant increase in MBC and MBN at the highest CDM rate (44.8 Mg ha^{-1}) compared to lower CDM rates considered in this study (Table 2); these are variables known to change rapidly in response to changes in soil organic matter management (Haynes, 1999). The significant increase in MBC and MBN from the two lower CDM rates to the highest CDM rate implies that higher CDM rates (e.g. ≥45 Mg ha^{-1}) may be required to induce rapid changes in microbial biomass in pasture soils such as those investigated here. The findings of Kushwaha et al. (2001) further confirm this idea by demonstrating that addition of organic residue increased the size of soil microbial biomass, while removal of organic residues had an opposite effect. Our results are similar to previous studies that demonstrate that changes in MBC and MBN in response to changes in organic matter management occur more rapidly than changes in total SOC (Carter, 1986; Haynes, 1999; Haynes and Beare, 1997).

In support of our second hypothesis, the inclusion of legume into grass-based pasture seemed to enhance microbial biomass. As hypothesized, the interseeding of alfalfa into pasture grasses resulted in higher MBC and MBN compared to CDM added at lower rates (Table 2). This is surprising due to the difficulty experienced in establishing alfalfa in a grass stand. Even after alfalfa was seeded

Table 5
Soil organic C and N distributions in aggregate-size fractions (g kg^{-1} sand-free aggregates) as affected by three composted dairy manure (CDM) levels and grass–legume treatment at two soil depths. >2000 μm = large macroaggregates, 250–2000 μm = small macroaggregates, 53–250 μm = microaggregates, <53 μm = silt plus clay fraction. Values in parenthesis are SD (n = 3).

Treatment	Depth	>2000 μm		250–2000 μm		53–250 μm		<53 μm	
		SOC	Total N	SOC	Total N	SOC	Total N	SOC	Total N
Control[a]	0–5 cm	57.2 (9.7)	4.9 (2.0)	58.1 (4.6)	3.8 (2.2)	50.5 (4.6)	3.7 (1.4)	28.5 (1.9)	1.6 (0.1)
Grass–legume[b]		63.4 (12.0) a[c]	5.6 (1.4) a	54.8 (9.1) a	4.5 (0.6) a	60.4 (27.4) a	5.4 (2.6) a	32.8 (2.4) a	2.80 (0.3) a
22.4		55.9 (7.3) a	4.7 (0.7) a	50.9 (1.1) a	4.3 (0.2) a	55.2 (10.2) a	4.5 (0.7) a	31.9 (2.1) a	2.97 (0.1) a
33.6		55.2 (7.8) a	5.3 (1.0) a	67.1 (10.6) a	5.7 (1.4) a	56.0 (3.5) a	4.8 (0.7) a	32.8 (2.8) a	2.94 (0.1) a
44.8		51.5 (8.2) a	3.7 (1.2) a	65.7 (22.0) a	5.0 (1.3) a	71.6 (24.6) a	9.0 (6.0) a	32.2 (2.4) a	3.05 (0.3) a
Mean		56.5 (8.8) A[d]	4.8 (1.1) A	59.6 (10.7) A	4.9 (0.9) A	60.8 (16.4) A	5.9 (2.5) A	32.4 (2.1) B	2.94 (0.2) A
Control	5–15 cm	45.2 (1.7)	3.8 (0.8)	39.5 (2.1)	3.7 (1.1)	43.4 (2.3)	4.1 (2.0)	28.7 (2.2)	2.1 (0.7)
Grass–legume		48.2 (3.4) b	5.3 (2.5) a	51.0 (10.5) b	4.3 (1.0) a	53.9 (14.6) a	4.4 (0.6) a	30.2 (2.3) a	2.69 (0.4) a
22.4		53.9 (7.6) b	4.7 (0.5) a	50.6 (10.4) b	4.8 (0.8) a	65.8 (21.6) a	6.1 (1.8) a	30.8 (3.1) a	2.87 (0.3) a
33.6		55.5 (4.9) b	4.7 (1.3) a	52.7 (16.0) b	4.8 (1.8) a	66.6(42.0) a	5.9 (3.3) a	31.5 (1.3) a	2.69 (0.6) a
44.8		64.6 (3.6) a	5.5 (1.6) a	61.1 (10.7) a	4.2 (1.0) a	67.3 (28.2) a	6.0 (2.4) a	32.4 (1.3) a	3.08 (0.3) a
Mean		55.6 (6.6) A	5.0 (1.5) A	53.9 (11.9) A	4.5 (1.1) A	63.4 (26.6) A	5.6 (2.0) A	31.2 (2.0) A	2.83 (0.4) A
ANOVA									
Treatment		*	ns	*	ns	ns	ns	ns	ns
Depth		ns	ns	ns	ns	ns	ns	*	ns
Treatment × depth		ns	ns	ns	ns	ns	ns	ns	ns

Level of significance: * $P<0.1$; ** $P<0.05$; $P<0.001$; ns, not significant.
[a] No-input control was not included in the statistical analysis because it was not replicated within the experimental plots.
[b] The grass–legume treatment had alfalfa (*Medicago sativa*) interseeded into a mixture of perennial grasses.
[c] Values within a column followed by common lowercase letter were not significantly different ($P<0.05$).
[d] Values followed by common uppercase letter were not significantly different ($P<0.05$) between the two sampling depths.

again in 2009, it contributed only 36% to the harvestable forage yield (data not shown). However, our results support findings from previous studies where legumes, including alfalfa, were shown to influence microbial biomass. Haynes and Beare (1997), for example, observed higher microbial biomass under legumes than grasses and attributed their observation to increased rhizodeposited C and N due to dying roots and sloughed-off nodules.

4.2. Effects of CDM and alfalfa interseeding on aggregate-size distribution

While the addition of CDM had no notable effect on soil aggregation at 0–5 cm depth, the proportion of macroaggregates (>250 μm) and MWD values were lower at 0–5 cm depth than at 5–15 cm (Table 4). This is in spite of the soil at the field site receiving CDM addition twice via surface broadcasting in spring and fall 2008 before sampling in summer 2009. In addition, the soil at the field site had not been tilled for several years, except in summer 2007 prior to planting of the experiment. So, the observation that macroaggregates and MWD values were lower at 0–5 cm than at 5–15 cm was unexpected. One possible explanation is that blanket CDM application may have caused changes in the exchange complex that resulted in easy dispersion of soil aggregates (Whalen and Chang, 2002). In studies conducted in Canada, Larney et al. (1994) and Bullock et al. (1999) observed fewer macroaggregates closer to the soil surface than in the subsurface layer and attributed their observation to freeze-thaw and wet-dry cycles. We speculate that the same might have occurred in this experiment. In Colorado, surface soils can be much drier than subsurface soil in the months of June to August (Damoff and Reynolds, 2004). This combined with soil rewetting due to frequent irrigation may have caused wet-dry cycles near the surface layer resulting in easy slaking of macroaggregates (Tisdall and Oades, 1982). While there appears to be greater potential for wet-dry and freeze-thaw cycles to have a dramatic effect on aggregation in the top few centimeters of soil in our climate (Degens and Sparling, 1995; Denef et al., 2001a,b), we acknowledge that this warrants further research.

Significant effects of the addition of CDM on soil aggregation were observed only at 5–15 cm depth (Table 4). Consistent with the CDM-induced increase in microbial biomass observed at the highest CDM rate (44.8 Mg ha^{-1}), a higher proportion of macroaggregates (>250 μm) and MWD values were also observed at the highest CDM rate compared to the lower CDM rates. Since both small and large macroaggregates had higher SOC content at 44.8 Mg CDM ha^{-1} compared to the other CDM rates considered in this study (Table 5), the greater proportion of macroaggregates observed at 44.8 Mg CDM ha^{-1} could be due to certain active organic carbon compounds such as POM from CDM, which may have acted as nucleation sites for the formation of macroaggregates (Chivenge et al., 2011). The positive correlation between large macroaggregates and MBC (Table 6) was evidence that microbial biomass had also played a role in the formation of large macroaggregates (Aoyama et al., 1999; Beare et al., 1997; Bossuyt et al., 2001; Drury et al., 1991; Edgerton et al., 1995; Haynes and Beare, 1997; Lynch and Bragg, 1985; Milne and Haynes, 2004; Robertson et al., 1991).

Similar to the highest CDM rate (44.8 Mg ha^{-1}), we observed a higher proportion of macroaggregates and MWD values under grass–legume treatment compared to the two lower CDM rates considered in this study (Table 4). Given the fact that there was less than 36% alfalfa stand in our grass–legume plots (data not shown), we did not expect the grass–legume treatment to have such a pronounced effect on aggregation in such a short period of time. However, this supports previous studies that demonstrate that the stability of macroaggregates can be modified after only a short period of alfalfa growth (Angers and Avon, 1990 cited in Campbell et al., 1993; Bronick and Lal, 2005). For example, Haynes and Beare (1997) concluded that soil aggregate stability was greatest due to higher

Table 6
Correlation coefficients (*R*) between measures of microbial biomass and soil aggregates. In each row, the correlation coefficient (*R*) is given with the P-value below it in italic.

Microbial biomass[a]	Soil aggregates		
	Large macroaggregates	Small macroaggregates	Microaggregates
MBC	0.81	0.44	−0.78
	0.002	*ns*	*0.003*
MBN	0.78	0.56	−0.84
	0.003	*0.063*	*0.001*

[a] MBC, microbial biomass C; MBN, microbial biomass N; ns, not significant.

rhizodeposited C and N under legumes, which also had higher fungal hyphal length in macroaggregates compared to pasture grasses without legumes. In our study, the SOC content of macroaggregates was not affected by the grass–legume treatment (Table 5). This makes it possible to exclude total amount of SOC as being the main factor in explaining differences observed between grass–legume treatment and the two lower CDM rates with respect to macroaggregates. This could be explained by the higher microbial biomass observed under grass–legume (Table 2) rather than total amount of SOC. The positive correlation between large macroaggregates and MBC further supports the hypothesis that rhizodeposition of C substrate for microbial growth is likely the major factor affecting macroaggregates (Haynes and Beare, 1997; Milne and Haynes, 2004). From soil aggregate stability point of view, our results suggest that interseeding alfalfa into pasture grasses can be more beneficial than the addition of CDM at lower rates (e.g. 22.4 Mg ha^{-1}).

5. Conclusion

While the results presented here suggest that the addition of a high quantity of CDM (e.g., 45 Mg ha^{-1}) and alfalfa interseeding to perennial pasture grasses influences short-term increase in microbial biomass and aggregate stability, the nature of the effects differ. The addition of a high quantity of CDM enhanced total SOC of macroaggregates, which likely resulted in the formation and stabilization of aggregates larger than 250 μm as reflected by the higher proportion of large macroaggregates. In the grass–legume treatment, on the other hand, the interseeding of alfalfa induced microbial biomass, which likely contributed to the formation and stabilization of large macroaggregates.

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