DISSERTATION

EVALUATION OF SOIL FERTILITY AND SOIL QUALITY ASPECTS OF ORGANIC PERENNIAL PASTURES AND ANNUAL FORAGE CROPS

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ABSTRACT

EVALUATION OF SOIL FERTILITY AND SOIL QUALITY ASPECTS OF ORGANIC PERENNIAL PASTURES AND ANNUAL FORAGE CROPS

In the United States, organic milk production has been one of the fastest growing segments of organic agriculture. Organic producers are required to manage soil fertility in a way that maintains soil organic matter. Animal manure contains essential plant nutrients such as nitrogen (N) that can be recycled beneficially on agricultural lands for enhancement of crop yield as well as soil quality by supplying soils with organic matter. While nutrients in synthetic fertilizers are readily available for plant uptake, not all N in manure is immediately available for plant uptake, and that organic-N must first be mineralized within a crop's growing season. While under-fertilization can result in reduced crop yield, loss of N into the environment can occur when N supplied by manure exceeds crop demand. Therefore, when fertility is maintained with manure, one of the challenges facing organic producers is how much should be applied to ensure an adequate N supply for optimum crop yield while reducing N loss to the environment. Due to the growing trend in organic dairy operations along Colorado's eastern High Plains, this study was conducted to determine whether using raw dairy manure (RDM) and composted dairy manure (CDM) is sufficient to meet the N needs of perennial grasses and annual forage crops. In addition, this study sought to evaluate how RDM and CDM managed for organic perennial grasses and annual forage crops impact soil quality.

The specific objectives of the study were to (i) estimate N mineralization (N_{min}) from a soil receiving CDM and subsequent N uptake by organically grown perennial forage grasses, (ii) to quantify N_{min} from a soil receiving CDM and RDM amendments and subsequent N uptake by organically grown annual forage crop, (iii) to evaluate how RDM and CDM amendments managed for organic annual forage crops and perennial pasture grasses impact the abundance, biomass and species-composition of earthworm communities in the short-term following implementation of management practices, and (iv) to evaluate the short-term impact of variable rate applications of CDM on soil microbial biomass carbon (MBC) and nitrogen (MBN) as well as aggregate stability under a perennial grass system.

Two field experiments were conducted to accomplish the above mentioned objectives. Experiment 1, hereafter 'perennial study', was initiated in 2008 on transitional organic land located at the Colorado State University Agricultural Research, Development and Education Center (ARDEC) south of Wellington, Colorado (40°39' N, 104°59' W). The soil at the field site was classified as a fine-loamy, mixed, mesic, Aridic Haplustalf. The experiment utilized a randomized complete block design with three replications. Plots measured 3 × 12 m each, and were seeded with perennial grass mixtures in September 2007.

Experiment 2, hereafter 'annual study', was initiated in 2008 on certified organic land located at the Colorado State University Horticultural Research facility northeast of Fort Collins, Colorado (40°36' N, 104°59' W). The soil was classified as a fine, smectitic, mesic, Aridic Argiustoll of the Nunn series. This study utilized a split-plot experimental design where the warm-season annual forage grass, teff (*Ergarostis tef*), and bare-fallow management were main plots and soil amendments (CDM, RDM, and control) were subplots.

To accomplish objective (i), a field incubation study was conducted at the perennial study site using an *in situ* intact core resin bag technique to estimate N_{min} under two perennial grass mixtures: (1) orchardgrass-smooth brome-meadow brome and (2) hybrid wheatgrass-tall fescue-hybrid brome. CDM was surface broadcast at 22.4 and 11.2 Mg ha^{-1} (wet weight) in 2008 and 2009, respectively. To accomplish objective (ii), a field incubation study was carried out at the annual study site and N_{min} was determined as described in objective (i). For this study, the application rates of CDM and RDM were designed to achieve approximately 123 and 56 kg total N ha^{-1} in 2008 and 2009, respectively. To accomplish the first part of objective (iii), earthworms were sampled from the annual study site in July 2009. To achieve the second part of objective (iii) and objective (iv), variable rate CDM applications of 0, 22.4, 33.6, and 44.8 Mg ha^{-1} (wet weight) were topdressed onto a grass mixture consisting of orchardgrass, smooth brome, and meadow brome. Earthworms and soil samples were collected from the perennial study site in July of 2009.

For N_{min} at the perennial site, results indicated that regardless of the grass mixture net soil N_{min} was higher in 2008 compared to 2009, even though soils were relatively drier and cooler. Net CDM N_{min} was very low in both years of the study, in some cases with slightly negative values, possibly suggesting N immobilization. Forage yield and N uptake of grasses that received CDM did not differ from those that received no CDM. Tissue N contents of grasses were below those considered optimal for forage grass production, possibly suggesting season-long N deficiency, especially in the second year of the study. Overall, this study indicated that CDM applied at the rates used in the present study might not meet the N needs of perennial forage grasses. Thus, higher quality CDM than the one used in the current study or alternatively other sources of N might be needed for optimum forage yield.

For N_{min} at the annual site, there was a significant ($p<0.05$) difference in mineralized N between RDM and CDM in 2008 within the teff; however, there was no significant difference in N_{min} between CDM- and RDM-amended soils under bare-fallow. Within the teff, RDM applied in the spring of 2008 resulted in N_{min} significantly greater than CDM and the unfertilized control. Of the total RDM N that was applied in the spring of 2008, about 59% was mineralized during the 2008 teff growing season. Teff dry matter yield and N uptake obtained from RDM-amended plots were also higher than those obtained from CDM-amended and the unfertilized control plots. In 2009, there was no significant difference in mineralized N between CDM and RDM. Overall, this study suggests that RDM can provide sufficient in-season plant available N to support high dry matter yields of annual forage crops such as teff, particularly in the year following application.

The earthworms identified from both the annual and perennial studies were composed entirely of the endogeic species *Aporrectodea rosea* (Savigny), *A. tuberculata* (Eisen), and *A. turgida* (Eisen), the first being found only in the perennial pasture. Regarding the annual study, RDM and CDM application did not influence earthworm abundance and biomass in teff plots. However, under bare-fallow plots, RDM and CDM both significantly ($p<0.05$) increased earthworm total abundance and biomass as compared to the control, the effect of the latter being greater than the former. In the case of the perennial study, a significant ($p<0.05$) increase in earthworm total abundance and biomass was observed at CDM application of 33.6 Mg ha^{-1} compared to the other rates used in this study. This study also demonstrated that total earthworm abundance and biomass were significantly ($p<0.05$) reduced at the highest CDM application rate (44.8 Mg ha^{-1}) which may be due in part to osmotic stress and dehydration resulting from larger

CDM application rates. The high CDM application rate (44.8 Mg ha^{-1}) increased soil electrical conductivity (EC = 0.68 dS m^{-1}) compared to the other rates used. A significant negative correlation was also found between EC and earthworm total abundance (R = -0.37, p = 0.002) and biomass (R = -0.29, p = 0.04). Overall, this study highlighted that endogeic earthworms can be negatively affected at concentrations often considered non-saline for most plants.

The study looking at soil microbial biomass and aggregate stability found MBC and MBN to be significantly ($p<0.05$) higher at the CDM application of 44.8 Mg ha^{-1} than at the other rates except for the 0 Mg ha^{-1} treatment of no CDM application, where alfalfa was interseeded into the perennial grass mixture. This trend was also reflected by increased aggregate stability at the 44.8 Mg ha^{-1} CDM application rate as compared to the other rates considered in this study. Interestingly, MBC and MBN as well as aggregate stability values obtained for the treatment with no CDM, where alfalfa was interseeded into the perennial grass mixture, matched those obtained at the high CDM application rate (44.8 Mg ha^{-1}). These results suggest that CDM application was just as important for improving microbial biomass and aggregate stability as was the addition of a legume (alfalfa) to grass-based pasture. Significant correlations were found between MBC and large (>2000 μm) macroaggregates (R = 0.81, p = 0.002), MBN and small (250-2000 μm) macroaggregates (R = 0.56, p = 0.06), as well as between microaggregates and MBN (R = -0.85, p = 0.001). These results provide some evidence of the possibility that higher microbial biomass resulting from the application of CDM at a relatively high rates (e.g., 44.8 Mg ha^{-1}) and alfalfa interseeded into a grass-based pasture resulted in the observed improved aggregate stability. Nevertheless, further investigations on this topic are warranted to confirm this.

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thanks also goes to Kathy Doesken and her wonderful husband Nolan Doesken. I thank you both for every single support you have offered me and my family alike from the very first day I arrived to Fort Collins. You are amazing, and it is my prayer that my heavenly God bless you so abundantly with everything you want in your life. There are two people who have had a massive influence on me and deserve even greater appreciation, for without their consistent moral support and prayer, I would not have achieved this. These are my own father, Taffe Hurisso, and my beloved wife, Seble Mamo Biykedagn. In fact one of the commandments of God says "Honor your father and your mother, so that you may live long in the land which the lord your God gives you". Seble, thank you very much also for taking care of our two lovely sons, Peniel and Ebenezer, who are the source of my inspiration and a true joy. Lastly, but certainly not least, I would like to thank Aurora Organic Dairy for funding this research and supporting the organic program at Colorado State University.

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CHAPTER 1: ESTIMATING NITROGEN MINERALIZATION FROM COMPOSTED DAIRY MANURE IN AN ORGANICALLY MANAGED PERENNIAL GRASS SYSTEM

Summary

Quantification of nitrogen (N) mineralized in manure can be a useful parameter for estimating its N supplying capacity for economic and environmental reasons. This study was conducted to determine N mineralization from composted dairy cow manure (CDM) applied on two different perennial grass mixes in northern Colorado. Intact soil cores contained in aluminum tubes and with ion-exchange resin bags attached at the bottom were incubated *in situ*. The tubes were amended with CDM or were part of control sets that received no CDM to monitor soil N mineralization. The CDM was surface broadcast in spring 2008 at 22.4 Mg ha^{-1} (on a wet basis). For the 2009 growing season, CDM was applied at 11.2 Mg ha^{-1} in the fall of 2008. Compared to 2009, net soil N mineralization was higher in 2008, even though soils were relatively drier and cooler. Despite net immobilization of N in 2008, there was net mineralization of N in 2009. Irrespective of the grass mixture type, apparent N recovery (ANR) in 2008 was higher than in 2009, suggesting that the grasses were more reliant on soil N than the CDM N input. Forage yield, N uptake, and tissue N concentrations of grasses that received CDM amendment were generally undifferentiated from those that did not. Our results suggest that CDM applied at these rates does not meet grass N needs, and that either high quality CDM or supplemental N from other readily available sources is required, at least on short-term basis. Surface broadcasting of CDM likely contributed to the delayed mineralization peak from 2008 to 2009.

1. INTRODUCTION

Organic milk production has been one of the fastest growing segments of organic agriculture in the U.S. in recent years (McBride and Greene, 2009). In the U.S., the area of certified organic pastureland has more than quadrupled, increasing from 200,880 ha in 1997 to 874,354 ha in 2008 (ERS, 2008). In Colorado, the organic dairy industry continues to expand, with the largest concentration of organic dairies being in the eastern High Plains. This trend is driving the need for organically grown forages in this region. It was estimated in 2007 that there were 48,954 hectares of certified pastureland in Colorado (ERS, 2008). The majority of irrigated grass pasture production in this area is from cool-season perennial grasses such as smooth and meadow bromegrass, orchardgrass, and to some extent fescues (Miller, 2010), mostly due to their potential to provide sufficient grazing for cattle.

Perennial grasses require sustained, season-long nitrogen (N) availability to remain productive (Lynch et al., 2004). Because synthetic N fertilizer is prohibited for use on organically certified land, organic farming systems rely on practices such as crop rotation or application of animal manures to maintain soil fertility. Manure can be used as a primary source of N in organic forage production (Hadas et al., 1996; Eghball et al., 2002; Muñoz et al., 2008). Unlike commercial fertilizers, not all N in manure is immediately available and thus the determination of application rates is not straightforward (Risse et al., 2006). Applying heavy rates of manure can lead to a buildup of soil N while underapplication can result in reduced crop yields (Van Kessel and Reeves III, 2002). Therefore, when fertility is maintained with manure, one of the challenges facing organic forage growers is how much should be applied to ensure an adequate supply of N for optimum forage yields while reducing N loss to surface and ground water.

To determine the appropriate rate of manure application, information on crop N requirement, soil N supply, and manure N supply is required (Van Kessel and Reeves III, 2002; Eghball et al., 2002). Manure N content and its availability depend on different factors such as cattle diet, species, and manure management (Gilbertson et al., 1979; Eghball et al., 2002; Muñoz et al., 2008). Composting manure is a useful technique as it improves the characteristics of manure by reducing its volume while increasing uniformity (Eghball and Power, 1999; Eghball et al., 2002). However, due to loss of the easily convertible N compounds during composting and the remaining N converting into chemically stable forms, composted manure N availability is often lower than that of fresh manure (Eghball et al., 1997; Eghball, 2000). In eastern Nebraska, Eghball (2000) measured N mineralization from raw and composted manure using the *in situ* resin method, and found that organic N mineralization from composted manure was about half (11%) of that for noncomposted manure (21%) during the corn growing season. Despite the number of field incubation studies (DiStefano and Gholz, 1986; Kolberg et al., 1997; Eghball, 2000; Hanselman et al., 2004; Wienhold et al., 2009) that have measured soil or manure N mineralization, no study has attempted to quantify N mineralization from composted dairy cow manure (CDM) in soils under organic perennial grass system.

Therefore, the objective of this study was to determine *in situ* N mineralization from CDM topdressed onto two different perennial grass mixes that were managed in accordance with organic protocols, and furthermore, to estimate apparent N recovery (ANR) from CDM by these two perennial grass mixes over two growing seasons.

2. MATERIALS AND METHODS

2.1. Site description

The study was conducted on transitional organic land which was in the process of certification and was located at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC), south of Wellington, CO (40°39′ N, 104°59′ W, elevation 1554 m). The climate is semiarid with mean annual precipitation of 330 mm; about 88% of this occurs between April and October. Mean monthly temperatures range from 0°C in January to 22°C in July. The soil at this site was classified as a fine-loamy, mixed, mesic, Aridic Haplustalf (NRCS, 1980). On 13 March 2008, eight soil subsamples were taken to a depth of 20 cm using soil probes and pooled for soil chemical analysis. The soil had the following initial properties: OM, 2.4%; NO_3-N, 15.4 mg kg^{-1}; Olsen P, 29 mg kg^{-1}; pH-H_2O (1:1 w/v), 8.3; and EC (1:1 w/v), 0.4 dS m^{-1}. Prior to this study, the site was in alfalfa which was killed using tillage in the summer of 2007. In fall 2007, the field was clean-tilled and a blanket application of 22.4 Mg ha^{-1} CDM was spread and incorporated by disking.

2.2. Experimental design

The experiment was laid out as a randomized complete block design with three replications. The experimental plots measured 3 m x 12 m and were planted with two perennial grass mixtures in the fall of 2007: (1) hybrid wheatgrass (*E. hoffmainni*), tall fescue (*F. arundinacea*), hybrid brome (*B. inermis x B. biebersteinii*), hereafter referred to as 'HWG-TF-HB', and (2) orchardgrass (*D. glomerata*), meadow brome (*B. biebersteinii*), smooth brome (*B. inermis*), hereafter referred to as 'OG-MB-SB'.

2.3. Field incubation experiment

The *in situ* N mineralization procedure used in our study was similar to that described by DiStefano and Gholz (1986). Aluminum tubes, 15 cm long and 5 cm internal diameter, were placed in a steel soil probe and inserted into the ground to a depth of ~ 15 cm using a Giddings

soil probe (Giddings Machine Co., Windsor, CO). The steel soil probe with intact soil core was withdrawn from the ground, and the soil filled aluminum tube was removed and prepared for reinsertion. Prior to reinsertion, a Lycra® bag containing 20 mL of ion exchange resins, consisting of equal amounts of anion- and cation-saturated resins (USF A-464D and USF C-211, respectively), was placed at the bottom of the tube where ~ 2 cm of soil had been removed. Resin bags were covered by nylon, which was then fastened to the bottom of the tube with duct tape. The entire unit was then reinserted back into the hole from which the soil came.

To compare N mineralization between the two grass mixes (HWG-TF-HB versus OG-MB-SB), twenty tubes were inserted in each plot on 22 April 2008. Before applying CDM to the plots, all tubes were first capped and CDM was then spread by hand uniformly across the plot area at 22.4 Mg ha^{-1} on wet weight basis (Table 1). Following CDM application, the caps were removed and 15 out of the 20 tubes were amended with CDM. Pre-weighed CDM amendment of 3.57 g (based on the 22.4 Mg ha^{-1} CDM application rate) was added to each tube in a 2-cm space that was left unfilled with soil for this purpose. The remaining 5 tubes that did not receive CDM amendment served as controls to monitor soil N mineralization. For the 2009 growing season, CDM was applied in fall 2008 at 11.2 Mg ha^{-1} (wet weight basis). In 2009, N mineralization tubes were inserted again on 22 April 2009, but no CDM amendments were added to the tubes as the soil already contained CDM from previous broadcast applications. As such, the mineralized N obtained from the second year study is attributable both to the initial spring 2008 application as well as the 11.2 Mg ha^{-1} CDM applied in fall 2008. In 2009, control tubes were inserted in the border plots where no CDM was applied.

Four tubes (three CDM-amended and one control) were randomly selected and removed from each plot at approximately 4-week intervals five-times during the growing season. In 2008,

tubes were retrieved on 23 May (30 d), 23 June (61 d), 23 July (91 d), 26 Aug. (126 d), and 27 Sept. 2008 (158 d). In 2009, tube sampling occurred on 23 May (30 d), 23 June (61 d), 23 July (91 d), 23 Aug. (122 d), and 23 Sept. 2009 (154 d). At each sampling date, resin bags from soil cores that remained in the field were also removed and replaced with fresh bags to avoid resin oversaturation, which could lead to loss of inorganic N (Giblin et al., 1994; Wienhold et al., 2009). Upon removal from the ground, tubes along with the resin bags were placed in a cooler and transferred to the lab.

Composted dairy manure application levels in this study are in keeping with the commonly used compost application practices in this area. The total amount of N applied in CDM was 235 kg ha^{-1} in 2008 and 65 kg ha^{-1} in 2009 (Table 1.1). Subsamples of CDM were collected from a stockpile of CDM obtained for the study and analyzed at the Colorado Analytical Laboratory (Brighton, CO) for selected properties (Table 1.2).

2.4. Chemical analyses

In the lab, resin bags and the soil tubes were stored at 4°C until analysis. The soil extracted from each tube was thawed prior to grinding, and crushed to pass through a 2-mm screen before laboratory analysis. A subsample of 10 g soil was placed in a 150 mL Erlenmeyer flask and extracted in 100 mL of 2 *M* KCl for 1 h on a shaker. For the resin portion, each intact resin bag was placed in a 250 mL Erlenmeyer flask and extracted in 50 mL 2 *M* KCl for 1 h. This procedure was repeated two more times (150 mL total volume). Such serial extraction resulted in only 76% (standard deviation = 11) and 82% (standard deviation = 5) of adsorbed NH_4-N and NO_3-N being recovered, respectively. The NH_4-N and NO_3-N concentrations obtained from these sequential extractions were corrected using these factors. Such sequential extraction was done on resin bags retrieved after 30 and 61 d incubations in 2008. All the remaining resin bags

were sequentially extracted six times using 50 mL 2 *M* KCl because the latter procedure resulted in 100% recovery. Extract solutions from soils and resin bags were filtered through pre-leached Whatman No. 40 filter paper, and a 20 mL aliquot sample was frozen (-20°C) until analysis. The concentrations of NH_4-N and NO_3-N in the extract were determined colorimetrically using an Alpkem Flow Solution IV Autoanalyzer (OI Analytical, College Station, TX).

2.5. Calculations

The NH_4-N and NO_3-N in the soil and resin of the incubation system will hereafter be referred to as soil inorganic N (soil N_{inorg}) and resin inorganic N (resin N_{inorg}), respectively. The N_{inorg} in each portion (soil or resin) was calculated as the product of its concentration by weight. Based on date of tube sampling, there were five incubation periods in 2008 and 2009. For example, mineralized N (hereafter N_{min}) at the 30 d incubation was calculated by adding both soil and resin N_{inorg} concentrations together (Eq. [1]). To calculate N_{min} at the 61 d incubation, resin N_{inorg} at the 30 d incubation was added to the combined amounts of N_{inorg} in soil and resin analyses at the 61 d incubation (Eq. [2]). The latter approach was continued throughout the remaining incubation periods.

$$N_{min}\ 1 = \text{Soil } N_{inorg} \text{ at 30 d incubation} + \text{Resin } N_{inorg} \text{ at 30 d incubation} \quad [1]$$

$$N_{min}\ 2 = (\text{Soil } N_{inorg} + \text{Resin } N_{inorg}) \text{ at 61 d incubation} + \text{Resin } N_{inorg} \text{ at 30 d incubation} \quad [2]$$

To calculate net N mineralization at each sampling time, the initial soil N_{inorg} at the beginning of the study was subtracted from the N_{inorg} at each sampling time (Hart et al., 1994; Eghball, 2000). It was calculated using Eq. [3].

$$\text{Net } N_{min} = N_{inorg\ (time=\ t)} - N_{inorg\ (time=\ 0)} \quad [3]$$

where *t* is tube sampling time and (0) refers to the initial time at the beginning of the study.

Net N_{min} in CDM was calculated as the difference between N_{inorg} in CDM-amended and control soils (Kaboneka et al., 1997). To express N_{min} as a percentage of total N added, the amount of N mineralized in CDM was divided by total N applied in CDM and the result was multiplied by 100 (Hanselman et al., 2004).

2.6. Forage yield and nitrogen uptake

Grass was cut six times (2 June, 18 June, 9 July, 23 July, 11 Aug., and 22 Sept.) in 2008 and five times (18 May, 19 June, 20 July, 18 Aug., and 1 Oct.) in 2009. For yield determination, a 1.5 x 12 m swath was cut from the middle of each plot. Subsamples (400 g) were oven-dried (55°C, 72 h), and ground to pass a 2-mm sieve. Ground samples were analyzed for total N using the modified Dumas combustion method (Etheridge et al., 1998) with a LECO C and N analyzer (Model CN 2000 TruSpec, LECO Corp., St. Joseph, MI). Nitrogen uptake was calculated for each cutting, and cumulatively for the entire growing season by multiplying dry matter (DM) yields from each harvest by total N concentration and summing the results for the entire harvest. These values were then used to determine apparent N recovery (ANR) of CDM based on the difference method (Helton et al., 2008) using Eq. [4].

$$\text{ANR } (\%) = \left[\frac{(\text{N uptake})_{\text{CDM}} - (\text{N uptake})_{\text{control}}}{\text{N applied in CDM}}\right] \times 100 \qquad [4]$$

where the CDM subscript refers to manured treatment.

Climatic data (precipitation and soil temperature) were obtained from a nearby weather station. Soil thermal units were calculated by summing mean daily temperatures above 0 °C between each tube sampling date (Honeycutt et al., 1999). Irrigation amounts were also recorded.

2.7. Statistical analyses

Two generalized linear mixed analysis of variance (ANOVA) models (PROC GLIMMIX procedure) were developed to analyze our data using SAS software version 9.2 (SAS Institute

Inc., 2008). In the first analysis, a split-plot repeated-measures ANOVA that included grass mixes as fixed whole-plots, amendment as the fixed split-plot, and sampling dates as the fixed repeated measures was used to determine effects of these factors on N mineralization. We developed a second GLIMMIX model using grass mixes as fixed main plots, amendment as fixed subplot, and cutting dates as fixed repeated measures to examine the effects of these factors on forage DM yield and N uptake. Block and any interaction with block were considered random effects. First order autoregressive covariance structure [type = AR (1)] was used to fit the repeated measures model.

F-tests that utilized Type III sums of squares were used to assess the significance of fixed effects. When effects were significant, separation of means was achieved using Tukey's adjustment to the LSMEANS. *F*-test values and LSMEANS comparisons were considered significant at the $p \leq 0.05$ level unless noted otherwise. Total DM yield and total N uptake were analyzed as separate subsets in order to make paired comparisons between grass mixes with and without CDM. The least significant difference (LSD) method was used to separate means when the *F*-test was significant at the $p < 0.05$ level unless stated otherwise.

3. RESULTS AND DISCUSSION

3.1. Climatic conditions

In 2008, mean daily soil temperatures (15 cm) ranged from -7 to 23°C with an overall mean of 13°C during the period when the tubes were in the ground. In addition to the 160 mm of precipitation that occurred at the site, the experimental plots received 245 mm of irrigation. In 2009, mean daily soil temperatures ranged from 3 to 21°C and averaged 15°C. A total of 147 mm of precipitation occurred in 2009, with the experimental plots receiving an additional 395

mm of irrigation. The combined amount of precipitation and irrigation was greatest during the 24 July to 26 August period: 196 and 301 mm in 2008 and 2009, respectively (Table 1.3).

3.2. Nitrogen mineralization in soil

There were no significant differences in mineralized N between grass mixes or amendment treatments (CDM versus control) in 2008 (Table 1.4). There was a significant sampling time effect ($p < 0.0001$). Averaged across grass mixes and amendment treatments, there was a significant decrease in cumulative N mineralized during the second sampling interval (61 d incubation). This decrease may have been due to loss of mineralized inorganic N as a result of denitrification. There was a heavy rainstorm that occurred at the study site on 22 May 2008. Based on personal observation, it was noted that the aluminum tubes were filled with water for few days after the rain and this likely caused dentrification. Since denitrifcation was not measured in this study, it is difficult to say with certitude whether denitrification was more pronounced in the tubes relative to the surrounding bulk soil. However, Kolberg et al. (1997) suggested that drainage could be impaired at the interface of soil in the cores and the top of the resin bags because of the difference in resin and soil particle sizes, thereby increasing the potential for denitrifcation. To elucidate this would require further research to compare moisture contents of cores upon their removal to that of the surrounding bulk soil.

Because of the lack of significant differences between the two grass mixes or amendment treatments with respect to N mineralization, this study compared 2008 versus 2009 differences separately for CDM and soil N mineralization. Overall soil net N mineralization was much greater in 2008, even though the soils were relatively drier and cooler compared to 2009 (Tables 1.4 and 1.5). It was speculated that residues from the alfalfa crop that was present in the years preceding the current experiment and other soil organic N sources (e.g. the initial compost

application in the fall of 2007) contributed to the observed greater soil N mineralization in 2008 (Table 1.4). This observation agrees with past research demonstrating that fields with a past history of alfalfa growth appear to result in greater soil inorganic N release (Fox and Piekielek, 1988; Mohr et al., 1999). Mohr et al. (1999) compared N release from alfalfa terminated by herbicide with that from tilled alfalfa in southern Manitoba, Canada, and found that tillage increased plant available N in the first spring after alfalfa termination. A similar result was reported by Westermann and Crothers (1993) who also observed greater N mineralization following tillage and incorporation of legume residues. It should be noted that in the present study, the site was in alfalfa that was killed by tillage in fall 2007. This likely enhanced N release from the alfalfa residue as well as previous compost, and thus, contributed to the greater soil N mineralization observed in 2008.

3.3. Nitrogen mineralization in CDM

The amount of N mineralized was not statistically different between the grass mixes in 2009. However, there were pronounced differences between CDM and soil N mineralization in 2009, with mineralized N averaging 18 versus 28 kg ha^{-1} in the CDM-amended and control soil, respectively, when averaged across grass mixes and sampling times (p = 0.01; Table 1.5). This indicates that there was net mineralization of CDM N (estimated as the difference in N_{inorg} between CDM-amended and control soil) in 2009 (Table 1.6). The mineralization of CDM N in 2009 is attributable to several possible causes, including (i) the wetter and warmer soil conditions as evidenced by the higher soil temperatures and greater amount of precipitation as well as irrigation (Table 3); and (ii) the increased importance of CDM as a source of mineralizable N in 2009, once residual soil N was mineralized.

There was net immobilization of CDM N in 2008 as indicated by negative mineralization values (Kaboneka et al., 1997) (Table 1.6). Biologically active soils are characterized by microbial immobilization of N from inorganic forms and remineralization of organic N compounds into inorganic N forms (Bonde and Rosswall, 1987). Thus, the negative mineralization values observed in 2008 were possibly due to immobilization of CDM N by soil microbes decomposing the applied CDM (Norman et al., 1990). Given that C and N have been shown to stabilize during composting (Castellanos and Pratt, 1981; DeLuca and DeLuca, 1997; Eghball, 2000; Eghball et al., 2002; Risse et al., 2006), it is likely that C and N were less labile immediately after CDM application and contributed to the observed net N immobilization in 2008 (Table 1.6). Since surface broadcasting of organic residues has been previously shown to minimize exposure of the residues to soil microbial populations (Cogle et al., 1987; Mohr et al., 1999), one would speculate that surface application of the CDM in this experiment contributed to the delayed peak in mineralization from 2008 to 2009. It is worth noting that the mineralized N in 2009 could have included some of the residual N from CDM that was applied in the spring of 2008.

Expressed as a percentage of total N added, about 6% of the CDM N applied was mineralized during the 2009 growing season (Table 1.7). Estimates of second year CDM N mineralization in the present study are lower than estimates of availability determined by other researchers (Motavallie et al., 1989; Klausner et al., 1994). Using the same *in situ* N mineralization measurement technique, Eghball (2000) reported higher N availability estimates (6 to 12%) for composted feedlot manure. Differences between the two studies could be attributed to the higher manure N application rates used in Eghball's (2000) study than the ones used in the present study. In the Eghball (2000) study, composted feedlot manure was applied

annually at different rates for a 3-yr period for a total of 109.2 Mg ha^{-1} on a dry basis (or 945 kg total N ha^{-1}) prior to initiation of the *in situ* experiment. Given the lower N mineralization obtained in the present study as well as past research suggesting that composting stabilizes nutrients, it is important to (i) consider high quality manure than the one used or (ii) additional N should be applied from other readily available sources to meet the N needs of perennial forage grasses.

3.4. Forage dry matter yield

Even though forage DM yields varied significantly ($p < 0.0001$) across cuttings in both years (Table 1.8), this paper will only discuss total DM yields (all harvests combined) since these were of primary importance. Total forage DM yield did not differ between grass mixes that either did or did not receive CDM in either year (Table 1.8). However, averaged across grass mixes, total annual forage yield was greater in 2008 than in 2009, averaging 9.9 versus 5.4 Mg ha^{-1}, respectively ($p < 0.0001$; Table 1.8). One possible explanation for this difference is the high initial soil fertility in 2008 due to the influence of previous legume growth and blanket compost application.

Averaged across amendment treatments, HWG-TF-HB yielded greater than that of OG-MB-SB in 2008, although this difference was significant only at $p = 0.06$ (Table 1.8). The yield superiority of HWG-TF-HB over that of OG-MB-SB in 2008 was possibly due to the superior performance of tall fescue in this environment. Based on visual observation, it was noted that tall fescue often dominated the sward in the HWG-TF-HB plots. The observation that tall fescue tended to have the highest yield compared to all other grasses (Table 1.8) is similar to the findings of Waldron et al. (2002), who evaluated cool-season perennial grasses including tall

fescue, orchardgrass, and meadow brome, and reported that tall fescue had superior forage yield over the other grasses.

3.5. Tissue N concentrations

In 2008, grass mixes that received CDM application appeared to have lower forage tissue N concentrations than those that did not (Table 1.9). The higher tissue-N concentrations observed in grasses that did not receive CDM amendment is likely due to high initial soil fertility (greater soil net N mineralization). On the other hand, lower tissue-N concentrations in grasses that received CDM was possibly due to net immobilization of N by microbes decomposing the applied CDM. In 2009, tissue-N contents were smaller compared to those in 2008 (Table 1.9). Furthermore, the tissue N concentrations of grasses found in this study were below those considered optimal for forage grass production possibly suggesting a season-long N deficiency (Dougherty and Rhykerd, 1985). Consequently, the tissue N data in this study show that additional N inputs from other sources may be needed to avoid potential yield reductions resulting from the lower N mineralization of CDM.

3.6. Forage nitrogen uptake

In both years, N uptake varied significantly only among cuttings when averaged across grass mixtures (Table 1.10). The uptake of N tended to be higher early in the growing season and decreased in rate during the latter stages of the growing season (Table 1.10). However, forage N uptake did not differ between grass mixes that either did or did not receive CDM. The lack of significant difference in N uptake was attributed to the lack of difference in forage DM yield between the grass mixes. When plant N uptake was summed across cuttings and compared between the two years, total N uptake was greater in 2008 than in 2009 (327 kg N ha^{-1} versus 122 kg N ha^{-1}, respectively; $p < 0.0001$; Table 1.10). It is likely that cultivation of pre-existing alfalfa

before the area was converted into a perennial pasture was followed by a flush of soil mineral N (Lynch et al., 2004). This along with residual N from the pre-plant compost application may have still been contributing to the soil N pool, and could partly explain the greater forage N uptake in 2008.

3.7. Apparent nitrogen recovery

Although the 2008 ANR from CDM obtained for HWG-TF-HB was lower than Helton et al. (2008), who on a soil similar to this study obtained first year ANR of 26% from CDM applied to coastal bermudagrass at 250 kg total N ha^{-1}, the ANR of 23% obtained in 2009 was well in agreement with the 22% ANR reported by Helton et al. (2008). In the present study, the estimate of ANR obtained in 2009 was higher than that in 2008 (23% versus 14%, respectively; Table 1.10); which suggests a gradual lowering of the soil N supply and a reduced influence of the previous alfalfa crop over time.

In contrast to HWG-TF-HB, apparent N recoveries from CDM by the OG-MB-SB were negative in both years (Table 10), which might indicate that OG-MB-SB relied more on soil N supply than the CDM N input. The magnitude of this dependence was more pronounced in 2008 than in 2009 (-22% versus -2%, respectively; Table 1.10). Less dependence of OG-MB-SB on soil N supply in 2009 might indicate, at least in part, that CDM had gradually become a more important source of N during the second crop season due to the gradual lowering of soil N supply once residual soil N was mineralized.

4. CONCLUSION

Irrespective of study year or grass mixture type, net mineralization of CDM N was generally lower compared to net soil N mineralization. The results of the present study are interesting in the fact that net mineralization of CDM N in the second crop season did not lead to increases in yield nor forage tissue N concentrations of grasses receiving CDM compared to those that did not. From an agronomic perspective, CDM application at rates used in the present study likely requires supplemental N from other sources or high quality CDM (e.g. low ash content, soluble salt content, high total N content, etc.) should be considered for optimal forage yields. The higher forage DM yield, tissue N contents, forage N uptake, and lower ANR from CDM during the 2008 growing season could be attributed to high initial soil fertility as a result of a pre-plant compost application, or legume growth before the site was converted into a perennial grass pasture.

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Table 1.1. Composted dairy manure application rate and mass of total C and N applied to the experimental plots before (fall 2007) and after forage planting (spring and fall 2008) at Agricultural Research, Development and Education Center (ARDEC) near Fort Collins, Colorado.

Parameter	Fall 2007	Spring 2008	Fall 2008	Total
Application rate (wet weight basis), Mg ha^{-1}	22.4	22.4	22.4	67.2
Total C, kg ha^{-1}	1662.3	2027.8	806.4	4496.5
Total N, kg ha^{-1}	139.7	235.4	65.2	440.3
C to N ratio	11.9	8.5	6.2	-

Table 1.2. Physical and chemical characteristics of composted dairy manure (CDM) applied in the experimental plots for 3 consecutive growing seasons.

Parameter	Summer 2007	Spring 2008	Fall 2008
Moisture, (%)	25.20	28.62	20.44
Total solids, (%)	74.80	71.38	79.56
Organic matter, (%)	14.10	17.20	6.84
Ash, (%)	60.70	54.18	72.72
Total N, g kg^{-1}	6.23	10.74	2.91
Organic N, g kg^{-1}	5.66	10.11	2.58
NH_4-N, g kg^{-1}	0.02	0.13	0.33
NO_3-N, mg kg^{-1}	0.55	0.50	0.01
Total P, g kg^{-1}	2.42	5.05	1.07
Total K, g kg^{-1}	6.39	13.21	4.65
EC‡, dS m^{-1}	2.13	5.14	2.66
pH-H_2O‡	7.94	8.62	9.09

‡ EC and pH were determined on 1:5 CDM to water ratio.

Table 1.3. Total precipitation, applied irrigation water, average soil temperature (15 cm), and soil thermal units during each incubation period in both experimental years (2008 and 2009).

	2008				2009				LTM‡	
	Total		Ave.	Soil	Total		Ave.	Soil		
Incubation period	Precip-itation	Irrig-ation	soil temp.	thermal units	Precip-tation	Irrig-ation	soil temp.	thermal units	Precip-tation	Soil temp.
	-----(mm)-----		------(°C)------		-----(mm)-----		------(°C)------		mm	°C
23-Apr to 23-May	10.7	53.3	1.3	61.5	36.1	38.1	9.5	285.0	45.9	8.9
24-May to 23-Jun	n.a.†	19.1	14.7	102.7	89.4	33.0	12.4	382.9	48.2	14.0
24-Jun to 23-Jul	3.1	133.4	17.7	532.2	31.0	108.0	17.2	515.6	28.5	18.0
24-Jul to 26-Aug	120.0	76.2	16.5	560.6	23.1	177.8	17.6	511.2	44.1	17.7
27-Aug to 27-Spet	25.9	16.5	13.1	417.6	3.3	76.2	15.8	491.2	28.2	13.7

‡ LTM, long term (1992 to 2009) mean for each incubation period.

† n.a. = data not available.

Table 1.4. Net N mineralization under two perennial grass mixes at five times during the growing season in 2008.

	Incubation period †				
Treatment‡	1	2	3	4	5
	----------kg N ha^{-1}----------				
HWG-TF-HB + control	39.2	9.9	39.7	36.8	101.0
HWG-TF-HB + CDM	21.8	15.5	25.2	45.6	84.0
OG-MB-SB + control	3.2	8.2	24.1	64.0	67.7
OG-MB-SB + CDM	15.7	8.9	34.5	49.4	71.8
			P > F		
Incubation period (I)			***		
1 (mean)			20.0 c¶		
2 (mean)			10.6 d		
3 (mean)			30.9 b		
4 (mean)			49.0 ab		
5 (mean)			81.1 a		
Grass-mix (G)			*NS*		
HWG-TF-HB (mean)			41.9		
OG-MB-SB (mean)			34.8		
Amendment (A)			*NS*		
Control (mean)			39.4		
CDM (mean)			37.3		
G × A			*NS*		
I × G			*NS*		
I × A			*NS*		
I × G × A			*NS*		

Level of significant: *P <0.1; **P <0.05; ***P <0.001; NS, not significant.

†The tube extraction days for incubation periods 1, 2, 3, 4, and 5 were 30, 61, 91, 126, and 158 days after insertion, respectively.

‡ CDM composted dairy manure; HWG-TF-HB, hybrid wheatgrass-tall fescue-hybrid brome; OG-MB-SB, orchardgrass-meadow brome-smooth brome.

¶ Incubation period means followed by a common letter were not significantly different ($P \leq 0.05$).

Table 1.5. Net N mineralization under two perennial grass mixes at five times during the growing season in 2009.

	Incubation period†				
Treatment‡	1	2	3	4	5
	----------kg N ha^{-1}----------				
HWG-TF-HB + control	4.5	8.8	23.8	26.5	25.8
HWG-TF-HB + CDM	8.1	16.3	29.8	44.0	45.4
OG-MB-SB + control	8.0	13.1	17.2	29.5	27.0
OG-MB-SB + CDM	10.0	20.9	31.5	36.0	33.9
			P > F		
Incubation period (I)			***		
1 (mean)			7.7 d§		
2 (mean)			14.8 c		
3 (mean)			25.6 b		
4 (mean)			34.0 a		
5 (mean)			33.0 ab		
Grass mix (G)			*NS*		
HWG-TF-HB (mean)			23.3		
OG-MB-SB (mean)			22.7		
Amendment (A)			**		
Control (mean)			18.4 b¶		
CDM (mean)			27.6 a		
G × A			*NS*		
I × G			*NS*		
I× A			*NS*		
I × G × A			*NS*		

Level of significant: *P <0.1; **P <0.05; ***P <0.001; NS, not significant.

† Tube extraction days for incubation periods 1, 2, 3, 4, and 5 were 30, 61, 91, 122, and 153 days after insertion, respectively.

‡ CDM composted dairy manure; HWG-TF-HB, hybrid wheatgrass-tall fescue-hybrid brome; OG-MB-SB, orchardgrass-meadow brome-smooth brome.

§ Incubation period means followed by different letters were significantly different (P<0.05).

¶ Amendment means followed by different letters were significantly different (P <0.05).

Table 1.6. The amount of N mineralized in composted dairy manure (CDM) after subtracting mineralized N in the control soil under two perennial grass mixes at five times during the growing season for 2 years.

	Incubation period‡				
Grass mix¶	1	2	3	4	5
	----------kg ha^{-1}----------				
			2008		
HWG-TF-HB	-17.4	5.6	-14.5	8.8	-17.0
OG-MB-SB	12.6	0.8	10.5	-14.6	4.0
			P > F		
Grass mix			*NS*†		
Incubation period			*NS*		
Grass mix X incubation period			*NS*		
			2009		
HWG-TF-HB	3.6	7.5	6.0	17.5	19.6
OG-MB-SB	2.0	7.8	14.4	6.4	6.9
			P > F		
Grass mix			*NS*		
Incubation period			*NS*		
Grass mix X incubation period			*NS*		

‡ The tube extraction days for incubation periods 1, 2, 3, 4, and 5 were 30, 61, 91, 126, and 158 in 2008; 30, 61, 91, 122, and 153 in 2009, respectively, from the tube insertion date.

¶ HWG-TF-HB, hybrid wheatgrass-tall fescue-hybrid brome; OG-MB-SB, orchardgrass-meadow brome-smooth brome.

† NS, not significant.

Table 1.7. Amounts of mineralized N as a percentage of total and organic N added in composted dairy manure (CDM) under two perennial grass mixes at five times during the growing season in 2008 and 2009.

	2008‡					2009‡				
	Incubation period†									
Grass mix¶	1	2	3	4	5	1	2	3	4	5
	(%)									
	Total N					Total N				
HWG-TF-HB	-7.2	2.3	-6.0	3.7	-7.1	1.2	2.5	2.0	5.8	6.5
OG-MB-SB	5.3	0.3	4.4	-6.1	1.7	0.7	2.6	4.8	2.1	2.3
	Organic N					Organic N				
HWG-TF-HB	-7.7	2.5	-6.4	3.9	-7.5	1.3	2.7	2.2	6.3	7.0
OG-MB-SB	5.6	0.3	4.6	-6.4	1.8	0.7	2.8	5.2	2.3	2.5

† The tube extraction days for incubation periods 1, 2, 3, 4, and 5 were 30, 60, 90, 120, and 150 in 2008; 30, 60, 90, 120, and 150 in 2009, respectively, from the tube insertion date.

‡ % N for the 2008 was calculated by dividing mineralized N at each sampling time by N applied in the spring of 2008 whereas 2009 numbers were divided by N applied in the spring and fall of 2008.

¶ HWG-TF-HB, hybrid wheatgrass-tall fescue-hybrid brome; OG-MB-SB, orchardgrass-meadow brome-smooth brome.

Table 1.8. Forage dry matter yields of two perennial grass mixes in response to composted dairy manure (CDM) at Agricultural Research, Development and Education Center (ARDEC) near Fort Collins, Colorado.

	2008							2009						
	Cutting†							Cutting†						
Treatment‡	1	2	3	4	5	6	Total	1	2	3	4	5	Total	2-yr avg
	(Mg ha^{-1})													
HWG-TF-HB + control	3.4	0.5	1.4	1.2	1.9	1.5	9.9	2.1	0.8	0.8	0.5	0.5	4.7	7.3
HWG-TF-HB + CDM	3.5	0.7	1.5	1.4	2.1	1.9	11.2	2.8	1.4	1.2	0.6	0.6	6.7	8.9
OG-MB-SB + control	3.6	0.4	1.4	1.4	2.0	1.0	9.8	2.1	0.7	1.2	0.4	0.6	5.1	7.5
OG-MB-SB + CDM	3.1	0.4	1.1	1.4	1.8	1.0	8.8	2.1	1.0	0.9	0.5	0.4	4.9	6.9
LSD ($\alpha = 0.05$)							2.51						2.43	2.44
	P > F							*P > F*						
Cutting (C)	***							***						
1 (mean)	3.40 a‡							2.28 a§						
2 (mean)	0.51 d							0.99 b						
3 (mean)	1.37 c							1.03 b						
4 (mean)	1.36 c							0.49 c						
5 (mean)	1.94 b							0.55 c						
6 (mean)	1.38 c							-						
Grass mix (G)	*							*NS*						
HWG-TF-HB (mean)	1.76 a¶							1.14						
OG-MB-SB (mean)	1.55 b							1.00						
Amendment (A)	*NS*							*NS*						
Control (mean)	1.65							0.98						
CDM (mean)	1.67							1.16						
G × A	*NS*							*NS*						
C × G	*NS*							*NS*						
C × A	*NS*							*NS*						
C × G × A	*NS*							*NS*						

Level of significant: *P <0.1; **P <0.05; ***P <0.001; NS, not significant.

‡ Cutting means followed by a common letter were not significantly different; ¶ Grass mix means with different lowercase letters were statistically different.

Table 1.9. Effects of composted dairy manure (CDM) on average tissue-N concentration of two perennial grass mixes.

Treatment†	N rate‡	2008	2009	2-yr avg
	(kg ha^{-1})	----------g N kg^{-1}----------		
HWG-TF-HB + control	0	33.4	24.9	29.1
HWG-TF-HB + CDM	300	32.6	25.3	29.0
OG-MB-SB + control	0	35.4	26.5	31.0
OG-MB-SB + CDM	300	32.3	24.8	28.6
			P > F	
Grass mix		*NS*	*NS*	*NS*
HWG-TF-HB (mean)		33.0	25.1	29.1
OG-MB-SB (mean)		33.9	25.9	29.8
Amendment		**	*NS*	*NS*
Control (mean)		34.4 a¶	25.7	30.1
CDM (mean)		32.4 b	25.1	28.8
Grass mix X amendment		*NS*	*NS*	*NS*

Level of significant: *P <0.1; **P <0.05; ***P <0.001; NS, not significant.

‡ Rate is a 2-yr total (235 and 65 kg ha^{-1} total N applied in 2008 and 2009, respectively).

¶ Amendment means with different letters were significantly different.

Table 1.10. Effects of composted dairy manure (CDM) on N uptake of two perennial grass mixes in the 2008 and 2009 growing seasons.

	2008							2009							
	N uptake							N uptake							
Treatment†	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 6	Total	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Total	2-yr total	2-yrN recovery
	---(kg ha^{-1})---														%
HWG-TF-HB + control	99	19	46	42	68	48	321	30	14	13	13	14	83	404	-
HWG-TF-HB + CDM	103	23	48	48	75	59	356	61	33	30	16	15	154	510	35
OG-MB-SB + control	109	13	54	53	79	33	341	50	19	30	12	18	128	469	-
OG-MB-SB + CDM	89	13	39	49	67	32	288	48	24	25	13	13	123	411	-19
LSD (α = 0.05)							99						81	178	
			P > F							*P > F*					
Cutting (C)			***							***					
1 (mean)			100.0 a‡							47.0 a‡					
2 (mean)			17.2 d							22.5 bc					
3 (mean)			46.5 c							24.3 b					
4 (mean)			47.8 c							13.4 c					
5 (mean)			72.2 b							14.7 bc					
6 (mean)			42.9 c							-					
Grass mix (G)			*NS*							*NS*					
HWG-TF-HB (mean)			56.4							23.7					
OG-MB-SB (mean)			52.4							25.1					
Amendment (A)			*NS*							*NS*					
Control (mean)			55.2							21.1					
CDM (mean)			53.7							27.7					
G × A			*NS*							*NS*					
C × G			*NS*							*NS*					
C × A			*NS*							*NS*					
C × G × A			*NS*							*NS*					

Level of significance: *$p \leq 0.1$; **$p \leq 0.05$; $p \leq 0.001$; ns = not significant.

Cutting means followed by a common letter were not significantly different

CHAPTER 2: ESTIMATING NITROGEN MINERALIZATION FROM RAW AND COMPOSTED DAIRY MANURE IN AN ORGANIC ANNUAL FORAGE SYSTEM

Summary

The objectives of this study were to quantify N mineralization (N_{min}) from manure and composted manure amendments and subsequent N uptake by organically grown forage. The annual forage grass, teff (*Eragrostis tef*), was grown on an irrigated clay soil in northern Colorado where composted dairy manure (CDM) or raw dairy manure (RDM) were applied to supply an annual N input of 123 and 56 kg total N ha^{-1} in the first (2008) and second (2009) cropping year, respectively. Bare-fallow and an unfertilized control treatments were included as well. An *in situ* intact soil core resin bag technique was used to estimate N_{min} three times (i.e., after 30, 60 and 70 days of incubation) during the growing season. Significant differences in N mineralization were restricted to the teff system in 2008. Within the teff system, the N_{min} from RDM-amended soil was significantly ($p< 0.05$) higher than that of CDM-amended and the unfertilized control soil at 30 and 70 d of incubation in 2008. The cumulative N_{min} during the 70 day incubation period in 2008 was 22.4 and 59% for CDM and RDM, respectively, of the total N applied in these amendments. Forage yields, N uptake, and recovery of N by teff were greater from RDM than CDM and the unfertilized control. The CDM amendment did not significantly increase N_{min}, forage yields, N uptake or N recovery by teff compared with the unfertilized control. In 2009, CDM, RDM and unfertilized treatments did not differ significantly from one another with respect to any of the parameter measured. First year results suggest that greater forage yields, recovery of N by teff, and N uptake responses to RDM compared to CDM reflect a higher N_{min} as well as relatively higher initial inorganic N concentration in RDM during the first production year.

1. INTRODUCTION

Organic milk production has been one of the fastest growing segments of organic agriculture in the U.S. in recent years (McBride and Greene, 2009). Although there has been a decline recently due to the current economic recession, the number of organic dairy operations has been increasing in eastern Colorado. This trend is driving the need for organically grown forages in this region. Most of these dairies are confronted with shortages of high-quality, locally-produced organic forage as there are not enough hectares in organic production to meet the demand. So the dairies are forced to truck in forage from locations as far away as Montana and Idaho. Many local farmers and growers are seeking alternative methods to produce organic forage and improve their income. One strategy would be for certified organic growers to incorporate organic forage production into organic vegetable rotations. This production system would allow organic vegetable growers to incorporate a forage crop into their yearly crop rotation and thereby increase farm profitability while providing local organic industries with necessary inputs. In light of this, a study was initiated at the Horticultural Research Center at Colorado State University in spring 2008 to evaluate the feasibility of organic forage production by incorporating an annual-warm season forage grass, teff (*Eragrostis tef*), into rotations with a spring planted vegetable crop.

Because synthetic N fertilizer is prohibited for use on organically certified land, organic farming systems rely on cultural practices such as crop rotation or application of animal manures to maintain soil fertility (ERS, 2008). Organic forage producers often use manure as a major source of N fertilizer (Hadas et al., 1996; Eghball et al., 2002; Muñoz et al., 2008). Nitrogen is frequently the most limiting nutrient for terrestrial plant productivity (Hart et al., 1994), and can have a profound consequence if under- or over-applied. Therefore, when soil fertility is

maintained with manure, it is essential to quantify manure N availability to ensure adequate N supply to crops in order to maximize yields while avoiding loss of N to the environment.

To determine the appropriate rate of manure application, information on crop type, soil N supply, and manure N supply is required (Eghball et al., 2002; Van Kessel and Reeves, 2002). Manure N content and its availability depend on several factors including animal diet, species, and manure management (Gilbertson et al., 1979; Eghball et al., 2002). A high percentage of N in manure is in the organic fraction while the proportion of inorganic N (NH_4-N and NO_3-N) is usually very low (Cabrera and Gordillo, 1995; Eghball et al., 2002; Miller et al., 2009). Thus, the supply of plant available N from manure depends mostly on the gradual release of N from the organic fraction (Miller et al., 2009). Since the mineralization of manure N is mediated by soil microbes, it is influenced by several factors including temperature, soil moisture, soil properties, and manure characteristics (Eghball et al., 2002). Past research has shown that estimates of N mineralization vary greatly with manure type (Eghball, 2000; Eghball et al., 2002). Composting manure is a useful technique as it improves the characteristics of manure by reducing its volume while increasing uniformity (Eghball and Power, 1999; Eghball et al., 2002). However, due to loss of volatile N compounds during composting and conversion of the remaining N into chemically stable forms, composted manure N availability is often lower than that of noncomposted manure (Cabrera and Gordillo, 1995; DeLuca and DeLuca, 1997; Eghball et al., 1997; Eghball, 2000; Eghball et al., 2002). For example, Eghball (2000) measured N mineralization from raw and composted manure during the growing season using the *in situ* resin method. He found that organic N mineralization from composted manure was about half (11%) of that for noncomposted manure (21%) during the corn growing season in eastern Nebraska.

Despite the number of previously published *in situ* incubation studies (DiStefano and Gholz, 1986; Kolberg et al., 1997; Eghball, 2000; Hanselman et al., 2004; Wienhold et al., 2009) that have measured soil or manure N mineralization, there are few studies that have attempted to quantify N mineralization from soils receiving raw or composted dairy manure under certified organic cropland. Therefore, the objective of this study was to compare *in situ* N mineralization from soil amended with raw and composted dairy manures applied to a warm-season annual forage grass, teff (*Eragrostis tef*), grown on a clay soil in northern Colorado. Furthermore, forage yield, apparent N recovery, and N uptake responses to composted dairy manure (CDM) and raw dairy manure (RDM) were assessed.

2. MATERIALS AND METHODS

2.1. Site description

This study was conducted at the Colorado State University (CSU) Horticultural Research Center located near Fort Collins, Colorado (40°36' N, 104°59' W, elevation 1524 m). The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980). The mean annual precipitation is 370 mm, and the mean summer and winter temperatures are 29°C and 5.6°C, respectively, at this location. The land was certified organic and it had previously been under a mixture of green manures including rye, peas, and vetch. It had no previous history of manure application in the years preceding the current experiment, except for one application in the fall of 2006 (22.4 Mg ha^{-1} composted poultry manure). On 17 March 2008 prior to the beginning of the present study, soil samples were collected to a 20 cm depth and analyzed at Ward Laboratories (Kearney, Nebraska) for selected properties. Organic matter content of the soil was 27 g kg^{-1}, extractable NO_3-N and P contents were 0.0376 and 0.038 mg kg^{-1},

respectively; soil pH and electrical conductivity (1:1 soil:water) were 8.1 and 0.97 dS m^{-1}, respectively.

2.2. Experimental design

The experiment was laid out in a split-plot design in which a warm-season annual forage crop or bare-fallow were the main plots with dairy manure form as the subplots. The main plots were 2.44 × 12.84 m and seeded with either teff (*Eragrostis tef*) or left bare (i.e., bare-fallow). The main plots were replicated three times, giving a total of 6 main plots. Each of the main plots contained three 2.44 × 4.28 m subplots in which three amendment treatments (manure form) were randomly assigned: (1) composted dairy manure (hereafter CDM), (2) raw dairy manure (hereafter RDM), or (3) no amendment as a control.

2.3. Amendment applications

On 8 June 2008, the quantity of CDM and RDM needed to obtain the desired application rates for each plot was hand-spread evenly on the surface of the treatment plots, and incorporated to a depth of approximately 15 cm by rototilling. In 2008, the CDM and RDM application rates were 11.4 and 51.7 Mg ha^{-1} respectively, on a wet weight basis (Table 2.1). For the 2009 field season, estimated N availability from first year CDM and RDM application was used as the primary determinant of application rate. That is, a total N credit of 20% from CDM and 40% total N from RDM that was expected to become available in the second year was taken into consideration when calculating the rate. Accordingly, the rates were 19.3 and 14.6 Mg ha^{-1} for CDM and RDM, respectively, and the CDM and RDM amendments were applied in the fall of 2008. The application rates in this study were designed to achieve approximately 123 kg total N ha^{-1} in the first year and 56 kg total N ha^{-1} in the second cropping year based on soil tests.

Manure and compost analysis was done at the Colorado Analytical Laboratory (Brighton, Colorado) before application each year (Table 2.2).

2.4. Cropping sequence

The cropping sequence was as follows: in 2008, teff was planted on 10 June and harvested on 18 August. Following teff, a lettuce crop was planted on 13 March 2009 and harvested on 5 June 2009. For the 2009 field season, teff was planted on 24 June and harvesting was completed on 20 August 2009. Teff, *Eragrostis tef*, is a warm-season annual grass native to Ethiopia, where it is used as a staple grain. In the U.S., however, teff is grown for forage and is gaining popularity with farmers across the U.S. as an alternative high-quality summer forage grass suitable for a wide range of livestock (Miller, 2010). In the fallow plots the soil surface was left bare and weeded three times throughout the summer. All plots were irrigated twice per week with 25.4 mm of water through an overhead sprinkler system in both years, while the lettuce crop was not irrigated.

2.5. Field incubation experiment

The *in situ* N mineralization procedure used in this study was similar to that described by DiStefano and Gholz (1986). Aluminum tubes, 15 cm long and 5 cm internal diameter, were placed in a steel soil probe and inserted into the ground to a depth of ~ 15 cm using a Giddings hydraulic soil probe (Giddings Machine Co., Windsor, CO). The steel probe with intact soil core was withdrawn from the ground, and the soil filled aluminum tube was removed and prepared for reinsertion. Prior to reinsertion, a Lycra® bag containing 20 mL of ion-exchange resins, consisting of equal amounts of anion- and cation-saturated (USF A-464D and USF C-211, respectively), was placed at the bottom of the tube where ~ 2-cm soil had been removed. Resin

bags were covered by nylon, which was then fastened to the bottom of the tube with duct tape. The entire unit was then reinserted into the hole from which the soil came.

On 12 June 2008, nine tubes were installed in each of the 18 treatment plots so that three tubes could be randomly selected and removed at approximately four-week intervals and three-times during the growing season. Tubes were retrieved on 12 July (30 d), 12 Aug. (61 d), and 22 Aug. 2008 (70 d). In 2009, because of rainy conditions in June, teff planting was delayed until the 24th of June, and tubes were installed on 1 July 2009. Only six tubes were installed in each of the 18 treatment plots, so that three tubes could be removed at approximately four-week intervals two-times during the growing season in 2009. Tubes were removed on 1 Aug. (30 d) and 31 Aug. 2009 (61 d). At each sampling date, resin bags from soil cores that remained in the field were also removed and replaced with fresh bags to avoid resin oversaturation, which could lead to loss of inorganic N (Giblin et al., 1994; Wienhold et al., 2009). Upon removal from the ground, tubes along with the resin bags were placed in a cooler and brought to the lab.

2.6. Chemical analyses

In the lab, resin bags and soil tubes were stored at 4°C until they were analyzed. The soil removed from each tube was thawed prior to grinding and ground to pass through a 2-mm screen before laboratory analysis. A subsample of 10 g soil was placed in a 150 mL Erlenmeyer flask and extracted in 100 mL of 2 M KCl for 1 h on a shaker. For the resin portion, each intact resin bag was placed in a 250 mL Erlenmeyer flask and extracted with 50 mL 2 M KCl by shaking for 1 h. This procedure was repeated five more times (Kolberg et al., 1997). Extract solutions from soils and resin bags were filtered through pre-leached Whatman No. 40 filter paper, and a 20 mL aliquot sample was frozen (-20°C) until analysis. The concentrations of NH_4-N and NO_3-N in the

extract were analyzed colorimetrically using an Alpkem Flow Solution IV Autoanalyzer (OI Analytical, College Station, TX).

2.7. Calculations

The NH_4-N and NO_3-N in the soil and resin of the incubation system will hereafter be referred to as soil inorganic N (soil N_{inorg}) and resin inorganic N (resin N_{inorg}), respectively. The N_{inorg} in each portion (soil or resin) was calculated as the product of its concentration by weight. For example, the amount of N mineralized (hereafter N_{min}) at 30 d incubation was calculated by adding both soil and resin N_{inorg} concentrations together (Eq. [1]). To calculate N_{min} at 61 d incubation, resin N_{inorg} at 30 d incubation was added to the combined amounts of N_{inorg} in soil and resin analyses at 61 d incubation (Eq. [2]). Finally, to determine N_{min} at 70 d incubation (this one was only for 2008), resin N_{inorg} at 30 and 61 d incubations was added to the combined amounts of N_{inorg} in soil and resin analyses at 70 d incubation (Eq. [3]).

N_{min} 1 = soil N_{inorg} at 30 d incubation + resin N_{inorg} at 30 d incubation [1]

N_{min} 2 = (soil N_{inorg} + resin N_{inorg}) at 61 d incubation + resin N_{inorg} at 30 d incubation [2]

N_{min} 3 = (soil N_{inorg} + resin N_{inorg}) + resin N_{inorg} at 30 d incubation + resin N_{inorg} at 61 d incubation [3]

To determine cumulative N_{min} at each incubation period, soil N_{inorg} that was present at the beginning of the study was subtracted from the amount of N_{min} at each incubation period (Hart et al., 1994; Eghball, 2000). The amount of N from unamended control plots provided an indication of the amount of soil N that was mineralized (Eghball, 2000).

The amount of N_{min} from manure was calculated as the difference between N_{inorg} in manured and control soils (Kaboneka et al., 1997). To express N_{min} as a percentage of added

CDM or RDM N, the quantity of N_{min} in CDM or RDM was divided by total N applied in these amendments and the result was multiplied by 100 (Hanselman et al., 2004).

2.8. Forage yield and nitrogen uptake

In 2008, teff was harvested on 18 August, while in 2009 harvesting was done on 20 August. For the determination of yield, teff was cut from the middle of each plot. **S**ubsamples (~ 500 g) were oven-dried to a constant weight (55°C, 72 h), and ground to pass a 2-mm screen. Ground samples were analyzed for total N using the modified Dumas method (Etheridge et al., 1998) with a LECO C and N analyzer (Model CN 2000TruSpec®, LECO Corp., St. Joseph, MI). Plant N uptake was calculated by multiplying dry matter (DM) yield by total N concentration. These values were then used to determine apparent N recovery (ANR) of manure by teff based on the difference method (Helton et al., 2008) using Eq. [4].

$$\text{ANR } (\%) = \left[\frac{(\text{N uptake})_{\text{manured}} - (\text{N uptake})_{\text{control}}}{\text{N applied in manure}}\right] \times 100 \quad [4]$$

2.9. Statistical analysis

A generalized linear mixed analysis of variance (ANOVA) model (PROC GLIMMIX) was developed to analyze the N_{min} data using SAS statistical software version 9.2 (SAS Institute, Inc., 2008). A split-plot repeated measures ANOVA that included forage treatments (teff and bare fallow) as fixed whole-plots, amendment treatments (control, CDM, and RDM) as the fixed subplots, and sampling dates as the fixed repeated measures was used to determine effects of these factors on N_{min} . The model included block and any interaction with block as random effects. First order autoregressive covariance structure [Type = AR (1)] was used to fit the repeated measures model. F-tests that utilized Type III sums of squares were used to assess the significance of fixed effects. When effects were significant, separation of means was achieved using Tukey's adjustment to the LSMEANS. *F*-test values and LSMEANS comparisons were

considered significant at the $P \leq 0.05$ level unless stated otherwise. The natural log transformation was applied as needed prior to analysis to meet the assumptions of ANOVA.

Teff dry matter yield and N uptake were analyzed using PROC GLM. Mean separation was performed using Tukey's adjustment to the LSMEANS statement and, differences having a probability level of < 0.05 were considered significant unless noted otherwise.

3. RESULTS AND DISCUSSIION

3.1. Climatic conditions

Precipitation data obtained from the Northern Colorado Water Conservancy District website for a weather station near the study site east of Fort Collins, Colorado show that a total of 111 mm of precipitation occurred during the field incubation period (12 June to 22 August) in 2008; and 167 mm during the 2009 field incubation period (1 July to 31 Aug. 2009). Air temperature averaged 20°C (range: 11 to 26°C) in 2008 during the period when the tubes were in the ground. In 2009, temperatures ranged from 14 to 24°C with an overall mean of 20°C.

3.2. Nitrogen mineralization

Since the soil was left bare in the bare-fallow plots, these soils were exposed to direct sun radiation and thought to be relatively prone to drying resulting from higher evaporative loss. Thus, it was hypothesized that compost and manure mineralization would be lower in bare-fallow plots due to hot and dry soil conditions in these plots compared to plots seeded with teff. Whereas in teff plots, higher mineralization was expected due to relatively moist soil conditions resulting from reduced evaporative loss as a result of ground cover provided by the teff. However, mineralized N did not differ between teff and bare-fallow plots ($p > 0.05$). While no measurement of soil moisture was taken in this study, the lack of significant difference in N_{min} between teff and bare-fallow plots in both years of the study is hypothesized to have been

influenced by the lack of substantial difference in soil moisture between teff and bare-fallow plots. This is because both teff and bare-fallow plots were irrigated at least twice per week in both 2008 and 2009 and this may have resulted in similar soil moisture condition in these plots.

Within the teff plots, the amount of mineralized N from CDM and RDM was compared and there was significant difference in N mineralized between these two soil amendments at 30 and 70 d of incubation in 2008 ($p = 0.002$). In contrast, there was no significant difference in mineralized N between CDM and RDM under bare-fallow at either of the incubation dates. Within the teff plots, RDM N_{min} at 30 d was significantly greater (63.3 kg N ha^{-1}) than CDM N_{min} (39.7 kg N ha^{-1}) and the unfertilized control (23.7 kg N ha^{-1}) in 2008 (Table 2.3). At 30 d in 2008, there was no significant difference in N_{min} between CDM and the unfertilized control. At 70 d in 2008, while N_{min} did not differ between RDM (47.5 kg N ha^{-1}) and the unfertilized control (31.4 kg N ha^{-1}), CDM N_{min} (21.4 kg N ha^{-1}) was significantly smaller compared to RDM N_{min}.

Raw dairy manure that was applied in the spring of 2008 had total N content of 2.4 g kg^{-1} (Table 2.2), and approximately 59% (i.e., the sum of N_{min} during incubation periods 1, 2 and 3) of this was mineralized in 2008 (Table 2.3). This is in agreement with the N availability assumption for fresh manures in the first year after application. What is known about N availability from animal manures is that 40-50% of the applied N would be plant available in the first year after application (Gilbertson et al., 1979; Eghball, 2000). In contrast, the total N content of the CDM amendment that was applied in the spring of 2008 was 10.7 g kg^{-1} (Table 2.2), and of this, 22.4% (i.e., the sum of N_{min} during incubation periods 1 and 2) was mineralized in 2008 (Table 2.3). The percentage of N mineralized from CDM is also in good agreement with trends previously established in the literature. According to (Gilbertson et al., 1979; Eghball, 2000),

20% of the N applied in composted manures is expected to mineralize and become plant available in the first year after application. Our study finding that estimated N_{min} from CDM in 2008 was lower than that from RDM (Table 2.3) is similar to findings of Castellanos and Pratt (1981), Eghball and Power (1999) and Eghball (2000), who reported that composted manures provide less available N compared to fresh noncomposted manures. Given that CDM had a relatively low C/N ratio (CDM that was applied in the spring of 2008 had C/N = 8; Table 2.2) as well as past research showing C and N stability during composting (Castellanos and Pratt, 1981; DeLuca and DeLuca, 1997; Eghball et al., 1997; Eghball, 2000; Risse et al., 2006), it is likely that C and N were more stabilized and less labile in the CDM amendment. This likely contributed to the observed lower N_{min} in plots that were treated with CDM than those treated with RDM (Table 2.3). Even though CDM and RDM rates were intended to provide similar amount of N (123 kg ha^{-1}) in the first year after application, only RDM-amended soil provided sufficient N in 2008 growing season (139 kg ha^{-1} over the entire 70 d period; Table 2.3). Although CDM-amended soil tended to provide less available N (86 kg ha^{-1} over the entire 70 d period; Table 2.3) than that of RDM-amended soil, this amount may be within acceptable range when taking into consideration the 20% N, which is expected to be plant available from composted manures in the year following application. Based on the results of this study, it is sufficient to say that both CDM and RDM might supply sufficient in-season plant available-N and that RDM has a higher fertilizer value than CDM, particularly during the first year following application.

In 2009, the second year of the study, no significant differences in N_{min} were found between soil receiving CDM and RDM at either of the incubation dates within the teff or bare-fallow (Table 2.3). Mineralized N from soil receiving either CDM or RDM was indistinguishable

from the unfertilized control. One of the reasons why there may have been no difference in N_{min} between CDM and RDM in 2009 was because there was probably less "carryover" of N from 2008-applied RDM into 2009 compared to 2008-applied CDM. In addition, greater rainfall activity in 2009 may have resulted in greater soil moisture conditions and greater microbial activity overall, thus confounding amendment treatments effects on N mineralization. It is also possible that any mineralized N from CDM and RDM that were applied in the fall of 2008 may have been either leached or used up by the spring planted vegetable crop, namely lettuce. Consequently, less N was likely carried over from fall-2008 applied CDM and RDM to 2009. This could be another possible explanation for the lack of significant difference in N mineralization between CDM and RDM in 2009.

Since a total N credit of 20% from CDM and 40% from RDM was expected to become available in the second year, CDM and RDM rates for the 2009 growing season were designed to achieve approximately 56 kg total ha^{-1} from these amendments. Compared to percentage N mineralized in 2008 from spring-2008 applied CDM and RDM, N_{min} estimates (percentagewise) from these amendments were smaller in 2009 (Table 2.4). For example, within the teff, 3.5% of total N added in CDM was mineralized 61 d after N_{min} tube installation, while 3.9% of total N added in RDM was mineralized 30 d after N_{min} tube installation. Within the bare-fallow, on the other hand, N_{min} values obtained for CDM were negative throughout the measurement period, probably indicating immobilization of soil N; however, there was a 4.4% RDM N_{min} 61 d after tube installation (Table 2.4). Since CDM and RDM were applied twice in this study (first in spring 2008 and then in fall 2008), both spring and fall 2008 applied N in these amendments was used for the purpose of calculating percentage N mineralized for the 2009. Therefore, the 2009 N_{min} values may have included some of the residual N from spring-2008 applied CDM and

RDM, in addition to N_{min} from fall-2008 applied CDM and RDM. Several hypotheses can be advanced to explain the observation that percentage N mineralized from CDM and RDM was lower for the 2009 than for the 2008. As discussed previously, it is likely that loss of N through leaching or up take of N by spring planted lettuce may have contributed to the lower CDM and RDM N_{min} observed in 2009. It should also be noted that low quality (e.g., high ash content; Table 2.2) CDM and RDM used for the second year study (i.e., fall-2008 application) likely contributed to the lower N mineralization from these amendments in 2009. Based on these results, therefore, it seems that manure quality should be considered when determining manure application rates. For practical purposes, this means that, it would be useful to consider manure with low ash content, pH, EC, and high total N content. Since this study did not measure N mineralization immediately after fall-2008 CDM and RDM application, it is also important to monitor N mineralization from fall applied CDM and RDM to assess how much N could be lost to the environment (e.g., via leaching) during an off-season.

3.3. Teff dry matter yield

In 2008, RDM resulted in significantly ($p = 0.04$) greater forage yields (4862 kg DM ha^{-1}) than CDM (4009 kg ha^{-1}) and the unfertilized control (3971 kg DM ha^{-1}) (Table 2.5). There was no significant yield difference between CDM and the unfertilized control. Raw dairy manure that was applied in the spring of 2008 had a slightly higher concentration of inorganic N (about 0.4 g kg^{-1} of the RDM N was NH_4-N; Table 2.2) than CDM, which might partly explain the greater teff yield observed in the RDM amended plots compared to those amended with CDM or the unfertilized control plots (Table 2.5). In addition, about 32% of the added total N with RDM was mineralized immediately after application (during the first 30 d of the incubation; Table 2.4). This also likely contributed to greater dry matter yield obtained for the RDM (Table 2.5). A

previous study conducted on N management of teff in the state of New York, USA showed that an N rate of 56 kg ha^{-1} broadcast at planting is required for optimum teff yield (Hunter et al., 2007). In the present study the amount of N mineralized in the RDM-amended soil during incubation period 1 (in the first thirty days after RDM application) alone was 63.3 kg N ha^{-1} (Table 2.3). Despite the fact that RDM N mineralized during period 2 (60 d) decreased compared with during periods 1 (30 d) and 3 (70 d), large dry matter yield of teff obtained for the RDM in year 2008 (Table 2.5) suggests that RDM can provide sufficient in-season plant available N to support high dry matter yields of warm-season annual forage crop such as teff, particularly in the first year following application. The observations that forage dry matter yields were higher for RDM than CDM are supported by previously published other annual forage studies (Stevenson et al., 1998; Helton et al., 2008).

In 2009, CDM and RDM did not differ significantly from one another with respect to forage dry matter yield (Table 2.5). Composted dairy manure and RDM failed to improve forage yields significantly compared with the unfertilized control. The lack of significant difference in forage dry matter yields between the CDM and RDM treatments in year 2009 may indicate that there was little residual N benefit from spring 2008 RDM N in comparison with CDM. Forage yields were lower in 2009 than in 2008 when averaged across the three amendment treatments (Table 2.5). The reduction in forage yields in 2009 is partly attributable to the shorter growing season (24 June to 20 Aug.) due to a decision to delay planting because of wet soils.

3.4. Nitrogen uptake

In 2008, there was no significant difference in N uptake between CDM and the unfertilized control (Table 2.5). However, there was an almost-significant trend of greater N uptake by teff for RDM compared to CDM treatment and the unfertilized control ($p = 0.06$).

Possible explanations for the greater N uptake response of the teff crop to RDM than CDM and the unfertilized control are that (i) RDM used in the 2008 N_{min} study had a slightly higher inorganic N concentration, mainly NH_4-N (Table 2.2); and (ii) following the application of RDM, about 59% of the added RDM N was mineralized over the 70 d period in 2008 and may have become available to the teff crop during the 2008 growing season (as compared with the 22% CDM N_{min}) (Table 2.4). In addition, RDM resulted in a greater forage dry matter yield in 2008 (Table 2.5), which would explain why N uptake response of the teff crop was greater to RDM than CDM as plant N uptake was estimated by multiplying dry matter yield by total N concentration in plant samples.

In 2009, there was no significant difference in teff N uptake between CDM and RDM amendments ($p > 0.05$; Table 2.5). Also, N uptake for CDM and RDM did not differ significantly from the unfertilized control. It is again likely that the absence of significant N uptake difference between CDM and RDM observed in 2009 was a result of less RDM N carry-over from spring 2008 applied RDM to the 2009 growing season as the spring 2008 RDM N might have been consumed immediately during the 2008 teff growing season. As discussed above, uptake of N by spring planted lettuce or loss of N through leaching from fall-applied CDM and RDM likely contributed to the lack of difference in teff N uptake between these two amendments. However, the observation that N uptake was similar for CDM and RDM in the present study was consistent with other published studies on dairy manure (Martin et al., 2006) and beef cattle manure (Miller et al., 2009).

3.5. Apparent nitrogen recovery

In the first forage production year (2008), ANR was -5% for CDM and 15% for RDM (Table 2.6). The lower (negative) ANR for the CDM treatment was probably due to lower N

mineralization and lower concentration of inorganic N in the CDM that was applied in the spring of 2008 compared to RDM. As a result, teff probably obtained more N directly from the soil pool. In contrast, the positive ANR for RDM might indicate that teff obtained some N from this applied source. This trend was consistent with the higher inorganic N concentration in the RDM that was applied in the spring of 2008 (Table 2.2) as well as greater N_{min} in this amendment (Tables 2.3 and 2.4). However, estimated ANR obtained for CDM was high (10%) compared to that obtained for the RDM treatment (ANR = -1%) in the second forage production year in 2009. Given that the inorganic N concentrations in the CDM and RDM that were applied in the fall of 2008 were almost similar (Table 2.2), this trend could not be explained by the inorganic N concentrations in the amendments and warrants further investigations. The low (negative) N recovery by the teff crop for RDM in year 2009 possibly indicates little residual N benefit from the previous (2008) applications of RDM. Cumulative ANR for was 6% for CDM and 9% for RDM (Table 2.5). The observation that recovery of N by teff was greater for RDM than CDM was consistent with findings of other annual forage studies utilizing beef cattle (Eghball and Power, 1999; Miller et al., 2009) and dairy manure (Lynch et al., 2004).

4. CONCLUSION

In the experimental plots seeded with teff, RDM resulted in significantly greater N mineralization in the first year following application (2008) than CDM and the unfertilized control. Although the observational nature of this study does not allow for determination of cause and effect, based on past research indicating a relationship between composting and C and N stability, it is likely that C and N in CDM were more stabilized and less labile (as partly indicated by lower C/N ratio of CDM) and this may have led to a slow CDM N_{min} as observed in this study. In contrast, the higher N_{min} from RDM-amended soil (approximately 59% of the added total RDM N was mineralized) compared to N_{min} from CDM-amended soil (22% of the added total CDM N was mineralized) in 2008 possibly suggests that RDM had more easily mineralizable organic N fractions, in addition to slightly higher proportion of inorganic N, than CDM. Greater forage dry matter yields, N uptake and ANR responses to RDM than CDM observed in 2008 reflect higher levels of initial RDM inorganic N concentration as well as greater N mineralization in this amendment during the first production year. The results of this study support the hypothesis that fresh manure such as RDM has a higher fertilizer value than composted manure such as CDM, particularly during the first year following application. It may well be that there was rapid consumption of spring 2008 RDM N in the 2008 teff growing season as well as uptake of N by spring planted lettuce or loss of N via leaching from fall-2008 applied CDM and RDM, so that less N was likely carried over to the 2009 growing season resulting in no significant difference in N mineralization between CDM and RDM amended soils in 2009, which was further reflected on forage dry matter yield and N uptake.

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Table 2.1. Treatments used, manure rate, and mass of total C and N applied during spring and fall 2008.

Treatment†	Treatment code	Rate‡		Total C applied		Total N applied	
		Spring 2008	Fall 2008	Spring 2008	Fall 2008	Spring 2008	Fall 2008
		-----Mg ha^{-1}-----		----------(kg ha^{-1})----------			
Teff with no amendment	T+C	0	0	0	0	0	0
Teff with CDM	T+CDM	11.4	19.3	1032	695	123	56
Teff with RDM	T+RDM	51.7	14.6	2082	856	123	56
Fallow with no amendment	F+C	0	0	0	0	0	0
Fallow with CDM	F+CDM	11.4	19.3	1032	695	123	56
Fallow with RDM	F+RDM	51.7	14.6	2082	856	123	56

† CDM, composted dairy manure; RDM, raw dairy manure; Fallow is a bare fallow.

Table 2.2. Physical and chemical characteristics‡ of manure and compost amendments applied in spring and fall 2008.

Parameter	Composted dairy manure (CDM)		Raw dairy manure (RDM)	
	Spring 2008	Fall 2008	Spring 2008	Fall 2008
Moisture, (%)	28.62	20.44	40.90	26.95
Total solids, (%)	71.38	79.56	59.10	73.05
Organic matter, (%)	17.20	6.84	7.65	11.14
Ash, (%)	54.18	72.72	51.45	61.91
C to N ratio	8.40	12.40	16.90	15.30
Total N, g kg^{-1}	10.74	2.91	2.38	3.85
Organic N, g kg^{-1}	10.11	2.58	1.99	3.56
NH_4-N, g kg^{-1}	0.13	0.33	0.36	0.28
NO_3-N, g kg^{-1}	0.50	0.01	0.03	0.003
Total P, g kg^{-1}	5.05	1.07	0.65	0.85
Total K, g kg^{-1}	13.21	4.65	3.05	4.17
EC (1:5 w/v), dS m^{-1}	5.14	2.66	4.42	2.44
pH-H_2O (1:5 w/v)	8.62	9.09	6.86	8.94

‡ All parameters are reported on a wet weight basis.

Table 2.3. Mineralized N in bare fallow and teff treatment plots amended with composted dairy manure (CDM), raw dairy manure (RDM), or untreated control soil at three sampling dates during teff growing season in 2008.

		2008			2009	
		Incubation period†				
Treatment	Amendment	1	2	3	1	2
		----------kg N ha^{-1}----------				
Teff	Control	23.7 b‡	13.5 b	31.4 ab	47.5	38.0
	CDM	39.7 b	25.0 b	21.4 b	42.0	44.2
	RDM	63.3 a	29.8 b	47.5 a	54.4	38.0
			P > F			*P > F*
Amendment (A)			***			NS
Incubation period (I)			**			NS
A× I			NS			NS
Bare-fallow	Control	30.2 a¶	37.6 ab	50.8 a	47.5	41.6
	CDM	31.9 ab	26.2 b	63.5 a	40.1	40.6
	RDM	55.7 a	43.3 ab	69.4 a	32.3	49.5
			P > F			*P > F*
Amendment (A)			NS			NS
Incubation period (I)			*			NS
A× I			NS			NS

Level of significance: *$P<$ 0.1; **$P<$ 0.05; ***$P<$ 0.001; NS, not significant.

‡ Within each column, values followed by different lowercase letters were significantly different ($P < 0.05$).

¶ Within each row, values followed by different lowercase letters were significantly different ($P<$ 0.1).

†Tube removal dates for incubation periods 1, 2, and 3 were 12 July (30 d), 12 Aug. (61 d), and 22 Aug. 2008 (70 d); 1 Aug. (30 d) and 31 Aug. 2009 (61 d), respectively, since tube installation date.

Table 2.4. Amounts of mineralized N as percentage of added N in manure or compost to teff and bare-fallow plots in 2008 and 2009.

		2008‡			2009‡	
		Incubation period†				
Treatment	Manure type	1	2	3	1	2
		----------(%)----------				
		Total N				
Teff	CDM	13.0	9.4	-8.1	-3.1	3.5
	RDM	32.2	13.3	13.3	3.9	0.0
Bare-fallow	CDM	1.4	-9.3	10.3	-4.1	-0.6
	RDM	19.1	4.6	15.1	-8.5	4.4
		Organic N				
Teff	CDM	13.9	10.0	-8.7	-3.3	3.8
	RDM	38.5	15.8	15.9	4.5	0.0
Bare-fallow	CDM	1.5	-9.9	11.0	-4.5	-0.6
	RDM	22.8	5.5	18.1	-9.8	5.1

†Tube removal dates for incubation periods 1, 2, and 3 were 12 July (30 d), 12 Aug. (61 d), and 22 Aug. 2008 (70 d); 1 Aug. (30 d) and 31 Aug. 2009 (61 d), respectively, since tube installation date.

‡ In 2008, % N was calculated by dividing the amount of N_{min} at each incubation period by the amount of total N added in CDM or RDM in spring 2008. Whereas in 2009, estimates of N_{min} were divided by the amount of N applied in manure or compost in spring and fall of 2008.

Table 2.5. Teff dry matter yield and N uptake responses to CDM and RDM amendments in 2008 and 2009.

						Apparent N recovery†		
	DM yield		N uptake					Cumulative
Amendment	2008	2009	2008	2009	Total	2008	2009	(2008-2009)
	----------kg ha^{-1}----------					----------%----------		
Control	3971 b‡	3135 a	111 b	68 a	179 a	-	-	-
CDM	4009 b	4282 a	105 b	85 a	190 a	-4.9	9.5	6.1
RDM	4862 a	3510 a	129 a	66 a	195 a	14.6	-1.1	8.9
			ANOVA					
Amendment	**	NS	*	NS	NS			

Level of significance: *P <0.1; **P <0.05; NS, not significant.

‡ Values in the same column, followed by a common letter were not significantly different.

† In 2008, % N recovery was calculated by dividing N uptake by the amount of total N that was added in the spring of 2008. Whereas 2009 and cumulative (2008-2009) N recoveries were calculated by dividing N uptake by the amount of total N that was applied in the spring and fall of 2008.

CHAPTER 3: EARTHWORM ABUNDANCE AND SPECIES COMPOSITION IN ORGANIC FORAGE PRODUCTION SYSTEMS OF NORTHERN COLORADO RECEIVING DIFFERENT SOIL AMENDMENTS

Summary

This study evaluated the effects of soil amendments on earthworm communities in organic annual forage and perennial pasture systems in northern Colorado. In the annual forage study, an annual warm-season grass, teff (*Eragrostis tef*) and bare-fallow were main plots and received one of three soil amendments: (1) composted dairy manure (CDM), (2) raw dairy manure (RDM), and (3) a control. For the perennial forage study, CDM was topdressed onto a grass mixture consisting of orchardgrass, smooth and meadow bromegrass at rates ranging from 0 to 44.8 Mg ha^{-1}. At both sites, earthworm and soil samples were collected in July 2009. The earthworms identified from both systems were composed of endogeic species *Aporrectodea rosea* (Savigny), *A. tuberculata* (Eisen), and *A. turgida* (Eisen). In the annual study, earthworm abundance did not differ between teff and bare-fallow. However, within the bare-fallow, earthworm abundance was significantly affected by soil amendment, with CDM averaging approximately 1.4 and 5.4 times greater earthworm abundance than RDM and the control, respectively. Earthworm abundance was found to be positively correlated with soil Cu ($R = 0.51$, $p = 0.03$) and K ($R = 0.58$, $p = 0.01$). In the perennial study, earthworm abundance tended to increase with an increase in the CDM rate to 33.6 Mg ha^{-1}. However, no further increase was observed when the CDM rate was increased to 44.8 Mg ha^{-1}. At this site, earthworm abundance was negatively correlated with EC ($R = -0.37$, $p = 0.02$). Results suggest that high quality (low C/N ratio) dairy manure is important for maintaining a high earthworm population. Higher manure rates appear to discourage earthworms, possibly due to salinity induced osmotic stress and dehydration.

1. **INTRODUCTION**

Because of its reliance on organic methods of soil fertility management, organic agriculture has been shown to lead to higher quality soil than conventional farming (Siegrist et al., 1998; Carpenter-Boggs et al., 2000). According to Siegrist et al. (1998), organically managed soils conserve a diverse group of soil fauna. Due to their contribution to total belowground biomass and influence on the functioning of soil ecosystems (Bohlen et al., 1997; Hendrix and Bohlen, 2002; Smith et al., 2008), earthworms are often described as one of the most important groups of soil fauna. Because of their influence on the physical, chemical, and biological properties of soil related to plant productivity (Lee, 1985; Edwards et al., 1995; Edwards and Bohlen, 1996 cited in Bohlen et al., 1997; Lavelle et al., 1997), increased abundance of earthworms is generally recognized by many as an indication of healthy and productive soils (Lee, 1995; Buckerfield et al., 1997; Doran and Safley, 1997 cited in Simonsen et al., 2010; Paoletti, et al., 1998).

Several studies have shown that organically managed fields fertilized with manure had higher earthworm biomass, density, and diversity than conventionally managed mineral-fertilized or unfertilized fields (Werner and Dindal, 1990; Pfiffner and Mäder, 1997 cited in Siegrist et al., 1998; Carpenter-Boggs et al., 2000; Jordan et al., 2004; Simonsen et al., 2010). Other previous studies have reported that earthworm abundance was related to the quality and quantity of organic soil amendments (Lofs-Holmin, 1983; Hendrix et al., 1992; Bohlen et al., 1997; Tian et al., 1997). Along the eastern plains of Colorado the organic dairy operation continues to expand, a trend that is driving the need for organically grown forages. Although it has declined recently due to the economic downturn, there were an estimated 48,954 hectares of certified organic pastureland in Colorado in 2007 (ERS, 2008). With this trend of increased

organic forage production, the question arises as to what effects this conversion will have on earthworm communities in this region.

This earthworm study was part of a larger study exploring two options: warm-season organic forage production in rotation with an organic spring vegetable, or organic perennial pasture production. The earthworm species present in Colorado agroecosystems are mainly European Lumbricids (Reynolds and Reeves, 2003; Damoff and Reynolds, 2004) that can be classified into three ecological groups depending on their feeding strategies: endogeic, anecic, and epigeic species, representing soil, soil and litter, and litter feeders, respectively (Bouché, 1977). The most recent surveys by Reynolds and Reeves (2003) and Damoff and Reynolds (2004) show that the following species are commonly found in northern Colorado: *Aporrectodea rosea* (Savigny), *A. trapezoides* (Duges), *A. tuberculata* (Eisen), *Lumbricus rubellus* (Hoffmeister), *Octolasion cyaneum* (Savigny), and *O. tyrtaeum* (Savigny). However, these studies focused on taxonomy and spatial distribution of earthworms, and information on their abundance or biomass is still lacking.

Thus, the present study investigated the effects of manure addition on earthworms in annual forages and perennial pastures managed in accordance with organic protocols. The specific objectives of this study were (1) to examine the effect of raw versus composted dairy manure on earthworm populations in an annual forage cropping system, and (2) to test the impact of surface broadcasting of composted dairy manure on earthworm populations in perennial pasture. Knowledge of how earthworms respond to changes in soil management practices is critical to the development of sustainable, organically-based systems of forage production, especially in light of the expected trend toward greater adoption of organic farming in this region.

2. MATERIALS AND METHODS

The study was conducted at two separate locations lying approximately 5 km apart. The following two sections (Sections 2.1 and 2.2) provide a brief description of the field experiments.

2.1. Annual forage study

The annual forage study, hereafter referred to as 'annual study', was conducted at Colorado State University Horticultural Research Center located northeast Fort Collins, Colorado (40°36' N 104°59' W). The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980). Mean summer and winter temperatures are 29°C and 6°C, respectively, at this location. The mean annual precipitation is 37 cm. The land was certified organic and a mixture of green manures including rye, peas, and vetch had been previously grown on the site. The field had no previous history of manure application in the years preceding the current experiment except for one application in the fall of 2006 (22.4 Mg ha^{-1} composted poultry manure).

The experiment was laid out using a split-plot design in which an annual forage crop and bare fallow, hereafter referred to as 'forage treatment', were the main plots with dairy manure type as the subplots. The main plots were 2.44 x 12.84 m and received one of two forage treatments: (1) teff (*Eragrostis tef*), or (2) fallow. The two forage treatments were replicated three times, giving a total of 6 main plots. Each of the main plots contained three 2.44 x 4.28 m subplots in which one of three amendment treatments were randomly assigned: (1) composted dairy manure (CDM), (2) raw dairy manure (RDM), or (3) no amendment as a control. The treatments considered in this study are listed in Table 3.1.

The quantity of wet CDM and RDM needed to obtain the desired application rates for each plot was hand-spread evenly on the surface of the treatment plots, and incorporated to a

depth of approximately 15 cm by rototilling. In spring 2008, the CDM and RDM application rates were 11.4 and 51.7 Mg ha^{-1}, respectively, on a wet weight basis. For the 2009 growing season, manure was applied in the fall of 2008 at a rate of 19.3 Mg ha^{-1} for CDM and 14.6 Mg ha^{-1} for RDM. The CDM and RDM application rates were designed to achieve approximately 123 kg total N ha^{-1} in the first year and 56 kg total N ha^{-1} in the second year according to soil tests. The characteristics of manure used in this study are listed in Table 3.2.

The cropping sequence was as follows: in 2008, teff was planted on 10 June and harvested on 18 August. Following teff, a lettuce crop was planted on 13 March 2009 and harvested on 5 June 2009. For the 2009 field season, teff was planted on 24 June and harvesting was completed on 20 August. Teff is a warm-season annual grass which is gaining popularity with farmers across the U.S. as an alternative high-quality summer forage grass suitable for a wide range of livestock (Miller, 2010). In the fallow plots, the soil surface was left bare and weeded three times throughout the summer. All plots were irrigated twice per week with 2.54 cm of water through an overhead sprinkler system. The lettuce crop was not irrigated.

2.2. Perennial pasture study

The perennial pasture study, hereafter referred to as 'perennial study', was conducted on transitional organic land which was in the process of certification and located at Colorado State University Agricultural Research, Development and Education Center (ARDEC) near Wellington, CO (40°39′ N, 104°59′ W). The soil was classified as a fine-loamy, mixed, superactive, mesic, Aridic Haplustalf (NRCS, 1980). Mean monthly temperatures at this location range from 0°C in January to 22°C in July. The mean annual precipitation is 33 cm; about 88% of which occurs between April and October. Prior to this study, the site was in alfalfa which was killed using tillage in the summer of 2007. Following the termination of alfalfa, the field was

clean-tilled, and a blanket application of 22.4 Mg ha^{-1} composted dairy manure (CDM) was spread and incorporated by disking.

The experiment was laid out as a randomized complete block design (RCBD) with three replications. Plots measured 3 x 12 m. On 5 September 2007, orchardgrass (OG) (*Dactylis glomerata*), meadow brome (MB) (*Bromus biebersteinii*), and smooth brome (SB) (*B. inermis*), hereafter referred to as 'OG-MB-SB', were seeded together with a no-till drill (Model 3P605NT, Great Plains Mfg., Inc., Salina, KS).

In spring 2008, plots received either a CDM treatment of 22.4 Mg ha^{-1}, or served as a control group that received no CDM. In fall 2008, the 22.4 Mg ha^{-1} CDM treatment was divided to include an 11.2 Mg ha^{-1} treatment and a 0 Mg ha^{-1} control giving three fertilization levels applied in triplicate, 0, 11.2, and 22.4 Mg ha^{-1}. The purpose of creating the 0 Mg ha^{-1} control was to observe nitrogen mineralization from previous CDM application. Additionally, a grass-legume treatment was established by interseeding alfalfa (*Medicago sativa*) into the plots that were part of a control group in spring 2008.

The treatments were the following three CDM levels: low, medium, and high. 'Low' = 22.4 Mg ha^{-1} (i.e., 22.4 Mg ha^{-1} CDM in spring 2008, and no CDM in fall 2008), 'medium' = 33.6 Mg ha^{-1} (i.e., 22.4 Mg ha^{-1} in spring 2008 + 11.2 Mg ha^{-1} in fall 2008), and 'high' = 44.8 Mg ha^{-1} (i.e., 22.4 Mg ha^{-1} in spring 2008 + 22.4 Mg ha^{-1} in fall 2008). Since all plots received a similar quantity of CDM in the spring of 2008, any significant differences in earthworm populations can, therefore, be attributed to the effects of CDM treatments established in 2009. The OG-MB-SB-alfalfa plots were considered as an unfertilized control because (1) no CDM was applied on these plots and (2) alfalfa contributed only 36% to the harvested forage yield in alfalfa grass-legume plots. The levels of CDM in this work are in keeping with those commonly

used in this area. The quantity of CDM needed to obtain the desired application rates for each plot was spread by hand uniformly across the plot area. Samples of CDM were analyzed at the Colorado Analytical Laboratory (Brighton, Colorado) and the characteristics of CDM are listed in Table 3.2.

2.3. Soil sampling and analyses

Soil sampling took place on two occasions: in the fall of 2008 prior to manure application and in the spring of 2009 before earthworm sampling. Eight subsamples per plot were collected to a depth of 20 cm using soil probes and pooled. All samples were air dried prior to grinding, to pass through a 2-mm sieve. Subsamples were sent to Ward Laboratories (Kearney, Nebraska) for soil analysis. Electrical conductivity (EC) was determined using an EC electrode (Bremner and Mulvaney, 1982) and a 1:1 ratio of soil to water. The pH was measured using a pH meter in a1:1 ratio of soil to water. Soil organic matter (SOM) was determined by the dry combustion method (Nelson and Sommers, 1996). Extractable NO_3^- and NH_4^+ were determined using a 2 *N* KCl extract (Keeney and Nelson, 1982). Phosphorus was determined by sodium bicarbonate ($NaHCO_3$) extraction and subsequent colorimetric analysis (Olsen et al., 1954). Potassium was extracted using NH_4OAc-EDTA (Chapman and Kelly, 1930). DTPA-extractable Zn, Fe, Mn, and Cu were determined according to Lindsay and Norvell (1978).

2.4. Earthworm sampling and identification

Earthworm sampling occurred from 8-15 July 2009. Within each plot, three soil blocks (20 x 20 x 20 cm) were excavated using a square shovel and earthworms were removed by hand-sorting. At both sites, to extract earthworms occurring below 20 cm, a solution of 27 ml of 37% aqueous formaldehyde in 10 liters of water (applied three times at 10-minute intervals) was poured in the bottom of pits from which soil samples had been taken for hand sorting (Hendrix et

al., 1992). Earthworms were counted in the field, placed in vials containing 70% ethanol and transferred to the lab for identification. All worms were washed clean of adhering soil, patted dry with a paper towel, weighed for total wet biomass, and preserved in 4% formaldehyde until identification (Fender, 1985). Identification was accomplished by using the keys of Fender (1985). Additional specimens were sent to the Oligochaetology Laboratory (Kitchener, Ontario, Canada) for further identification. After identification, earthworms were oven-dried at 60°C for 24 h to obtain total dry weight (Bohlen et al., 1997). All earthworm species were combined for analysis and reported as total abundance (no. m^{-2}) and biomass (g m^{-2}).

2.5. Statistical analyses

Data were analyzed using SAS software version 9.2 (SAS, Institute, 2008). Total abundance and biomass data of the annual study were analyzed using PROC MIXED for a split-plot design with forage treatments (teff and fallow) and amendments (CDM, RDM, and control) as factors. In the mixed model, forage and amendment treatments were fixed effects, and block and any block interactions were random effects. The interaction between forage treatment and block was used as the error term for determining the significance of forage treatment main effects. Total abundance and biomass data of the perennial study were analyzed using PROC GLM for a one-way randomized complete block design with the CDM rate (control, low, medium, and high) as a factor. Mean separation was achieved using the least significant difference (LSD) option of LSMEANS statement. Treatment differences were considered significant at $p < 0.05$ unless stated otherwise.

Simple Pearson correlation analysis was also performed to determine relationships between total abundance and biomass of earthworms and soil properties. Significant predictors

were selected by further subjecting significant correlations to multiple linear regression analysis with stepwise model selection that used a $p < 0.05$.

3. RESULTS

3.1. Total abundance and biomass of earthworms in annual forages

The earthworms recovered from the annual study belonged to two endogeic species: *A. tuberculata* and *A. turgida.* They were found mostly at the 0-20 cm depth. Very few (about 5%) of these species were recovered below 20 cm, only in the control plots.

Earthworm total abundance ranged from 19 to 103 individuals m^{-2} in the bare-fallow treatment (Fig. 3.1A), while total earthworm dry biomass ranged from 2.1 to 13 g m^{-2} (Fig. 3.1B). Whereas in the teff treatment, total earthworm abundance ranged from 26 to 53 individuals m^{-2}, with total dry biomass ranging from 5.2 to 7.6 g m^{-2}. Earthworm total abundance or biomass did not differ between bare-fallow and teff treatments. There was a significant effect of amendment treatment ($p = 0.04$). Since the forage by amendment interaction was also significant ($p = 0.02$), further analysis to compare amendment treatments separately for forage treatment was run. This analysis revealed that differences were only significant for the bare-fallow treatment. Earthworm total abundance was lowest in the control bare-fallow plots, where earthworm total abundance was 3.8 and 5.4 times lower than in RDM- and CDM-treated bare-fallow plots, respectively. Bare-fallow plots treated with CDM had on average 42% higher earthworm total abundance than those treated with RDM ($p = 0.02$; Fig. 3.1A).

3.2. Total abundance and biomass of earthworms in perennial pasture

The earthworms collected from the perennial pasture belonged to three endogeic species, the above two species plus *A. rosea*. All of them were recorded within the 20 cm soil depth. No worms were recovered below 20 cm. Earthworm total abundance tended to increase with an

increase in CDM application rate to 33.6 Mg ha^{-1} (Fig. 3.2A), but no further increase was observed when the rate was augmented to 44.8 Mg ha^{-1}. However, differences were only significant between the medium rate of CDM and other rates (Fig. 3.2A). Earthworm total abundance was on average 35, 43, and 71% higher, respectively, in the medium rate of CDM compared to the high rate, low rate, and control (Fig. 3.2A). Although earthworm total abundance was not significantly different, but higher earthworm total abundances were observed in the low and high rates of CDM treatments compared to the control.

Earthworm total abundance and biomass were highly correlated ($R = 0.8$, $p = 0.003$). Consequently, total biomass showed the same pattern as total abundance in relation to CDM treatments. Earthworm total biomass ranged from 18.1 g m^{-2} in the control to 46.3 g m^{-2} in the medium CDM rate (Fig. 3.2B). The medium rate of CDM had 156, 76, and 48% higher, respectively, total biomass when compared to the control, low and high CDM rates. Additionally, the high CDM rate had on average 1.7 times greater earthworm total biomass than the control ($p = 0.001$).

3.3. Effects of manure type on soil properties in annual forages

The initial soil properties for both fields studied can be considered homogenous. So, any significant changes in soil parameters can be attributed to the effects of manure addition. Both in the annual and perennial studies, no significant treatment differences in soil measurements made in the fall of 2008 were found. Because treatment differences were more pronounced in the spring 2009 sampling, only the 2009 soil data are reported in this paper. For all soil parameters, the effect of forage treatment (fallow versus teff) was not significant. Also, no significant forage by amendment interaction was found for any soil parameter. Thus, for all soil parameters mean separation was done by comparing the amendment means (only amendment effect was

significant) averaged across fallow and teff treatments. When averaged across the forage treatment, soil collected from CDM-treated plots had significantly higher Zn, P, and K compared to the control plots, with concentrations averaging 1.20, 12.2 and 532 mg kg^{-1} versus 1.0, 10.8 and 498 mg kg^{-1} in the CDM and control treatments, respectively (Table 3.3). Cu and Zn were significantly higher (1.2 and 1.1 mg kg^{-1}, respectively; Table 3.3) in soils of RDM-treated plots than in the control soil. Soil K was significantly higher in the CDM-treated plots than in the RDM-treated plots, with soil K level averaging 532 versus 497 mg kg^{-1}, respectively (Table 3.3).

3.4. Effects of manure application rate on soil properties in perennial pasture

Soil organic matter tended to increase with an increase in CDM application rate (Table 3.4). However, the only significant difference was for the highest rate, which had higher SOM content (approximately 2.9%; Table 3.4) compared to the control and the low CDM rate. Additionally, the highest rate of CDM also had the highest EC (0.68 dS m^{-1}; Table 3.4) compared to other rates considered in this study (Table 3.4). Electrical conductivity of soil measured in the control soil was surprisingly high (0.65 dS m^{-1}; Table 3.4) and did not differ from the high CDM rate. Relative to other CDM application rates considered in this study, the low rate had the lowest soil K (405 mg kg^{-1}; Table 3.4).

3.5. Correlations between earthworms and soil properties

In the annual study, earthworm total abundance and biomass were positively correlated with K ($R = 0.58$, $p = 0.01$ for abundance and $R = 0.51$, $p = 0.03$ for biomass; Table 3.5) and Cu ($R = 0.51$, $p = 0.03$ for abundance and $R = 0.48$, $p = 0.05$ for biomass; Table 3.5). Both K and Cu emerged as significant predictors according to the results of the stepwise regression analysis.

Of the soil properties measured in the perennial study, only soil pH and EC showed significant correlation with earthworms (Table 3.5). EC was negatively correlated with both total

abundance (R = -0.37, p = 0.02) and biomass (R = -0.29, p = 0.04), whereas pH was negatively correlated with total biomass (R = -0.54, p = 0.07). These two soil variables (EC and pH) could be inter-related, and identifying the variable truly related to earthworm abundance or biomass can be difficult. Stepwise regression analyses can be helpful in this respect, and suggested that EC was the more important predictor of abundance and biomass of earthworms.

4. DISCUSSION

Earthworms identified in the present study consisted entirely of endogeic species. These species were also the most common in previous earthworm studies in this area (Reynolds and Reeves, 2003; Damoff and Reynolds, 2004). Of the three species (*A. rosea*, *A. tuberculata* and *A. turgida*) identified in this study, *A. rosea* was found only in the perennial study. This was probably due to surface broadcasting of compost and the absence of soil disturbance at this site. Lofs-Holmin (1983) also observed *A. rosea* to build up a large population in grass leys in Sweden, especially when farmyard manure was spread on the surface. No epigeic or anecic species were found in either study. The epigeic species *Lumbricus rubellus* had previously been found at other study sites not far from where the experimental sites were located (Smith, 2002). *Lumbricus terrestris* had also been found in northern Colorado (Reynolds and Reeves, 2003; Damoff and Reynolds, 2004). In consideration of the occurrence of epigeic (L. *rubellus*) and anecic (L. *terrestris*) species in previous studies and surveys in this region (Smith, 2002; Reynolds and Reeves, 2003; Damoff and Reynolds, 2004), it seemed reasonable to expect their presence in these study plots.

Surface soils can be quite dry and hot during the summer months in Colorado (Damoff and Reynolds, 2004). Although measurements of soil moisture were not taken, it was noted at the time of earthworm sampling that the fields were dry. Thus, it is likely that earthworms were in a

quiescent state or forced to retreat to deeper soil horizons, and thus, did not respond to the formalin extraction (Chan, 2004; Damoff and Reynolds, 2004). Given the dynamics in earthworm populations with time (sampling was done only once), one would expect the July sampling to underestimate the abundance, biomass, and species-composition of earthworm communities present in these study areas due to the hot and dry conditions. Therefore, it is important for future studies to include multiple earthworm sampling to deal with temporal distribution and variability.

Although tillage is known to have an adverse impact on earthworms (Lee, 1985; Werner and Dindal, 1990; Buckerfield et al., 1997; Jordan et al., 1997; Paoletti et al., 1998; Hubbard et al., 1999), a review by Chan (2001) suggests that the effect of tillage depends on the earthworm functional group. As all species recovered in the present study were exclusively endogeic, the high rates of soil disturbance in the systems studied likely contributed to the dominance by the genus *Aporrectodea*, which is relatively tolerant of agricultural activities such as disk tilling (Smith et al., 2008). Further evidence supporting this hypothesis is previous research by Nuutinen (1992) and Springett (1992) who reported that the endogeic species *A. caliginosa* responded most favorably to tillage. They also reported that the abundance of anecic species *L. terrestris* declined due to conventional tillage. The greater number and biomass of endogeic species in the tilled soil was attributed to the large amount of organic matter ploughed under thereby providing a greater food supply for the earthworms (Springett, 1992). In the current study, the annual study site was tilled twice per forage crop produced, while the perennial study site was tilled only once during the establishment of the grasses. However, long-term cultivation of the area before it was converted into a perennial pasture may have contributed to dominance

by the endogeic species. At both sites, forage was cut and removed. This reduced the availability of surface litter and likely discouraged the epigeic and anecic species (Nuutinen, 1992).

The greater earthworm total abundance and biomass (Figs. 3.1A and 3.1B) in bare-fallow plots treated with CDM compared to those treated with RDM was in contrast to our hypothesis. Due to the greater amount of C input through RDM, earthworm abundance and biomass was expected to be greater in plots treated with RDM. The total mass of C applied through RDM was almost double (2938 kg ha^{-1}; Table 3.1) that applied in the CDM. However, the higher numbers and biomass of earthworms were associated with the amendment that was low in C content. Given that endogeic earthworms, especially the genus *Aporrectodea*, have been shown to be sensitive to harmful chemical inputs, particularly Cu (Paoletti et al., 1998), one would expect elevated Cu levels to have a negative impact on earthworms. Although RDM was relatively higher in Cu than CDM, even though this was without significance, earthworms were positively correlated with Cu. Thus, the lower earthworm populations observed in the RDM-treated bare-fallow plots (Figs. 3.1A and 3.1B) are unlikely to have resulted from Cu; rather it seems that a factor other than copper may be responsible for reduced earthworm populations observed in the bare-fallow plots amended with RDM. The NH_3-N content in the RDM that was applied in the spring and fall of 2008 combined was 42% higher than that in the CDM (644 vs. 453 mg kg^{-1}, respectively; Table 3.2). It has been reported that ammonia and ammonia-based fertilizers adversely affect earthworm populations (Soil Quality Institute, 2001). This suggests that the higher ammonia-N content in the RDM than CDM likely contributed to reduced earthworm total abundance and biomass observed in the RDM-amended bare-fallow plots. Although no lignin analysis of the manure was done, RDM may have had higher lignin content and likely provided a low quality food source for earthworms. This may have led to scarcity of food and could thus

partly explain the lower earthworm abundance and biomass in the RDM-treated bare-fallow plots. Given that CDM had a relatively low C/N ratio (approximately 9; Table 3.1) as well as previous research suggesting earthworms' preference for high quality (low C/N ratio) food (Lofs-Holmin, 1983; Hendrix et al., 1992; Bohlen et al., 1997; Tian et al., 1997; Hubbard et al., 1999), it is likely that CDM provided a high quality food supply and consequently led to greater earthworm total abundance and biomass observed in the CDM-treated bare-fallow plots. Although the endogeic species encountered in this study might actually be favored by manure with a low C/N ratio, the relatively small difference in the ratio between CDM and RDM would mean that this factor can only partially explain the observed variation in earthworm total abundance in the bare-fallow treatment. Although a positive correlation between soil K and earthworm total abundance and biomass was observed, it is not clear if this relationship was reflecting some other property (e.g. osmotic pressure or water availability). However, it is likely that higher soil K in manured plots was the result of K release from manure due to earthworm feeding (Basker et al., 1992).

Although total earthworm abundance and biomass appeared to increase with an increase in composted dairy manure application rates (Figs. 3.2A and 3.2B), significant differences were observed only between the medium (33.6 Mg ha^{-1}) and other application rates considered in this study. This observation was in contrast to the hypothesis that earthworm abundance and biomass would respond linearly to increasing C inputs (Hendrix et al., 1992) when a soil is exposed to a range of CDM additions. Soil organic matter content at the medium application rate was approximately 2.7% (Table 3.4), and did not differ from the SOM content at the other manure application rates. This clearly suggests that other factors other than SOM are responsible for the greater number and biomass of earthworms observed at the 33.6 Mg ha^{-1} manure application rate.

The absence of correlation between SOM and earthworm total abundance or biomass further supports the hypothesis that SOM is not a key factor in explaining differences among the CDM application rates in earthworm total abundance and biomass. Nonetheless, this does not mean that SOM is not having an effect on earthworm numbers or biomass. Surface broadcasting of CDM at 33.6 Mg ha^{-1} may have played more of a mulching effect than serving as a food source, thus reducing the risk of damage by heat or water stress (Jordan, et al., 2004; Fonte et al., 2009), and consequently contributed to the observed greater earthworm total abundance and biomass (Figs. 3.2A and 3.2B). Although this could not be verified because soil moisture and temperature were not measured, the build-up of earthworm populations following compost addition has previously been attributed to improvement of soil microclimate (Tian et al., 1997; Werner and Dindal, 1990; Hubbard et al., 1999).

The decline in earthworm total abundance and biomass (Figs. 3.2A and 3.2B) when the CDM application rate was augmented to 44.8 Mg ha^{-1} was unexpected but not surprising. It is possible that manure application at 44.8 Mg ha^{-1} contributed to elevated concentrations of Cu and consequently resulted in low earthworm total abundance and biomass observed at this application rate. In a previous study, Paoletti et al. (1998) found that the abundance and biomass of endogeic species were severely reduced by Cu. They attributed this to the greater exposure of endogeics to Cu in different forms and concentration as they mainly feed on decomposed organic matter. Another possible explanation is that the highest manure application rate (44.8 Mg ha^{-1}) increased salinity (0.68 dS m^{-1}; Table 3.4), thereby affecting the availability of soil moisture and thus contributed to the decreased earthworm total abundance and biomass observed at this rate. A similar result was reported by Owojori et al. (2009) who found that *A. caliginosa* was negatively affected at 0.52 dS m^{-1}. The negative correlation between EC and earthworm total

abundance and biomass in this study further supports the hypothesis that the relative decrease in earthworm total abundance and biomass at the 44.8 Mg ha^{-1} manure application rate may have resulted, at least in part, from salinity induced osmotic stress and dehydration on earthworms since their body is mostly water. The higher EC (0.65 dS m^{-1}; Table 3.4) associated with the 0 Mg ha^{-1} treatment of no CDM application, which had alfalfa in the grass mixture, is difficult to explain and warrants further investigation.

Of the soil properties considered in this study, only a few were significantly correlated with earthworm total abundance or biomass. A similar result was reported by Siegrist et al. (1998) who found a non-significant correlation between organic C and biomass and density of earthworms in organic farmyard manure treated plots in Switzerland. Other researchers also reported similar results to these (Rossi et al., 1997; Nuutinen et al., 1998). It is likely that a period of two years is not enough for both soil properties and earthworm populations to change in response to changes in management practices. The results of the present study and those of others emphasize the need for cautious interpretation of results from short-term studies. Therefore, for future studies, it is important to consider soil characteristics that better reflect new additions of organic matter and change more rapidly (e.g., soil microbial biomass) instead of total soil C content, which changes relatively slowly (Haynes, 1999).

5. CONCLUSION

This study showed that manure (CDM and RDM) addition did not have a significant effect on earthworm total abundance and biomass in the plots with teff. However, in the bare-fallow treatment, the addition of RDM increased earthworm total abundance and biomass compared to the control, but not as substantially as when compared to CDM. It was suggested that high quality manure (i.e., manure with low NH_3-N content, lignin content, C/N ratio, and low Cu content) is important for supporting high earthworm populations. More study on the critical C/N ratio that stimulates feeding of endogeic species are still needed to better understand the effects of manure quality on these species. The results from the perennial pasture study did not support the hypothesis that larger manure application rates increase SOM and consequently encourage higher earthworm abundance and biomass. Overall, the results indicate that larger manure application rates can lead to considerably lower earthworm total abundance and biomass. It was hypothesized that larger CDM application rates lead to high metal inputs such as Cu, which at elevated concentrations can be harmful to earthworms. Furthermore, larger CDM application rates increase soil salinity thereby causing dehydration of earthworms (osmotic stress) and consequently results in low earthworm populations. Further study is needed to elucidate the exact effects manure quality and quantity on endogeic earthworm species.

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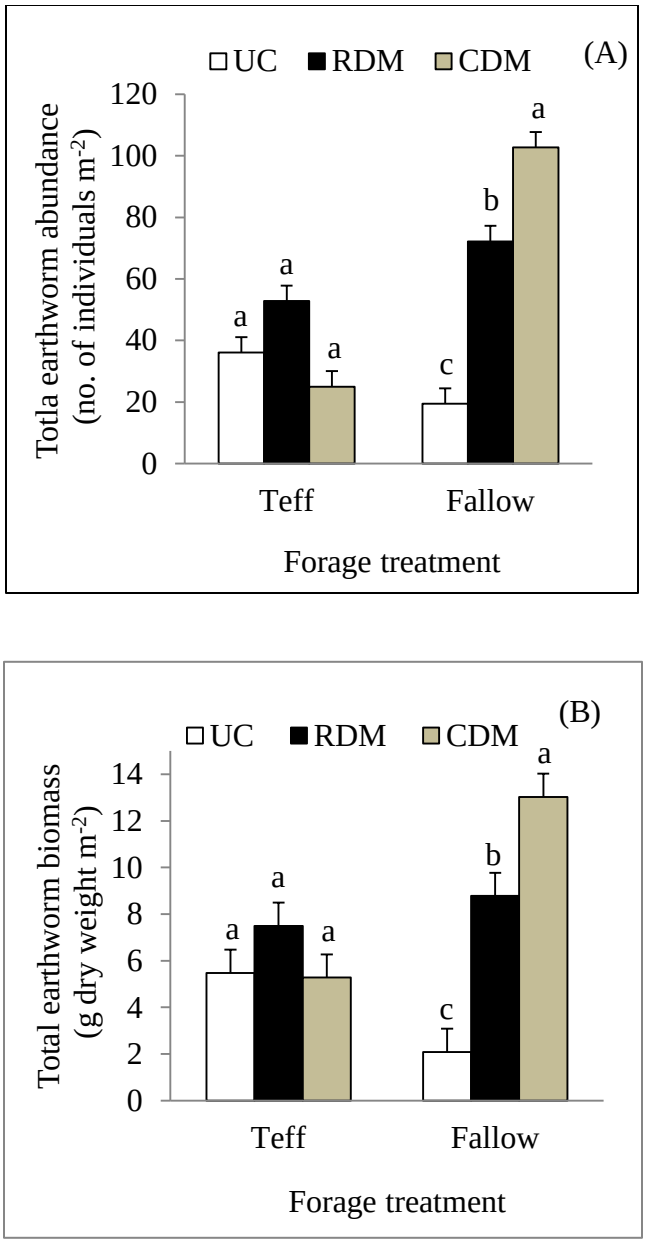
FIGURE CAPTIONS

Figure 3.1. Total earthworm abundance (A) and biomass (B) to 20 cm soil depth as affected by teff (*Eragrostis tef*) and fallow treatments amended with composted dairy manure (CDM), raw dairy manure (RDM), and unfertilized control (UC). Different letters above error bars indicate significant differences ($P < 0.05$) among soil amendment treatments within a forage treatment.

Figure 3.2. Earthworm total abundance (A) and biomass (B) to 20 cm soil depth as affected by composted dairy manure (CDM) applied at different rates to a perennial pasture consisting of orchardgrass, smooth and meadow bromegrass. Values followed by different lowercase letters are significantly different from one another. Bars represent standard errors.



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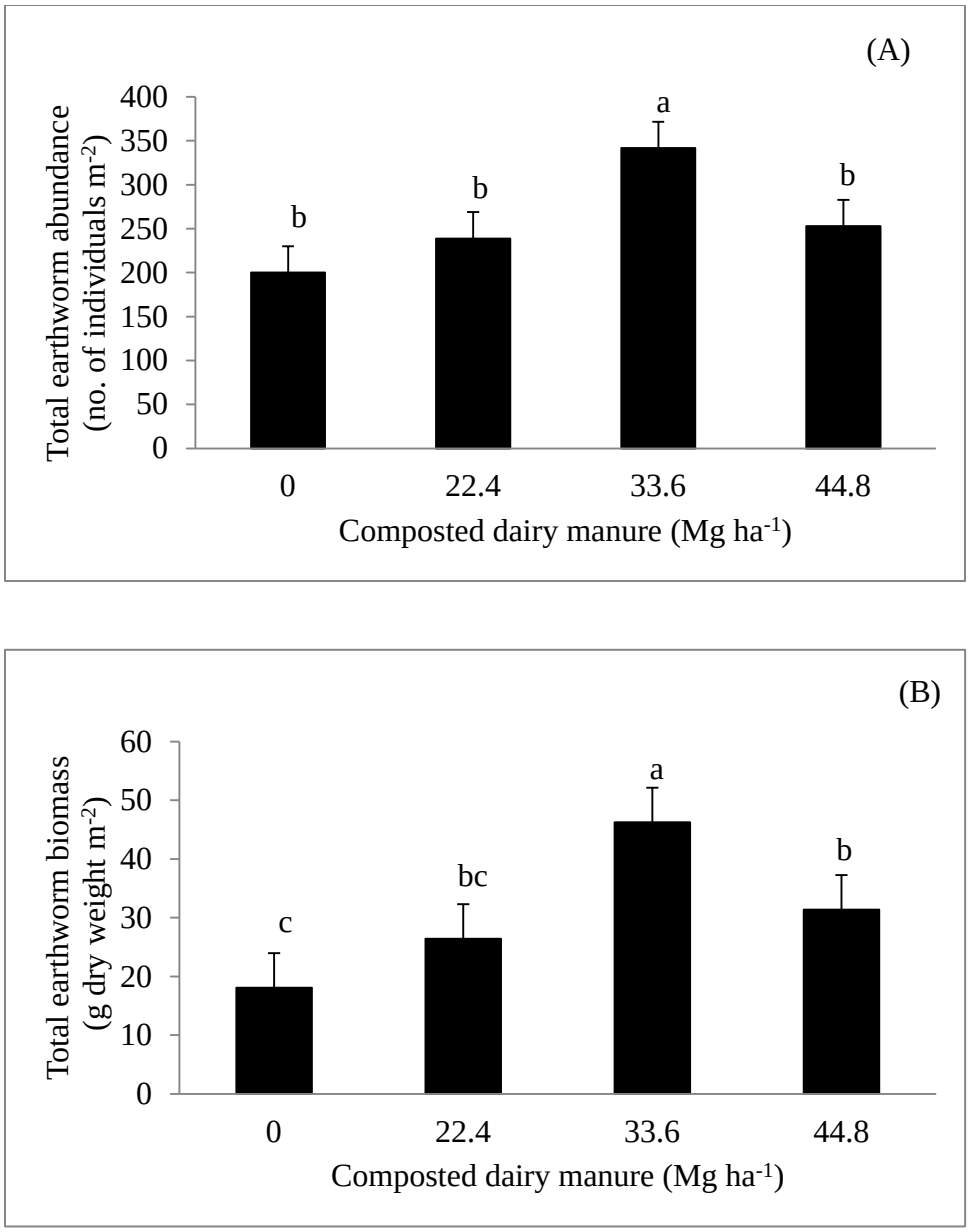

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Table 3.1. Treatments used, manure rate, and cumulative mass total C and N applied to experimental plots in two (spring and fall 2008) applications of dairy manure.

Experiment	Treatment†	Rate	Treatment code	Cumulative total C applied	Cumulative total N applied	C/N ratio
		Mg ha^{-1}		----------(kg ha^{-1})----------		
1. Annual	Teff + control	0		0	0	-
	Teff + CDM	30.7		1727	180	9.6
	Teff + RDM	66.3		2938	181	16.2
	Fallow + control	0		0	0	-
	Fallow + CDM	30.7		1727	180	9.6
	Fallow + RDM	66.3		2938	181	16.2
2. Perennial	OG-MB-SB/alfalfa	0	Control	0	0	-
	OG-MB-SB CDM	22.4	Low	2028	240	8.5
	OG-MB-SB + CDM	33.6	Medium	2438	274	8.9
	OG-MB-SB + CDM	44.8	High	2834	305	9.3

† OG, orchardgrass; MB, meadow brome; SB, smooth brome. CDM, composted dairy manure; RDM, raw dairy manure; Fallow, bare fallow.

Table 3.2. Characteristics of dairy manure used in the experimental plots for two seasons (spring and fall 2008) in the annual forage and perennial pasture studies.

Experiment			variable‡											
	Time†	Type§	Mois-ture	Ash	OM	C/N	pH	EC	NH_3-N	NO_3-N	Orga-nic N	Total N	Total P	Total K	Total C
			----------(%)--------					dS m^{-1}	----(mg kg^{-1})----		----------(g kg^{-1})----------				
1. Annual	Spring	CDM	29	54	17.2	8	8.6	5.1	127	503.0	10.0	11.0	5.1	13.2	91
(incorporated)		RDM	41	52	7.7	17	6.9	4.4	361	31.4	2.0	2.4	0.7	3.1	40
	Fall	CDM	20	73	6.8	12	9.1	2.7	326	6.7	2.6	2.9	1.1	4.7	36
		RDM	27	62	11.1	15	8.9	2.4	283	3.3	3.6	3.9	0.9	4.2	59
2. Perennial	Spring	CDM	29	54	17.2	8	8.6	5.1	127	503.0	10.0	11.0	5.1	13.2	90
(topdressed)	Fall	CDM	20	73	6.8	12	9.1	2.7	326	6.7	2.6	2.9	1.1	4.7	36

† Time, time of application of manure (for 2008 field season, CDM and RDM were applied in spring 2008; while for 2009 field season the application was carried out in fall 2008).

§ Type: CDM = composted dairy manure, RDM = raw dairy manure.

‡ OM = organic matter; EC = electrical conductivity.

Table 3.3. Soil properties (0-20 cm) measured in teff and bare fallow treatments as affected by composted dairy manure (CDM), raw dairy manure (RDM), and control. The data presented are only for amendment treatment averaged across forage treatment. ANOVA results test simple effects of forage and amendment treatments and their interaction with respect to soil properties.

Amendment	Manure rate	Soil properties†							
		SOM	pH	EC	K	Cu	P	Zn	NO_3^--N
	($Mg\ ha^{-1}$)	(%)		($dS\ m^{-1}$)	----------($mg\ kg^{-1}$)----------				
Control	0	2.6 a‡	8.23 a	1.13 a	498 b	1.03 b	10.8 b	1.0 b	9.5 a
CDM	30.7	2.5 a	8.20 a	1.15 a	532 a	1.15 ab	12.2 a	1.2 a	7.1 a
RDM	66.3	2.5 a	8.20 a	1.16 a	497 b	1.20 a	11.5 ab	1.1 a	8.3 a
		P > F							
Forage (F)		NS	NS	NS	NS	NS	NS	NS	NS
Amendment (A)		NS	NS	NS	**	**	*	**	NS
F x A		NS	NS	NS	NS	NS	NS	NS	NS

Level of significance: * = $P < 0.1$; ** = $P < 0.05$; NS = not significant.

‡ Means within a column, followed by a common lowercase letter are not statistically different.

† SOM = soil organic matter; EC = electrical conductivity.

Table 3.4. Soil properties (0-20 cm) measured in the perennial study as influenced by composted dairy manure (CDM) application rates. ANOVA results test CDM rates with respect to soil chemical properties.

		Soil properties‡						
Treatment	Rate†	SOM	pH	EC	K	P	NO_3^--N	NH_4^+-N
	(Mg ha^{-1})	(%)		(dS m^{-1})	----------(mg kg^{-1})----------			
Control	0	2.63 b¶	8.20 a	0.65 a	474 a	27.3 a	2.9 a	3.1 a
Low	22.2	2.67 b	8.23 a	0.57 b	405 b	25.0 a	2.2 a	4.1 a
Medium	33.6	2.73 ab	8.27 a	0.53 b	469 a	28.7 a	1.5 a	3.3 a
High	44.8	2.87 a	8.23 a	0.68 a	467 a	32.7 a	1.7 a	4.4 a
					P > F			
Treatment		*	NS	*	**	NS	NS	NS

Level of significance: * = $P < 0.1$; ** = $P < 0.05$; NS = not significant.

† Rate, CDM application rates on wet weight basis.

¶ Means in the same column, followed by the same letter are not statistically different.

‡ SOM = soil organic matter; EC = electrical conductivity.

Table 3.5. Linear correlation coefficients (R) between measures of selected soil properties and earthworm total abundance and biomass. In each row, the correlation coefficient (R) is given with the P-value below it in italics.

Experiment	Measures of earthworms	Soil properties‡					
		K	P	SOM	EC	pH	Cu
1. Annual	Total abundance	0.58	0.29	- 0.27	0.34	0.32	0.51
		0.01	*NS*	*NS*	*NS*	*NS*	*0.03*
	Total biomass	0.51	0.19	- 0.33	0.27	0.19	0.48
		0.03	*NS*	*NS*	*NS*	*NS*	*0.05*
2. Perennial	Total Abundance	- 0.18	0.04	0.04	- 0.37	0.34	-
		NS	*NS*	*NS*	*0.02*	*NS*	
	Total biomass	- 0.46	-0.05	- 0.04	- 0.29	- 0.54	-
		NS	*NS*	*NS*	*0.04*	*0.07*	

‡ SOM = soil organic matter; EC = electrical conductivity; NS = not significant.

CHAPTER 4: EFFECTS OF DAIRY MANURE AND ALFALFA ON SOIL MICROBIAL BIOMASS AND AGGREGATE STABILITY IN AN ORGANICALLY MANAGED GRASS-BASED PASTURE

Summary

Due to its influence on water infiltration, aeration, erosion and workability of soil, aggregate stability is an important characteristics of soil. Understanding how soil management impacts aggregation will aid in making management decisions to enhance soil stability. This study sought to evaluate the effect of composted dairy manure (CDM) and alfalfa on soil microbial biomass C (MBC) and N (MBN) and water stability of aggregate distribution in an organic perennial grass system. Composted dairy manure was applied at rates of 0, 22.4, 33.6, and 44.8 Mg ha^{-1}(wet weight), where the 0 Mg ha^{-1} treatment was a grass/alfalfa mixture. Soil MBC and MBN as well as aggregate stability were measured in samples collected from the 0-5 and 5-15 cm soil depths. Soil samples were separated into four aggregate size classes by wet-sieving: >2000, 250-2000, 53-250, and <53 μm. Microbial biomass C, MBN, and aggregate stability (i.e., proportions of the >2000 and 250-2000 μm fractions) were higher in the high CDM application rate (44.8 Mg ha^{-1}) as well as in the 0 Mg CDM ha^{-1} treatment containing alfalfa than in the low (22.4 Mg ha^{-1}) and medium (33.6 Mg ha^{-1}) CDM application rates. Significant correlations were found between large macroaggregates (>2000 μm) and MBC (R = 0.81, p = 0.002), small macroaggregates (250-2000 μm) and MBN (R = 0.56, p = 0.06), as well as between microaggregates (53-250 μm) and MBN (R = -0.85, p = 0.001). These correlations suggest that higher microbial biomass resulting from the application of CDM at a higher rate (44.8 Mg ha^{-1}) and alfalfa interseeding in a grass-based pasture resulted in the observed improved aggregate stability in these two management treatments. Further investigations on this topic are needed to elucidate the exact effects of CDM and alfalfa on soil microbial biomass and aggregate stability.

1. INTRODUCTION

Organic milk production has been one of the fastest growing segments of organic agriculture in the U.S. in recent years (McBride and Greene, 2009). The organic dairy industry, with typically higher input costs and an inherent susceptibility to public scrutiny, is under pressure to increase its reliance on grazed forages (Booher, 2010). In the U.S., the area of certified organic pastureland has more than quadrupled, increasing from 200,880 ha in 1997 to 874,354 ha in 2008 (ERS, 2008). The majority of irrigated grass pasture production in the western Great Plains is from cool-season perennial grasses including orchardgrass and smooth and meadow bromegrasses (Miller, 2010). Perennial grasses require sustained, season-long nitrogen availability to remain productive (Lynch et al., 2004). Because synthetic N fertilizers are prohibited for use on organically certified land, organic farming systems rely on cultural practices such as crop rotation or application of animal manures to maintain soil fertility (ERS, 2008). In many cases, the fertility needs of organic pasture are met by utilizing dairy manures, composts, or legumes. Since animal manures supply both organic C and mineral nutrients (Barkle et al., 2000), they are likely to exert immediate and wide ranging effects on soil biological and physical quality and C sequestration greater than synthetic inorganic fertilizers (Min et al., 2003). Therefore, both biological and physical properties of soil are essential to evaluate soil quality changes in response to organic amendments.

The term “soil quality” integrates biological, chemical, and physical soil properties as they respond to management practices (Islam and Weil, 2000). Since soil quality cannot be measured directly, it is often inferred from management-induced changes in soil properties (Min et al., 2003). Unlike soil organic C that changes slowly (Haynes, 1999), soil microbial biomass has been shown to change rapidly in response to soil management practices and is recognized as

an early and sensitive indicator of soil quality change (i.e., input or loss of soil organic matter) in agroecosystems (Carter, 1986; Sparling, 1992; Kennedy and Papendick, 1995; Islam and Weil, 2000; Haynes, 1999). It has been suggested that soil microbial biomass, in addition to total C, are important to sustain biologically mediated soil macroaggregation and stability processes (Islam and Weil, 2000). Previous research has shown that addition of fresh organic material can create hot spots of microbial activity where new soil aggregates are developed (Guggenberger et al., 1999; Aoyama et al., 2000; De Gryze et al., 2005). Through processing of organic material and production of organic binding agents, microbial biomass has been shown to play an important role in the formation and stabilization of soil aggregates (Tisdall and Oades, 1982; Aoyama et al., 2000). Stable aggregates, in turn, are thought to protect organic matter physically by making it inaccessible for further decomposition (Tisdall and Oades, 1982; Elliott, 1986; Six et al., 1998; Bossuyt et al., 2004; Pulleman and Marinnsen, 2004). This contributes to decreased decay, and thus, increased C residence time in soils (Six et al., 2002).

Due to its influence on water infiltration, aeration, microbial activity, nutrient dynamics, erosion and workability of soil, aggregate stability is an important characteristics of soil (Campbell et al., 1993). Since animal manure is a source of organic matter and contains polysaccharides and other aliphatic and aromatic compounds that can bind to soil particles and create organo-mineral complexes important for flocculating aggregates (Tisdall and Oades, 1982; Angers and N'Dayegamiye, 1991), its application to agricultural land is generally expected to promote soil aggregation. In addition, manure is a source of energy and nutrients for soil microorganisms and plant roots that produce extracellular polysaccharides, which are known to flocculate soil minerals into aggregates (Tisdall and Oades, 1982). The proportion of large macroaggregates (>2000 μm) is the most important fraction to evaluate when considering the

effects of management practices on soil aggregation, because it exerts a strong influence on the mean weight diameter (MWD), which is a comprehensive index for evaluating soil aggregation (Jiao et al., 2006; Lichter et al., 2008). Previous studies have shown manure addition to significantly improve soil aggregation, particularly macroaggregates (>2000 μm), when compared with chemical fertilizers (Min et al., 2003; Mikha and Rice, 2004). For example, Min et al. (2003) found a 30% increase in aggregate stability for dairy manure slurry (with C and N input levels of 32674 and 3124 kg ha^{-1}, respectively) compared to control. They also reported that dairy manure slurry increased aggregate stability by 18% over NH_4NO_3 fertilizer (with an N input level of 1232 kg ha^{-1}) applied to orchardgrass on a silt-loam soil. Similarly, Mikha and Rice (2004) found the proportion of >2000 μm soil aggregates to be 74% higher in a cattle manure treatment than NH_4NO_3 fertilizer treatment when both were applied at the same N rate of 168 kg ha^{-1} yr^{-1} to a silt-loam soil. Several studies have reported soil aggregation, particularly of the >2000 μm aggregates, to increase with increasing manure application rates (Min et al., 2003; Whalen et al., 2003; Gulde et al., 2008). Grandy et al. (2002) and Wortmann and Shapiro (2008) also found the proportion of >2000 μm soil aggregate fraction to be greater in composted feedlot manure-amended soil than unamended control soil. Contrary to the findings of the above mentioned studies, Whalen and Chang (2002) reported a decline in the proportion of macroaggregates upon repeated manure application. Most notably, they observed a decrease in larger (>7.1 mm) dry-sieved aggregates, particularly in the 0-5 cm depth of irrigated soils that received >60 Mg ha^{-1} yr^{-1} of cattle feedlot manure in Canada. The authors hypothesized that dispersion of soil colloids resulting from monovalent cations, primarily Na^+ and K^+, present in the animal manure contributed to the breakdown of larger soil macroaggregates.

Most aggregate stability studies, such those mentioned above, focused on either conventional or no-till agroecosystems. However, very few studies have evaluated the impact of dairy manure application on aggregate stability in certified organic perennial pasture systems. The overall objective of this study was to evaluate the effect of composted dairy manure application on soil microbial biomass, water-stable aggregate size distribution, and C and N concentrations of aggregate size fractions in an organically managed perennial grass system. Given the trend toward greater adoption of organic farming in this area, knowledge of how soil microbial biomass and soil aggregate stability change in response to management practices will help in identifying best management practices to maintain a sustainable, organic forage production system.

2. MATERIALS AND METHODS

2.1. Description of study area

This study was initiated in 2008 on transitional organic land (in the process of certification) located at the Colorado State University Agricultural Research Development and Educational Center (ARDEC), northeast of Fort Collins, Colorado (40°39′ N, 104°59′ W, elevation 1554 m). The soil at the experimental site is classified as a fine-loamy, mixed, superactive, mesic, Aridic Haplustalf with a 1 to 2% slope (NRCS, 1980). The climate of the area is classified as semi-arid with precipitation of 330 mm yr^{-1}; about 88% of this occurs between April and October. Mean monthly temperatures at this location range from 0°C in January to 22°C in July. Before the current experiment was established in the spring of 2008, the site was in alfalfa which was killed using tillage in the summer of 2007. Following the termination of alfalfa, the field was clean-tilled and received a uniform application of composted

dairy manure (CDM) at 22.4 Mg ha^{-1}(wet weight). The CDM was incorporated by disking immediately after application.

2.2. Experimental design

The experiment utilized a randomized complete block design (RCBD) with three replications. Each block contained four plots, each measuring 3 × 12 m. In September of 2007, experimental plots were seeded with a mixture of orchardgrass (*Dactylis glomerata*), meadow brome (*Bromus biebersteinii*), and **s**mooth brome (*B. inermis*) using a no-till drill (Model 3P605NT, Great Plains Mfg., Inc., Salina, KS). In April of 2008, 3 out of 4 plots in each block received CDM at 22.4 Mg ha^{-1} (wet weight). The remaining plot was interseeded with alfalfa (*Medicago sativa*) to establish a legume treatment (seeding rate: 9 kg ha^{-1}). Visual inspection and quantification of alfalfa in spring 2008 revealed that establishment of alfalfa was inadequate. In 2009, the single CDM treatment (22.4 Mg ha^{-1}) from 2008 was divided to create three CDM application rates: 0, 11.2, and 22.4 Mg ha^{-1} (wet weight). For the 2009 growing season, CDM was applied in October of 2008. Alfalfa was also replanted in March of 2009 because of inadequate stands resulting from grass competition.

This approach resulted in CDM treatments of: 22.4 Mg ha^{-1} (i.e., 22.4 Mg ha^{-1} applied only in April of 2008), 33.6 Mg ha^{-1} (i.e., 22.4 Mg ha^{-1} applied in April of 2008 plus 11.2 Mg ha^{-1} applied in October of 2008), and 44.8 Mg ha^{-1} (i.e., 22.4 Mg ha^{-1} applied in April of 2008 plus 22.4 Mg ha^{-1} applied in October of 2008). Since all plots received the same CDM application rate in 2008, any significant differences in soil microbial biomass and aggregate size distribution can, therefore, be attributed to the effects of CDM treatments established in 2009. Alfalfa plots were considered as unamended control plots (0 Mg CDM ha^{-1}) because no CDM was applied on these plots. The quantity of CDM needed to obtain the desired application rates was broadcast by

hand across the plot area. Composted dairy manure application rates in this study were in keeping with those commonly used in this area. Samples of CDM were analyzed at the Colorado Analytical Laboratory (Brighton, Colorado). Selected characteristics of CDM are listed in Table 4.1.

2.3. Soil microbial biomass

Soil samples were taken from the 0-5 and 5-15 cm soil depths on 8 July 2009. Soil microbial biomass C (MBC) was measured by determining dissolved organic C in chloroform fumigated and non-fumigated samples using a method described by Horwath and Paul (1994). Briefly, two subsamples (15 g each) of field moist soil were weighed, and the first subsample soil was immediately extracted with 75 mL 0.5 *M* K_2SO_4 (5:1, v/w). The second subsample was fumigated with ethanol-free chloroform for 5-days. After the 5 d incubation period, the soil was extracted using the same method as the non-fumigated counterpart. Dissolved organic C was analyzed with a Shimadzu TOC Analyzer (Model TOC-V Series, Shimadzu, Kyoto, Japan). Microbial biomass C was then calculated as: MBC = E_C/K_C, where E_C is the difference between C extracted with K_2SO_4 from fumigated and non-fumigated soils and K_C (extractable part of microbial biomass C after fumigation) was 0.44 (see Section 2.4). Microbial biomass N (MBN) was determined as dissolved N in the K_2SO_4 extracts. Subtraction of K_2SO_4 extractable N of the non-fumigated samples from fumigated samples permitted the quantification of the flush of dissolved N, which was divided by a K_N (extractable part of microbial biomass N after fumigation), given by the equation: $K_N = -0.014 (C_F/N_F) + 0.39$, where C_F and N_F were dissolved organic C and dissolved N extracted by 0.5 *M* K_2SO_4 from fumigated samples, respectively (Voroney and Paul, 1984). All the results are expressed in μg C or N g^{-1} of dry soil.

2.4. Carbon use efficiency

Carbon utilization efficiency is defined as the efficiency with which carbon taken up by the microbial community is converted into microbial biomass (Steinweg, et al., 2008). Cellobiose (a glucose disaccharide derived from the partial hydrolysis of cellulose) was used as substrate to measure the amount of carbon assimilated or lost as CO_2 using the method described by Steinweg et al. (2008). Briefly, six replicate samples (30 g each) were weighed and placed in plastic specimen cups (100 mL, 6.5 cm diameter). One and a half mL of cellobiose solution (20 mg mL^{-1}) was added to the surface of each sample, resulting in an addition rate of 421 μg C g^{-1} soil. This rate was intended to add sufficient cellobiose to induce microbial uptake and respiration of the substrate without inducing a significant increase in population size. Three of the 6 replicate samples received cellobiose, while the remaining three control samples received 1.5 mL of deionized (DI) water to maintain soil moisture content equivalent to that of amended soils.

Each sample was split into two 10 g and one 5 g sample, and placed into separate jars. Soil respiration and cellobiose remaining were measured in the 5 g samples immediately after addition, then after 4 and 20 h following addition. Along with measuring the CO_2 respired and the amount of cellobiose remaining in the soil, microbial biomass was also measured using the two 10 g samples by the chloroform-fumigation extraction technique (see Section 2.3). Soil respiration was monitored by analyzing CO_2 concentrations in gas samples taken from the headspace of the jars using a LI-6252 CO_2 Analyzer (LI-COR Bioscience, Lincoln, NE). Subtraction of the non-amended samples from their amended counterparts permitted the quantification of cellobiose-derived CO_2.

Cellobiose remaining was determined using the sulfuric acid-anthrone method for water-soluble carbohydrates (Brink et al., 1960), in which soil extracts are hydrolyzed by addition of

sulfuric acid, reacted with anthrone, and measured photometrically to determine total carbohydrate concentration. Aliquots (5 g) of soil were extracted with 50 mL of DI water in 125 mL Erlenmeyer flasks and shaken for 30 min; the suspension was poured into 50 mL round-bottom centrifuge tubes, balanced, and centrifuged for 10 min at 10,000 rpm. A 25 mL extract of supernatant was transferred to a 100 mL volumetric flask and diluted to 100 mL with DI water. The extract solutions were stored at 4°C before analysis. Five mL of standard, blank (DI water), or soil extract were transferred to a glass test tube. Ten mL of anthrone-sulfuric acid reagent (0.2% anthrone in 95% (v/v) sulfuric acid) were rapidly added to the glass test tubes. The solution was mixed and then allowed to cool for 10 min. After cooling, 10 mL of the solution were transferred to glass cuvettes. The absorbance was read at 625 nm in a spectrophotometer and converted to cellobiose concentration using a standard calibration curve. Soil cellobiose concentrations were determined using the following equation:

$$\mu g \text{ cellobiose } g^{-1} \text{ moist soil} = A \times s \times \frac{(100 \text{ mL})\ (50 \text{ mL extract})}{(25 \text{ mL extract})\ (5 \text{ g moist soil})}$$

where A is the solution absorbance at 625 nm and s is the slope ($\mu g\ mL^{-1}\ nm^{-1}$) of the standard curve.

Out of the total cellobiose added (421 $\mu g\ C\ g^{-1}$ soil), the amount of cellobiose-C lost as CO_2 was 103.7 $\mu g\ C\ g^{-1}$ soil and cellobiose remaining (anthrone positive) was 9.9 $\mu g\ C\ g^{-1}$ soil, resulting in non-microbial C of 113.6 $\mu g\ C\ g^{-1}$ soil. Thus, the amount of total C (cellobiose + soil) assimilated by the soil microbes was estimated by difference to be 307.4 $\mu g\ C\ g^{-1}$ soil (i.e., 421 $\mu g\ C\ g^{-1}$ – 113.6 $\mu g\ C\ g^{-1}$). Out of the 307.4 $\mu g\ C\ g^{-1}$ soil, 135.8 $\mu g\ C\ g^{-1}$ soil was found to be due to cellobiose, which was obtained by subtracting K_2SO_4 extractable C of the non-amended control samples (275.3 $\mu g\ C\ g^{-1}$ soil) from cellobiose-amended samples (411.1 $\mu g\ C\ g^{-1}$ soil) following the chloroform fumigation extraction. Thus, the correction factor was estimated to be 0.44 (i.e., 135.8 $\mu g\ C\ g^{-1}$ divided by 307.4 $\mu g\ C\ g^{-1}$). Since results for the 20 h measurements

were most consistent, only data for the 20 h measurement was used to calculate the correction factor.

2.5. Aggregate separation

Separation of aggregate size fractions was achieved using the wet-sieving method described by Elliott (1986). Field moist soil was passed through an 8-mm mesh sieve before air-drying. Prior to wet-sieving, subsamples of 100 g air-dried soils were quickly submerged in deionized water (slaked) on top of a 2000-µm sieve for 5 min. Separation of aggregate size fractions was achieved by manually moving the sieve approximately 3 cm up and down 50 times during a period of 2 min. After the 2 min cycle, the stable >2000 µm aggregates were back washed off the sieve into an aluminum pan. Floating organic material (>2000 µm) was decanted and discarded because this large organic material is, by definition, not considered SOM (Six et al., 1998). The process of aggregate separation continued, and water plus soil that passed through the 2000 µm sieve was poured onto the next sieves (250 µm and 53 µm sieves) and the procedure was repeated, but floating material was retained in this case. The aggregates were oven dried (105°C), weighed, and stored in glass jars at room temperature. Four size fractions were obtained: >2000 µm = large macroaggregates; 250-2000 µm = small macroaggregates; 53-250 µm = microaggregates; and, <53 µm = silt plus clay fraction. Based on the size distribution of slaked aggregates, mean weight diameter (MWD) of aggregates was calculated as:

$$MWD = (f_{>2000\mu m} \times 5) + (f_{250\text{-}2000\mu m} \times 1.125) + (f_{53\text{-}250\mu m} \times 0.1515) + (f_{<53\mu m} \times 0.0265)$$

where f is the proportion of soil weight recovered in the size fraction after wet-sieving with a size given in the subscript. The numbers 5, 1.125, 0.1515 and 0.0265 are mean diameters (mm) of each size fraction.

2.6. Carbon and nitrogen analysis

Carbon and nitrogen concentrations in the aggregate-size fractions were measured with a LECO C and N analyzer (Model CN 2000 TruSpec®, Leco Corp., St. Joseph, MI). Since there is no binding of organic matter with sand particles, it is necessary to correct for the sand content when comparing the C and N concentrations of aggregate-size fractions among different treatments (Elliott et al., 1991). Sand-free C and N concentrations (g kg^{-1} sand-free aggregates) were calculated as follows (Six et al., 1998):

$$\text{Sand-free } (\text{C or N})_{\text{fraction}} = \frac{(\text{C or N})_{\text{fraction}}}{1 - (\text{sand proportion})_{\text{fraction}}}$$

where the sand proportion of each aggregate-size fraction was determined by weighing and drying (105°C, 24 h) the >53 μm material that remained on a 53 μm sieve after dispersing the aggregates (2-5 g) in 10-25 mL sodium hexametaphosphate (5 g L^{-1}) (Mikha et al., 2005).

2.7. Statistical analyses

A mixed model (PROC MIXED) analysis of variance (ANOVA) for a randomized split-plot design with CDM application rate (0, 22.4, 33.6, and 44.8 Mg ha^{-1}) as the main effect and sampling depth (0-5 and 5-15 cm) as the split effect was used to analyze data using SAS software version 9.2 (SAS Institute, 2002). The model included CDM application rate, sampling depth, and CDM application rate by sampling depth interaction as fixed variables and block as a random variable. When significant effects were detected ($\alpha = 0.05$), means were separated using Tukey's HSD mean separation test. In addition, simple Pearson correlation analysis was also performed to determine the relationship between soil aggregate size fractions and MBC and MBN. Significant predictors were selected by computing a multiple linear regression analysis, across CDM application rates and sampling depths, with stepwise model selection that used a $p < 0.05$ unless noted otherwise.

3. RESULTS

3.1. Soil microbial biomass

In the 0-5 cm soil depth, MBC ranged between 137 μg C g^{-1} at the 22.4 Mg ha^{-1} CDM application rate and 346 μg C g^{-1} with no CDM application treatment (i.e., 0 Mg ha^{-1}), where alfalfa was interseeded into the grass mixture (Table 4.2). Microbial biomass C was significantly affected by CDM application rate ($p < 0.0001$) and sampling depth ($p = 0.01$) with a significant ($p = 0.03$) application rate by sampling depth interaction (Table 4.2). In the 0-5 cm soil depth, the two lower CDM levels (i.e., 22.4 and 33.6 Mg ha^{-1}) had a lower MBC content (137 and 171 μg C g^{-1} soil, respectively) compared to the high CDM level (i.e., 44.8 Mg ha^{-1}), which had 265 μg C g^{-1} soil (Table 4.2). Interestingly, the MBC measured in the 0 Mg ha^{-1} treatment with no CDM that had alfalfa in the grass mixture (346 μg C g^{-1} soil; Table 4.2) matched the MBC content obtained for the high CDM application rate. Microbial biomass C did not change significantly from the 22.4 to 33.6 Mg ha^{-1} CDM application rate.

Microbial biomass C tended to decrease with sampling depth (Table 4.2). The pattern for MBC across the four CDM application rates at the 5-15 cm depth was similar to that in the 0-5 cm depth. In the 5-15 cm soil depth, the high CDM application rate had a higher MBC content (214 μg C g^{-1}) than the two lower CDM application rates, which had 152 and 146 μg C g^{-1} soil, respectively (Table 4.2). Likewise, there was no significant difference in MBC between the 44.8 Mg ha^{-1} and no CDM treatment that contained alfalfa in the mixed grass stand. There was no significant change in soil MBC between the two lower CDM application rate treatments in the 5-15 cm soil depth.

Microbial biomass C and MBN were highly correlated ($R = 0.87$, $p < 0.001$). Consequently, soil MBN showed the same pattern as MBC in relation to CDM application rate. In the 0-5 cm soil depth, MBN was significantly greater at the high CDM application rate (44.8

Mg ha^{-1}) with 94 μg N g^{-1} soil (Table 4.2) compared with the two lower CDM application rates (22.4 and 33.6 Mg ha^{-1}), which had 32 and 30 μg N g^{-1} soil, respectively. Microbial biomass N was also higher (110 μg N g^{-1} soil; Table 4.2) in the treatment with no CDM that contained alfalfa in the grass mixture than the two lower CDM application rates; however, MBN did not differ between the 0 Mg ha^{-1} treatment with alfalfa and the high CDM application rate (44.8 Mg ha^{-1}). Similar trends were evident for MBN in the 5-15 cm soil depth. The high and no CDM treatments had higher MBN content (67 and 72 μg N g^{-1} soil, respectively) than the two lower CDM application rates, which had 43 and 46 μg N g^{-1} soil, respectively (Table 4.2). Microbial biomass N was not different between the two lower CDM application rates at either of the sampling depths.

3.2. Whole soil characteristics

Variable rate applications of CDM had no significant impact on the stocks of organic C and N at either of the sampling depths (Table 4.3). The top 0-5 cm soil depth had significantly higher organic C content than that of the 5-15 cm soil depth (Table 4.3).

3.3. Aggregate stability

Aggregate stability among CDM application rates was evaluated based on MWD of aggregates (Table 4.4). No significant differences were detected among the CDM application rates with respect to MWD in the 0-5 cm depth. Whereas in the 5-15 cm depth, the high CDM application rate (44.8 Mg ha^{-1}) increased MWD by 53 and 72% over the medium (33.6 Mg ha^{-1}) and low (22.4 Mg ha^{-1}) CDM application rates, respectively (Table 4.4). The treatment with no CDM application that had alfalfa in the grass mixture also increased MWD by 92 and 70%, respectively, compared with the 22.4 and 33.6 Mg ha^{-1} CDM application rates (Table 4.4). The MWD did not differ between the 22.4 and 33.6 Mg ha^{-1} CDM application rates.

The proportions of water-stable large (>2000 μm) and small (250-2000 μm) macroaggregates tended to be lower in the 0-5 cm depth than in the 5-15 cm depth (Table 4.4). However, significant influences of CDM application rates on water-stable large and small macroaggregates were observed only for the 5-15 cm soil depth. Under the high CDM application rate (44.8 Mg ha^{-1}), large and small macroaggregates together accounted for 39% of total soil weight in the 5-15 cm depth. In the no CDM application treatment with alfalfa present, large and small macroaggregates together constituted 42% of the total soil weight in the 5-15 cm depth. In the 5-15 cm depth, large macroaggregates were found to be significantly higher (18% of total soil weight; Table 4.4) at the high CDM application rate (44.8 Mg ha^{-1}) than at the other rates except for the no CDM treatment with alfalfa, where large macroaggregates made up 21% of total soil weight. Small macroaggregates were also higher (20% of total soil weight; Table 4.4) at the high CDM application rate (44.8 Mg ha^{-1}) than at the low rate (22.4 Mg ha^{-1}); however, the proportion of small macroaggregates did not differ between the high CDM application rate and the treatment where alfalfa was present. Microaggregates (53-250 μm) tended to be higher in the 0-5 cm depth than in the 5-15 cm depth; however, significant differences among CDM application rates were observed only in the 5-15 cm soil depth (Table 4.4). In the 5-15 cm depth, microaggregates tended to be lower at the high CDM rate (53.7 g 100 g^{-1} soil) and at the no CDM treatment with alfalfa (50 g 100 g^{-1} soil) compared with the low and medium rates, which had 68 and 65 g 100 g^{-1}soil, respectively (Table 4.4). The silt plus clay fraction (<53 μm) did not differ significantly among CDM application rates at either of the sampling depths.

3.4. Aggregate carbon and nitrogen concentrations

Sand-free total C concentrations of wet-sieved aggregates tended to decrease with depth, but differences in total C contents between the 0-5 and 5-15 cm depths were not significant (Table 4.5). Composted dairy manure application had no significant influence on sand-free total C contents within any of the aggregate-size fractions at either of the sampling depths. There tended to be higher sand-free total N contents of the aggregate-size fractions in the 0-5 cm depth than 5-15 cm depth (Table 4.5). However, these differences were not significant. No significant influences of CDM application on sand-free total N content were observed within any of the aggregate-size fractions at either of the sampling depths.

3.5. Correlations between soil microbial biomass and soil aggregates

To determine the relationship between soil microbial biomass and water-stable soil aggregates, large (>2000 μm) and small (250-2000 μm) macroaggregates and microaggregates (53-250 μm) were regressed against MBC and MBN in the total soil (Table 6). Significant positive correlations were found between large macroaggregates and MBC (R = 0.81, p = 0.002), large macroaggregates and MBN (R = 0.78, p = 0.003), small macroaggregates and MBN (R = 0.56, p = 0.06), microaggregates and MBC (R = -0.78, p = 0.003), as well as between microaggregates and MBN (R = -0.85, p = 0.001). To identify significant predictors of micro- and macroaggregates, stepwise multiple regression analysis was computed across CDM application rates. In spite of the moderate number of data points (sample size = 12), there were significant regression models. Stepwise regression selected only MBC as the significant regressor against large macroaggregates, which explained 65% (p = 0.002; Table 4.6) of the variability in >2000 μm sized aggregates. Whereas MBN was selected as the significant predictor of small macroaggregates, and explained 31% (p = 0.06; Table 4.6) of the variability in 250-2000 μm sized aggregates. Only MBN emerged as the significant predictor for changes in

the proportion of microaggregates after stepwise model selection, explaining 72% (p = 0.001; Table 4.6) of the variability in 53-250 μm soil aggregates.

4. DISCUSSION

This study was conducted to evaluate the impact of management-induced (mainly manipulation of CDM application rates) changes on soil quality, specifically the size of soil microbial biomass and aggregate stability, in an organically managed perennial grass system. Soil organic C and N change slowly with changes in soil management practices, and measuring such changes accurately against high background levels of C and N already present in an organic system can be difficult (Haynes, 1999). In the present study, management treatments (i.e., CDM applications) were implemented in 2008, that is, only one year before the time of sampling. Therefore, the lack of significant differences in total C and N of the bulk soil among CDM application rate treatments was possibly due to the short-term duration of the study. Nonetheless, a significant difference in total C of the bulk soil was observed between the two depths sampled. The higher whole soil organic C at the 0-5 cm depth (Table 4.3) reflects the high topsoil SOM of pasture soils resulting from the lack of soil mixing (Elliott, 1986; Six et al., 2004).

Although changes in soil organic C and N in response to soil management practices tend to be longer-term (Haynes, 1999), it was possible to observe significant influences of CDM application rates on soil MBC and MBN in this experiment. For example, soil MBC and MBN were greater at the high CDM application rate (44.8 Mg ha^{-1}) as well as with no CDM application, which had alfalfa in the grass mixture, as compared to the other rates (Table 4.2). In 2008, the first year of this study, all of the experimental plots had the same basic composition of grasses (a mixture of orchardgrass, smooth and meadow bromegrass) since the presence of alfalfa was inadequate resulting from grass competition. However, in the second year of the

experiment (2009), alfalfa was reseeded into the grass mixture in the 0 Mg CDM ha^{-1} treatment, and became a substantial component in many plots contributing up to 36% to the harvestable forage yield (data not shown). Therefore, the greater soil MBC and MBN contents observed in the treatment with no CDM application may be a result of the decision to include alfalfa in this treatment. This observation is generally supported by previously published work of Haynes and Beare (1997) that legumes are useful in supporting higher soil microbial biomass, particularly in N deficient soils. Haynes and Beare (1997) used an N deficient soil for their study and suggested that higher N content of rhizodeposited organic material (e.g., dying roots and sloughed-off nodules) from the legumes is likely to have been a major difference leading to a larger and differing microbial population under the legumes than the non-legumes. This trend was further reflected in aggregate stability, in that the MWD of aggregates and the proportion of macroaggregates (>2000 μm and 250-2000 μm) in the no CDM treatment with alfalfa was higher than at the low and medium CDM application rates (22.4 and 33.6 Mg ha^{-1}), especially in the 5-15 cm depth (Table 4.4). Soil microbial biomass derives a majority of energy from decomposing readily available C (Aoyama et al., 2000; Bossuyt et al., 2001; Six et al., 2002). So the greater soil MBC and MBN observed at the 44.8 Mg ha^{-1} CDM application rate may suggest an abundance of readily available C in this treatment. By increasing significantly from the low and medium C input levels (22.4 and 33.6 Mg ha^{-1}) to the high level (44.8 Mg ha^{-1}), the results of this study suggest that soil MBC and MBN can be used as sensitive indicators of soil quality changes, specifically changes in SOM availability, even shortly after the implementation of management practices. The findings of Kushwaha et al. (2001) confirm this idea by demonstrating that substrate addition to soil increased the size of microbial biomass, while depletion or reduction of substrate level had an opposite effect. The fact that changes in soil

microbial biomass C occur more rapidly than changes in soil organic C in response to soil management practices was also reported by several other workers (Carter, 1986; Haynes and Beare, 1997; Haynes, 1999).

The soil at the study site was not tilled, except prior to planting of the experiment in the summer of 2007. Moreover, the soil received two CDM applications via surface broadcasting in the spring and fall of 2008 before sampling in the spring of 2009. Yet, the MWD of aggregates and the proportions of large (>2000 μm) and small (250-2000 μm) macroaggregates were smaller in the surface layer (0-5 cm depth) than in the lower layer (5-15 cm depth) (Table 4.4). In contrast, the proportion of microaggregates (53-250 μm) increased significantly in the 0-5 cm depth compared to that in 5-15 cm depth (Table 4.4). There are two possible explanations for the MWD and macroaggregates being lower in the 0-5 cm soil depth. First, aggregates closer to the soil surface were likely disrupted by farm vehicles used to irrigate and harvest the plots to a greater extent than aggregates located deeper within the soil matrix (Whalen and Chang, 2002). For example, in the current study, the experimental plots were irrigated frequently (once per week) using a linear move sprinkler system and plots were harvested five to six times per year. Second, studies conducted in semiarid prairies of Canada found that aggregates in the top 5 cm of soil are made unstable through freezing and thawing, wetting and drying as well as freezing and drying (Larney et al., 1994; Bullock et al., 1999), suggesting that the same might have occurred in this experiment. Surface soils can be much drier than subsoil in the months of June through August in Colorado (Damoff and Reynolds, 2004). Thus, it is likely that soil drying during summer months along with soil wetting resulting from frequent irrigation promoted wet-dry cycles in this area thereby enhancing the slaking of large and small macroaggregates, particularly in the surface layers (Tisdall and Oades, 1982). Although there appears to be greater

potential for wet-dry and freeze-thaw cycles to have a dramatic effect on aggregate-size distribution in the top few centimeters of soil in this region (Degens and Sparling, 1995; Denef et al., 2001a,b), further investigations are needed to confirm this possibility.

Because significant effects of different rates of CDM application on aggregate size distribution were observed only in the 5-15 cm depth (Table 4.4), the reminder of this discussion is based on results obtained from this soil depth. For example, the proportion of large macroaggregates in the 5-15 cm depth tended to be higher for the 44.8 Mg CDM ha^{-1} treatment than for the 22.4 and 33.6 Mg CDM ha^{-1} treatments, whereas small macroaggregates increased significantly at the high CDM application rate (44.8 Mg ha^{-1}) as compared to the low CDM application rate (22.4 Mg ha^{-1}) (Table 4.4). The total C content of the >2000 μm sized aggregates did not significantly differ among CDM application rate treatments (Table 4.5). Similarly, the concentration of total C in the aggregates of 250-2000 μm was similar across all CDM application rates (Table 4.5). This makes it possible to exclude total C as a causal factor to explain the observation that macroaggregates (>2000 μm and 250-2000 μm) increased significantly from the 22.4 to 44.8 Mg ha^{-1} CDM application rate. Therefore, it seems that factors other than total C content are responsible for the observed differences in aggregation between CDM application rates. It is possible that the high C input level (i.e., 44.8 Mg CDM ha^{-1}) had increased soil microbial activity as compared to the two lower C input rates (i.e., 22.4 and 33.6 Mg CDM ha^{-1}), as indicated by higher MBC and MBN values (Table 4.2). There was evidence of a positive relationship between large macroaggregates and MBC ($R = 0.81$, $p = 0.002$) as well as between small macroaggregates and MBN ($R = 0.56$, $p = 0.06$) in this study. Although MBN did not account for a large proportion of the variability in the small macroaggregate fraction ($r^2 = 0.31$, Table 4.6), these relationships were meaningful. It is likely that the addition of CDM at

44.8 Mg ha^{-1} may have encouraged fungal growth, and the fungal hyphae would have facilitated temporary aggregation (Oades, 1984; Oades and Waters, 1991; Golchin et al., 1994; Puget et al., 1995; Haynes and Beare, 1997; Guggenberger et al., 1999; Kushwaha et al., 2001; Milne and Haynes, 2004; De Gryze et al., 2005), which resulted in the higher proportions of large and small macroaggregates (Table 4.4) observed in this treatment. More studies are needed to investigate whether the observed increase in large and small macroaggregates under high CDM application rates was caused by manure induced fungal growth or was related to hydrophobic organic compounds such as lipids originating from manure, which have been proposed as agents that improve wet-aggregate stability in manure-amended soils (Aoyama et al., 1999; Six et al., 2004).

Since manure additions have been shown to increase the proportion of >2000 μm aggregates (Whalen et al., 2003; Mikha et al., 2004; Gulde et al., 2008; Wortmann and Shapiro, 2008), the higher proportion of large macroaggregates found at the high CDM application rate (44.8 Mg ha^{-1}) as compared to the low and medium CDM application rates (i.e., 22.4 and 33.6 Mg ha^{-1}; Table 4.4) is, therefore, not unexpected. In contrast to trends observed at the low and medium CDM application rates, but more in line with results of the high CDM application rate, the treatment with no CDM application, which had alfalfa in the grass mixture, resulted in high aggregate stability (higher MWD and higher proportions of large and small macroaggregates; Table 4.4), suggesting that the inclusion of alfalfa in the grass mixture had apparent beneficial effects on aggregate stability in this study. This might be a little bit surprising because alfalfa has a tap-root system as opposed to the fibrous root systems of grasses, which are known to enhance soil aggregation. Further, it was noted that the presence of alfalfa was inadequate during the growing season in 2008, and alfalfa was actually reseeded in March 2009. However, the advantage of legumes, such as alfalfa, in increasing aggregate stability has been documented by

several studies (Tisdall and Oades, 1982; Angers and Avon, 1990 cited in Campbell et al., 1993; Haynes and Beare, 1997; Bronick and Lal, 2005), and was attributed to the stimulatory effects of legumes on microbial biomass. For example, Haynes and Beare (1997) reported higher aggregate stability under legumes than non-legumes in a greenhouse study that compared the effect of the growth of different grasses and legumes. They postulated that the rhizosphere microbial population of leguminous plants differed in some way to that of non-legumes (possibly due to the higher N content of rhizodeposited material) and that this contributed to the higher measured aggregate stability. Further, Haynes and Beare (1997) found fungal hyphal length in aggregates under legumes to be four times that in non-legumes. The findings that MBC and MBN contents were greater in the treatment with no CDM application that contained alfalfa than all the other treatments (except for the 44.8 Mg ha^{-1} treatment; Table 4.2), as well as the positive correlation observed between MBC and large macroaggregates and also between MBN and small macroaggregates (Table 4.6) further support the hypothesis that the addition of alfalfa to grass-based pasture enhanced the formation of macroaggregates via an increase in soil microbial biomass in the rhizospehere of legumes. Based on the observations and recorded soil microbial biomass as well as water-stable aggregate size distribution results of the present study, one can deduce that using either high CDM application rates (e.g., 44.8 Mg ha^{-1}) or the addition of legumes such as alfalfa to grass-based pasture are both equally important management practices for improving soil aggregation.

In this study, significant positive correlations of soil MBC (with >2000 μm aggregates) and MBN (with 250-2000 μm aggregates), and also negative correlations between microaggregates and soil MBN were found (Table 4.6). This fits with observations of Kushwaha et al. (2001) who also found macroaggregates (>300 μm) to be positively correlated with both

MBC and MBN, while microaggregates (<300 μm) were found to be negatively correlated with MBC and MBN. Similarly, Min et al. (2003) also reported a significant positive correlation between aggregate stability and soil MBC. The results of the present study generally corroborate the findings of past research indicating a relationship between short-term increases in soil aggregate stability and increases in microbial biomass C and N (Lynch, 1984; Drury et al., 1991; Robertson et al., 1991; Haynes and Francis, 1993; Min et al., 2003; Wortmann and Shapiro, 2008). This study suggests that management practices involving the use of relatively high CDM application rates may thus offer a means of improving the stability of aggregates; however, the addition of alfalfa to a grass-based pasture was almost as significant as the high CDM application rates on aggregate stability.

It has been well documented in the literature that soil organic matter binds mineral particles together into stable aggregates (Tisdall and Oades, 1982) and in turn, stable aggregates can physically protect soil organic matter against rapid decomposition by making it less accessible to microbial attack (Elliott, 1986; Six et al., 1998; Bossuyt et al., 2004; Pulleman and Marinnisen, 2004). It has been suggested that C and N concentrations tend to increase with aggregate size (Six et al., 1998; Six et al., 2004). In the present study, however, there were no significant differences in aggregate C and N concentrations across CDM application rates (Table 4.5). Also, the C and N concentrations did not change among aggregate size fractions within any of the CDM application rates. The lack of significant differences in aggregate C and N concentrations may be due in part to large and unequal variability in total C and N concentrations of the aggregate size fractions. Because of the lack of significant differences in aggregate total C and N contents, these data provide no indication of accumulation of C and N within specific soil aggregate size fractions. Further studies involving density fractionation of different pools of

organic matter in aggregate size fractions are warranted to better understand C and N sequestration dynamics.

5. CONCLUSION

The addition of composted dairy manure and alfalfa interseeding to grass-based pasture was found to increase soil microbial biomass C and N, the proportion of large (>2000 μm) and small (250-2000 μm) macroaggregates as well as MWD of aggregates. The results of this study suggest (i) that addition of larger CDM application rates (e.g., 44.8 Mg ha^{-1}) and legumes such as alfalfa can significantly increase soil microbial biomass in a perennial grass system in the first 2-yr after application; (ii) that this increased microbial biomass appears to be linked with a higher proportion of macroaggregates, probably because microbial processing of added organic matter yielded organic binding agents that are critical to the formation and stability of soil aggregates. In addition, the results of this study support the conclusion that soil microbial biomass can be used as an early measure of changes in soil quality in response to management practices. It remains to be determined whether CDM application is contributing to the accumulation of C and N within specific soil aggregate size fractions. Future studies need to consider measurements of N in the rhizosphere of alfalfa as well as fungal hyphae length in aggregates affected by alfalfa and CDM amendment to better understand the effects of these management practices on aggregate stability.

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Table 4.1. Physical and chemical characteristics of CDM† applied to an organic perennial forage production system.

Parameter	Summer 2007	Spring 2008	Fall 2008
Moisture, (%)	25.20	28.62	20.44
Total solids, (%)	74.80	71.38	79.56
Organic matter, (%)	14.10	17.20	6.84
Ash, (%)	60.70	54.18	72.72
Total N, g kg^{-1}	6.23	10.74	2.91
Organic N, g kg^{-1}	5.66	10.11	2.58
NH_3-N, g kg^{-1}	0.02	0.13	0.33
NO_3-N, g kg^{-1}	0.55	0.50	0.01
Total P, g kg^{-1}	2.42	5.05	1.07
Total K, g kg^{-1}	6.39	13.21	4.65
EC‡, dS m^{-1}	2.13	5.14	2.66
pH-H_2O‡	7.94	8.62	9.09

† CDM, composted dairy manure.

‡ EC and pH were determined on 1:5 CDM to water ratio.

Table 4.2. Soil microbial biomass C and N as affected by CDM application rate treatments at 0-5 and 5-15 cm depths. Standard deviations listed in parenthesis (n = 3).

	Soil microbial biomass (μg g^{-1})			
	Chloroform Labile C		Chloroform Labile N	
Treatment	0-5 cm	5-15 cm	0-5 cm	5-15 cm
Mg CDM ha^{-1}				
0	346.1 (62.7) a‡	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†	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.

‡ Values followed by the same lowercase letter in the column indicate that the means of soil microbial C or N were not significantly different (at P <0.05 according to t-test).

† Values followed by the same uppercase letter indicate that the means of soil microbial biomass C or N were not significantly different (at P <0.05 according to t-test) between the two sampling depths.

Table 4.3. Total C and N (g kg^{-1} soil) for whole soil across four CDM‡ application rate treatments and two depths. Standard deviations listed in parenthesis (n = 3).

	Total C		Total N		C/N ratio	
Treatment	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
Mg CDM ha^{-1}						
0	23.9 (3.9) a†	19.9 (1.4) a	1.74 (0.1) a	1.59 (0.2) a	13.7 (1.9) a	12.7 (2.0) a
22.4	23.6 (3.8) a	19.3 (1.8) a	1.72 (0.4) a	1.73 (0.3) a	13.8 (0.8) a	11.3 (1.8) a
33.6	22.8 (1.5) a	21.1 (1.5) a	2.79 (1.8) a	1.59 (0.1) a	10.2 (4.9) a	13.4 (2.0) a
44.8	23.2 (3.1) a	19.7 (1.0) a	2.50 (1.3) a	2.47 (1.6) a	10.7 (4.3) a	9.8 (4.3) a
Mean	23.4 (3.1) A¶	20.0 (1.4) B	2.20 (0.9) A	1.80 (0.6) A	12.1 (3.0) A	11.8 (2.5) A
	ANOVA		ANOVA		ANOVA	
Treatment	NS		NS		NS	
Depth	*		NS		NS	
Treatment × depth	NS		NS		NS	

Level of significance: *P < 0.1; **P < 0.05; ***P < 0.001; NS, not significant.

‡ CDM, composted dairy manure.

† Values followed by the same lowercase letter in the column indicate that the means of total C, total N or C/N ratio were not significantly different (at P <0.05 according to t-test).

¶ Values followed by the same uppercase letter indicate that the means of total C, total N or C/N ratio were not significantly different (at P <0.05 according to t-test) between the two sampling depths.

Table 4.4. Water-stable aggregate size distribution and MWD‡ across four CDM† application rate treatments and two depths. >2000 μm = large macroaggregates; 250-2000 μm = small macroaggregates; 53-250 μm = microaggregates; <53 μm = silt plus clay fraction. Standard deviations listed in parenthesis (n = 3).

		Aggregate-size distribution (g 100 g^{-1} soil)				
Treatment	Depth	>2000 μm	250-2000 μm	53-250 μm	< 53 μm	MWD
Mg CDM ha^{-1}						
0	0-5 cm	9.6 (1.1) a§	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¶	14.6 (1.6) B	66.6 (2.6) A	7.5 (1.0) A	0.83 (0.12) A
0	5-15 cm	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) ab	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.

‡ MWD = mean weight diameter.

† CDM = composted dairy manure.

§ Values followed by the same lowercase letter in the column indicate that the means of aggregate-size distribution were not significantly different (at $P < 0.05$ according to Tukey's HSD mean separation test) between CDM application rate treatments.

¶ Values followed by the same uppercase letter in the column indicate that the means of aggregate-size distribution were not significantly different (at $P < 0.05$ according to Tukey's HSD mean separation test) between the sampling depths.

Table 4.5. Total C and N concentrations for aggregate-size fractions (g kg^{-1} sand-free aggregates) across four CDM† application rate treatments and two depths. >2000 μm = large macroaggregates, 250-2000 μm = small macroaggregates, 53-250 μm = microaggregates, <53 μm = silt plus clay fraction. Standard deviations listed in parenthesis (n = 3).

		>2000 μm		250-2000 μm		53-250 μm		< 53 μm	
Treatment	Depth	Total C	Total N	Total C	Total N	Total C	Total N	Total C	Total N
Mg CDM ha^{-1}									
0	0-5 cm	63.4 (12.0) a‡	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¶	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
0	5-15 cm	48.2 (3.4) a	5.3 (2.5) a	53.0 (10.5) a	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) a	4.7 (0.5) a	56.6 (10.4) a	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 (15.9) a	4.7 (1.3) a	56.7 (16.0) a	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 (15.5) a	5.5 (1.6) a	49.1 (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 (10.6) A	5.0 (1.5) A	53.9 (11.9) B	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	NS	NS
Depth		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.

†CDM, composted dairy manure.

‡ Values followed by the same lowercase letter in the column indicate that the means of C or N concentration were not significantly different (at $P < 0.05$ according to Tukey's HSD mean separation test) between CDM application rate treatments.

¶ Values followed by the same uppercase letter in the column indicate that the means of total C or N concentration were not significantly different (at $P < 0.05$ according to Tukey's HSD mean separation test) between the sampling depths.

Table 4.6. Regression parameters (A, intercept; B, regression coefficient; r, correlation coefficient) reflecting relationships between soil variables and micro- and macroaggregates (n = 24). MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; microaggregates = 53-250 μm; small macroaggregates = 250-2000 μm; large macroaggregates = >2000 μm.

Parameters			A	B	S.E. of B	r^2
y		x				
Large macroaggregates	vs.	MBC	5.34	0.084	0.0196	0.65**
Large macroaggregates	vs.	MBN	8.03	0.262	0.066	0.61**
Small macroaggregates	vs.	MBC	12.94	0.036	0.024	0.19 NS‡
Small macroaggregates	vs.	MBN	13.39	0.147	0.069	0.31*
Microaggregates	vs.	MBC	73.53	-0.118	0.029	0.61**
Microaggregates	vs.	MBN	70.64	-0.413	0.081	0.72***

Level of significance: * $P < 0.1$; ** $P < 0.05$: *** $P < 0.001$.

‡ NS, not significant.

CHAPTER 5: CONCLUSION AND FUTURE DIRECTIONS

This study sought to evaluate soil fertility and quality aspects of organic perennial and annual forage crops. Specifically, the study (i) determined nitrogen (N) mineralization from a soil receiving composted dairy manure (CDM) in two organically managed perennial grass mixtures: orchardgrass-smooth brome-meadow brome, and hybrid wheatgrass-tall fescue-hybrid brome; (ii) evaluated and compared under field conditions N mineralization from a soil receiving CDM versus raw dairy manure (RDM) under an annual warm-season grass, teff (*Eragrostis tef*), and bare-fallow plots; (iii) investigated how different soil amendments managed for organic perennial grasses and annual forage crops impact the abundance, biomass, and species composition of earthworm communities in the short-term following the implementation of management practices; and finally (iv) assessed the short-term impact of variable rate applications of CDM on soil microbial biomass and aggregate stability under a perennial grass system.

Objective (i) determined whether CDM is sufficient to meet the N needs of perennial forage grasses, particularly when it is surface broadcast at the rates considered in this study. This is of particular interest in organic systems where the use of synthetic fertilizers on organically certified land is prohibited. As shown in chapter 1, depending on the grass mixture type, the percentage of N mineralized from CDM that was applied in the spring of 2008 ranged from 6 to 12%. Whereas the percentage of N mineralized in the second year (2009) of the study was 13 to 18%; however, the percentage of N mineralized in 2009 could have included some of the residual N from spring 2008 applied CDM. Clearly, little available N was being provided to the forage grasses, when taking into consideration the 20% expected N availability from composts. Secondly, forage yield and N uptake of grasses that received CDM were indistinguishable from

those that did not. Further, tissue-N concentrations of grasses were below those considered optimal for forage grass production, especially in the second year of the study, suggesting season-long N deficiency. Therefore, the work reported here showed that applying CDM at the rates used in the present study might not meet the N needs of perennial forage grasses, and that higher rates or other sources of N might be needed. If N mineralization begins at a C/N ratio of about 30:1, the C/N ratio of CDM used in this study was low enough (the CDM had C/N ratio < 10) to promote sufficient N mineralization. The low N mineralization found in this study, however, supports previous studies showing that the release of N from composted manures is slow, especially in the first two years after application. It remains to be determined whether this observed low compost mineralization was due to the stability of C and N compounds (i.e., C and N were more stable and less labile), or whether surface broadcasting of the CDM caused the delay in peak N mineralization. Also, any contribution of microbial N immobilization and denitrification losses to low compost mineralization needs to be accounted for in future studies in order to better understand the causes of low N mineralization from CDM.

In the case of objective (ii), the underlying hypothesis was that N mineralization from a soil receiving RDM would be higher than a soil receiving CDM, and that this mineralization would be higher under teff than bare-fallow. As described in chapter 2, CDM and RDM were incorporated in this study by tilling unlike the perennial pasture study (Chapter 1) where CDM was surface broadcast without incorporation. As expected, RDM was found to have higher N mineralization than the CDM in the first year following application; however, RDM N mineralization under the teff was not different from that under bare-fallow. In this study, CDM and RDM rates were intended to provide approximately similar amounts of available N (i.e., 123 kg total N ha^{-1}) in the first year of application. This study estimated first-year N mineralization to

be 22 and 59% of total added N for CDM and RDM, respectively. As suggested in chapter 2, RDM appeared to have a higher fertilizer value in the year following application than that of CDM. The results of this study agree with those of previous studies in that N availability is higher for manures than for composted manures. Overall, this study suggests that both CDM and RDM might provide sufficient in-season plant available N to support high dry matter yields of annual forage crop such as teff, especially during the first year following application, when taking into consideration the 20 and 40% N availability expected from composted and raw manures, respectively. Considering the lower N_{min} (only 22% of total CDM N that was applied in the spring of 2008 was mineralized during the 2008 teff growing season compared with the nearly 60% RDM N_{min}), it is sufficient to say that CDM may not have supplied the needed N to the forage crop, namely teff (*Eragrostis tef*). Given the fact that CDM was tilled-in in annual forage production system, the sum effects of CDM organic fractions and immobilized N may eventually become important after several years of repeated CDM applications. In the meantime, it would be important to accurately determine N release from CDM in order to account for the contribution of residual (carryover) effects on N mineralization. This is particularly important to precisely predict the need for further N fertilization, and ultimately to improve manure management and optimize forage yield.

In line with objective (iii), it was hypothesized that CDM and RDM would affect the abundance and biomass of earthworm communities differently in teff and bare-fallow management. Also, it was hypothesized that variable rate applications of CDM would affect earthworm populations differently in a perennial grass system. With respect to the first hypothesis, this study found that CDM and RDM both increased the abundance and biomass of earthworms as compared to the control in the bare-fallow plots, the effect of the former being

greater than the latter. As suggested in chapter 3, the higher earthworm abundance and biomass observed in the CDM-amended soil in bare-fallow plots could be due in part to high quality food (i.e., low C/N ratio) provided by this amendment; however, the difference in C/N ratio between CDM (CDM had C/N ratio = ~ 9) and RDM (RDM had C/N ratio = 16) was probably too small to totally base the effects observed in this study on the C/N ratio alone. It was also suggested that the relatively higher ammonia-N content in the RDM (644 mg kg^{-1}) than CDM (453 mg kg^{-1}) likely contributed to the lower abundance and biomass of earthworms observed in the RDM-amended soil in the bare-fallow plots. As discussed in chapter 3, given the sensitivity of endogeic species to metal inputs (in particular to Cu), the positive correlation between Cu and earthworm abundance and biomass in this study was totally unexpected, and hence, warrants further investigations. Since a critical C/N ratio for earthworm feeding has not been identified, a laboratory incubation study involving manure with different C/N ratios applied on soils collected from the same study area and involving endogeic species would be interesting to clarify the effect of manure quality (mainly C/N ratio) on earthworm feeding.

With respect to the second hypothesis of objective (iii), this study found earthworm abundance and biomass to be greater at the 33.6 Mg ha^{-1} CDM application rate than the other rates (0, 22.4, and 44.8 Mg ha^{-1}) considered. Given the lack of correlation between soil organic matter and earthworm populations as shown in chapter 3, it was considered likely that a microclimate effect where the CDM addition modified soil temperature and moisture played a significant role in the differences observed. This study also highlighted that higher CDM application rates may pose significant implications for soil fauna. Most notably, the application of CDM at 44.8 Mg ha^{-1} reduced both abundance and biomass of earthworms as shown in chapter 3. The high CDM application rate (44.8 Mg ha^{-1}) resulted in higher soil electrical

conductivity (EC) than the other application rates used (except for the 0 Mg ha^{-1}). This provides some evidence of the possibility that the observed lower earthworm abundance and biomass may have resulted, at least in part, from direct effects of salts on earthworms resulting from larger CDM application rates. The negative correlation between EC and earthworm abundance and biomass supports this hypothesis. The higher EC observed in the treatment with no CDM application (0 Mg ha^{-1}), which had alfalfa in the grass mixture, is difficult to explain and warrants further investigation. As shown in chapter 3, soil EC values observed in this study, 0.5 – 0.7 dS m^{-1}, were generally below the threshold of 0.8 – 1.0 dS m^{-1} (soil:water, 1:1), above which the growth and activity of plants and microorganisms can be significantly altered. Further, soils with EC < 2 dS m^{-1} are usually classified as non-saline (or "normal soils") for most plants; however, the results of the present study suggest that endogeic species can be negatively affected at concentrations considered being non-saline for many plants (0.68 dS m^{-1}). Further investigations of this topic are warranted. For example, a greenhouse trial where earthworms with endogeic feeding strategies were exposed to soils across a range of salinity levels commonly found in soils will be interesting to assess the effect of soil salinity on earthworm populations.

With regard to objective (iv), the research question addressed was how variable rate (0, 22.4, 33.6, and 44.8 Mg ha^{-1}) applications of CDM managed for perennial grasses would impact soil microbial biomass and aggregate stability. This study found soil microbial biomass C (MBC) and N (MBN) to be greater when CDM was applied at a relatively higher rate (44.8 Mg ha^{-1}). As shown in chapter 4, this trend was further reflected in higher aggregate stability where the CDM application of 44.8 Mg ha^{-1} resulted in higher mean weight diameter (MWD) of aggregates as well as greater proportions of large (>2000 μm) and small (250-2000 μm) macroaggregates.

Even more striking in this study was the fact that CDM application was just as important to maintaining high aggregate stability as was the addition of a legume (alfalfa) into the perennial forage grass system. The 0 Mg ha^{-1} treatment of no CDM application, which had alfalfa interseeded in a mixture of perennial grasses, resulted in soil microbial biomass and aggregate stability values similar to those obtained in the highest CDM application rate. In 2009, the second year of this study, the percent alfalfa in these plots was about 36%. As discussed earlier in chapter 4, there were significant positive relationships between MBC and large macroggregates as well as between MBN and small macroaggregates. For example, MBC accounted for a large proportion of the variability ($r^2 = 0.65$, $p < 0.05$) in large macroaggregates. These results generally corroborate the findings of previous studies indicating a relationship between short-term increases in soil microbial biomass and aggregate stability. This study suggests that management practices involving CDM application of 44.8 Mg ha^{-1} or alfalfa interseeded into a perennial pasture can possibly offer a means of improving aggregate stability via promoting higher soil microbial biomass in a relatively short (2 yr) period of time after implementation. Further investigation of the effect of CDM on macroaggregation could be assessed by incubating 250 μm sieved air-dried soil mixed with and without CDM for a given period of time to allow the formation of sufficient aggregates followed by air drying, slaking and wet sieving of the soil.

APPENDIX – Organic Protocols

The National Organic Program (NOP) guidelines are posted on the USDA's website www.ams.usda.gov/nop or http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=3f34f4c22f9aa8e6d9864cc2683cea02&tpl=/ecfrbrowse/Title07/7cfr205_main_02.tpl.

Although the website is useful in terms of the actual regulations, it can be difficult to sort through since everything is written in government language, not "farmer language." However, information regarding what natural substances are prohibited and what synthetic substances are allowed for crop production can be found in Subpart G (aka "The National List of Allowed and Prohibited Substances") available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=3f34f4c22f9aa8e6d9864cc2683cea02&tpl=/ecfrbrowse/Title07/7cfr205_main_02.tpl.

In a nutshell, organic regulations or protocols regarding organic seeds/seedlings, pest control or fertilizer products can be summarized as follows:

- Organic seeds must be used unless the particular variety one needs is not available as organic seed (must document proof of searching at least 3 companies). If organic seeds cannot be found, one may use non-organic UNTREATED seed (price is NOT considered an acceptable barrier to foregoing organic seed in favor of untreated non-organic seed). The information on seed sources for the West can be found at https://attra.ncat.org/attra-pub/organic_seed/search_results.php?sType=reg&region=5.

- If seedlings are to be used instead, they must be organic from the start or if they are perennial seedlings, they must be managed organically for the past year before their produce/crops may be considered organic.

- Production materials must meet NOP requirements; e.g. treated lumber is not allowed in greenhouse areas in contact with plants/crops.

- Any pest control or fertilizer products must be allowed by the "National List." If one needs to source approved materials, brand name or generic products can be searched at the OMRI (Organic Materials Review Institute) website http://www.omri.org/omri-lists.