THESIS

SOIL CARBON AND NITROGEN POOLS UNDER PERENNIAL FORAGE

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2010

COLORADO STATE UNIVERSITY

January 14, 2010

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DWI P. WIDIASTUTI ENTITLED SOIL CARBON AND NITROGEN POOLS UNDER PERENNIAL FORAGE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

SOIL CARBON AND NITROGEN POOLS UNDER PERENNIAL FORAGE

Global warming is a critical concern that influences environmental conditions all over the world. Agriculture plays a significant role in climate change, both in increasing and alleviating atmospheric CO_2 emissions. Soil plays an important role in the global C cycle for C sequestration. Carbon sequestration is a means to reduce CO_2 in the atmosphere, thus alleviating global warming. Soil organic matter (SOM) plays an important function in enhancing soil quality and at the same time serves as a C sink to store C in soil. In the long run, the process of accumulation of SOM can enhance C sequestration. Small alterations in C and N pools in world soils can affect atmospheric CO_2, CH_4, and N_2O concentrations. Agricultural practices such as deforestation, biomass burning, cultivation, rice paddies, residue management, fertilizer application, and farming systems can alter soil C and N pools. However, several management improvements such as fertilizer use, organic amendments, improved grazing management, cropping system intensification, tillage intensity reduction, perennial vegetation reversion, conversion from cultivation to native vegetation, sowing of legumes and grasses, earthworm introduction, and irrigation may also have considerable impact on grassland ecosystems through enhancing soil organic carbon (SOC).

Crop diversity such as forage type, and the use of legumes and dairy compost as N-sources may affect SOM, particularly soil C fractionation. The objective of this study was to evaluate the effect of legume inter-planting and compost application on soil C and N pools at different depths under a perennial grass mix.

Soil samples were collected from the Colorado State University Agricultural Research Development and Education Center (ARDEC) in fall 2008 and 2009 from 0-5 and 5-10 cm depths. The soil series was a Fort Collins sandy clay loam (fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) with a typical grassland pedon (Davis et al., 2006). Treatments were laid out in a Randomized Complete Block Design with two factors and three replications. The first factor was the variety of perennial forage: Hybrid Wheatgrass-Tall Fescue-Hybrid Brome (HWG-TF-HB), Tall Fescue (TF), and Orchardgrass-Meadow Brome-Smooth Brome (OG-MB-SB). N-sources were the second factor, in fall 2008: sainfoin, dairy compost (22.4 Mg ha^{-1} ; 240.6 kg N ha^{-1} ; 1,924.8 kg C ha^{-1}), alfalfa, and alfalfa+dairy compost (22.4 Mg ha^{-1} ; 240.6 kg N ha^{-1} ; 1,924.8 kg C ha^{-1}), while in fall 2009: sainfoin, dairy compost (0, 11.2, 22.4 Mg ha^{-1} ; 0, 32.6 kg N ha^{-1}, 65.2 kg N ha^{-1} ; 0, 391.2 kg C ha^{-1}, 782.4 kg C ha^{-1}), alfalfa, and alfalfa+dairy compost residue. Parameters for this study were total, resistant, slow, and active C and N pools. Total C and N were determined by dry combustion, inorganic C was measured using a modified pressure calcimeter method (Sherrod et al., 2002), recalcitrant C and N by acid hydrolysis, mineralizable C by analysis of CO_2 evolution within 28 days of incubation, and mineralizable N by chemical extraction using 2 *M* KCl before and after incubation. Data were analyzed by analysis of variance ($P < 0.05$) and least significant differences post hoc test.

In fall 2008, there was no significant effect of grass mix on total organic C, resistant, slow, or active C pools. The grass mix did affect the resistant, slow, and active N pools in the 5-10 cm depth, but did not affect total N. Both the highest resistant and active N pools were found in tall fescue, 26.0% and 2.3%, respectively; and the highest slow N pool was found in OG-MB-SB (74.3%). In fall 2009, the grass mix did not have any significant effect on C and N pools, with the exception of the active N pool in the deeper layer; the highest active N pool (1.1% of total N) was measured in the HWG-TF-HB and tall fescue treatments.

N-source only had a significant effect on the active C pool at the 5-10 cm depth in fall 2008. Alfalfa resulted in a significantly greater active C pool in the deeper depth (5% of total C). However, there was a significant effect of N-source on total, resistant, and slow C pools in the 0-5 cm depth in fall 2009. The greatest resistant C pool was measured in dairy compost 22.4 Mg ha^{-1} (44.5%), the greatest slow C pool was found in sainfoin (61.4%), and the highest total organic C was in dairy compost 0 Mg ha^{-1} (16.70 g C kg^{-1}).

Depth significantly affected C and N pools. In fall 2008 and 2009, depth consistently had a very significant effect on total organic C and total N. Total organic C and total N were consistently greater in the 0-5 cm layer. In addition, the active C pool declined with depth, while the active N pool increased with depth. In general, the C and N proportions followed a trend of slow pool > resistant pool > active pool. In fall 2008 and 2009, the slow N pool was greater than the slow C pool; however, active and resistant C pool levels were higher than comparable N pools.

In fall 2008, the highest cumulative C mineralization over 28 days of incubation was measured in the OG-MB-SB grass mix (0.65 g C kg^{-1}) and alfalfa+dairy compost N-source (0.66 g C kg^{-1}). In the second year, the three grass mixes were not different in cumulative mineralizable C, while N-source slightly influenced cumulative mineralizable C with the greatest cumulative mineralizable C found in the compost treatment applied at 11.2 Mg ha^{-1}. The shallow depth had greater cumulative mineralizable C in both fall 2008 and 2009. Potential mineralizable C (C_o) in the 0-5 cm layer was significantly higher than in the 5-10 cm layer in fall 2008. Overall, the results of cumulative C mineralization in this two year study indicated that a 28 day period for laboratory incubation was insufficient to decompose all available C in SOM.

Depth was the only significant factor affecting cumulative C mineralization in the two years over a 28 day incubation, in which the shallow depth had the greater cumulative C mineralization. Overall, there was no significant effect of grass mix or N-source on C mineralization rate at either depth in this two year study. Depth consistently affected the C mineralization rate, in that the 0-5 cm layer had a significantly higher C mineralization rate over a 28-day incubation, with the exception of no significant difference on day 2 of fall 2008. The C mineralization rate tended to decline during the 28 day incubation.

Organic N fertilizer (legume, dairy compost, or manure) should be used to enhance SOC. Identification of a locally adapted legume variety and optimization of agricultural practices should be considered for legume-grass mix establishment.

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ACKNOWLEDGEMENTS

I wish to gratefully acknowledge those who helped me with this endeavor. First and foremost among these is my advisor, Dr. Jessica G. Davis. I want to thank for her great support, patience, and faith in me, especially when I wondered in my first challenging year whether I could survive in my graduate study or not and my second year of study whether I could complete my research project or not after twice changing the topic of my research. Dr. Davis convinced me that I could complete my graduate study. She has been an inspiring mentor and source of strength for me during the past two years, and has taught me much about agriculture in the West which is completely different from agriculture in my country, Indonesia. In addition, Dr. Davis taught me how to conduct good research and be a great scientist. I could not have asked for a better advisor and I am forever grateful for all she has done for me.

In addition, my graduate committee has played a very important role in the completion of this thesis. I would like to thank Dr. Maysoon M. Mikha for sharing her excellent expertise and support and Dr. Yaling Qian for providing helpful comments as members of my committee.

I would also like to thank Dr. Joe E. Brummer for his great support with my field data, Dr. Eldor A. Paul for his expertise, Dan Reuss and Colin Pinney for their laboratory experience and assistance with LECO TruSpec CN and LI-COR infrared gas analyzer and acid hydrolysis, and Dr. Tatang M. Ibrahim for his inspiring motivation in my graduate study.

In a very real way, this project would never have happened without the extensive help, hard work, inspiration and friendship of many others, including (in alphabetical order by last name): Karen Allison, Addy Elliott, Trish Babbitt, Sarah Berg, Matt Booher, Dr. Greg Butters, Dr. Pat Byrne, Can Xing, Kathy Doesken, Nolan Doesken, Dan Goldhamer, Michelle Haddix, Michael Herder, Dani Herrero, Tunsisa Hurisso, Khishigbayar Jamiyansharav, Kierra Jewel, Jennifer Matsuura, Dan McCue, Jacob McDaniel, Matswene Moshia, Dr. Gary Peterson, Sarah Rappe, Dr. Jim Self, Dr. Mary Stromberger, Hannah Walters, Jacob Walker, Dr. Dwayne Westfall, and Lorraine Voss. You have all helped to carry me through these past two years. Thank you for your assistance and for your friendship. And most importantly, grateful thanks to my parents and my entire family, for providing my early education's good start, never-ending support, and prayer.

Finally, I am indebted to The Ford Foundation International Fellowships Program that gave me the opportunity to pursue my graduate study in The U.S., the Aurora Dairy Project for financial support of this project, the Ford Foundation staff in New York, Tammy Langan and Maura Cassells, and in Jakarta, Mira Sambada, Marni Andriani, and Nurwening Saragih, and the Office of International Programs at Colorado State University, Christy Eylar and Lisa Pappas, for their support and assistance.

Just as it takes a community to raise a child, it took a whole community to help me through this challenging but exciting period of my life. I extend my grateful and humble thanks to all these people and organizations for helping and supporting me during my graduate work. I was able to accomplish this work because of the help and support from each person and organization I have mentioned, and to the countless others whom I did not mention specifically, but to whom I am equally indebted.

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Chapter 1

INTRODUCTION

Soil organic matter (SOM), the result of decaying biomass in the soil environment, plays a significant role in enhancing soil quality in terms of its chemical, physical, and biological properties. It accomplishes this through its cation exchange capacity, buffering capacity, chelation, water retention, formation of stable micro-aggregates, and its provision of C sources for microbes. It also provides nutrient rich substrates for plant growth.

According to Van Veen and Kuikman (1990), soil structure directly impacts the physical and biological properties of soil and is also has a significant impact on SOM decay. In addition, soil structure, in terms of form and stability, has direct effects on a wide range of processes that influence crop growth and sustainability of agricultural production (Hamblin, 1985; Letey, 1985; Juma, 1993).

Global warming is a critical concern that influences environmental conditions all over the world. Agriculture plays a significant role in climate change, both in increasing and alleviating atmospheric CO_2 emission. Currently, the world's wetlands have been estimated to be a C sink of about 0.08 Pg C yr^{-1} and a source of 0.055 Pg C yr^{-1} (Lal et al., 1995). Net global C retention was estimated to be 0.057 to 0.083 Pg yr^{-1} in wetland peats. Agricultural conversion of peatlands altered the global C cycle. By 1980, agricultural drainage of peatlands in North America was resulting in a total C shift of 0.063-0.085 Pg C yr^{-1}; in addition, peat combustion created an emission of 0.032-0.039 Pg C yr^{-1}

Several studies have shown that stored C has been depleted in recent centuries, primarily in cultivated soils. In one semi-arid agro-ecosystem, organic C sequestered in the 0-25 cm stratum was measured to be 32.3 Mg C ha^{-1} under no-tillage and 26.9 Mg C ha^{-1} under disk tillage management (Bono et al., 2008). Furthermore, Bono et al. (2008) also found that soil C level was significantly higher ($P<0.01$) under no tillage compared to conventional tillage at 0-5, 5-10, and 10-15 cm soil layers. Tillage affects the annual CO_2-C emission from soil depending on environmental conditions. In some cases, higher respiration rates were reported in plowed soils, which resulted in more C turnover in cultivated soils (Kessavalou et al., 1998; Curtin et al., 2000; Franzluebbers et al., 1998). Uncultivated, i.e. no-tillage or perennially cropped, soils generally have slower rates of residue decomposition (Fortin et al., 1996). On the other hand, under different environmental conditions, labile C compounds accumulate in no-till soil, and mineralization of C can result in greater CO_2-C effluxes (Alvarez et al., 1995, 1998; Franzluebbers et al.,1995; Wagal et al., 1998). Spargo et al. (2008) indicated that continuous no-tillage had a C sequestration rate of about 0.308 Mg C ha^{-1} yr^{-1} at 0-15 cm depth, which predominantly occurred in the surface 7.5 cm of that 15 cm depth. In addition, there were significant increases in soil C in the 0-2.5, 0-7.5, and 0-15 cm depths, i.e. 27%, 14%, and 5%, respectively.

Carbon sequestration is a way to reduce CO_2 in the atmosphere since CO_2 is a significant greenhouse gas attributed with half of current global warming (Flach et al., 1997). Total anthropogenic CO_2 emissions increased during the 1980s, 1990s, and 2000-2005, i.e. 6.3, 8.0, and 9.0 Pg yr^{-1}, respectively (Lal and Follett, 2009). Terrestrial ecosystems are one of the four principal global C pools. The uptake of CO_2-C by the terrestrial biosphere and the ocean was estimated to be about half of the total emissions (Lal and Follett, 2009). In the 1990s, CO_2-C sequestration was estimated to be 1 Pg C yr^{-1} higher than during the 1980s (IPCC, 2007). In spite of that, there are some concerns that the atmospheric CO_2 uptake capacity of the terrestrial ecosystem is declining (Canadell et al., 2007).

Soil plays an important role in the global C cycle due to its function to sequester C in the terrestrial ecosystem. Soil C sequestration implies a managerial intervention to enhance the total soil C pool in the top 2 m depth in order to transfer atmospheric C to the soil C pool (Lal and Follett, 2009). Improving agricultural practices in the United States could reduce total C emissions by almost 10% (FAO, 2001).

SOM can function as a C sink to store C in soil rather than releasing it into the atmosphere as a greenhouse gas. In the long run, the process of accumulation of SOM can enhance C sequestration.

Carbon and Nitrogen Pools Affect Greenhouse Gas Emissions

According to Duxbury (1991), there are four forms of C pools in soil, i.e. active or labile pool or also known as mineralizable pool, slowly oxidized pool, very slowly oxidized pool, and passive or recalcitrant or resistant pool. The active C pool is readily oxidizable C that is influenced by residue inputs and climate and agronomic factors (management). The slowly oxidized C pool is generally found in macro-aggregates and is controlled by soil aggregation and mineralogy and agronomic factors (tillage); while the very slowly oxidized C pool exists within micro-aggregates and is affected by water stable micro-aggregates with little influence from agronomic factors. These slowly and very slowly oxidized C pools together represent the intermediate C pool. Clay mineralogy controls the recalcitrant C pool; in addition, microbial decomposition may have an influence on this pool.

The estimation of relative C amounts in several soil types indicates that the active pool is the most transient pool (Eswaran et al., 1995). The highest concentration of active C pool is mostly located in the top 20 cm, and this pool is the most easily oxidized and also the most easily lost through erosion. Furthermore, Eswaran et al. (1995) asserted that depth plays a role with an exponential decrease in C with depth in most soils. The soil surface, containing the most active pool, is susceptible to be lost through erosion under poor land management. The C loss is estimated to be 50 to 80% of the total organic C in the top one meter of soil.

World soils also comprise about 95 Tg of nitrogen (Lal et al., 1995). This N pool is the major source of N_2O and other N gases (N_xO) contributing to global warming, in particular, due to inorganic N fertilizers applied to cultivated soils. Almost 80 percent of N stored in soil is located in the top 10 cm of the soil surface (Lal et al., 1995).

Total C and N pools represent the sum of the active, intermediate, and resistant C and N pools. Small alterations in C and N pools in world soils can affect the atmospheric CO_2, CH_4, and N_2O concentrations. Agricultural practices such as deforestation, biomass burning, cultivation, rice paddies, residue management, fertilizer application, and farming systems can alter soil C and N pools.

Factors Affecting Carbon and Nitrogen Pools

Soil fertility depletion as a result of soil mismanagement tends to render soils as the primary source of greenhouse gases (Lal et al., 1995). In tropical ecosystems, soil degradation contributes 130 Tg C yr^{-1} (Lal et al., 1995). Indeed, it decreases the net primary productivity of land, which is the rate of C uptake by plants/biota from the atmospheric C pool.

Management improvements such as fertilizer use, organic amendments, improved grazing management, cropping system intensification, tillage intensity reduction, perennial vegetation reversion, conversion from cultivation to native vegetation, sowing of legumes and grasses, earthworm introduction, and irrigation have considerable impact on grassland ecosystems through significant gains in SOC (about 3 Mg C ha^{-1} within a decade) (Janzen et al., 1998). These SOC changes were predominantly observed in labile C fractions.

Nitrogen fertilizer application can stimulate litter production, thereby enhancing soil C accumulation. The increasing productivity of tallgrass prairie due to N fertilization has resulted in increased soil C levels by 1.6 Mg ha^{-1} (Rice, 2000). Another study also documented increased soil C in the top 10 cm of a sandy loam soil following four years of 34 kg N ha^{-1} annual application to a mixture of cool-season grasses (Reeder et al., 1998). However, emission costs also have to be considered, such as emissions from N fertilizer manufacturing (Conant et al., 2001; Oenema et al., 1997; Schlesinger, 1999). Lee and Dodson (1996) found that N fertilizer application of 70 kg N ha^{-1} yr^{-1} could lead to C sequestration of 0.16 Mg C ha^{-1} yr^{-1}. Net C sequestration was eventually reduced to 0.06 Mg C^{-1} yr^{-1} due to 1.4 kg C emission per kilogram fertilizer N manufactured.

The use of synthetic N fertilizer has to be managed through a site-specific assessment of soil N availability. In a study assessing the impact of synthetic N fertilizer application over 40-50 yr indicated that N fertilization rates exceeded the grain N removal by 60 to 190% (Khan et al., 2007). There was also an increasingly large C residue incorporation; however, it resulted in a net decline of soil C, particularly in a corn-soybean or corn-oats-hay rotation and the decline was greater in the 0-46 cm depth than the surface 0-15 cm (Khan et al., 2007). This phenomenon was explained by Khan et al. (2007) as related to the historical use of crop rotation accompanied by manure application prior to synthetic N fertilizer development. The first decade of commercial N fertilizer use showed a small increase in soil C from previously unamended plots, followed by a considerable decline of soil C over 50 yr. This created a concern due to the disappearance of the C accumulated from manure within the plow layer by 2005. N fertilizer tends to increase biomass production, but not C sequestration, unless the N fertilizer can increase heterotrophic soil microorganism use of C derived from crop residues or SOM (Khan et al., 2007). Over-fertilization beyond the crop N requirement creates some concerns, such as low economic profitability of crop production, higher rate of microbial oxidation of residue C and native SOC, anthropogenic N_2O production as a potent greenhouse gas, and NO_3^- pollution of ground and surface waters (Khan et al, 2007; McSwiney and Robertson, 2005).

The effects of N deposition on plant litter and soil organic matter decomposition vary depending on the stage of decomposition (Berg and Matzner, 1997). Furthermore, Berg and Matzner (1997) concluded that N additions promote the initial decomposition of fresh litter, but in later stages, N application suppresses humus decay. There were also significant negative correlations between N concentrations in humus and respiration rate. Thus, there was probably a N sink in humus that initially was assumed to be a steady state N pool. N deposition results in small contributions to C sequestration due to immobilization in the uppermost soil and the forest floor (Nadelhoffer et al., 1999). N input might affect SOM cycling in a contrasting way (Hagedorn et al., 2003). Higher mineral N inputs tend to retard decomposition of older litter and recalcitrant organic matter due to ligninolytic enzyme suppression of soil microbes and chemical stabilization (Fog, 1988; Green et al., 1995; Magill and Aber, 1998). Elevated N depositon significantly enhanced mineralization of more than 4 yr old SOM in the clay and silt fractions; thus, it preserved native and stable SOM. This SOM is composed of microbially derived, strongly decomposed, and hence, stabilized organic compounds (Christensen, 1992; Guggenberger et al., 1994).

C sequestration rate was highest in the top 10 cm of soil during the first forty years after management improved treatments (Conant et al., 2001). Converting cropland into the Conservation Reserve Program for five years demonstrated that perennial grasses have significantly increased SOC stocks across the Great Plains and the western Corn Belt of the United States (Follett et al., 2001). Grassland vegetation was also predicted to be a greater C sink, since it could increase C uptake under conditions of elevated CO_2 as shown in tall grass prairie which responded to CO_2 enrichment by increasing C uptake (Hungate et al., 1997).

Crop diversity affects soil organic matter. Research has shown that in agricultural systems, the amount and turnover of soil organic matter can be influenced by diverse management practices (Paustian et al., 1997). Furthermore, Paustian et al. (1997) and Chan (1997) asserted that perennial forage increases organic C levels in surface soil layers. Pasture had greater organic C levels at 20-40 and 40-60 cm depths than in an annual crop rotation in a long-term rotation study (Gentile et al., 2004). In another study, it was found that perennial grasses induced 2.7 times greater storage of soil C compared to annual grasses (Mapfumo et al., 2002).

Sustainable production of grassland ecosystems aims to maintain SOM. Management practices in these ecosystems are designed to increase forage production that might eventually enhance SOM for sequestering atmospheric C (Conant et al., 2001). Crop residue management plays an important role in SOC dynamics due to the fact that it is the direct source of the SOC pool containing about 45% C on a dry-weight basis (Follett, 2001; Lal, 1997; Lal et al., 2004). Therefore, the above-ground crop residue represents potential SOC storage (Lal, 1997). On the other hand, increasing SOC pool size through technology adoption has a positive impact on agronomic productivity (Lal and Follett, 2009).

Objective and hypotheses

In spite of the former studies of soil C and N pools under different cropping systems, little is known about crop management practices, especially those involving a variety of forages and N-sources as fertility treatments that might affect C and N pools in semi-arid regions. Therefore, the objective of this study is to evaluate the effect of legume inter-planting and compost applications on soil C and N pools at different depths under a perennial grass mix.

The hypotheses of this study are:

Ho: null hypotheses

1. Grass mix has no effect on C and N pools.
2. Fertility treatment (N-sources), i.e. legume inter-seeding and dairy compost application, does not affect C and N pools.
3. Depth does not have a significant effect on C and N pools.
4. Combining alfalfa inter-seeding and dairy compost application does not affect C and N pools.

5. Rate of dairy compost application does not affect C and N pools.

Ha: alternative hypotheses

1. Grass mix will affect C and N pools.
2. Fertility treatment (N-sources), i.e. legume inter-seeding and dairy compost application, affects C and N pools.
3. Depth affects C and N pools.
4. Combining alfalfa inter-seeding and dairy compost application affects C and N pools.
5. Rate of dairy compost application affects C and N pools.

Chapter 2

MATERIALS AND METHODS

Study Site Description

The study site was located in north-central Colorado at the Agricultural Research, Development, and Education Center of Colorado State University (ARDEC) (Township 8N, Range 68W, and the SW quarter of section 15) north of Fort Collins, Colorado (Davis et al., 2006). The soil series was a Fort Collins sandy clay loam (fine-loamy, mixed, superactive, mesic Aridic Haplustalfs) on a 2% slope with a typical grassland pedon (Davis et al., 2006). Soil properties in the surface horizon were 550 g sand kg^{-1}, 160 g silt kg^{-1}, 290 g clay kg^{-1}, pH 7.9, EC of 1.19 dS m^{-1}, 18 g OM kg^{-1}, cation exchange capacity (CEC) of 24.2 cmoles kg^{-1}, and 44 g $CaCO_3$ kg^{-1} (Davis et al., 2006).

The climate for this study location is semi-arid. The total annual precipitation at ARDEC from 1992-2009 ranged from 121.2 to 532.1 mm with fairly evenly distributed rainfall throughout the year, and the mean annual temperature ranged from 7.5 to 9.4 ^{o}C (Colorado Climate Center, 2010). The most recent three years of climatic data at ARDEC (from 2007-2009 when the study took place) showed the total annual precipitation to range from 272.0 to 285.0 mm, and the mean annual temperature ranged from 8.4 to 9.1 °C (Colorado Climate Center, 2010).

Experimental Design

<u>The first year (2008)</u>

The treatments for this experiment were designed in a Randomized Complete Block Design (RCBD) with two factors. The first factor in this experiment was variety of perennial grass forages:

- Hybrid Wheatgrass-Tall Fescue var. Fawn-Hybrid Brome var. Bigfoot (HWG-TF-HB),
- Tall Fescue var. Fawn (TF), and
- Orchardgrass var. Crown royale-Meadow Brome var. Paddock-Smooth Brome var. Lincoln (OG-MB-SB).

N-source was the second factor:

- Sainfoin var. SandHills,
- Dairy compost (22.4 Mg ha^{-1}; 240.6 kg N ha^{-1}; 1,924.8 kg C ha^{-1}),
- Alfalfa var. Ranger, and
- Alfalfa var. Ranger + dairy compost (22.4 Mg ha^{-1} ; 240.6 kg N ha^{-1} ; 1,924.8 kg C ha^{-1}).

The ratio of grass mix to legume was 75:25. The planting date was on Sept 5, 2007, while compost application was on April 22, 2008.

The second year (2009)

In the second year, the second factor (N-source) included some additional treatments:

- Sainfoin,
- Dairy compost (0 Mg ha^{-1} ; 0 Mg N ha^{-1} ; 0 Mg C ha^{-1}),
- Dairy compost (11.2 Mg ha^{-1} ; 32.6 kg N ha^{-1} ; 391.2 kg C ha^{-1})
- Dairy compost (22.4 Mg ha^{-1} ; 65.2 kg N ha^{-1} ; 782.4 kg C ha^{-1}),
- Alfalfa, and
- Alfalfa + dairy compost (22.4 Mg ha^{-1} ; 65.2 kg N ha^{-1}; 782.4 kg C ha^{-1}) residue applied in spring 2008.

Compost was applied on October 23, 2008.

Soil Sampling

Soil samples were taken on September 29, 2008 and September 4, 2009 at depths of 0-5 cm and 5-10 cm. A target population and population units were identified for estimation of parameters (Gilbert, 1987). This perennial study represented the target population which consisted of 96 plots (population units), but there were only 36 plots (fall 2008) and 54 plots (fall 2009) available for selection and measurement based on the objective of this particular study; these plots became the sampled population. Furthermore, Gilbert (1987) identified population units as certain amounts (aliquots) of soil taken from a field that undergo specified preparatory procedures, such as drying, grinding, and so on, and that will be analyzed for some parameters according to a specified procedure. Those parameters in this study were total, resistant, slow, and active (mineralizable) C and N.

Two collection sets of 72 (in the first year) and 108 (in the second year) representative, composite soil samples were taken as outlined in Appendix B. A composite soil sample consisting of 3 subsamples was taken (~500 grams total in fall 2008 and ~300 grams total in fall 2009) from each plot. The soil sample pattern was diagonal (avoiding the edge of the plot) within a 12.2 m x 3.05 m plot. The two soil layers were put into separate paper bags, and labeled. The three subsamples were mixed thoroughly into one homogeneous composite sample. The composite soil samples were prepared for analysis by crushing and air drying before being passed through 8- and 2-mm sieves (Swift, 1996) and then stored at room temperature (25 ^oC).

Total Carbon and Nitrogen

Total C and N were determined with the dry combustion method. The principle of this method is based on oxidation of organic C and thermal decomposition of carbonate minerals in a medium-temperature resistance furnace. Then liberated CO_2 was measured spectrophotometrically (Nelson and Sommers, 1996).

Total C and N analyses were performed before and after acid hydrolysis using a LECO TruSpec CN (Leco Corp., St. Joseph, Michigan, USA). Soil used for total C and N analysis was air-dried and ground to a fine powder (diameter around 100-200 microns); results were corrected for soil moisture content (oven-dried weight).

The total C used in the calculation was in the form of total soil organic C (SOC). To determine SOC, the total C was corrected by subtracting carbonate carbon (inorganic C) as shown in the following equation (Nelson and Sommers, 1996):

organic C, % = % total C - % inorganic C

The soil reaction was determined by electrometric measurement using a pH meter with a glass electrode-calomel electrode system as described by Thomas (1996). Ten g air-dried, sieved (< 2mm) soil was mixed with 10 mL deionized water for pH measurement (Thomas, 1996). Inorganic C (carbonates) was measured in soil samples with a pH ≥ 7.2 (Mikha et al., 2005) using a modified pressure calcimeter method (Sherrod et al., 2002).

Mineralizable Carbon

The conversion of an element from an organic to an inorganic form is known as mineralization (Paul and Clark, 1989). Mineralizable C or sometimes called active C is defined as the CO_2 release from metabolizing organisms (Zibilske, 1994), comparable to soil respiration as the CO_2 produced from all soil metabolic activity (Lundegardh, 1927). However, soil respiration also includes O_2 uptake, which was not accommodated by the general mineralization term. Bioavailable or active C in soils can be determined by CO_2 measurements (Evans et al., 2001).

USA). The static method for determining CO_2 uses sealed chambers for soil incubation in the laboratory. Direct analysis was used for determining CO_2 by an infrared gas analyzer (IRGA). The incubation chambers were aerated frequently to avoid unnaturally high CO_2 accumulations and depleted O_2 in the incubating soil.

Hundred-gram portions of air-dried soil sieved through an 8-mm sieve were placed into a plastic specimen cup (10 cm tall x 10 cm diameter) of known weight. The soil water content was adjusted to field capacity by addition of deionized (DI) water (gradual rewetting to avoid flush of CO_2) at the beginning of the laboratory incubation study. Field capacity was based on gravimetric water contents. Half of the water was added evenly over the soil surface using a syringe, and samples were returned to the cooler for two days. After two days, the sample was taken out of the cooler, and then the sample was mixed and shaken horizontally and gently by hand. The rest of the water was added gently to the sample and returned to the cooler for another two days. After two days, the sample was taken out of the cooler and mixed gently as in the previous step.

The specimen cups containing 40 g of wet soil were placed in $\pm$ 1 liter (the volume of each Mason jar was measured precisely) wide mouth Mason jar containing 20 mL of water. The 20 mL of water in the jar was used for minimizing the loss of moisture from the soil in the plastic specimen cup. The Mason jar lids were fitted with rubber septa to allow headspace sampling of CO_2. Then all Mason jars containing soil samples and control (no soil) were incubated at 25 ± 1 °C in a dark room for 28 days.

Head space CO_2 was sampled from each Mason jar using a series A-2 Pressure-Lok® precision analytical syringe (VICI Precision Sampling Inc., Baton Rouge, LA, USA). In order to assure a representative sample, the jar air was mixed with a 50-mL syringe 4 times prior to sampling since CO_2 is heavier than air. Analysis of CO_2 concentration was performed using a LI-COR IRGA (infrared gas analyzer) CO_2 Analyzer (model LI-6252, LICOR, Lincoln, NE). After each sampling, in order to avoid anaerobic conditions, the incubation jars were aerated for 10 minutes (by removing lids) to achieve at least 16-17% O_2. Carbon dioxide evolved was determined at 2, 5, 7, 10, 14, 21, and 28 days after initiation of incubation. After each CO_2 measurement, the water in the jar was replaced with 20 mL fresh DI water. The weight of the cup + soil was monitored weekly to ensure a constant water content during the incubation period. The water content of the soil was adjusted by weighing the samples and drop wise addition of the required amount of DI water. In order to correct for the ambient CO_2 in the laboratory atmosphere, there were four control jars without soil (only an empty cup on 20 mL water). Control CO_2 measurements were averaged and subtracted from treatment data. The conversion of CO_2 evolution from laboratory incubation to mineralizable C per gram of soil is shown in Appendix A.

Mineralizable Nitrogen

Inorganic N (NH_4-N and NO_3-N) was determined for each grass mix, N-source, and depth at 0 and 28 days of laboratory incubation. The laboratory incubation was conducted under optimum conditions for N mineralization. A 15-g subsample of moist (field-capacity), sieved (< 8 mm) soil (before and after laboratory incubation) was extracted with 75 mL of 2 *M* KCl and shaken for 30 min (Davidson et al., 1989). Then, the extract was filtered through a Whatman no. 42 filter paper, and the supernatant was stored at -20 °C in a freezer to prevent further microbial processes until it was ready to be analyzed. Two days before analysis, the frozen samples were thawed by moving them to a refrigerator (4 °C). The instrument used for NH_4-N and NO_3-N colorimetric determination was an autoanalyzer (Flow Solution IV, O-I-Analytical) (Bundy and Meisinger, 1994). Inorganic N concentrations were expressed on an oven-dry basis.

Net nitrogen mineralization or active-pool N was estimated by the following equation (Hart et al., 1994):

$$\text{N mineralization} = (NH_4^+\text{-N}+NO_3^-\text{-N})_{t+28} - (NH_4^+\text{-N}+NO_3^-\text{-N})_t \quad [1]$$

where t+28 represents measurement on 28 day of laboratory incubation (after incubation), whereas t was on 0 day of laboratory incubation (before incubation). A negative value for net N mineralization indicates net immobilization.

Carbon Mineralization Model

The rate of C mineralization is proportional to the amount of potentially mineralizable C in the soil, and the pattern of C mineralization typically follows first-order kinetics. Similar to the N mineralization model described by Stanford and Smith (1972) and Molina et al. (1980), the Marquardt option of SAS PROC NLIN, a one-factor model for nonlinear curve fitting (SAS Institute, 2009) was used to estimate a C mineralization potential value (C_0) and rate constant (k) as in the following model:

$$C_m = C_0\,(1 - e^{-kt}) \qquad [2]$$

where C_m is mineralized C (g C kg^{-1}); C_0 is potentially mineralizable C (g C kg^{-1}) and rate k is rate constant (d^{-1}); t is time (d).

Resistant Carbon and Nitrogen

Resistant C and N were determined by the acid hydrolysis method (Plante et al., 2006). A 0.5-g sieved (2 mm), air-dried soil was refluxed at 95°C for 16 hours in 25 mL 6 *M* HCl. Reflux is a technique used in laboratory distillations involving the condensation of vapors and the return of this condensate to the system from which it originated. Inside the column, the downflowing reflux liquid provides cooling and condensation of the upflowing vapors thereby increasing the efficiency of the distillation column. After refluxing, the suspension was filtered and washed with deionized water over a glass-fiber filter. The residue was oven-dried at 60°C and weighed. C and N contents in the residue were determined by the dry combustion method using a LECO TruSpec CN (Leco Corp., St. Joseph, Michigan, USA). The hydrolysability of samples is expressed as the percentage of non-hydrolysable C or N (%NHC or %NHN), and was calculated using the following equation, which accounts for mass loss of the sample during hydrolysis and incomplete recovery during filtration.

$$\%NHC,\ \%NHN = \frac{\left[\frac{g\ C,\ N}{kg\ sample}\right]_{after} \ x\ \frac{mass_{\ after}}{mass_{\ before}}}{\left[\frac{g\ C,\ N}{kg\ sample}\right]_{before}} \qquad [3]$$

where the terms represent the total organic C or total N concentrations and masses before and after acid hydrolysis.

Intermediate Carbon and Nitrogen

Intermediate C and N, also known as slow C and N pools, were determined by calculation, based on the following equation according to Paul et al., (2006):

Slow C, N = 100% (as total C, N) - %(resistant + active) C, N pools [4]

Statistical Analysis

Differences in C and N pools, i.e. total, resistant, active, and slow within and between depths and among grass mixes and N-sources were tested employing analysis of variance (ANOVA) by using PROG GLM procedure in SAS 9.1 (SAS Institute, 2009). Treatment means were compared using least significant difference (LSD) post hoc test (n=3, P<0.05).

Chapter 3

RESULTS AND DISCUSSION

Effect of Grass Mix

Grass mix did not have any significant effect on total organic C (TOC), in either the 0-5 cm or the 5-10 cm depth in 2008 (Table 1). The TOC content ranged from 14.59 to 15.18 g kg^{-1} in the 0-5 cm depth, and this depth had significantly greater TOC than the 5-10 cm depth within the same grass mix. The order of TOC in the 0-5 cm depth was TF > OG-MB-SB > HWG-TF-HB, and this was in agreement with the significantly higher total annual dry matter (DM) yields for TF in the 2008 field season (Booher, 2010). The greater TOC of TF was also in concordance with its significantly higher C/N ratio in fall 2008 (Appendix A). In this study, the resistant C pool represented 39.0 to 42.5% to the TOC, slow C pool ranged from 53.0 to 56.4%, and the active C pool comprised 4.0 to 5.0% of TOC across the 0-5 and 5-10 cm depths.

Resistant, slow, and active N pools were affected by grass mix in the 5-10 cm depth (Table 1). However, there was no grass mix effect on total N. The total N ranged from 1.75 to 1.80 g kg^{-1} in the 0-5 cm depth and 1.57 to 1.65 g kg^{-1} in the 5-10 cm depth. Overall, there was an indication of higher total N in the shallow depth. This was similar to results from a study that evaluated the effect of conventional and conservation tillage on the quantity and distribution of C, light fraction C, and N in soil (Ding et al., 2002). The total N and SOC were significantly greatest in the top 0-5 cm especially in the conservation tillage treatment, and then it decreased with depth (Ding et al., 2002). This was probably due to SOC enrichment in the surface zone as concluded by Novak et al. (1996); in particular, perennial forage can contribute to SOC and total N due to its large root mass in shallow depths. Moreover, no tillage was used in this perennial study, and a blanket application of compost was made prior to planting, and this would preserve more soil C and N compared to conventional tillage in an annual forage. The resistant pool was affected by grass mix in the 5-10 cm depth, in which Tall Fescue and HWG-TF-HB had significantly higher resistant-pool N (26 and 25.8%, respectively, of total N) than the OG-MB-SB grass mix (23.8%). On the other hand, the grass mix of OG-MB-SB (74.4% of total N) had the greatest slow-pool N percentage in the 5-10 cm depth, and tall fescue had the highest active-pool N (2.3%) among all grass mixes.

In fall 2009, grass mix did not have any significant effect on C and N pools, except the active-pool N in the 5-10 cm depth (Table 2). In general, similar to 2008, TOC and total N contents were significantly higher in the 0-5 cm depth than in the 5-10 cm depth within the same grass mix. However, there was a tendency for the TOC in the second year in both depths to be lower than in the first year. This was a result of the lower forage yield in the second year (Booher, 2010). A study evaluating forage rotation (bromegrass and alfalfa) and N fertilization also found that the majority of soil organic C (59.6-66.1%) was in the 0-20 cm depth, in spite of significant differences in the 30-40 cm depth (Moulin et al., 2002). Follett et al. (2009) also indicated that the top 100 cm

Effect of N-source

In fall 2008, the N-source only had a significant effect on the active C pool in the deeper layer (5-10 cm) (Table 3). Specifically, alfalfa had the highest active C pool (5% of TOC) although it was not different from alfalfa+dairy compost (4.8% of TOC). The legumes, i.e. sainfoin and alfalfa, did not grow very well in the first year (Booher, 2010), and the dairy compost had low nutrient content. The C/N ratio of SOM is typically between 10 and 12 for plant available nutrient uptake (Allison, 1973). The dairy compost applied in spring 2008 had a C/N ratio of 8. This indicates that the N content in the compost was available to be taken up by plants; however, the C and N content were low (TOC was 8.59% and total N was 1.07%) (Appendix B). Therefore, the legumes and compost treatments did not contribute much N for enhancing plant growth in this soil that also had low organic matter (Appendix B). This condition resulted in no significant difference among most C and N pools. However, within the same N source, TOC and total N was significantly greater in the 0-5 cm depth (13.84 to 16.00 g C kg^{-1} and 1.68 to 1.83 g N kg^{-1}) compared to the 5-10 cm depth (11.89 to 13.71 g C kg^{-1} and 1.51 to 1.69 g N kg^{-1}). The higher total N found in dairy compost 22.4 Mg ha^{-1} and alfalfa+dairy compost 22.4 Mg ha^{-1} (1.83 g N kg^{-1}) was in agreement with the low C/N ratio of the dairy compost in fall 2008 (Appendix A). The lower C/N ratio could indicate higher N availability from the dairy compost.

In fall 2009, the N-source had a significant effect on total, resistant, and slow C pools but no significant effect on N pools (Table 4). This was due to the fact that the compost quality was lower in the second year (total C was 3.49% and total N was 0.29%)

(Appendix B). However, legume establishment was somewhat better in the second year. In the fourth and fifth cutting, alfalfa contributed 21.9% and 14% of harvested yield; whereas, sainfoin did not grow at all in the second year, and the sainfoin plots were contaminated with alfalfa that contributed to 3.1 and 1.6% of harvested yield. Similar to fall 2008, the total C and N contents were significantly greater in the 0-5 cm depth (13.44 to 16.70 g C kg^{-1} and 1.56 to 2.2 g N kg^{-1}, respectively). The higher total C of dairy compost 0 Mg ha^{-1} in the top layer 0-5 cm was in concordance with the significantly higher C/N ratio of forage in dairy compost 0 Mg ha^{-1} application rate in fall 2009 (Appendix A). Total N accumulation at the soil surface was due to organic N sources and possibly due to greater crop production that resulted in surface-residue accumulation and subsequent decomposition at the soil surface (Ismail et al., 1994). In the final two cutting dates of fall 2009, alfalfa had significantly higher average DM yield (647.9 kg ha^{-1}) than sainfoin (463.0 kg ha^{-1}) (Booher, 2010). The highest TOC content (16.70 g kg^{-1}) was in dairy compost 0 Mg ha^{-1}, and the lowest total C occurred in the sainfoin, dairy compost 22.4 Mg ha^{-1}, and alfalfa treatments (Table 4). The dairy compost analysis shows that the second year of the study utilized lower quality compost than in the first year (Appendix B).

Dairy compost at the 22.4 Mg ha^{-1} application rate resulted in the highest resistant C pool in the 0-5 cm depth, i.e. 44.5% of TOC, although it was not different from dairy compost 11.2 Mg ha^{-1} (39.8%). On the other hand, the dairy compost 22.4 Mg ha^{-1} treatment had the lowest slow C pool, i.e. 50.7% of TOC, and it was not different from dairy compost 11.2 Mg ha^{-1} (55.7%).

The resistant N pool ranged from 18.8 to 24.1%, the slow N pool was 74.9 to 80.2%, and the active N pool was 0.6 to 1.2% on a total N basis across 0-5 cm and 5-10 cm depths. However, there were no statistical differences among N-sources that affected those N pools.

Effect of Depth

In fall 2008, depth had a significant effect on TOC and total N pools (PR < 0.0001) (Table 1). However, there was no significant effect of depth on slow, active, and resistant C and N pools (Fig. 1).

A significant effect of depth was found for TOC and total N, which had a greater concentration in the 0-5 cm depth. This was probably due to the residue effect and the shallow root system of perennial grasses (Tables 1 and 3). Similarly, in a study for assessing total C content in croplands and grasslands in the Texas High Plains, the total C decreased with depth, and the highest total C was observed in the 0-2 cm depth (Unger, 2009). Franzluebbers (2009) also estimated SOC sequestration under conservation tillage and pasture management and found that the shallow depth (0-5 cm) conserved greater SOC than the deeper depths (5-12.5 and 12.5- 20 cm).

The amount of TOC in this study was in agreement with results from Paul et al. (1997 and 2006). Overall, the proportion of C and N pools, regardless of grass mix, N-source, and depth, showed a trend of slow pool > resistant pool > active pool. This may be due to the fact that the decomposition of the compost applications was incomplete. In the short term, there were probably some soluble compounds that were used by soil microbes and some compost had not yet decomposed. The amount and order of slow, resistant, and active C pools from TOC (on a percent basis) as found in this study was similar to results from Paul et al. (1997 and 2006). In subsurface soils, the slow pool represented the largest pool size (Paul et al., 2006). While the active pool was generally a small percentage of TOC C and varied with soil type (Paul et al., 2006). In general at both depths, the slow pool N percentage was greater than the slow pool C percentage; however, the resistant and active pool C percentages were higher than for N. This may be related to the N-source contributions in this study producing a large amount of undecomposed soluble N compounds.

In the second year, depth consistently played a significant role in TOC and total N (PR < 0.0001 in Table 2), in addition to active-pool C (PR < 0.0001) and N (PR 0.0004) (Table 2). Both TOC and total N were significantly greater in the 0-5 cm layer than in the deeper layer (Tables 2 and 4).

The active C pool was significantly higher (4.2%) in the 0-5 cm depth than in the 5-10 cm depth (2.7%) (Fig. 2) due to the higher activity of soil microorganisms in the shallow depth. The higher active C pool in the shallow depth is probably due to its aerobic condition that increases the activity of decomposer microbes. In addition, higher potential activity of mineralizable C may occur under optimum temperature and moisture conditions (Franzluebber, 2009). Woods (1989), Hook et al. (1991), Follett et al. (2009), and Novak et al. (2007) also reported that the top layer contained biologically active organic matter in greater amounts than in the deeper layers.

This phenomenon of exponential reduction in soil C with depth is commonly found (Eswaran et al., 1995). The highest soil C concentration, especially the active C pool, is generally in the top 20 cm depth (Eswaran et al., 1995).

On the contrary, the active-pool N was significantly greater in the 5-10 cm layer (1.1%) compared with the 0-5 cm depth (0.8%). This is probably due to the fact that in the surface 0-5 cm layer, mineralizable N was affected by temperature and soil alkalinity (pH ~ 8.2); therefore, the plant available N, especially NH_4^+, was lost from the shallow layer by volatilization. Similar to the fall 2008 data, there were no significant effects of depth on slow or resistant C and N pools in fall 2009 (Fig. 2).

The proportion of C and N pools regardless of grass mix, N-source, depth, or year effects, tended to have slow pool > resistant pool > active pool. This result was in concordance with the result from a study of soil C pools determined by light fraction and incubation-based methods in an alfalfa ecosystem, in which the slow C pool was the greatest pool, followed by the resistant C pool, and the smallest portion was the active C pool (Grandy and Robertson, 2007). Furthermore, Grandy and Robertson (2007) asserted that a part of the slow pool C was located within aggregates, which provided physical protection. Over time, increases in active-pool C followed the breakdown of aggregates resulting in the release of the physically protected slow C pool and its rapid decomposition; thus, the slow C pool was transferred into the active C pool. Therefore, our results can also be explained similarly, in that the decomposition was still ongoing from the transfer of the slow C pool to the active C pool. In both years, the depth effect was consistently greater in the slow N pool than the slow C pool; however, active and resistant-pool C levels were higher than the corresponding N pools. This may be due to the more soluble C compounds being used by microbes and resulting in higher active and resistant C.

C Mineralization

C mineralization measured through incubation procedures contributes to increased knowledge on a diversity of topics such as (i) the physiological status or catabolic potential of soil microbial populations; (ii) decomposition of specific organic substrates and soil C availability; (iii) soil biomass size and activity; (iv) the relative contribution of microbes, fauna, and abiotic sources (carbonate minerals dissolution); and (v) pool sizes and fluxes organic matter (Robertson et al., 1999; Zibilske, 1994). Incubation has been found to be a good approach for quantification of the active C fraction (Paul et al., 2006).

The means of cumulative C mineralization increased throughout a 28 day incubation in fall 2008 (Fig 3 and 4). The linear trend of cumulative C mineralization indicates that decomposition was still ongoing. The initial flush of CO_2 evolution was on the second day and was then followed by a sharp decline for all grass mixes and N-sources (data was not reported); while in another study using a 30-day laboratory incubation, the initial flush was seen on the third day (Kaboneka et al., 1997). This phenomenon is commonly thought to be due to the rapid decomposition of readily decomposable, water-soluble, low molecular weight components of applied organic materials (Reinertsen et al., 1984; Ajwa and Tabatabai, 1994).

In the first five days of incubation, there were only slight differences in cumulative C mineralization among grass mixes. Tall Fescue and OG-MB-SB grass mixes had the same cumulative C mineralization at two and five days, 0.08 and 0.16 g C kg^{-1} soil, respectively, which was higher than C mineralization in HWG-TF-HB grass mix, 0.07 and 0.15 g C kg^{-1} soil, respectively. Finally, over 28 days of incubation the highest cumulative C mineralization was in OG-MB-SB grass mix (0.65 g C kg^{-1}), followed by tall fescue (0.64 g C kg^{-1}), and HW-TF-HB (0.59 g C kg^{-1}).

N-source did not show any effect on cumulative C mineralization in the first two days of incubation. All N-sources had the same mineralizable C, 0.08 g C kg^{-1}. There were two groups on the five days incubation that had the same mineralizable C. Sainfoin and dairy compost 22.4 Mg ha^{-1} obtained 0.15 g C kg^{-1}; whereas, alfalfa and alfalfa+dairy compost 22.4 Mg ha^{-1} resulted in slightly higher mineralizable C (0.16 g C kg^{-1}). At seven days, those two N-sources, alfalfa and dairy compost, still had the same amount of mineralizable C (0.22 g C kg^{-1}). Over 28 days of incubation, the highest cumulative C mineralization was in the order of alfalfa+dairy compost (0.66 g C kg^{-1}) > dairy compost or alfalfa (0.63 g C kg^{-1}) > sainfoin (0.58 g C kg^{-1}). Sainfoin had significantly lower yield compared to alfalfa (Booher, 2010); therefore the yields are likely reflected in the low C mineralization rates in the sainfoin treatment.

The dairy compost+alfalfa treatment had higher C mineralization than the dairy compost or alfalfa treatments alone. This phenomenon indicated a possible synergism of dairy compost application in inter-seeded alfalfa that increased activity of soil microorganisms in this fertile soil ecosystem.

The surface layer (0-5 cm) tended to have greater cumulative C mineralization than the 5-10 cm layer (Fig. 4). On days two and five of the incubation, the depths were very similar, i.e. 0.08 and 0.07 g C kg^{-1}, respectively, and 0.16 and 0.15 g C kg^{-1}, respectively. However, on the 28^{th} day, the 0-5 cm depth showed a higher amount of cumulative mineralizable C (0.69 g C kg^{-1}) than the 5-10 cm depth (0.56 g C kg^{-1}).

In the second year, the three grass mixes were not different in cumulative mineralizable C (Fig. 5A). N-source tended to have an effect on means of cumulative mineralizable C, although the pattern of the chart was similar among all N-sources (Fig. 5). The slope of cumulative mineralizable C as a function of time increased following day 14. In the first week of incubation, the decomposition was fast, indicated by the steep slope of cumulative C as a function of time, and then in the second week, the decomposition was slower indicating the adaptive or lag phase of microbial growth (Bottomley, 2005). Following the second week, the cumulative C mineralization showed an increasing rate of decomposition representing the period when the microbes reached an unlimited growth stage (Bottomley, 2005). Beginning on day 2 of the incubation, each N-source had different cumulative mineralizable C in the order of dairy compost 0 and 11.2 Mg ha^{-1} (0.08 g C kg^{-1}) > dairy compost 22.4 Mg ha^{-1}, sainfoin, and alfalfa+dairy compost residue (0.07 g C kg^{-1}) > alfalfa (0.06 g C kg^{-1}). After 28 days of incubation, the order of cumulative mineralizable C was similar, with the exception that dairy compost 22.4 Mg ha^{-1} resulted in a higher cumulative mineralizable C than dairy compost 0 Mg ha^{-1}. This could be due to the C in the dairy compost (22.4 Mg ha^{-1}) being released over time; however, the dairy compost 11.2 Mg ha^{-1} had the highest cumulative C mineralization. Additional time may be needed for the higher rate of compost (22.4 Mg ha^{-1}) to decompose due to its greater organic matter content. In fall 2009, the addition of dairy compost as a N-source mixed with a legume such as alfalfa+dairy compost residue had higher cumulative C mineralization compared with the legume (alfalfa alone). Final cumulative mineralizable C after 28 days followed this trend: dairy compost 11.2 Mg ha^{-1} (0.51 g C kg^{-1}) > dairy compost 22.4 Mg ha^{-1} (0.49 g C kg^{-1}) > dairy compost 0 Mg ha^{-1} (0.48 g C kg^{-1}) > sainfoin (0.44 g C kg^{-1}) > alfalfa+dairy compost residue (0.43 g C kg^{-1}) > alfalfa (0.42 g C kg^{-1}).

Depth had a significant effect in 2009; the surface layer had higher cumulative mineralizable C compared with the 5-10 cm layer (Fig. 6). The trend was not similar between these depths. The 0-5 cm depth showed a high activity of decomposition as shown by the steeper slope; while the 5-10 cm depth was almost linear over the 28 day incubation period. After two days of incubation, the 0-5 cm layer had 0.10 g C kg^{-1}, and the 5-10 cm depth had 0.05 g C kg^{-1}, whereas, after 28 days of incubation, C mineralization was 0.62 and 0.30 g C kg^{-1}, respectively. Cumulative mineralizable C was almost double in the 0-5 cm depth than in the deeper layer.

During fall 2008, potential mineralizable C (C_o) in the 0-5 cm layer was significantly higher than in the 5-10 cm layer (Table 5). The 28 day period was insufficient to decompose all available C in soil organic matter. Therefore, the models did not converge in fall 2009, particularly in the 5-10 cm depth. The available data for potential mineralizable C and mineralizable rate constant were only in the 0-5 cm depth, i.e. 400.1 g C kg^{-1} and 0.42, respectively.

Effect of Grass Mix, N-source, and Depth on Cumulative C Mineralization

There was no effect of grass mix or N-source on cumulative C mineralization over a 28 day incubation in fall 2008 or 2009 (Tables 6, 7, and 8). The only significant factor affecting cumulative C mineralization in the two fall growing seasons was depth (PR < 0.0001 in Table 6). The significantly greater cumulative C mineralization was found in the shallow layer (0-5 cm) in both fall 2008 and 2009 (Fig. 7). The cumulative C mineralization over a 28 day incubation was an actively decomposed C pool that occurs more optimally under aerobic conditions in the shallow layer. The same phenomenon was found in other studies by Woods (1989), Hook et al. (1991), Follett et al. (2009), and Novak et al. (2007).

Effect of Grass Mix on Mineralizable C Rate

In fall 2008, the C mineralization rate was high on the first two days of incubation, and then it went down, especially after day 7, at both 0-5 and 5-10 cm depths (Fig. 8). From day 5 to 7, there was an increasing C mineralization rate at the 0-5 cm depth and a stagnant rate at 5-10 cm depth. Overall, in fall 2008, there was no significant effect of grass mix on C mineralization rate in either depth (0-5 cm and 5-10 cm).

Similar to fall 2008, there was a higher C mineralization rate in the first two days of incubation in fall 2009. After that, the mineralizable C rate went down dramatically during the following 14 days of incubation at both the 0-5 cm and 5-10 cm depths (Fig. 9). In general, after 28 days of incubation, the C mineralization rate was stagnant or declining. In the second year, there was also no significant effect of grass mix on C mineralization rate in either depth (0-5 cm and 5-10 cm).

Effect of N-source on Mineralizable C Rate

In general, the effect of N-source on C mineralization rate in both depths (0-5 cm and 5-10 cm) in fall 2008 and 2009 (Fig. 10 and 11) was similar to the pattern of C mineralization rate discussed above (Fig. 8 and 9), i.e. it declined over 28 days of incubation, except there was an increase in day 7 in the 0-5 cm depth. There was also no significant effect of N-source on the C mineralization rate at 0-5 cm or 5-10 cm depths in either fall 2008 or 2009.

Effect of Depth on Mineralizable C Rate

Depth was the only significant factor influencing C mineralization rate in this two year study. Indeed, there was a significantly higher C mineralization rate in the shallow depth in both fall 2008 and 2009 over a 28 day incubation, with the exception of no significant difference on day 2 of fall 2008 (Fig. 12).

Table 1. Effect of grass mix on soil carbon and nitrogen pools in fall 2008.

Grass mix	Depth (cm)	Total (g kg^{-1})		Resistant pool (%)		Slow pool (%)		Active pool (%)	
		C[¶]	N	C	N	C	N	C	N
HWG-TF-HB[†]	0-5	14.59A[‡]	1.77A	41.6	24.3	53.9	73.8	4.4	1.8
	5-10	13.32B	1.59B	41.3	25.8a[§]	54.6	72.4b	4.0	1.7b
Tall Fescue	0-5	15.18A	1.80A	39.0	26.0	56.4	72.1	4.7	2.0
	5-10	12.30B	1.57B	40.8	26.0a	54.5	71.7b	4.8	2.3a
OG-MB-SB	0-5	14.72A	1.75A	40.1	24.8	54.9	73.2	5.0	2.1
	5-10	12.78B	1.65B	42.5	23.8b	53.0	74.3a	4.5	1.8b
		PR > F							
0-5 cm		0.69	0.84	0.66	0.38	0.70	0.34	0.22	0.08
5-10 cm		0.26	0.45	0.87	0.03	0.86	0.02	0.08	0.01
Depth		< 0.0001	< 0.0001	0.35	0.69	0.47	0.69	0.19	0.96

[†]HWG-TF-HB, Hybrid Wheatgrass-Tall Fescue-Hybrid Brome; OG-MB-SB, Orchard grass-Meadow Brome-Smooth Brome.

[‡]Depths with different uppercase letters within each grass mix are significantly different (ANOVA); $P < 0.05$.

[§]Grass mixes with different lowercase letters within each depth are significantly different (ANOVA); $P < 0.05$.

[¶]Total organic carbon

Table 2. Effect of grass mix on soil carbon and nitrogen pools in fall 2009.

Grass mix	Depth (cm)	Total (g kg^{-1})		Resistant pool (%)		Slow pool (%)		Active pool (%)	
		C[¶]	N	C	N	C	N	C	N
HWG-TF-HB[†]	0-5	14.47A[‡]	1.66A	38.2	24.7	57.5	74.5	4.3A	0.8A
	5-10	11.26B	1.36B	39.5	21.5	57.7	77.4	2.7B	1.1a[§]B
Tall Fescue	0-5	14.79A	1.92A	38.9	20.4	57.0	78.8	4.1A	0.8A
	5-10	11.15B	1.38B	42.2	21.3	55.0	77.6	2.8B	1.1aB
OG-MB-SB	0-5	15.20A	1.74A	37.0	21.8	58.8	77.5	4.2A	0.7A
	5-10	10.95B	1.39B	41.3	20.3	56.0	78.8	2.7B	0.9bB
		PR > F							
0-5 cm		0.48	0.42	0.60	0. 12	0.64	0.11	0.86	0.57
5-10 cm		0.68	0.87	0.66	0.77	0.65	0.69	0.88	0.007
Depth		< 0.0001	< 0.0001	0.07	0.28	0.41	0.38	< 0.0001	0.0004

[†]HWG-TF-HB, Hybrid Wheatgrass-Tall Fescue-Hybrid Brome; OG-MB-SB, Orchard grass-Meadow Brome-Smooth Brome.

[‡]Depths with different uppercase letters within each grass mix are significantly different (ANOVA); $P < 0.05$.

[§]Grass mixes with different lowercase letters within each depth are significantly different (ANOVA); $P < 0.05$.

[¶]Total organic carbon

Table 3. Effect of N-source on soil carbon and nitrogen pools in fall 2008.

N-source	Depth (cm)	Total (g kg^{-1})		Resistant pool (%)		Slow pool (%)		Active pool (%)	
		C[§]	N	C	N	C	N	C	N
Sainfoin	0-5	14.31A[†]	1.74A	40.6	24.5	54.9	73.5	4.4	2.0
	5-10	13.71B	1.65B	39.8	25.2	56.3	72.8	3.9c[‡]	2.0
Dairy compost 22.4 Mg ha^{-1}	0-5	15.18A	1.83A	42.1	26.4	53.2	71.5	4.7	2.1
	5-10	13.37B	1.69B	37.8	24.9	58.1	73.1	4.1bc	2.0
Alfalfa	0-5	13.84A	1.68A	41.5	24.9	53.5	73.2	5.0	1.9
	5-10	11.89B	1.51B	44.7	25.8	50.4	72.5	5.0a	1.7
Alfalfa+dairy compost 22.4 Mg ha^{-1}	0-5	16.00A	1.83A	36.7	24.2	58.7	74.0	4.6	1.9
	5-10	12.23B	1.57B	43.8	25.0	51.4	72.8	4.8ab	2.1
		PR > F							
0-5 cm		0.09	0.31	0.39	0.38	0.36	0.32	0.39	0.18
5-10 cm		0.06	0.11	0.29	0.75	0.20	0.92	0.02	0.23

[†]Depths with different uppercase letters within each N-source are significantly different (ANOVA); $P < 0.05$.

[‡]N-sources with different lowercase letters within each depth are significantly different (ANOVA); $P < 0.05$.

[§]Total organic carbon

Table 4. Effect of N-source on soil carbon and nitrogen pools in fall 2009.

N-source	Depth (cm)	Total (g kg^{-1})		Resistant pool (%)		Slow pool (%)		Active pool (%)	
		C[§]	N	C	N	C	N	C	N
Sainfoin	0-5	14.03bc[‡]A[†]	1.63A	34.5b	20.1	61.4a	79.1	4.2A	0.8A
	5-10	10.77B	1.33B	31.4	24.1	53.5	74.9	2.7B	1.0B
Dairy compost 0 Mg ha^{-1}	0-5	16.70aA	1.92A	35.6b	22.2	60.5a	77.1	3.9A	0.7A
	5-10	11.74B	1.45B	23.1	20.3	60.5	78.6	2.7B	1.1B
Dairy compost 11.2 Mg ha^{-1}	0-5	15.25abA	2.20A	39.8ab	23.0	55.7ab	75.8	4.5A	1.2A
	5-10	11.06B	1.34B	26.8	21.6	51.8	77.3	3.0B	1.1B
Dairy compost 22.4 Mg ha^{-1}	0-5	14.28bcA	1.60A	44.5a	24.0	50.7b	75.3	4.8A	0.7A
	5-10	11.48B	1.40B	27.7	20.4	53.0	78.6	2.6B	1.0B
Alfalfa	0-5	13.44cA	1.56A	38.1b	22.4	57.8a	76.9	4.1A	0.7A
	5-10	10.60B	1.38B	21.2	18.8	59.8	80.2	2.7B	1.0B
Alfalfa+dairy compost residue	0-5	15.22abA	1.74A	35.9b	22.1	60.4a	77.3	3.7A	0.6A
	5-10	11.06B	1.36B	22.9	21.0	58.6	77.8	2.7B	1.2B
		---------- PR > F ----------							
0-5 cm		0.01	0.24	0.02	0.85	0.01	0.83	0.15	0.08
5-10 cm		0.26	0.57	0.28	0.48	0.26	0.48	0.32	0.48

[†]Depths with different uppercase letters within each N-source are significantly different (ANOVA); $P < 0.05$.

[‡]N-sources with different lowercase letters within each depth are significantly different (ANOVA); $P < 0.05$.

[§]Total organic carbon

Table 5. Potential mineralizable C (C_o) and mineralizable rate constant (k_c) in fall 2008 and 2009.

Depth	2008		2009[†]	
	C_o	k_c	C_o	k_c
0-5 cm	158.7a[‡]	0.36	400.1	0.42
5-10 cm	113.6b	0.16	-	-

[†]In 2009, only half of the models at the 0-5 cm depth converged, and very few of the models at the 5-10 cm depth converged. Therefore, data for 2009 from the 5-10 cm depth are not shown.

[‡]Values with different lowercase letters between depths are significantly different (ANOVA); $P < 0.05$.

Table 6. Effect of grass mix on cumulative C mineralization over a 28 day incubation in fall 2008 and 2009.

Grass mix	Depth (cm)	Cumulative C Mineralization (g C kg^{-1}) Fall 2008	Fall 2009
HWG-TF-HB§	0-5	0.65 A†	0.61 A
	5-10	0.54 B	0.31 B
Tall Fescue	0-5	0.70 A	0.61 A
	5-10	0.57 B	0.31 B
OG-MB-SB	0-5	0.73 A	0.63 A
	5-10	0.57 B	0.30 B
		---------- PR > F ----------	
0-5 cm		0.26	0.85
5-10 cm		0.33	0.77
Depth		< 0.0001	< 0.0001

§HWG-TF-HB, Hybrid Wheatgrass-Tall Fescue-Hybrid Brome; OG-MB-SB, Orchard grass-Meadow Brome-Smooth Brome.

†Depths with different uppercase letters within each grass mix are significantly different (ANOVA); $P < 0.05$.

Table 7. Effect of N-source on cumulative C mineralization over a 28 day incubation in fall 2008.

Grass mix	Depth (cm)	Cumulative C Mineralization (g C kg^{-1})
Sainfoin	0-5	0.63 A[†]
	5-10	0.53 B
Dairy compost 22.4 Mg ha^{-1}	0-5	0.72 A
	5-10	0.54 B
Alfalfa	0-5	0.69 A
	5-10	0.58 B
Alfalfa+dairy compost 22.4 Mg ha^{-1}	0-5	0.73 A
Compost	5-10	0.58 B
		-------------PR > F -------------
0-5 cm		0.35
5-10 cm		0.27

[†]Depths with different uppercase letters within each N-source are significantly different (ANOVA); $P < 0.05$.

Table 8. Effect of N-source on cumulative C mineralization over a 28 day incubation in fall 2009.

Grass mix	Depth (cm)	Cumulative C Mineralization (g C kg^{-1})
Sainfoin	0-5	0.58 A†
	5-10	0.30 B
Dairy compost 0 Mg ha^{-1}	0-5	0.64 A
	5-10	0.32 B
Dairy compost 11.2 Mg ha^{-1}	0-5	0.69 A
	5-10	0.33 B
Dairy compost 22.4 Mg ha^{-1}	0-5	0.68 A
	5-10	0.30 B
Alfalfa	0-5	0.56 A
	5-10	0.28 B
Alfalfa+dairy compost residue	0-5	0.56 A
	5-10	0.30 B
		----------------PR > F -------------
0-5 cm		0.13
5-10 cm		0.09

†Depths with different uppercase letters within each N-source are significantly different (ANOVA); $P < 0.05$.

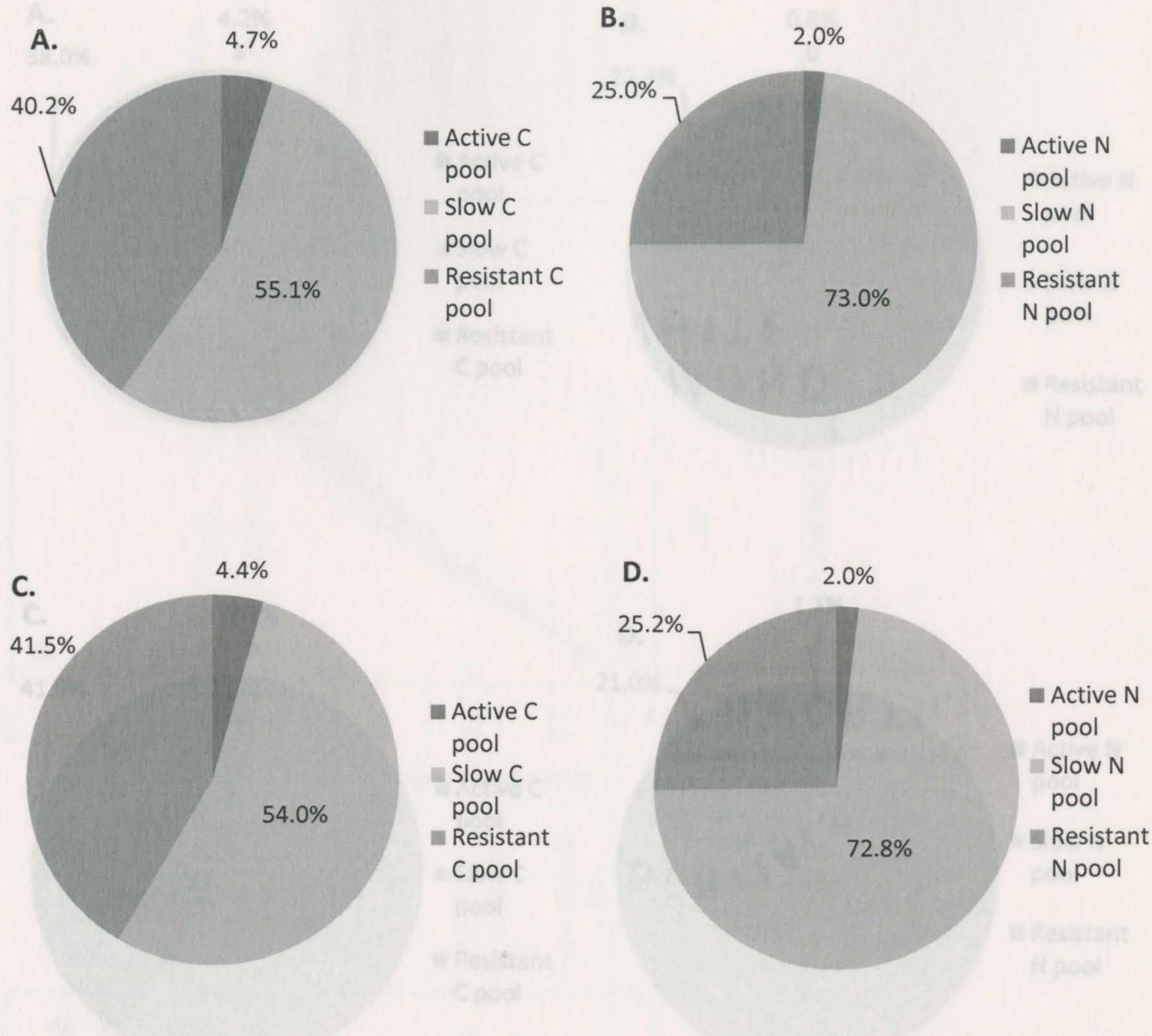

Fig. 1. Soil nutrient pools in fall 2008. A. Carbon at 0-5 cm depth; B. Nitrogen at 0-5 cm depth; C. Carbon at 5-10 cm depth; D. Nitrogen at 5-10 cm depth.

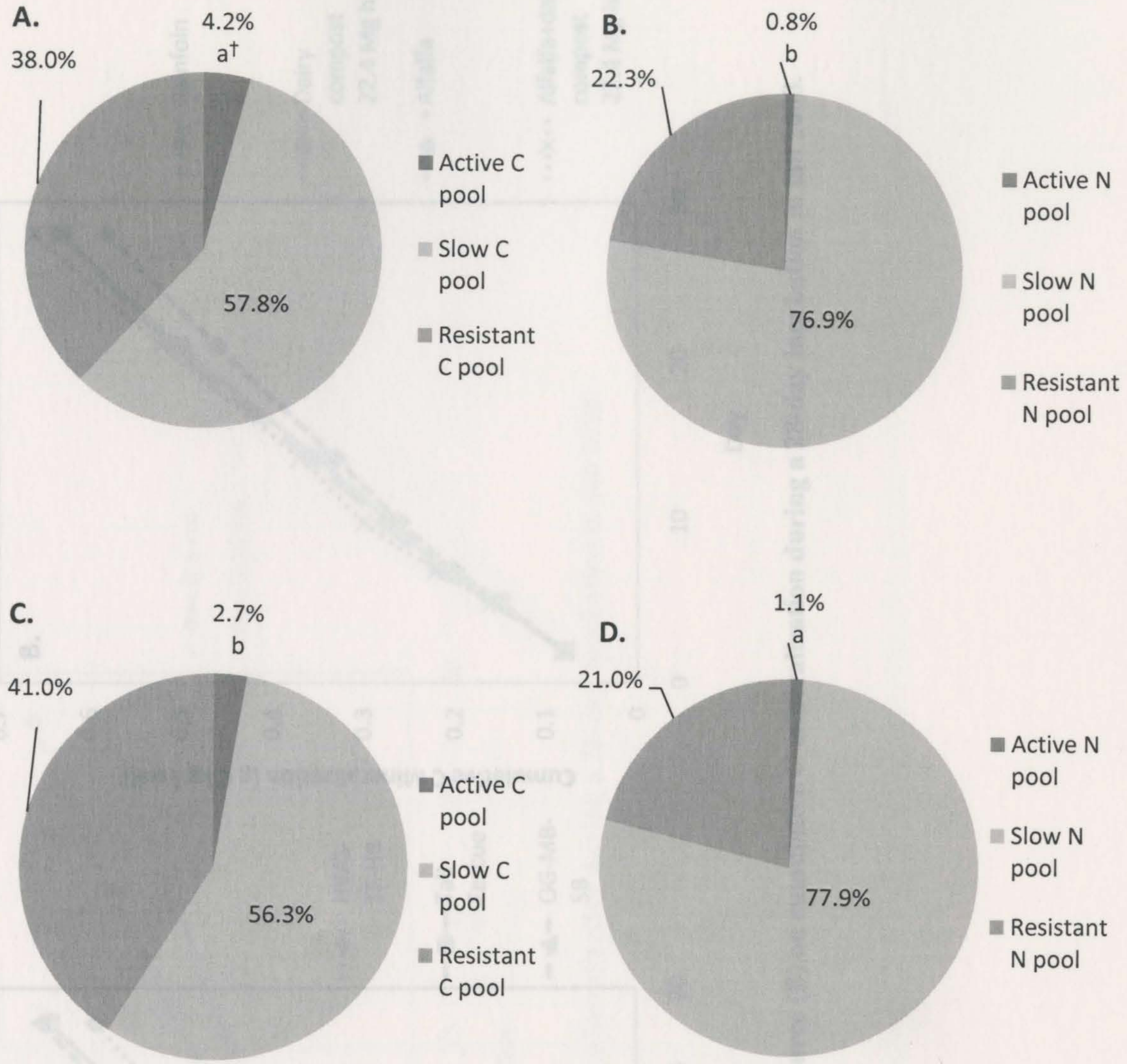

Fig. 2. Soil nutrient pools in fall 2009. A. Carbon at 0-5 cm depth; B. Nitrogen at 0-5 cm depth; C. Carbon at 5-10 cm depth; D. Nitrogen at 5-10 cm depth.
†Values with different lowercase letters between depths within each pool are significantly different (ANOVA); *P* < 0.05.

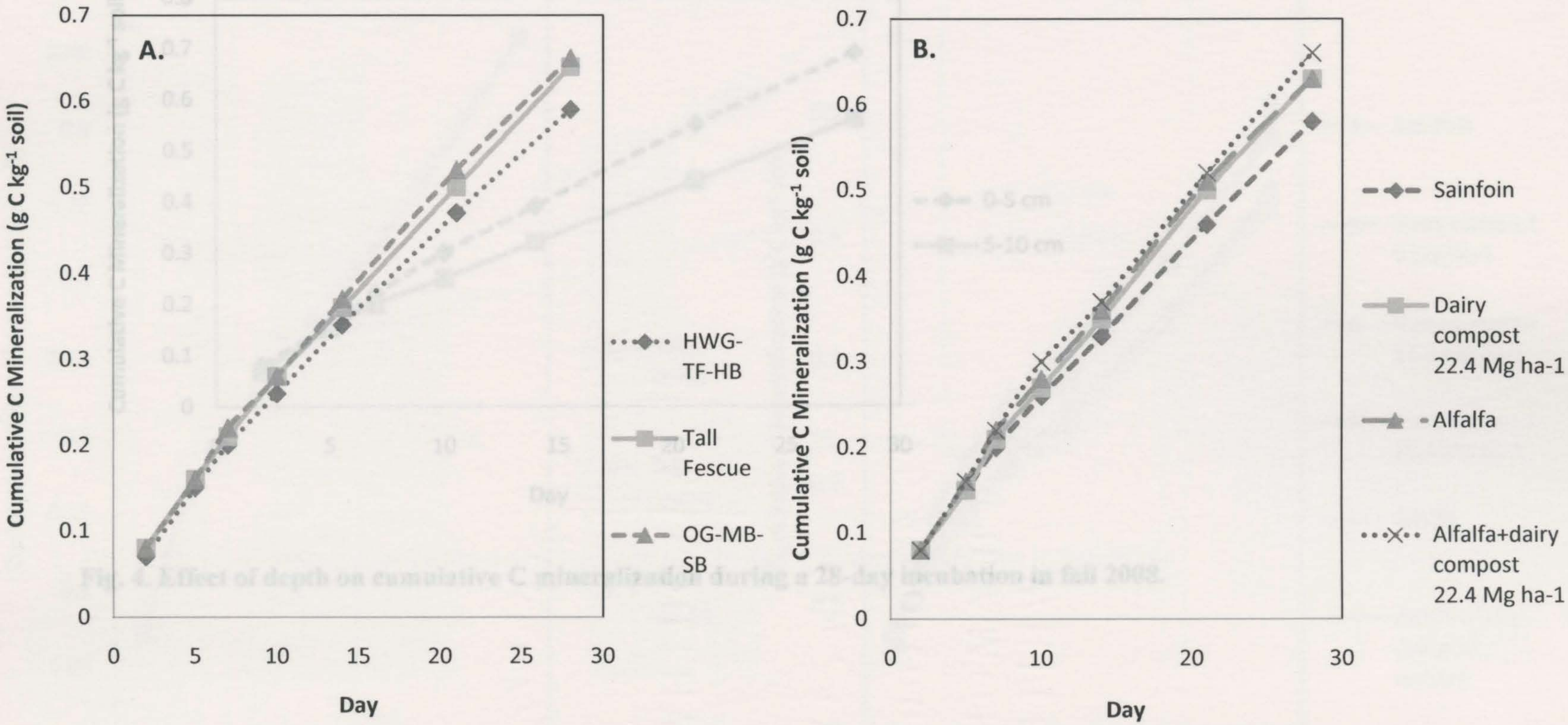

Fig. 3. Effect of grass mix (A) and N-source (B) on cumulative C mineralization during a 28-day incubation in fall 2008.

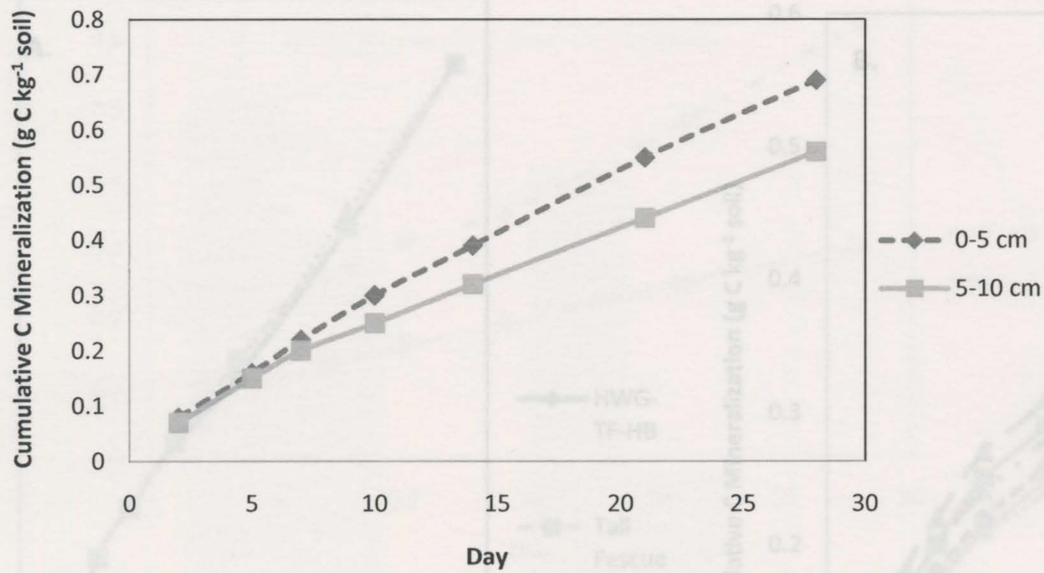

Fig. 4. Effect of depth on cumulative C mineralization during a 28-day incubation in fall 2008.

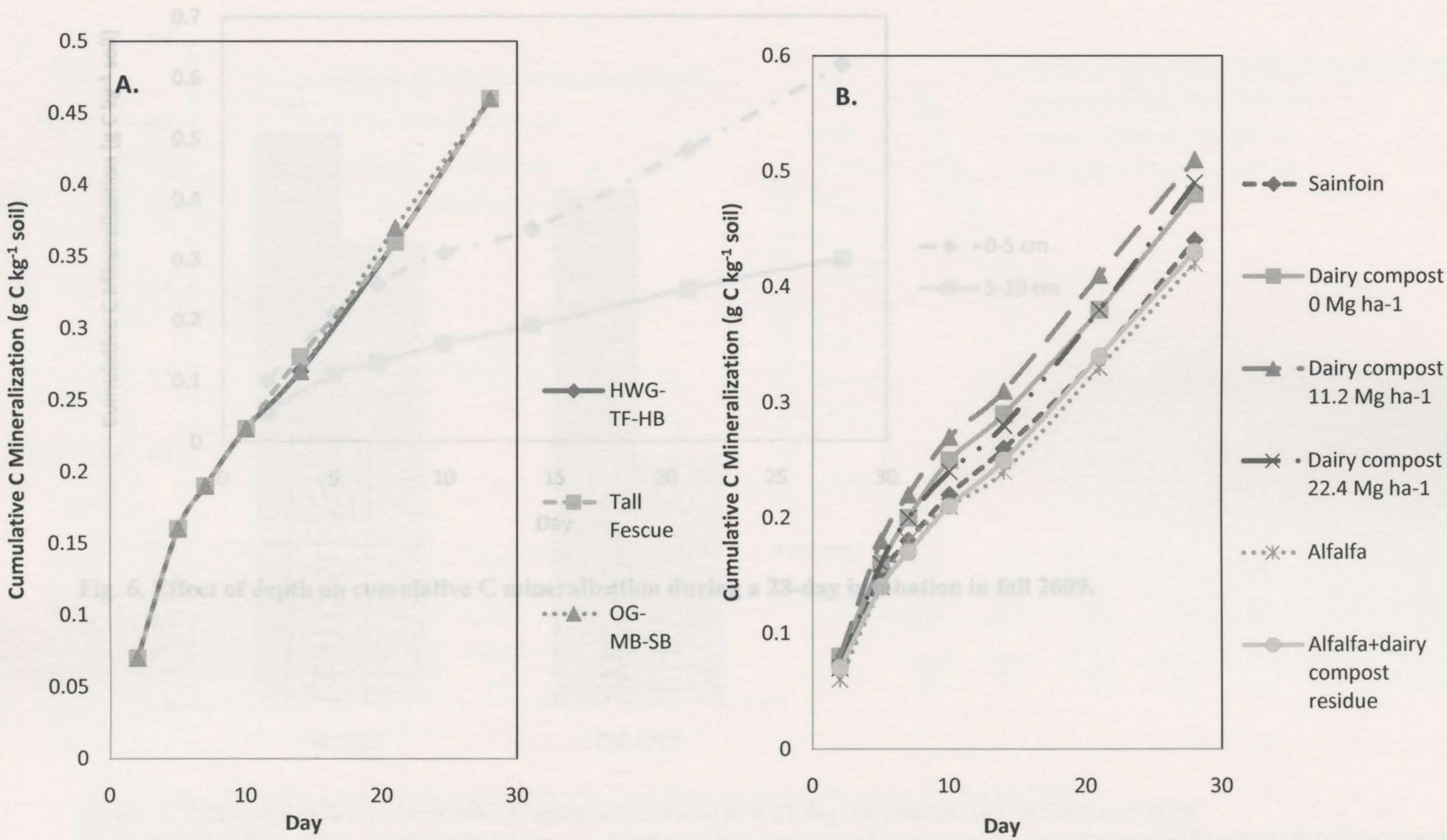

Fig. 5. Effect of grass mix (A) and N-source (B) on cumulative C mineralization during a 28-day incubation in fall 2009.

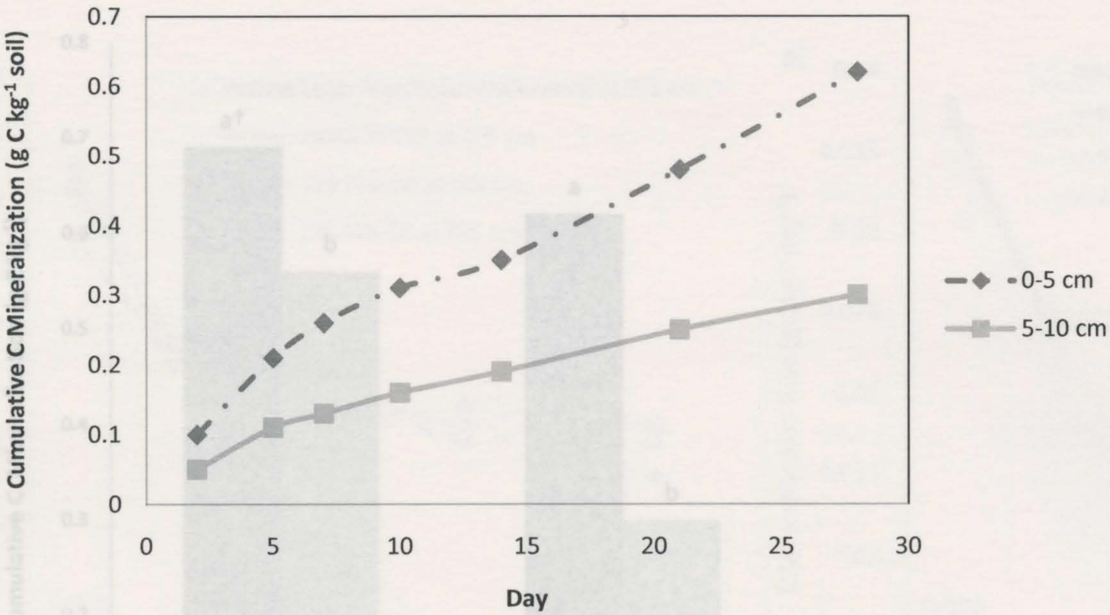

Fig. 6. Effect of depth on cumulative C mineralization during a 28-day incubation in fall 2009.

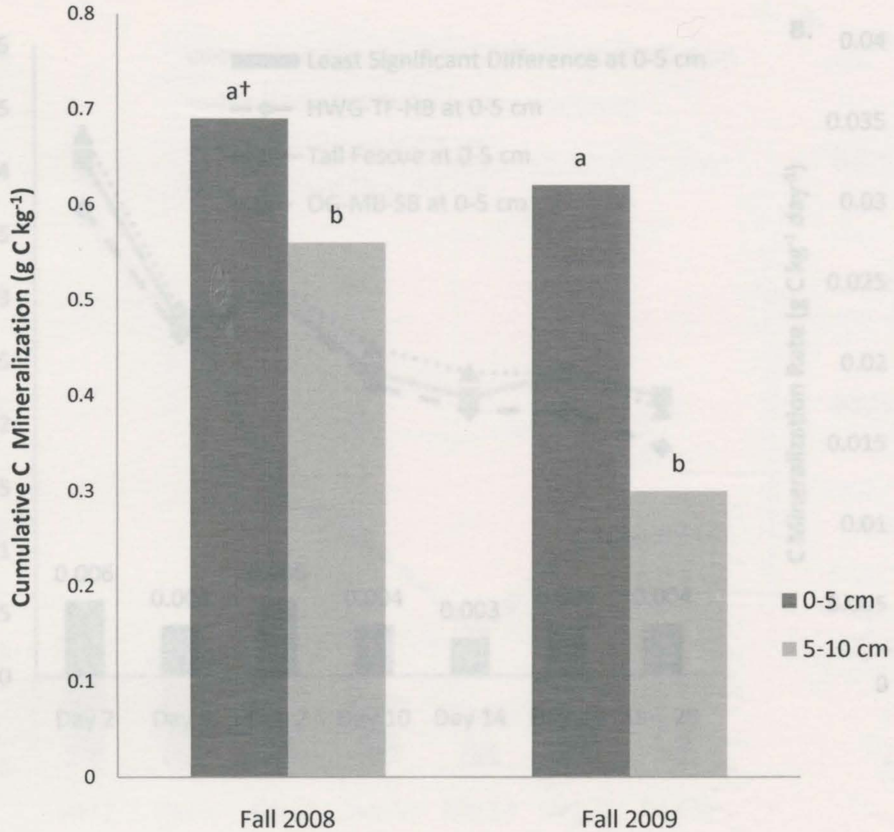

Figure 7. Effect of depth on cumulative C mineralization over a 28 day incubation in fall 2008 and 2009.
†Values with different lowercase letters between depths within each growing season are significantly different (ANOVA); *P* < 0.05.

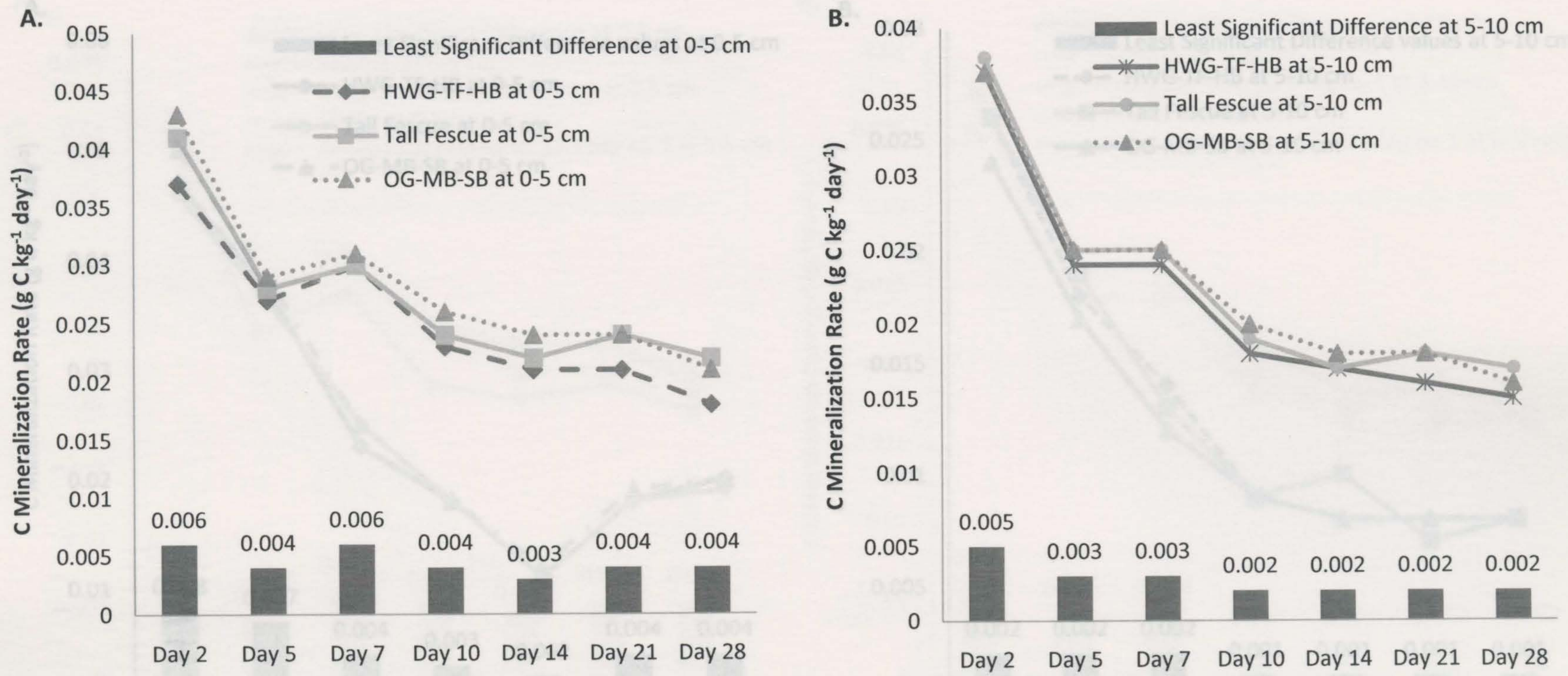

Fig. 8. Effect of grass mix on C mineralization rate at 0-5 cm (A) and 5-10 cm (B) in fall 2008.

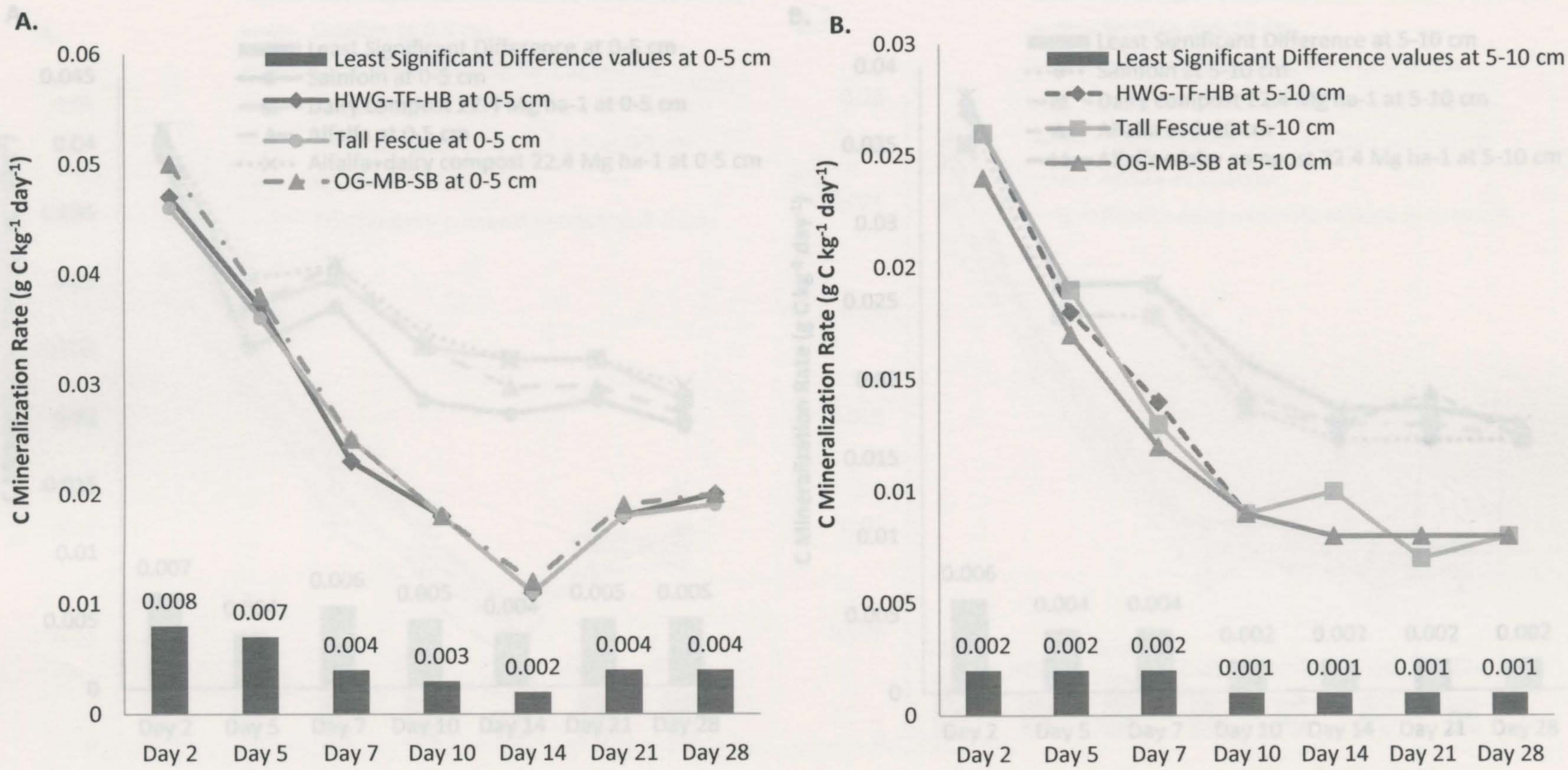

Fig. 9. Effect of grass mix on C mineralization rate at 0-5 cm (A) and 5-10 cm (B) in fall 2009.

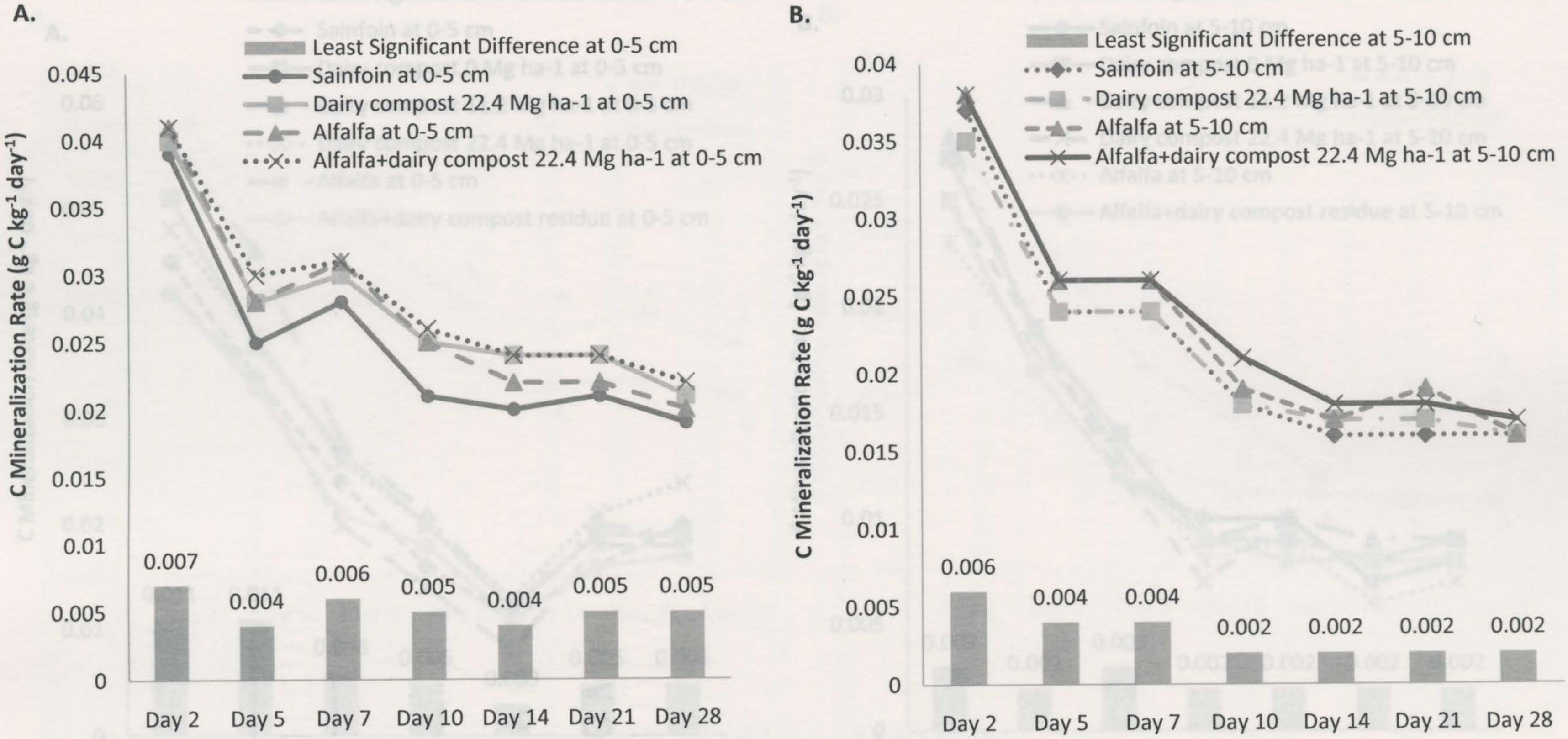

Fig. 10. Effect of N-source on C mineralization rate at 0-5 cm (A) and 5-10 cm (B) in fall 2008.

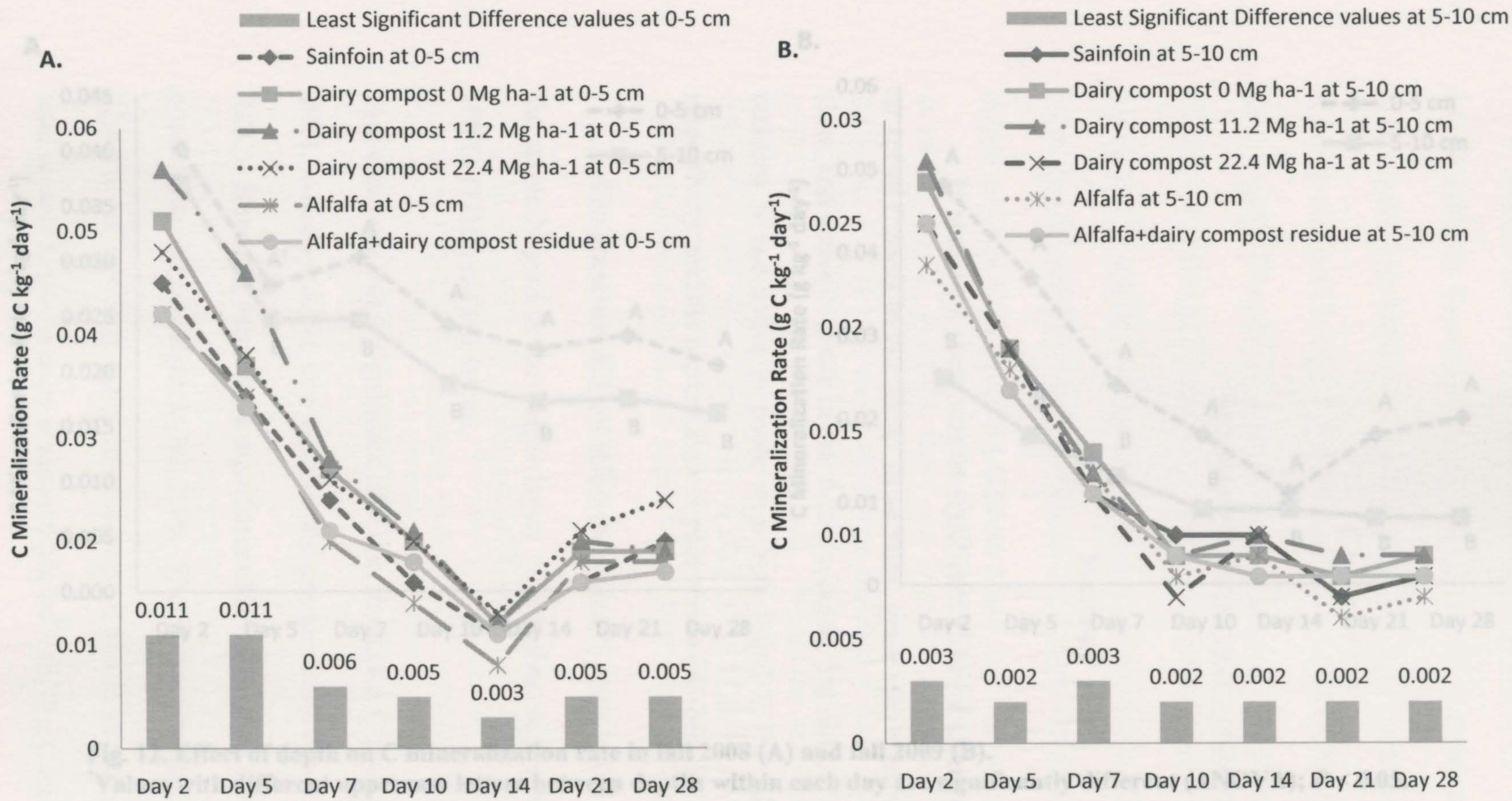

Fig. 11. Effect of N-source on C mineralization rate at 0-5 cm (A) and 5-10 cm (B) in fall 2009.

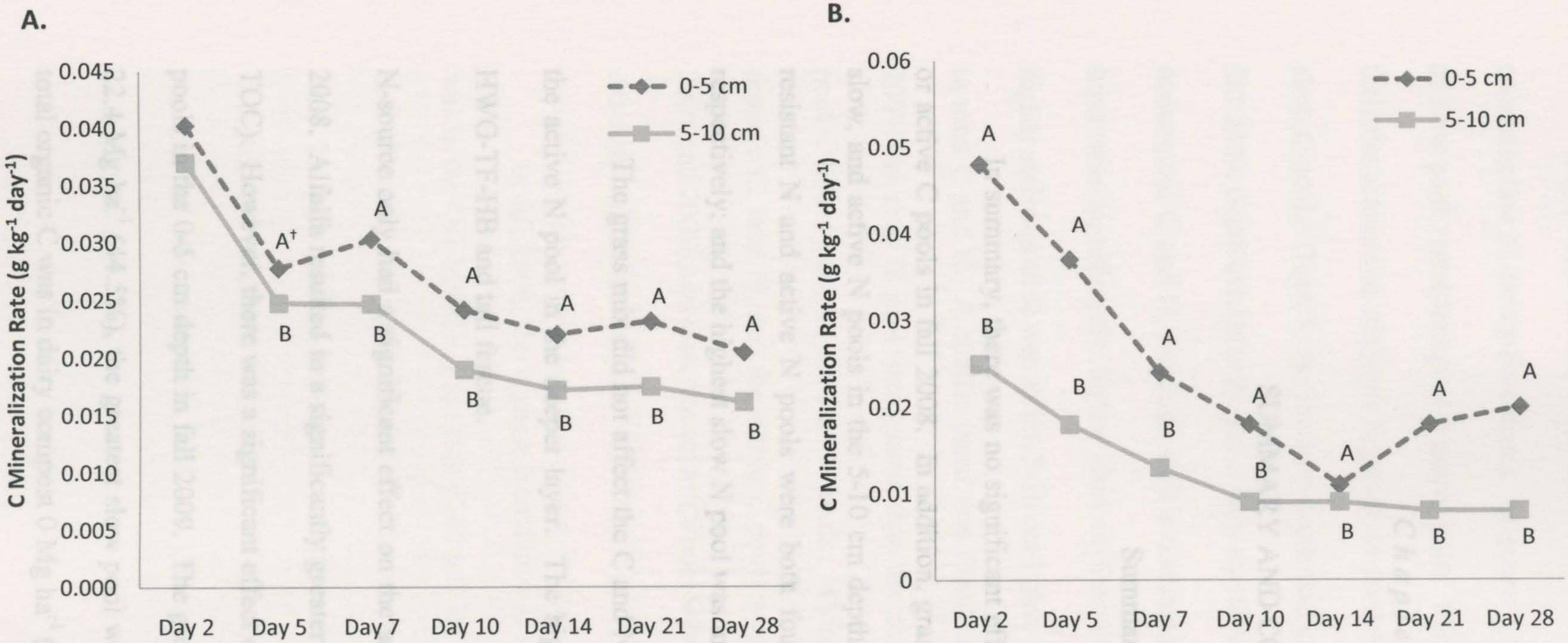

Fig. 12. Effect of depth on C mineralization rate in fall 2008 (A) and fall 2009 (B).
†Values with different uppercase letters between depths within each day are significantly different (ANOVA); $P < 0.05$.

Chapter 4

SUMMARY AND CONCLUSIONS

Summary

In summary, there was no significant effect of grass mix on TOC, resistant, slow, or active C pools in fall 2008. In addition, grass mix significantly affected the resistant, slow, and active N pools in the 5-10 cm depth, but did not affect total N. The highest resistant N and active N pools were both found in the tall fescue, 26.0% and 2.3%, respectively; and the highest slow N pool was in OG-MB-SB (74.3%).

The grass mix did not affect the C and N pools in fall 2009, with the exception of the active N pool in the deeper layer. The highest active N pool (1.1%) was found in HWG-TF-HB and tall fescue.

N-source only had a significant effect on the active C pool at the 5-10 cm depth in fall 2008. Alfalfa resulted in a significantly greater active C pool in the deeper depth (5% of TOC). However, there was a significant effect of N-source on total, resistant, and slow C pools in the 0-5 cm depth in fall 2009. The greatest resistant pool was in dairy compost 22.4 Mg ha^{-1} (44.5%), the greatest slow pool was in sainfoin (61.4%), while the greatest total organic C was in dairy compost 0 Mg ha^{-1} (16.70 g C kg^{-1}).

Depth significantly affected total organic C in fall 2008, which had greater C concentration in the shallow depth. In general, the proportion of C and N showed a trend of slow pool > resistant pool > active pool. The active and resistant C pools were higher than the active and resistant N pools; in contrast, the slow N pool was greater than the slow C pool. Overall, the shallow depth had greater C pools than the deeper depth. In fall 2009, depth consistently had a very significant effect on total organic C in addition to active-pool C and N, although with a contrasting pattern. The active C pool in the top layer was significantly higher than in the deeper layer; conversely, the significantly higher active-pool N was in the 5-10 cm layer. The 0-5 cm layer was consistently higher in total C and N. A similar trend was found in the second year. In both fall 2008 and 2009, the slow N pool was greater than the slow C pool; however, active and resistant-pool C levels were higher than N pools. Within the same grass mix and N-source, total organic C, total N, and the active C pools were higher in the 0-5 cm depth in both fall 2008 and 2009; however, the active N pool showed a significantly higher %N in the 5-10 cm depth in the second year.

In fall 2008, the highest cumulative C mineralization over 28 days of incubation was in the OG-MB-SB grass mix (0.65 g C kg^{-1}), followed by tall fescue (0.64 g C kg^{-1}), and HWG-TF-HB (0.59 g C kg^{-1}). The N source effect on cumulative C mineralization over 28 days was in the order of alfalfa+dairy compost mix (0.66 g C kg^{-1}) > dairy compost and alfalfa as a single N source (0.63 g C kg^{-1}) > sainfoin (0.58 g C kg^{-1}). At the end of the incubation, the 0-5 cm depth had a higher amount of cumulative mineralizable C.

In the second year, the three grass mixes were not different in cumulative mineralizable C during the 28 day incubation. Cumulative mineralizable C was slightly influenced by grass mix and N-source, in which the slope of cumulative mineralizable C increased following day 14. Final cumulative mineralizable C followed this trend: dairy compost 11.2 Mg ha^{-1} > dairy compost 22.4 Mg ha^{-1} > dairy compost 0 Mg ha^{-1} > sainfoin > alfalfa+dairy compost residue > alfalfa. The fall 2009 data had results similar to fall 2008; in particular, the shallow depth had almost double the cumulative mineralizable C than the deeper depth. In addition, the 0-5 cm depth also showed a faster decomposition rate compared to the 5-10 cm depth.

During fall 2008, potential mineralizable C (C_o) in the 0-5 cm layer was significantly higher than in the 5-10 cm layer. While in fall 2009, there was limited data for potential mineralizable C and mineralizable rate constant in the 5-10 cm depth, and statistical analysis could not be performed.

Depth was the only significant factor affecting cumulative C mineralization in the two fall growing seasons over a 28 day incubation, and the shallow depth had the greater cumulative C mineralization. At both the 0-5 and 5-10 cm depths in fall 2008 and 2009, grass mix and N-source did not affect the C mineralization rate. Generally, the highest rate occurred in the first two days of incubation, and then it went down. In this two year study, depth consistently affected the C mineralization rate, in that the 0-5 cm layer had a significantly higher C mineralization rate over the 28 day incubation, with the exception of no significant difference on day 2 of fall 2008.

Conclusions

In conclusion, grass mix and N source, especially dairy compost, affected the C and N pools; however, results varied from year to year. There was no distinct pattern in grass mix or N-source impact on C and N pools. In fall 2009, the additional dairy compost application rate treatments did not show any significant impact on the C and N pools. The only factor that seemed to have a significant effect on C and N pools in fall 2008 and 2009 was depth, in that, the shallow depth had predominantly greater C and N pools, with the exception of active-pool N.

The linear trend of cumulative C mineralization implied that decomposition was still ongoing. Therefore, a 28-day period for laboratory incubation was insufficient to decompose all available C in soil organic matter.

In order to enhance SOC and in the long run soil C sequestration, the use of organic N fertilizer is recommended, such as using legume, dairy compost, or manure. Locally adapted legume varieties should be considered for inter-seeding with grass mix. Alfalfa was proven to be a good and locally adapted legume in this perennial study; however, it needs time to establish, and then it can grow very well to enhance SOC. Improvement in agricultural practices for legume establishment such as more frequent irrigation may improve legume productivity.

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APPENDIX A

Table A-1. Sample calculation of CO_2 evolution.

Parameter	Symbol	Equation
Moles of CO_2-C in 1 L	A	N = PV/RT
% mL CO_2 standard tank	B	0.1055
μg CO_2-C mL^{-1} air of standard tank	C	(B/100)*(A/1)*(1/1000)*12000000
Milliliters of CO_2 injected gas from standards	D	Standards: 2, 4, 6, 8, 10
μg CO_2-C mL^{-1} air of standard	E	D*C
IRGA readings from standards or samples	F	F
Correlations	G	x-values (F); y-values (E) of standards
Slope	H	Slope(x-values (E);y-values (F)) of standards
Intercept	I	Intercept(x-values (E); y-values (F)) of standards
Milliliters of CO_2 injected from samples	J	Samples: vary
CO_2 evolve of sample (μg mL^{-1})	K	I+(H*F)
Average of CO_2 evolve of control (μg mL^{-1})	L	L
Jar volume (mL)	M	M
Oven-dried soil weight (g)	W	W
CO_2 evolve (μg g^{-1} soil)	O	((M-(N/ρ))*(K-L))/W
Mineralizable C (μg g^{-1} soil)	Q	((K-L)*M)/W
Mineralizable C rate (μg g^{-1} day)	S	Q/days of incubation

N = Number of moles
P = Pressure standard (0.843 bar)
V = Volume of gas (1 L)
R = Universal gas constant (0.0831 L bar K^{-1} mol^{-1})
T = temperature standard of the incubation room (293 °K = 25°C)
0.1055 = in this study: 0.1055% mL CO_2 in the standard tank, this number varies depending on the standard tank in a laboratory
B/100 = % mL CO_2 standard tank
A/1 = Moles of CO_2-C in 1 L
1/1000 = Moles of CO_2-C in 1 L converted to mL unit
12,000,000 = 12 is an atomic weight of C and 1,000,000 is a conversion from g $mole^{-1}$ to μg $μmole^{-1}$ unit
ρ = soil particle density (2.65 g mL^{-1})
W = in this study: 40 g field capacity moist soil corrected to oven-dried soil

Table A-2. Effect of grass mix on C to N ratio in 2008.

Grass mix	Cutting 1	Cutting 2	Cutting 3	Cutting 4	Cutting 5	Cutting 6	Average
HWG-TF-HB§	15.70	11.94	12.23 b†	12.28 a	12.40 a	14.50 ab	13.18 a
Tall Fescue	16.02	13.20	13.48 a	12.58 a	12.72 a	15.00 a	13.84 a
OG-MB-SB	15.21	12.15	11.12 c	11.11 b	11.25 b	12.65 b	12.25 b
	----------	----------	----------	PR > F	----------	----------	----------
Type III SS	0.42	0.23	0.0017	0.0305	0.0048	0.11	0.0036

§HWG-TF-HB, Hybrid Wheatgrass-Tall Fescue-Hybrid Brome; OG-MB-SB, Orchard grass-Meadow Brome-Smooth Brome.

†Grass mixes with different lowercase letters are significantly different (ANOVA); $P < 0.05$.

Table A-3. Effect of grass mix on C to N ratio in 2009.

Grass mix	Cutting 1	Cutting 2	Cutting 3	Cutting 4	Cutting 5	Average
HWG-TF-HB[§]	22.16 b[† ¶]	18.71 a	18.26 a	15.71 b	16.48 a	16.99 a
Tall Fescue	24.63 a	20.20 a	18.67 a[¶]	16.41 a	17.16 a	17.71 a
OG-MB-SB	18.75 c	17.03 b	17.01 b[¶]	14.25 c	14.75 b	15.42 b
	----------	----------	---------- PR >	F ----------	----------	----------
Type III SS	< 0.0001	0.0025	0.0007	< 0.0001	< 0.0001	< 0.0001

[§]HWG-TF-HB, Hybrid Wheatgrass-Tall Fescue-Hybrid Brome; OG-MB-SB, Orchard grass-Meadow Brome-Smooth Brome.

[†]Grass mixes with different lowercase letters are significantly different (ANOVA); $P < 0.05$.

Table A-4. Effect of N-source on C to N ratio in 2008.

Grass mix	Cutting 1	Cutting 2	Cutting 3	Cutting 4	Cutting 5	Cutting 6	Average
Sainfoin	15.79	12.35	12.32	12.13	12.35 a[†]	14.36	13.22
Dairy compost 22.4 Mg ha^{-1}	15.20	12.47	11.74	11.40	11.38 b	12.94	12.52
	PR > F						
Type III SS	0.33	0.65	0.69	0.15	0.01	0.16	0.11

[†]N-sources with different lowercase letters are significantly different (ANOVA); $P < 0.05$.

Table A-5. Effect of N-source on C to N ratio in 2009.

Grass mix	Cutting 4‡	Cutting 5	Average
Sainfoin	16.04 a†	16.76 a	17.50 a
Dairy compost 0 Mg ha^{-1}	16.14 a	17.22 a	18.39 a
Dairy compost 22.4 Mg ha^{-1}	16.28 a	16.62 a	18.20 a
Alfalfa	14.46 b	15.20 b	14.83 b
Alfalfa+dairy compost residue	14.35 b	14.86 b	14.61 b
	---------------------------- PR > F	---------------------------	
Type III SS	< 0.0001	< 0.0001	< 0.0001

†N-sources with different lowercase letters are significantly different (ANOVA); $P < 0.05$.
‡No data available for first 3 cuttings.

APPENDIX B

Table B-1. Compost analysis of spring 2008 application.

Parameter	As received basis	Dry matter basis
Total solids (%)	71.38	100.00
Moisture (%)	28.62	0.00
Organic matter (%)	17.20	24.10
Ash (%)	54.18	75.90
Soluble salts 1:5 (mmhos/cm)	5.14	-
pH 1:5 (units)	8.62	-
Total N (%)	1.074	1.504
Organic N (%)	1.011	1.416
NH_4-N (%)	0.0127	0.018
NH_4-N (ppm)	127.2	178.2
NO_3-N (%)	0.0503	0.0704
NO_3-N (ppm)	502.7	704.3
Total P (%)	0.505	0.708
Total P_2O_5 (%)	1.162	1.628
Total K (%)	1.321	1.851
Total K_2O (%)	1.585	2.221
C/N ratio	8	8
NH_4-N/NO_3-N ratio	0.3	0.3

Table B-2. Compost analysis of fall 2008 application.

Parameter	As received basis	Dry matter basis
Total solids (%)	79.56	100.00
Moisture (%)	20.44	0.00
Organic matter (%)	6.84	8.60
Ash (%)	72.72	91.40
Soluble salts 1:5 (mmhos/cm)	2.66	-
pH 1:5 (units)	9.09	-
Total N (%)	0.291	0.366
Organic N (%)	0.258	0.324
NH_4-N (%)	0.0325	0.041
NH_4-N (ppm)	325.5	409.1
NO_3-N (%)	0.0007	0.0008
NO_3-N (ppm)	6.7	8.5
Total P (%)	0.107	0.134
Total P_2O_5 (%)	0.245	0.308
Total K (%)	0.465	0.584
Total K_2O (%)	0.558	0.701
C/N ratio	12	12
NH_4-N/NO_3-N ratio	48.4	48.4

Table B-3. Soil analysis prior to the perennial forage study.

Parameter	Replicate 1	Replicate 2	Replicate 3
Soil pH 1:1	8.2	8.3	8.3
Soluble salts 1:1 (mmhos/cm)	0.76	0.63	0.71
Excess lime rating	High	High	High
Organic matter LOI (%)	2.5	2.4	2.2
FIA NO_3 (ppm N)	18.8	13.3	14.1
Depth 0-8 in:			
NO_3 (lbs N/A)	45	32	34
Method P (ppm P):			
O-P	37	32	17
M-P3	62	64	28
Ammonium acetate:			
K (ppm)	441	405	356
Ca (ppm)	4463	4385	4428
Mg (ppm)	554	536	519
Na (ppm)	70	70	69
Ca-P sulfate (ppm S)	94	83	86
DTPA:			
Zn (ppm)	2.35	2.52	1.80
Fe (ppm)	9.9	10.5	9.5
Mn (ppm)	3.9	3.7	3.4
Cu (ppm)	1.11	1.36	1.27
Sum of cations (me/100g)	28.4	27.7	27.7
% base saturation:			
H	0	0	0
K	4	4	3
Ca	79	79	80
Mg	16	16	16
Na	1	1	1

center of
fire hydrant
to SW
corner:
263' 7"

W

Rep 1

HWG - TF - HB Alfalfa Plot 4	HWG - TF - HB Sainfoin Plot 3	OG - MB - SB Sainfoin Plot 2	OG - MB - SB Alfalfa Plot 1
OG - MB - KBG Birdsfoot Trefoil Plot 5	OG - MB - KBG Plot 6 10 lb.	Tall Fescue White Clover Plot 7	OG - MB - SB White Clover Plot 8 BROM
HWG - TF - HB Alfalfa Plot 12	OG - MB - SB Birdsfoot Trefoil Plot 11	OG - MB - KBG White Clover Plot 10	HWG - TF - HB Plot 9 20 lb.
HWG - TF - HB Plot 13 0 lb.	OG - MB - KBG Plot 14 0 lb.	Tall Fescue Plot 15 20 lb.	Tall Fescue Plot 16 10 lb.
OG - MB - KBG Plot 20 20 lb.	HWG - TF - HB White Clover Plot 19 BROM	OG - MB - SB Alfalfa Plot 18	OG - MB - SB Plot 17 20 lb.
OG - MB - KBG Sainfoin Plot 21	OG - MB - SB Plot 22 0 lb.	OG - MB - KBG Alfalfa Plot 23	Tall Fescue Sainfoin Plot 24
Tall Fescue Plot 28 0 lb.	HWG - TF - HB Birdsfoot Trefoil Plot 27	OG - MB - SB Plot 26 TUBE 10 lb.	OG - MB - KBG Alfalfa Plot 25
Tall Fescue Alfalfa Plot 29	HWG - TF - HB Plot 30 TUBE 10 lb.	Tall Fescue Birdsfoot Trefoil Plot 31	Tall Fescue Alfalfa Plot 32

Rep 2

Fig. B-1a. Field lay-out of the perennial forage study.

S — N

HWG - TF - HB Alfalfa Plot 36	HWG - TF - HB Plot 35 20 lb.	OG - MB - KBG Alfalfa Plot 34	Tall Fescue Birdsfoot Trefoil Plot 33
OG - MB - SB Sainfoin Plot 37	Tall Fescue Plot 38 20 lb.	OG - MB - SB White Clover Plot 39 BROM	Tall Fescue Alfalfa Plot 40
Tall Fescue Plot 44 0 lb.	OG - MB - SB Alfalfa Plot 43	OG - MB - SB Plot 42 20 lb.	HWG - TF - HB White Clover Plot 41 BROM
HWG - TF - HB Sainfoin Plot 45	Tall Fescue Sainfoin Plot 46	HWG - TF - HB Birdsfoot Trefoil Plot 47	OG - MB - KBG Plot 48 10 lb.
OG - MB - SB Plot 52 0 lb.	OG - MB - SB Alfalfa Plot 51	HWG - TF - HB Plot 50 TUBE 10 lb.	OG - MB - KBG White Clover Plot 49
IRRIGATION	WHEEL	TRACK	################
OG - MB - KBG Birdsfoot Trefoil Plot 53	Tall Fescue White Clover Plot 54	OG - MB - SB Birdsfoot Trefoil Plot 55	HWG - TF - HB Alfalfa Plot 56
OG - MB - KBG Alfalfa Plot 60	HWG - TF - HB Plot 59 0 lb.	OG - MB - KBG Sainfoin Plot 58	OG - MB - SB Plot 57 TUBE 10 lb.
Tall Fescue Alfalfa Plot 61	OG - MB - KBG Plot 62 20 lb.	Tall Fescue Plot 63 10 lb.	OG - MB - KBG Plot 64 0 lb.

Rep 3

Fig. B-1b. Field lay-out of the perennial forage study (continued).

west post of entrance gate to SW corner: 332 ft.

HWG - TF - HB White Clover Plot 68 BROM	OG - MB - SB Plot 67 0 lb.	HWG - TF - HB Plot 66 20 lb.	OG - MB - KBG Plot 65 10 lb.	
OG - MB - SB Sainfoin Plot 69	Tall Fescue Plot 70 0 lb.	OG - MB - KBG White Clover Plot 71	Tall Fescue Plot 72 10 lb.	
OG - MB - KBG Alfalfa Plot 76	Tall Fescue Sainfoin Plot 75	HWG - TF - HB Alfalfa Plot 74	OG - MB - KBG Plot 73 0 lb.	
Tall Fescue Birdsfoot Trefoil Plot 77	OG - MB - SB Plot 78 20 lb.	OG - MB - SB Alfalfa Plot 79	OG - MB - SB White Clover Plot 80 BROM	
OG - MB - KBG Alfalfa Plot 84	OG - MB - SB Birdsfoot Trefoil Plot 83	OG - MB - SB Plot 82 TUBE 10 lb.	HWG - TF - HB Plot 81 TUBE 10 lb.	
OG - MB - KBG Plot 85 20 lb.	OG - MB - KBG Birdsfoot Trefoil Plot 86	Tall Fescue Alfalfa Plot 87	Tall Fescue White Clover Plot 88	
HWG - TF - HB Sainfoin Plot 92	Tall Fescue Plot 91 20 lb.	OG - MB - SB Alfalfa Plot 90	Tall Fescue Alfalfa Plot 89	
HWG - TF - HB Alfalfa Plot 93	HWG - TF - HB Birdsfoot Trefoil Plot 94	HWG - TF - HB Plot 95 0 lb.	OG - MB - KBG Sainfoin Plot 96	↑ 10' ↓

E

⟵ 40' ⟶

Compost	
No-compost	

Dwi's sampling plots:

HWG-TF-HB (2008 & 2009)
Tall Fescue (2008 & 2009)
OG-MB-SB (2008 & 2009)
only 2009 sampling

HWG	Hybrid Wheatgrass
TF	Tall Fescue
HB	Hybrid Brome
OG	Orchardgrass
KBG	Kentucky Bluegrass
SB	Smooth Brome
BROM	Bromide

Fig. B-1c. Field lay-out of the perennial forage study (continued).