ECOSYSTEM RESPONSE OF COOL-SEASON GRASS PASTURE TO MANAGEMENT FOR LIVESTOCK IN SOUTHERN WISCONSIN

by

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A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Environment and Resources)

at the

UNIVERSITY OF WISCONSIN-MADISON

2009

DEDICATION

To my best friend and wife, Tish, whose undying love and support has been my foundation. You are me, and I am you.

To my parents, Larry and Margaret, who instilled in me the importance of education, and gave me the opportunity to experience the natural world.

To my children, Wes, Ian, and Garrett, who allow me to appreciate the wonders of life.

To Joey and Pete, you know what you do.

ACKNOWLEDGEMENTS

As I come to the culmination of this journey, there are many who deserve acknowledgement for their assistance. Thanks to my wife Tish for her unselfish support of hugs, kisses, cards, and encouraging words. But just as importantly, many thanks for the slack she has picked up as I spent time in the field, lab, and writing this dissertation. I would also like to recognize to her, what it means to have a mate that is there for me forever, for always, and no matter what. Thanks LOVE!

Many thanks to Dr. Randy Jackson, Dr. Claudio Gratton, and Dr. Michael Bell – the agroecology cluster hire – for initiation of this project, and to the University of Wisconsin College of Agriculture and Life Sciences Interdisciplinary Hatch Grant and The Center for Integrated Agricultural Systems for project funding.

Thanks to Franbrook Farm for being the idyllic pastoral setting, and to all the critters that graced me with their presence as I sampled their habitat – even their non-demonic interference. A special thanks to Ms. Cow #83 – a friendlier example of your species I have not met. When you went snooping in Clifford the big red van, licked the mirrors, or presented your head for me to scratch, you provided me with many hours of satisfaction. To all the "little guys" gallivanting in the field, picturing your unfettered enthusiasm will always make me smile. To farm manager Ralph Seigenthaler, thanks for applying my grazing treatments, keeping the farm and animals in great shape, for being an all-around great guy, and in general just keeping an eye on the place.

I am very grateful to my labmates – Julie Doll, Ellen Hamingson, Emma Bouressa (Keyser), Herika Kummel, Andy Jakubowski, David Duncan, Gregg Sanford, Laura Smith,

Craig Maier, and Sue Chamberlain – who have been great sounding boards, and with whom waxing esoteric on all things ecological has been most enlightening and pleasurable.

Thanks to all who have worked on this project either in the field or lab – John Albright, Jon Bleier, Liz Froelich, Trish Oates, Kelly Forman, John Harrigan, Andy Jakubowski, Nate Hoftiezer, Joey Pfaller, Ana Metscher, Elke Dougherty, Genya Erling, Devin Wixon-Keller, and Ashley Bennett. Special thanks go to Dr. Dan Undersander and Kevin Silviera in the Forage Analysis lab for assistance with NIR. Also, special thanks to Dr. Teri Balser, Kevin Budsberg, Jessica Mentzer-Gutknecht, Devin Wixon-Keller, and Jenny Kao-Kniffen of the Microbial Ecology lab for assistance with PLFA/FAME extraction and analysis.

I am very grateful to my committee - Dr. Teri Balser, Dr. Jess Gilbert, Dr. Emily Stanley, and Dr. Dan Undersander - for their support and contribution to my graduate formation and for the time they have dedicated to this project.

And most importantly, to my advisor and friend Randy Jackson, go raibh míle maith agat! His gentle yet professional style has punctuated my time here with encouragement, while at the same time challenging me to “embrace uncertainty”. I believe that I have become a better scientist as a result. With his guidance, the lab is filled with curiosity for the natural world, is grounded in practical agronomic and ecological theory, and is a vibrant and fulfilling place to do research.

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ABSTRACT

Livestock production for meat and dairy represent major agroecosystems in Wisconsin and comprise much of the State's landscape. Many farmers in Wisconsin are concerned about the effects of agricultural practices that are the result of the industrial model, especially the role of confinement operations where feed is mechanically harvested and transported to a centralized location where livestock spend most if not all of their time. Farmers' desire to remain profitable while at the same time practicing environmentally sound management techniques has led to a growing phenomenon in the upper Midwest, management-intensive rotational grazing. But while the social benefits and economic flexibility of rotational grazing are increasingly well established, its agroecological consequences remain mostly anecdotal. I employed a randomized complete block field experiment to test specific ecological and agronomic research questions under four pasture management practices typical of the upper Midwest – management-intensive rotational grazing, continuous grazing, haymaking, and land that has been set aside with no agronomic management. First, I compared forage production, forage quality, and belowground net primary production finding forage production was significantly greater under management-intensive rotational grazing when compared to continuous grazing, mechanical harvest for hay, and no agronomic management. Forage quality was also significantly higher under management-intensive rotational grazing, while belowground net primary production was significantly lower under grazed treatments compared to non-grazed treatments. Second, I used an ecosystem carbon balance approach to quantify the net ecosystem C accumulation or loss of pastures under the treatments described above. Management-intensive rotational grazing lost significantly less C in 2006 than all other treatments, while in 2007 MIRG was the only

treatment that was a sink for C. The potential for sequestering C in perennial pastures exists, but if considered at scales broader than the pasture boundary, import and export of C need to be taken into account. And lastly, I used a hybrid phospholipid fatty acid and fatty acid methyl ester analysis to assess microbial biomass, fungal-to-bacterial ratios, and guild composition in the surface 15-cm of soils under the four pasture treatments. Microbial community structure was assessed with principal components analysis, and regression trees and linear mixed-effects models were used to explore factors related to microbial community response to management. There was a general effect of grazing on microbial communities, but different grazing management (i.e., management-intensive rotational grazing vs. continuous grazing) did not affect microbial community composition or biomass. Production increases with management-intensive rotational grazing likely stem from the direct effect of defoliation on plant developmental stage and community composition rather than indirectly via microbial mediated feedbacks.

INTRODUCTION

"Grazing is the meeting of cow and grass."

Andre Voisin (1959)

Throughout history, grassland and grazing have played a prominent role in the life of humans, from shepherds with their flocks chasing the next bloom of grass and indigenous Americans following bison herds across the plains, to early settlers importing grass seed to plant down and raise the family livestock. Grasslands and grazing are as intrinsic to agrarian agriculture as flight is to the bird. Domesticated livestock were of such importance to the early colonists of North America that enclosing land with fencing to contain them became a justification for acquiring land through the Lockeian tenet of improvement by labor (1984). And while the colonies became a land of fields and fences, movement to settle the West became intrinsically linked with American independence and divorce from the influence of Europe (Turner, 1894). As settlers moved into Wisconsin, much of the landscape was converted to pasture and crop fields. Of particular interest was the migration into the upper Midwest of dairy and cheese making interests, which by the early 1920s had supplanted New England and New York as the centers of manufacture, making the area "America's dairyland" (Lewthwaite, 1964; Cross, 2001).

"Management-intensive grazing is not for every producer. It will not instantly provide wealth and leisure or solve all the problems livestock producers face. Some experienced graziers say it takes three years of observation and manipulation of soil, plant, and animal resources to really begin to manage them well. During these years there will be countless challenges and necessary adjustments. Every attempt to prepare for potential problems will make the transition smoother. An assumption that the system can continually be improved will help the manager to identify weak areas early. Being alert for difficulties ensures that they can be addressed before they become serious."

National Sustainable Agriculture Information Service (ATTRA Publication #IP086)

Management Intensive Rotational Grazing (MIRG), which entails livestock grazing in relatively small paddocks at high densities, but for short durations, is an agriculture movement that has been adopted by a large number of dairy and beef farmers in Wisconsin. It has been adopted with the desire not only to improve their relationship with the land, but also to educate through the exchange of technology and information the benefits of the grazier lifestyle (Hassanein and Kloppenburg, 1995; Paine et al., 2000). In his seminal book, *Grass Productivity*, Voisin (1959) points out that rotational grazing "… is not a new idea: its principles were known but have been forgotten". In fact, some of the earliest writings attributed to the concept of rotational grazing were penned by Scottish agriculturist James Anderson in 1777 and by French agronomist and botanist Jean-Francois Rozier in 1786 (Voisin, 1959). And, although Voisin was a strong advocate for rotational grazing in the late 50s and early 60s, he died before his grazing principles were recognized in the United States. In fact, upon querying range scientists while on a tour of American universities in 1978, noted holistic management advocate Allan Savory found that not one of these scientists had read Voisin's book. And even though Voisin's grazing principles did not gain early favor in the United States, (it was almost 30 years before his work would impact grazing in the U.S.) they were put into practice in other countries. Of these, the

most notable was New Zealand where managing livestock using rotational grazing remains a cornerstone of their dairy industry (Murphy, 1991).

For much of the early 20th century, Wisconsin dairy farmers used pastures as a primary food source for their livestock, but early scientific efforts at maximizing production using grazing management principles neglected the critical element of time (Voisin, 1959). The length of time and the timing of repeated exposure to defoliation are critical in maintaining plant, and therefore pasture, health. As demand for dairy products intensified during the last century, poor management of pasturage reduced the ability of graziers to compete with those farms applying new scientific knowledge of feeding stored feed such as alfalfa and corn silage (Liebhart, 1993). Agricultural research trials directed farmers to adopt feeding stored forage and grain to livestock not only in the winter, but also in the summer as a means of maximizing milk production per acre. For example, Larsen (1959) was promoting the adoption of feeding stored forage as one of the "…more efficient ways of utilizing summer forage", and to overcome the "extravagant" use of the cow as a harvesting unit, "… [more] efficient methods of harvesting must come into use if herd expansion is to take place without a large increase in farm size".

"Environmental issues in agriculture, particularly actual or proposed regulations on chemical use and large confinement animal feeding operations, have become increasingly important during this decade."

Program on Agricultural Technology Studies
University of Wisconsin – Madison (Research Report No. 5)

The structure of the Wisconsin dairy industry was driven by the hegemony of the Land Grant University during the 1950s – 1980s. The small- to mid-sized confinement dairy (under 200 cows) became the industry standard as pasture-based research was shifted to confinement

systems after 1950 (Fales, 1992; Hassanein, 1999). With the decline of commodity prices in the 1980s, economic sustainability of industrial agriculture as a whole came under scrutiny. Along with economic concern, there were concerns for the environmental sustainability of systems and processes related to agriculture. Negative environmental consequences of high input homogeneous cropping systems were hard to overlook. Burgeoning debt coupled with low commodity prices had effects on rural communities that were staggering, with farm abandonment and foreclosures reaching an all time high. For the first time in U.S. history, the total of interest payments on farm loans exceeded total net farm income in1984 (USDA, 1987). Farm closures had a trickle-down effect, negatively impacting the manufacture and sale of farm machinery, and inputs such as seed, fertilizer, herbicides and pesticides. Unable to recover capital investments, rural banks went into receivership. As more and more farmers were forced out of business, negative impacts were also felt by small town enterprises, which saw their profits plummet (Lasley, 2003). As a result, the farmers that had leveraged their farms to seek an advantage through economies of scale, had adopted many of the new technologies, and had observed the admonition to plant crops "fence row to fence row", became especially vulnerable to depreciating land values, reduced capital flow, and financial encumbrance (Bultena et al., 1986).

"I get asked more questions that I can't answer than I can answer about rotational grazing…There's been no research done."

University of Wisconsin agronomist
(as quoted in Hassanein 1999)

By the early 1990s the events described above precipitated changes in the Wisconsin dairy industry that were embraced by farmers in distinctly different ways. While some farm

managers have maintained their role as a mid-sized confinement operations, a farm model that has undergone rapid growth has been increased industrialization (Brock and Barham, 2009). This has manifested itself in the use of updated technology and expanded land base to produce feed for an increasingly larger herd sizes (greater than 200 cows). A third alternative, posited as a possible escape from the industrial "treadmill", is skilled management of resources and information that are not subject to an increase in cost to the farm (Cochrane, 1993). In Wisconsin, this alternative management style and information exchange has been adopted by smaller farms with owners seeking alternative methods to increase production and economic flexibility, reduce environmental and health problems associated with industrial farming, and increase life-style satisfaction (Ostrom and Jackson-Smith, 2000). Many of these farmers were responding to dissatisfaction with the role of public agricultural research and the efficacy of Cooperative Extension to meet increasing demand for non-agribusiness solutions (Buttel, 2003). In the 1990s a new style of intense pasture management manifested itself in the form of MIRG, which while not complex in theory, requires the complex knowledge of local pasture plant communities, soils, climate and weather, types of livestock, and marketing opportunities (Hassanein and Kloppenburg, 1995; Paine et al., 2000).

Knowledge transfer from state and federal farm agencies, traditional cooperatives, Cooperative Extension, and other sources of agricultural information was incomplete for farmers entertaining a switch to MIRG (Hassanein and Kloppenburg, 1995; Paine et al., 2000). Instead, farmers already using, or considering MIRG, turned to sustainable farming networks that were strictly farmer initiated and farmer led. Following a conference sponsored by the Rodale Institute in 1984, four farmers initiated and formed the first Wisconsin grazing network with the expressed goal of developing methods of evaluating technologies, encouraging information

exchange between farmers, and forging relationships with University research personnel (Paine et al., 2000).

The number of grazier networks has grown from 1 in 1986, to 23 in 2000 (Paine et al. 2000), and while these networks and the exchange of ideas has flourished, there are recognizable holes in the research base and the flow of this information to grass based farmers (Jackson, 2006). Based on a literature review done by the University of Wisconsin's Center for Integrated Agricultural Systems (CIAS), academic literature related to MIRG exploded in the 1990s and the current decade, but there are noticeable shortcomings in current research (Mariola et al., 2005). For example, they state that data summaries to date focus mainly on the link between grazing and human nutrition, or between grazing and water quality. Also, in a list of Wisconsin grazing research, peer-reviewed grazing literature comprised 54 papers, of which 13 fell under the general description of environment/nutrition, but most of these papers dealt only with wildlife and/or wildlife habitat (see Jackson, 2006). More recently, a report by CIAS on the effects of grazing on Wisconsin's environment produced 33 peer-reviewed articles, of which 11 were focused on the upper Midwest. Forage production and quality were at the core of 2 studies, 3 were centered on carbon storage/loss, while the remaining dealt with stream erosion or wildlife - mainly bird and bird nesting habitat (Taylor and Neary, 2008b). Identified as an area of concern by all authors was the paucity of studies addressing rotational grazing and its impact on the environment, and yet the number of farms employing grazing as a management strategy has increased from 7.3% in 1993 to 26% in 2005 (Brock and Barham, 2009).

While MIRG's benefits for production economics are increasingly well established, farmers using MIRG realized cost reductions per milk hundred weight equivalent (CWT EQ) when compared to confinement operations. Although milk production per cow was lower with

grazing, the reduction in overall farm operating costs makes MIRG economically competitive with confinement operations (Kriegl and McNair, 2005). However, its agronomic and ecological consequences remain mostly anecdotal, and the scientific basis for how management affects ecosystem production and function is lacking. We identified three primary areas of need to extend our knowledge on the effects of management on pasture in a subhumid climate: 1) forage production and quality, 2) carbon accumulation or loss, i.e., net ecosystem carbon balance, and 3) soil microbial biomass and community composition.

Forage production and quality

Productivity of pastures in much of the upper Midwest is limited to April through October, so promoting high quality forage production for summer grazing and winter storage is critical to dairy and beef farm profitability. Grazing systems are less important or not important in regulating forage production and quality in rangelands of the West. The ecological underpinning is that plant-herbivore and plant-plant interactions are decoupled. However, in grasslands of the upper Midwest, plant community dynamics may behave in an equilibrium manner (Woodis and Jackson, 2008). If so, defoliation by large herbivores may impact plant-plant interactions and drive plant community composition through competitive advantage. As a management tool, managing the temporal and spatial distribution of livestock may be beneficial to improving production. In Chapter 1, I examined the effects of intensively managing grazing pressure (MIRG), in comparison with less intensive continuous grazing (CONT) on forage production and quality. What is novel about this study is that we held stocking rate constant between grazing treatments. This was done to assess the impacts of managing spatial and temporal defoliation directly to herbivore directed defoliation. Also, to compare other

management strategies typical of the upper Midwest, we did this in comparison with haymaking (HARV), and land removal from management (NONE).

Net ecosystem carbon balance

Human activities and land use practices have altered local, regional, and global C cycles (Vitousek et al., 1997). The terrestrial biosphere is a known sink for CO_2 (IPCC 2001), but annual C balance is strongly dependent on management (Skinner, 2008). Strategies that increase pasture productivity and improve forage quality are thought to provide opportunities to increase C storage. Return of nutrients and plant-derived C in the form of manure, and the quality and distribution of the C inputs can influence C sequestration (Schnabel et al., 2001). Pastures that are hayed or continuously grazed with low stocking densities are thought to have less C sequestration potential, while MIRG is thought to enhance C sequestration. In Chapter 2 we used an ecosystem carbon balance approach to quantify the net ecosystem C accumulation or loss in subhumid pastures. As markets for carbon credits develop and expand accurate estimates of carbon storage or loss will be necessary to inform policy and the monetary value of effective land contracts (Chapin et al., 2006; Smith et al., 2007).

Soil microbial biomass and community composition

The effects of grazing management on ecosystem structure and function are highly variable. However, in more mesic climates management of the timing and spatial distribution of livestock is of paramount importance to maintaining or improving net primary production. In these environments, grazing may enhance pasture production directly via effects on plant developmental stage and community composition (Wardle et al., 2004) and/or indirectly by making nutrients more available to plants from excreta and enhanced litter quality leading to

accelerated nutrient mineralization (Frank and Groffman, 1998). Numerous studies have shown that grazing addition, or increasing the intensity of grazing may result in changes in total soil microbial biomass and changes in microbial community composition that may impact C and N mineralization rates and seemingly promote aboveground biomass production (Klumpp et al., 2007). Conversely, cessation or reduction of grazing intensity leads to reductions in key ecosystem processes (Bardgett et al., 1996; Bardgett et al., 2001; Grayston et al., 2004). In Chapter 3, I test the extent to which pasture management affects soil microbial biomass and communities, and explore potential predictors of community patterns.

The research goals of this study were to: 1) provide a scientific basis from which farmers can inform decisions about how best to manage rural landscapes under the mounting pressure to simultaneously produce, maintain, and develop ecosystem services such as air, soil and water quality, biodiversity and aesthetics, and 2) contribute to the basic science of agronomy and ecology by generating knowledge about management effects on pasture ecosystems that will assist agencies, and policymakers.

LITERATURE CITED

Bardgett, R.D., Hobbs, P.J., Frostegard, A. 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils* 22, 261-264.

Bardgett, R.D., Jones, A.C., Jones, D.L., Kemmitt, S.J., Cook, R., Hobbs, P.J. 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. *Soil Biology & Biochemistry* 33, 1653-1664.

Brock, C., Barham, B. 2009. Farm structural change of a different kind: Alternative dairy farms in Wisconsin - graziers, organic and Amish. *Renewable Agriculture and Food Systems* 24, 25-37.

Bultena, G., Lasley, P., Geller, J. 1986. The farm crisis - Patterns and impacts of the financial distress among Iowa farm families. *Rural Sociology* 51, 436-448.

Buttel, F.H. 2003. Envisioning the Future Development of Farming in the USA: Agroecology Between Extinction and Multifuntionality. New Directions in Agroecology Research and Education, University of Wisconsin - Madison.

Cochrane, W.W. 1993. The Development of Agriculture: A Historical Analysis. University of Minnesota Press, Minneapolis, MN.

Cronon, W. 1984. Changes in the Land." Indians, Colonists and the Ecology of New England. Hill and Wang, New York.

Cross, J.A. 2001. Geographical record note change in America's dairyland. *Geographical Review* 91, 702.

Fales, S.L., S. A. McMurry, McSweeny, W. T. 1992. The role of pasture in northeastern dariy farming: historical perspectives, trends, and research imperatives for the future. *In*: Sims, T. (Ed.), Agricultural Research in the Northeastern United States: Critical Review and Future Perspectives. American Society of Agronomy, Madison, WI, pp. 111-132.

Frank, D.A., Groffman, P.M. 1998. Ungulate vs. landscape control of soil C and N processes in grasslands of Yellowstone National Park. *Ecology* 79, 2229-2241.

Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L., Clegg, C.D., Ritz, K., Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J., Millard, P. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. *Applied Soil Ecology* 25, 63-84.

Hassanein, N., Kloppenburg, J.R. 1995. Where the grass grows again: Knowledge exchange in the sustainable agriculture movement. *Rural Sociology* 60, 721-740.

Jackson, R.D. 2006. Grazing research town hall meeting at Agronomy/soils field day. Grass Clippings. Center for Integrated Agricultural Systems, University of Wisconsin - Madison, pp. 5-7.

Klumpp, K., Soussana, J.F., Falcimagne, R. 2007. Effects of past and current disturbance on carbon cycling in grassland mesocosms. *Agriculture Ecosystems & Environment* 121, 59-73.

Kriegl, T., McNair, R. 2005. Patures of plenty: Finanacial performance of Wisconsin's grazing dairy farms. Center for Integrated Agricultural Systems, University of Wisconsin-Madison.

Larsen, H.J. 1959. Methods of Forage Utilization in the Midwest. *Journal of Dairy Science* 42, 574-578.

Lasley, P., Hanson, M. 2003. The Changing Population of the Midwest: A Reflection on Opportunities. *In*: Walzer, N. (Ed.), The American Midwest: Managing Change in Rural Transition. M. E. Sharpe, Inc., Armonk, NY.

Lewthwaite, G.R. 1964. Wisconsin Cheese and Farm Type: A Locational Hypothesis. *Economic Geography* 40, 95-112.

Liebhart, W.C. 1993. Farmer experience with rotational grazing: A case study approach. *In*: Liebhart, W.C. (Ed.), The dairy debate: Consequences of bovine growth hormone and rotational grazing technologies. University of California, Sustainable Agriculture Research and Education Program, Davis CA.

Mariola, M.J., Stiles, K., Lloyd, S. 2005. The social implications of management-intensive rotational grazing: an annotated bibliography. Center for Integrated Agricultural Systems, University of Wisconsin - Madison.

Murphy, B.M. 1991. Greener pastures on your side of the fence: Better farming with Voisin grazing management. 3rd ed. Arriba Publishing, Colchester, VT.

Ostrom, M., Jackson-Smith, D. 2000. The Use and Performance of Management Intensive Rotational Grazing Among Wisconsin Dairy Farms in the 1990s. Program on Agricultural Technology Studies, University of Wisconsin - Madison. Research Report No. 8.

Paine, L.K., Klemme, R.K., Undersander, D.J., Welsh, M. 2000. Wisconsin's Grazing Networks: History, Structure, and Function. *Journal of Natural Resources and Life Sciences Education* 29, 60-67.

Schnabel, R.R., Franzluebbers, A.J., Stout, W.L., Sanderson, M.A., Stuedemann, J.A. 2001. The Effects of Pasture Management Practices. *In*: Follett, R.F., J. M. Kimball, and R. Lal (Ed.), The Potential off U. S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect. Lewis Publishers, Boca Raton, pp. 291-322.

Skinner, R.H. 2008. High biomass removal limits carbon sequestration potential of mature temperate pastures. *Journal of Environmental Quality* 37, 1319-1326.

Taylor, J., Neary, S. 2008. How does managed grazing affect Wisconsin's environment? Center for Integrated Agricultural Systems, University of Wisconsin - Madison.

Turner, F.J. 1894. The significance of the frontier in American history. U.S. Government Printing Office Washington, pp. 199, 201, 226-227.

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M. 1997. Human domination of Earth's ecosystems. *Science* 277, 494-499.

Voisin, A. 1959. Grass Productivity. Philosophical Library Inc., New York.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H. 2004. Ecological linkages between aboveground and belowground biota. *Science* 304, 1629-1633.

Woodis, J.E., Jackson, R. D. 2008. Subhumid pasture plant communities entrained by management. *Agriculture, Ecosystems and Environment* 129, 83-90.

Chapter 1. Management-intensive rotational grazing promotes higher productivity and forage quality of subhumid pastures

ABSTRACT

Management-intensive rotational grazing is used by many farmers seeking to balance profitability, environmental stewardship, and quality of life. Productivity of pastures in much of the upper Midwest is limited to April through October, so promoting high quality forage production for summer grazing and winter storage is critical to dairy and beef farm profitability. We conducted a field experiment on pastures dominated by Kentucky bluegrass (*Poa pratensis* L.), orchardgrass (*Dactylis glomerata* L.), meadow fescue (*Festuca elatior* L.), perennial ryegrass (*Lolium perenne* L.), and white clover (*Trifolium repense* L.) to compare forage production, relative forage quality, and belowground net primary production under management-intensive rotational grazing, continuous grazing, haymaking, and land that has been set aside with no agronomic management. Grazing treatments were implemented with similar stocking rates, and all treatments were established in May 2005 in a randomized complete block design with three blocks. In 2005, it was apparent that much of the biomass in the continuously grazed pastures was being refused by livestock. We found potential utilizable forage was significantly greater under management-intensive rotational grazing when compared to the other treatments. For each treatment year relative forage quality was also significantly higher in management-intensive rotational grazing. In 2006 belowground net primary production in the surface 15-cm was significantly lower under grazed treatments compared to non-grazed treatments. Substantially higher belowground net primary production was produced under all treatments in 2007, with the management removal treatment producing significantly more biomass than the grazed and hayed treatments. While all management treatments had a negative impact on

belowground production, our results point to management-intensive rotational grazing as a viable grazing management alternative to continuous grazing and haymaking in terms of both forage production and quality.

INTRODUCTION

Non-equilibrium models of vegetation change are frequently promoted for arid and semi-arid grasslands because equilibrium models inadequately describe plant community dynamics (Westoby et al., 1989; Jackson and Bartolome, 2002; Bestelmeyer et al., 2003). A recent review by Briske et al. (2008) showed that grazing systems were less important than grazing intensity to plant community response in rangelands of the West. However, in mesic grasslands of the upper Midwest, Woodis and Jackson (2008a) showed that plant community dynamics behave in an equilibrium way - biotic factors such as grazing management drive vegetation composition. If so, enhanced forage production and quality may be achieved by delaying plant maturation, reducing shading of young leaves, excluding unpalatable species, and altering nutrient cycling through management (Schuman et al., 1999; Bardgett and Wardle, 2003). In grasslands of mesic climates where weather is more predictable than in arid and semi-arid regions, limited research, observation, and anecdote have indicated that rotational grazing promotes productivity. When compared to continuous grazing, management-intensive rotational grazing is credited with efficient use of land (Phillip et al., 2001), greater livestock weight gain and parasite reduction (Papadopoulos et al., 1993), and increased pasture productivity (Paine et al., 1999; Fales et al., 1995).

Linking aboveground net primary production to belowground plant response to grazing is thought to be essential for long-term sustainability of production (DeAngelis and Huston 1993). While aboveground production may be improved, grazing may have a negative effect on root systems (Milchunas and Lauenroth, 1993). Grasses respond to defoliation by increasing C allocation to new leaves while decreasing allocation to roots (Detling et al., 1979). Repeated

frequent simulated grazing resulted in decreased root biomass, root N reserves and, C allocation to leaves was enhanced at the expense of root biomass (Turner et al., 1993; Schuman et al., 1999). But root biomass may affected differentially along a grazing chronosequence, and root production may be less affected with adequate rest periods between defoliation events (Holland and Detling, 1990).

Management-intensive rotational grazing has been adopted by a large number of dairy and beef farmers in Wisconsin and the upper Midwest. Where grazing was once considered the antithesis of technological innovation, it is now an established practice (Taylor and Foltz, 2006; Hassanein and Kloppenburg, 1995). This management strategy entails livestock grazing in relatively small paddocks at high densities, but for short durations. In a management-intensive rotational grazing system, large pastures are divided into smaller paddocks and once a sward height objective is reached in the paddock being grazed, the herd is moved to the next paddock. Management-intensive rotational grazing has been publicized as beneficial to both farmers and livestock, for social, economic, and production (both quantity and quality) benefits (Paine et al., 2000). And, while this type of grazing operation typically results in lower livestock production and output when compared to confinement systems, it requires lower capital inputs, thereby decreasing the economic risk and increasing the flexibility and profit of the individual dairy farmer (Frank, 1995). Despite the potential advantages of management-intensive rotational grazing, Paine et al. (1999) reported that ~ 690 thousand pasture hectares in Wisconsin remain in unmanaged continuous grazing.

Improved pasture is land that has been sown with preferred forage and legume species, and is maintained with management inputs; irrigation, fertilizer, and periodic cultivation to maintain or re-establish preferable species. Management of improved pasture typically includes

nitrogen fertilization. Introduced cool-season grass and forb species are managed for production yield and forage quality, and are harvested by livestock, mechanical means, or both (Schnabel et al., 2001). Mechanically harvested forage is used for winter feeding or as a major feeding component in confinement operations.

Within the North Central Region of the United States, which contains ~ 9 M ha of pasture, Wisconsin contains just less than 1 M ha of permanent improved pasture and cropland that is used exclusively as pasture for grazing livestock (Vough, 1990). Jackson-Smith et al. (1996) estimated that ~ 15% of all dairy operations in Wisconsin maintained some form of grazing as a management strategy. In 2006, an updated grazing study indicated this estimate had grown to ~ 25% (Taylor and Foltz, 2006). In addition to dairy operations, the Wisconsin beef industry is also flourishing with ~ 23% of the state's total cattle population (Taylor and Lehmkuhler, 2008). Taylor and Lehmkuhler (2008) also report that ~ 40% of beef farmers rotate their cattle through pasture, ~33% continuously graze, and the remainder use confinement systems.

The objectives of this study were to test how pasture management practices typical of the upper Midwest – management-intensive rotational grazing, continuous grazing, haying, and no agronomic management - affect pasture yield, quality, and root production. Our hypotheses were that management-intensive rotationally grazed plots would have 1) greatest forage production, 2) highest forage quality characteristics for livestock consumption, and 3) greater belowground net primary production than continuous grazing, but not haymaking and land removed from agronomic management.

METHODS

Study site – experimental design

This study was conducted at the Franbrook Farm, a research property in south central Wisconsin, USA (lat 42°44'16.65"N, long 89°45'13.27"W). The climate is continental with warm summers and cold winters. Average temperatures range from ~ -7° C in January to 22 °C in July. For our study period (2006 to 2007) mean temperature range was 0 to 22 and -5 to 21 °C, respectively. Mean annual precipitation is ~ 900 mm, of which ~ 100 mm is from snow. Approximately two-thirds of the annual precipitation falls during the growing season, April to October. Yearly precipitation totals for 2006 and 2007 were 691 and 583 mm, respectively. The research area is ~ 26 ha of valley bottom pasture dominated by cool-season grasses. Soils in this area are ~ 90 cm deep and are classified as Otter silt loam (Cumulic Endauolls), Arenzville silt loam (Typic Udefluvent), and Huntsville silt loam (Cumulic Hapludolls) (USDA, NRCS, 2007). In the top 15-cm of soil; bulk density, pH, organic carbon, and total nitrogen are 1.15 g cm^{-3}, 6.8 (H_2O), 4.2, and 0.31% respectively. Elevation range at the farm is 265 to 320 m above sea level.

In April 2005, a randomized complete block (RCBD) field experiment with three blocks and four treatments was established. The four treatments mimicked management-intensive rotational grazing (MIRG), continuous grazing (CONT), periodic harvesting of pasture forages for hay (HARV), and management removal (NONE) (Fig. 1). Beginning in May 2005, MIRG paddocks (0.6 ha) were grazed by separate 25 cow-calf pair herds (1 pair = 1.3 animal units [AU]) at high animal stocking rates (i.e., instantaneous stocking rate of 54 AU ha^{-1}) for a brief duration of ~ 2 d (i.e., a stocking rate of 108 AU d $month^{-1}$), and then allowed to rest for 28-29 d (Paine et al. 1999). Continuous pastures (8.1 ha) had lower instantaneous stocking rates (i.e., 4

AU ha^{-1}), but pastures were rested only during the 2 d that the herd was confined to the paddocks, resulting in a comparable stocking rate to MIRG (i.e., a stocking rate of 112 AU d $month^{-1}$). Plant biomass was mechanically harvested and removed in May and mid-August 2006, and May and late July 2007 from the HARV plots (0.3 ha) to mimic the making of hay typical to confinement operations. Finally, we set aside 0.3 ha plots for no agronomic management (NONE) to mimic a conservation reserve program site. To represent standard management practice, granular ammonium phosphate (11-44-0) fertilizer was applied at the University of Wisconsin Extension recommended rate (57 kg N ha^{-1}) to all treatment areas except NONE at the beginning of June in 2005, 2006, and 2007.

Vegetation sampling

Starting in May 2006 and continuing through October 2007, aboveground net primary production (ANPP) was estimated monthly for both MIRG and CONT. In the HARV plots, biomass production was estimated for three growth periods: April through May, June to mid-July in 2006 and June through July in 2007, and from the end of the second growth period through October. Biomass in the NONE treatment was estimated for three growing periods: April through June, July through September, and September through October. Biomass was estimated using Leaf Area Index (LAI), which is defined as the amount of leaf area in a canopy per unit ground area. In 2005, at multiple time points throughout the season, LAI was calculated as a function of intercepted photosynthetically active radiation (PAR). Intercepted PAR was determined by measuring incoming PAR above and below the leaf canopy in randomly placed 0.1-m^2 quadrats with an AccuPAR LP-80 Ceptometer (Decagon Devices, Inc., Pullman, WA [see Campbell and Norman, 1989]). Biomass was harvested from these quadrats, dried at 60° C

for 48 h, and weighed. An allometric equation was developed from the relationship between LAI measured and biomass (r^2=0.72). Production in MIRG paddocks was calculated as the difference between pre-grazing event measurements and post grazing measurements of the previous grazing event. For CONT pasture, grazing exclusion cages were randomly located and moved each month. Biomass was estimated inside and outside the cage, and monthly production was calculated as the difference in biomass estimated from inside the cage minus biomass estimated outside the cage.

At the beginning of the season in all treatments, and during the season and at the end of the season in NONE, live and dead fractions were calculated using a line-point transect. Five 10-m transects were randomly located in each treatment, and point determinations of live or dead were made at each decimeter resulting in 100 point determinations in each treatment at each sampling date. Biomass estimates were adjusted by multiplying total biomass by percentage of live biomass to reflect incremental growth of live biomass for the given growth period. In order to quantify potential forage for utilization by livestock in the grazing treatments, five randomly placed 1-m × 20-m band transects were sampled for each season (spring, summer, and fall) to determine refused and non-utilized forage. Production in our study is defined as potential utilizable forage (PUF), and was quantified by incorporating the estimates of refused and non-utilized biomass into our estimates of total production.

Belowground net primary production (BNPP) was estimated in 2006 and 2007 from root in-growth cores (Fahey 1999). Five 5-cm diameter × 15-cm deep mesh cores containing a neutral soil medium (75% field soil and 25% sand) were installed within each treatment at the beginning of the growing season (April). The cores were harvested at the end of the season

(October), and the roots were washed free of soil over a 1-mm sieve, dried at 60 °C for 48 h, and weighed.

To estimate forage quality, grab samples of biomass were harvested monthly from April to October in 2006 and 2007. In order to compare forage *on offer*, or forage that grazing animals would be exposed to at the time they were turned into the pasture, samples were taken at any given time for CONT (monthly at the same time as biomass estimates), and immediately before animals were turned into the paddock for MIRG (sampling in the MIRG paddocks was done at the same time as pre-grazing biomass estimates were made). Five random samples of standing biomass were harvested from each treatment, composited, and then dried at 65 °C for 48 h. Samples were ground through a 1-mm screen in a Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ), and analyzed for forage quality attributes at the University of Wisconsin Forage Lab (Madison, WI) by Near Infrared Reflectance Spectroscopy (NIRS) using a Foss NIR Model 6500 (Foss NIRSystems Inc., Laurel, MD). We report forage quality as Relative Forage Quality (RFQ), which is a single numeric index that reflects the sum of forage quality attributes measured in a forage sample. Relative forage quality is calculated from predicted values of voluntary forage intake, and the estimated available energy derived from that forage (Appendix A). In this way, it is similar to the more common Relative Feed Value (RFV) that was developed to market forages and to be used as a forage education tool (Rohweder et al., 1978). But, because RFQ is applicable over a greater range of forage types and improves estimation of dry matter intake of high-quality grasses, it is the preferable index to use for cool season grasses (Moore and Undersander, 2002).

Soil cores to 15-cm depth were used to estimate nitrogen (N) availability. Duplicate 10 g subsamples were weighed out, with: one subsample for immediate inorganic N extraction in 2 *M*

KCl, and one that was aerobically incubated for a period of seven days and then extracted to measure net N mineralization following Robertson (1999). Extracts were frozen prior to colorimetric NH_4^+ (method #12-107-06-2-A) and NO_3^- (method #12-107-04-1-B) determination on a Lachat QuikChem flow injection analyzer (Lachat Instruments, Milwaukee, WI).

Cover was estimated in late July (2005 through 2007) and late September (2006 and 2007). Five 10-m transects were randomly located within each treatment plot. At 50-cm intervals along each transect a sharpened point was lowered from above the vegetation and the first plant species intercepting the point was recorded (Heady et al., 1959). Absolute cover was calculated by dividing species intercepts by total intercepts. The cover estimates by species were categorized into the following functional groups, C3 cool-season grasses, perennial legumes, and non-leguminous forbs.

Statistical analysis

Averages calculated from sub-samples within each experimental unit (paddock) were used in ANOVA linear mixed-effects (LME) modeling using the maximum likelihood algorithm. All modeling was done using S-Plus 7.0 (Insightful Corp., Seattle, WA, 2005). Saturated models were constructed to analyze response variables (y) as a function of management treatments (T_j), once we accounted for the random effect of block (b).

$$y_{ijk} = \beta_0 + T_j + (b)_k + \varepsilon_{ijk}$$

$i = (1,\ldots, 12), j = (1,\ldots, 4), k = (1, 2, 3)$

$b_k \sim N(0, \sigma_1^2), \varepsilon_{ijk} \sim N(0, \sigma^2)$

To test the significance of the fixed effect, we dropped the treatment term and compared a model with only the intercept term to the model including the treatment term using likelihood

ratios (Crawley, 2002). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criterion (AIC) was retained. If not, we selected the simpler model. If treatment was significant, treatment levels were sequentially collapsed and subsequent models were compared with likelihood ratio tests using the same approach of model selection. Separate model selection procedures were run for each year (2006 and 2007) to test for treatment effects on PUF, BNPP, and RFQ. To test for treatments effects on PUF and RFQ by season, separate models were run for each season within year, spring (April through May), summer (June through August), and fall (September through October).

RESULTS

Potential utilizable forage

The ANOVA LME showed that treatment had a significant effect on total PUF production in both 2006 and 2007 (model 1; Table B1 and 2). For 2006, model comparisons collapsing treatment levels showed that none of the treatments could be combined without significantly increasing residual deviance (models 3 - 8; Table B1). For 2007 CONT and HARV were not different, but all other model combinations were not warranted (models 3 - 7; Table B2). Total production of PUF was greatest in MIRG for both years, while the NONE treatment had the lowest total production (Fig. 2).

Treatment had a significant effect on PUF for each season within year (models 9, 15, and 22; Table B1, and 8, 15, and 21; Table B2), but comparison of total production within season was less consistent than year (Fig. 2). Total PUF production in spring 2006 was greatest in CONT, while in 2007 it was greatest in the treatment plots of MIRG, CONT, and HARV (models 11 - 14; Table B1). The most notable spring pattern was total PUF in the MIRG

treatment increased from 325 g m^{-2} in 2006 to 459 g m^{-2} in 2007 (41%), while production in the other treatments either decreased (CONT and NONE) or stayed the same (HARV) (Fig. 2).

Summer 2006 saw a 115% increase in PUF over spring under the MIRG treatment (325 g m^{-2} to 698 g m^{-2}), while CONT, HARV, and NONE production were all less than their spring totals (Fig. 2). Summer totals for 2007 were less definitive than 2006 with the greatest changes over spring occurring as a substantial production increase under HARV, a marked decrease under CONT and NONE, and the maintenance of spring production levels throughout the summer under the MIRG treatment (Fig.2). Model comparisons for 2006 showed MIRG PUF was significantly greater than all treatments (models 17 - 21; Table B1). However this pattern differed slightly in 2007, with MIRG and HARV showing significantly greater PUF than CONT and NONE (models 17 - 20; Table B2).

Model comparisons for fall showed PUF was significantly greater under MIRG and CONT in 2006, and under MIRG in 2007 (models 24 -27; Table B1, and 23 - 27; Table B2). Potential utilizable forage production in fall under each treatment was less than their spring and summer totals (Fig 2). Of note is the low variability - when compared to the other treatments - in production under the MIRG treatment across seasons in 2007.

Forage quality on offer

In 2006 and 2007, there was a significant treatment effect on RFQ (model 1; Table B3 and B4). Model comparisons for 2006 showed that RFQ was significantly different for all treatment comparisons (models 3 - 8; Table B3). Relative forage quality was highest under the MIRG treatment (Fig. 3). For 2007 the pattern was similar in that MIRG had the highest RFQ, and MIRG and NONE were significantly different from each other. However, unlike 2006 the

CONT and HARV treatments were not different (models 3 - 7; Table B4). After MIRG the highest values of RFQ were realized under CONT and HARV (Fig. 3).

As we might expect due to the first flush of growth, treatment comparisons showed spring RFQ values in both years were not different, except under the NONE treatment for 2006 (models 10 - 12; Table B3, and model 9; Table B4) (Fig. 3). When RFQ was modeled for summer 2006, estimates under MIRG and HARV treatments were significantly greater than CONT and NONE (models 15 - 18; Table B3) (Fig. 3). For summer 2007, RFQ was highest in the MIRG treatment followed by HARV, CONT, and NONE, respectively (Fig. 3). All treatments were significantly different from each other (models 12 - 17; Table B4). Comparisons of fall RFQ values showed significantly higher RFQ under the MIRG treatment in both years (models 21 - 25; Table B3, and 20 - 24; Table B4). The pattern of fall RFQ between treatments was consistent for both years (Fig. 3).

Belowground net primary production

In 2006 and 2007, there was a significant treatment effect on BNPP (model 1 and 7; Table B5). Treatment comparisons for 2006 showed that MIRG and CONT were not different, and HARV and NONE were not different (models 3 - 6; Table B5), and belowground production was significantly greater in the HARV and NONE treatments. This pattern was repeated in 2007 for the grazing treatments, but unlike 2006 the HARV treatment was significantly less productive than NONE (models 9 - 11; Table B5). In 2007, belowground production was greatest under NONE (Fig. 4), and all treatments had greater BNPP than 2006 with increases of 121, 81, 75, and 35% for MIRG, NONE, CONT, and HARV, respectively.

Vegetation cover

Estimates of cover by functional group showed no significant directional trends between years for all treatments for except for an increase in grass cover in the HARV treatment (p=0.04)(Fig. 5). At the species level, the CONT treatment contained significantly greater Kentucky bluegrass cover (p<0.05) while containing significantly less orchard grass cover (p=<0.05) than all other treatments. Also, the proportion of bare ground was significantly greater in the CONT treatment (p=<0.05)(Table 1).

Net nitrogen mineralization

Average net N mineralization across the 2006 and 2007 growing seasons was significantly affected by treatment (p=0.02). Rate of N mineralization was 0.31 ± 0.04, 0.22 ± 0.02, 0.08 ± 0.07, and -0.04 ± 0.15 µg N gds-1 d-1 (mean ± SE), for CONT, MIRG, HARV, and NONE, respectively (Fig. 6).

DISCUSSION

Potential utilizable forage

Our estimates of greater total PUF under MIRG for both 2006 and 2007 support the hypothesis that cool-season grass pasture is sensitive to timing, and frequency of grazing. This is an indication that the relationship between plant production and foliar removal is affected by the distribution of grazing pressure in space and time for a given stocking rate, and is beneficial as a production strategy over stocking rate alone in this mesic setting. Our results compare favorably with a mensurative study in Wisconsin by Paine et al. (1999) who compared these with a survey approach, and a clipping study by Volesky et al. (2007) that found significantly more forage available under frequent clipping (average clipping frequency over the growing season 17.8

times) to 21-cm stubble height when compared to less frequent clipping (6.7 times) to 7-cm stubble height.

Directional shifts in functional groups or decreased absolute cover of preferred species, could affect forage production and quality (Deak et al., 2009). The directional trends in plant cover by functional group in our study showed no pattern between years except for an increase in grass cover in the HARV treatment (Fig. 5). This supports ecosystem stability and equilibrium models of herbivore-plant and plant-plant interaction dynamics, e.g., selective grazing by livestock dampens plant-plant competition, giving less palatable species an advantage when grazing pressure is low on an areal basis, a condition found in continuously grazed pastures (Milchunas and Lauenroth, 1993; Derner and Hart, 2007). In contrast, in the MIRG and HARV treatments competitive interactions were heightened. Homogeneous biomass removal left all plants in a state of being equally capable of responding to resources availability, and thus homogeneous compensatory growth. Management for short duration grazing events at high intensity may increase forage production by reducing the ability of the animals to graze selectively (Bardgett et al., 1998).

Managed grazing had seasonal benefits as well and it is likely that graziers will realize greater PUF and higher RFQ during the summer months. Most of the total seasonal growth of cool-season grasses occurs in the spring; as much as 60% of growth is attained by July (Riesterer et al., 2000), so the ability to manage for adequate forage production and quality through the summer is a key challenge (Paine et al., 1999). Cool-season grasses require as much as 2.5 times longer for re-growth in summer as they do in spring; a condition commonly referred to as the *summer slump* (Smart et al., 1995; Paine et al., 1996; Balasko, 2003; Brink et al., 2007). Others have reported production peaks early in the growing season with a decline in production as the

growing season progresses (Phillip et al., 2001; Paine et al., 1999; Popp et al., 1995). Our results were similar with the notable exception of greater summer production under MIRG. In our experiment, summer PUF under MIRG was significantly greater than CONT in both years (Fig. 2). This result portends a positive production benefit to rotational graziers during the summer slump when forage production traditionally suffers in a continuously grazed system. More impressive, this result occurred when both years had precipitation totals that were less than average, and in 2007 when lower than average precipitation total was coupled with a 2-week drought in late July.

We observed no difference in PUF between defoliation treatments in fall of 2006, and production declined relative to both spring and summer. In 2007 there was a significant fall production advantage under MIRG. This was surprising given the droughty conditions of summer and that there were no differences in soil moisture status between treatments (data not shown). A possible explanation is that MIRG maintained the vegetation in the re-growth phase giving the plant community an advantage when conditions subsequently became favorable for growth. While available soil water and ambient temperature may affect growth within a season, i.e., the *summer slump*, our treatment differences appear to be driven by herbivore-plant interactions. Strategically managing the timing, duration, and intensity of grazing may allow graziers to capitalize on the seasonal growth patterns of cool-season grasses.

Relative forage quality

As grazing farmers seek to balance profitability despite lower farm output compared to confinement operations, forage quality becomes increasingly important for livestock performance, and for lowering feed costs by reducing the need for supplements (Parker et al.,

1992). Forage utilization and livestock performance are known to increase as forage quality increases. A RFQ estimate of 137 is the level at which forage is deemed medium quality. At this level, growing cattle would gain 0.6 kg d^{-1} and lactating cows would produce 10 kg milk d^{-1} (Moore and Undersander, 2002). Our results show that MIRG was the only treatment that consistently met this target value, with the exception of spring and summer 2006 (Fig. 2). This supports our second hypothesis that MIRG would positively benefit forage quality.

Forage quality under various defoliation regimes can fluctuate between seasons (Paine et al., 1999; Popp et al., 1997; White et al., 2004; Volesky, 2007). Popp et al. (1997) found that quality of forage declined with season in alfalfa-grass pastures in Canada, and in New Zealand pastures dominated by perennial rye (*Lolium perenne* L.). White et al. (2004) documented that forage quality was highest in the fall when compared to summer, but neither Popp nor White found differences in quality between continuous and rotational grazing systems. Our results do not directly support these findings. Relative forage quality estimates did not differ between treatments in spring (Fig. 3), and this was not surprising given that spring growth in all treatments results in new foliar tissue of high nutrient concentrations. In contrast, summer and fall RFQ estimates were highest under MIRG (Fig. 3), which is an important finding for graziers because during the *summer slump* MIRG treatments not only offer a greater quantity of forage, but forage that is of higher quality. This supports MIRG as a viable alternative grazing management to CONT and HARV in terms of forage quality. Even in years when precipitation was less than normal, MIRG produced forage that was of consistent quality for livestock performance and profitability.

It has been suggested that optimization of forage production and quality is a function of defoliation timing relative to growth state of the vegetation (see Turner et al., 1993), exclusion of

less palatable species (Menke and Bradford, 1992; Bardgett et al., 1998), and increased nutrient cycling (Frank and Groffman, 1998; Conant et al., 2003; Le Roux et al., 2003). Our results support this, as significant differences in PUF and RFQ were most likely influenced by 1) maintenance of the plant community in a homogeneous vegetative state - variability in sward height was almost double in CONT plots when compared to the MIRG plots (data not shown), 2) functional group stability (Fig. 5), 3) retention of taller bunch grasses such as orchard grass (Table 1), and 4) increased rates of net N mineralization (Fig. 6).

Root production

Grazing lands in the temperate latitudes are thought to contain a significant portion of the terrestrial carbon (C) sink, and understanding root contribution to this pool is important as it represents a major organic C input in grasslands (Reeder et al., 2001). A reduction in root production under grazing lands may represent a major pathway for C loss. Also, linking the effects of grazing on plant production belowground is thought to be essential for long-term sustainability of aboveground production (DeAngelis and Huston, 1993). However, the effects of defoliation on root growth are complex (McNaughton et al., 1998). In our study no differences in BNPP were found between grazing treatments, but we did find substantially less root biomass under disturbed treatments when compared to the NONE plots (Fig. 4). Our hypothesis that MIRG would promote root production relative to CONT was not confirmed.

Grasses respond to defoliation by increasing C allocation to new leaves while decreasing allocation to roots (Detling et al., 1979), and there is some evidence from simulated grazing trials that repeated frequent defoliations result in decreased root biomass, root N reserves, and increased C allocation to leaves (Turner et al., 1993; Schuman et al., 1999). Grazing intensity, time between grazing events, plant community composition, and grazing tolerance of the plant

community have been shown to impact belowground biomass production both positively (see review by Milchunas and Lauenroth, 1993), negatively (Ferraro and Oesterheld, 2002; Holland et al., 1992; Guitian and Bardgett, 2000), and in a neutral manner (Milchunas and Lauenroth, 1993; Johnson and Matchett, 2001). Our results compare favorably with the results of Woodis and Jackson (2008b) who found smooth brome (*Bromis inermis* Lyess), a major component of many cool-season pastures, was affected negatively by both intensity and frequency of clipping relative to an unclipped control, but not studies such as Biondini et al. (1998) that compared the effect of stocking rate in northern mixed-grass prairie and found BNPP was not affected by moderate grazing when compared to ungrazed sites, but that BNPP decreased as intensity of grazing increased. For contrast, Turner et al. (1993) found no effect of grazing or mowing on BNPP in tallgrass prairie dominated by warm-season grasses.

Reduced BNPP under the grazing and haying treatments relative to the NONE treatment may reflect 1) alteration of vegetation structure and composition (see Holland et al., 1992 for references), 2) accelerated rates of carbon and nitrogen cycling reducing the need for root growth to compensate for lower N availability (Fig.6) (Bardgett et al., 1998; McNaughton et al., 1998), and 3) fine root turnover (Pucheta et al., 2004). It is most likely the result of all of the above mechanisms, coupled with differential carbon allocation of photosynthate; as a result of frequent defoliations photosynthates are directed to rebuilding aboveground biomass rather than to belowground root production and accumulation of reserves (Holland et al., 1992).

Management Implications

In their review of experiments comparing rotational grazing strategies with continuous grazing, Briske et al. (2008) reported that rotational grazing systems can be a viable management

option in arid and semi-arid grazing land, but that stocking rate was the most important management tool regardless of grazing strategy. They concluded that managed grazing systems are not superior to continuous grazing in stimulating plant production or animal performance, for the fundamental reason that management is unable to balance maintenance of leaf area for production while at the same effectively utilizing production for sufficient animal performance. This view is well established for arid and semi-arid rangelands (Barnes et al., 2008). For mesic systems, what has not been well established is whether stocking rate alone or controlling the distribution of grazing pressure in space and time is beneficial for forage production and quality. In fact, the evidence is equivocal, with some researchers reporting little or no benefit from rotational systems when compared to continuous grazing (Dale et al., 2008; Popp et al., 1997; Bransby, 1991), while others have found production benefits in livestock weight gain and parasite reduction (Papadopoulos et al., 1993), increased pasture productivity (Paine et al., 1999; Fales et al., 1995), and land use efficiency (Phillip et al., 2001).

Our results provide evidence against the argument that stocking rate alone is the key to managing mesic plant communities through grazing. In our system where abiotic factors were not limiting for plant growth, plant-plant and herbivore-plant interactions appear to be tightly coupled, and to be the main drivers of community structure by dampening competitive advantage (Wiens, 1984). Management of the spatial distribution of livestock and timing of defoliations positively impacted biomass production, increasing forage production and quality in both years (Figs. 2 and 3) while having a negative impact fine root production (Fig. 4). These results support the hypothesis that our pasture behaved as an equilibrium ecosystem. It then follows that in a pasture systems with equilibrium dynamics, carefully managing grazing pressure may be beneficial as a production strategy over stocking rate alone. Our results are an indication that

with grazing management, land managers have the opportunity to manipulate the plant community response toward management objectives.

SUMMARY

In the upper Midwest, adoption of managed grazing systems has relied on mensurative studies and anecdotal evidence to support claims that they result in superior production and forage quality. Our goal was to quantify forage production and quality in a more rigorous experimental framework. We compared pasture management strategies typical of the upper Midwest with particular focus on the effects of grazing strategy on biomass production and forage quality. We held stocking rate constant between grazing strategies to appropriately examine the effects of spatial and temporal control of defoliation on forage production and quality, and root production.

The results of this study demonstrate that mesic pastures behave as equilibrium ecosystems and, to enhance forage production and quality, MIRG is a beneficial management strategy for mesic pastures. Where abiotic factors are not limiting for plant growth, plant-plant and plant-herbivore interactions are tightly coupled and managing grazing frequency, timing, and intensity of defoliations confers an advantage to forage production and quality over stocking rate alone.

LITERATURE CITED

Balasko, J. A., Nelson, C. J. 2003. Grasses for northern areas. *In:* R. F. Barnes, C. J. Nelson, M. Collins and K. J. Moore (Ed.). Forages: An Introduction to Grassland Agriculture. Ames, IA: Iowa State Univ. Press. p. 125–143.

Bardgett, R. D., Wardle, D. A., Yeates, G. W. 1998. Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biology & Biochemistry* 30, 1867-1878.

Barnes, M. K., Norton, B. E., Maeno, M., Malechek, J. C. 2008. Paddock size and stocking density affect spatial heterogeneity of grazing. *Rangeland Ecology & Management* 61, 380-388.

Bestelmeyer, B. T., Brown, J. R., Havstad, K. M., Alexander, R., Chavez, G., Herrick, J. 2003. Development and use of state-and-transition models for rangelands. *Journal of Range Management* 56, 114-126.

Biondini, M. E., Patton, B. D., Nyren, P. E. 1998. Grazing intensity and ecosystem processes in a northern mixed-grass prairie, USA. *Ecological Applications* 8, 469-479.

Bransby, D. I. 1991. Biological implications of rotational and continuous grazing: A case for continuous grazing. *In* Proc. Am. Forage Grassl. Conf.; Columbia, MO: Am. Forage Grassl. Counc. Gergetown, TX p. 10-14.

Brink, G. E., Casler, M. D., Hall, M. B. 2007. Canopy structure and neutral detergent fiber differences among temperate perennial grasses. *Crop Science* 47, 2182-2189.

Briske, D. D., Derner, J. D., Brown, J. R., Fuhlendorf, S. D., Teague, W. R., Havstad, K. M., Gillen, R. L., Ash, A. J., Willms, W. D. 2008. Rotational grazing on rangelands: Reconciliation of perception and experimental evidence. *Rangeland Ecology & Management* 61, 3-17.

Campbell, G. S., Norman, J. M. 1989. An introduction to environmental biophysics. New York: Springer Verlag.

Cherney, D. J. R., Cherney, J. H. 2005. Forage yield and quality of temperate perennial grasses as influenced by stubble height. Online. *Forage and Grazinglands*. doi:10.1094/FG-2005-0215-01-RS.

Conant, R. T., Six, J., Paustian, K. 2003. Land use effects on soil carbon fractions in the southeastern United States. I. Management-intensive versus extensive grazing. *Biology and Fertility of Soils* 38, 386-392.

Crawley, M. 2002. Statistical Computing: An Introduction to Data Analysis Using S-Plus. John Wiley and Sons, West Sussex, England.

Dale, A. J., Mayne, C. S., Laidlaw, A. S., Ferris, C. P. 2008. Effect of altering the grazing interval on growth and utilization of grass herbage and performance of dairy cows under rotational grazing. *Grass and Forage Science* 63, 257-269.

DeAngelis, D. L., Huston, M. A. 1993. Further considerations on the debate over herbivore optimization theory. *Ecological Applications* 3, 30-31.

Deak, A., Hall, M. H., Sanderson, M. A. 2009. Grazing Schedule Effect on Forage Production and Nutritive Value of Diverse Forage Mixtures. *Agronomy Journal* 101, 408-414.

Derner, J. D., Hart, R. H. 2007. Grazing-induced modifications to peak standing crop in northern mixed-grass prairie. *Rangeland Ecology & Management* 60, 270-276.

Detling, J. K., Dyer, M. I., Winn, D. T. 1979. Net photosynthesis, root respiration, and regrowth of bouteloua-gracilis following simulated grazing. *Oecologia* 41, 127-134.

Fahey, T. J., Bledsoe, C. S., Day, F. P., Ruess, R. W., Smucker, A. J. (Ed.). 1999. Fine root production and demography. New York: Oxford University Press.

Fales, S. L., Muller, L. D., Ford, S. A., Osullivan, M., Hoover, R. J., Holden, L. A., Lanyon, L. E., Buckmaster, D. R. 1995. Stocking rate affects production and profitability in a rotationally grazed pasture system. *Journal of Production Agriculture* 8, 88-96.

Ferraro, D. O., Oesterheld, M. 2002. Effect of defoliation on grass growth. A quantitative review. *Oikos* 98, 125-133.

Frank, D. A., Groffman, P. M. 1998. Ungulate vs. landscape control of soil C and N processes in grasslands of Yellowstone National Park. *Ecology* 79, 2229-2241.

Frank, G. R., Klemme, R., RajBhandary, B., Tranel, L. 1995. Economics of alternative dairy grazing scenarios. Dep. of Agric. Econ., University of Wisconsin - Madison. Managing the farm 28(3).

Guitian, R., Bardgett, R. D. 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant and Soil* 220, 271-277.

Hassanein, N., Kloppenburg, J. R. 1995. Where the grass grows again: Knowledge exchange in the sustainable agriculture movement. *Rural Sociology* 60, 721-740.

Heady, H. F., Gibbens, R. P., Powell, R. W. 1959. A comparison of the charting, line intercept, and line point methods of sampling shrub types of vegetation. *Journal of Range Management* 12, 180-188.

Holland, E.A., Detling, J.K. 1990. Plant-response to herbivory and belowground nitrogen cycling. *Ecology* 71, 1040-1049.

Holland, E. A., Parton, W. J., Detling, J. K., Coppock, D. L. 1992. Physiological-responses of plant-populations to herbivory and their consequences for ecosystem nutrient flow. *American Naturalist* 140, 685-706.

Jackson, R. D., Allen-Diaz, B. 2006. Spring-fed wetland and riparian plant communities respond differently to altered grazing intensity. *Journal of Applied Ecology* 43, 485-498.

Jackson, R. D., Bartolome, J. W. 2002. A state-transition approach to understanding nonequilibrium plant community dynamics in Californian grasslands. *Plant Ecology* 162, 49-65.

Jackson-Smith, D., Barham, B.L., Nevius, M., Klemme, R. 1996. Grazing in Dairyland: the use and performance of Management Intensive Rotational Grazing among Wisconsin dairy farms. Program on Agricultural Technology Studies. Agricultural Technology and Family Farm, University of Wisconsin - Madison, Institute Technical Report No. 5.

Johnson, L. C., Matchett, J. R. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. *Ecology* 82, 3377-3389.

McNaughton, S. J., Banyikwa, F. F., McNaughton, M. M. 1998. Root biomass and productivity in a grazing ecosystem: The Serengeti. *Ecology* 79, 587-592.

Le Roux, X., Bardy, M., Loiseau, P., Louault, F. 2003. Stimulation of soil nitrification and denitrification by grazing in grasslands: do changes in plant species composition matter? *Oecologia* 137, 417-425.

Menke, J., Bradford, G. E. 1992. Rangelands. *Agriculture Ecosystems & Environment* 42,141-163.

Milchunas, D. G., Lauenroth, W. K. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63, 327-366.

Moore, J. E., Undersander, D. J. 2002. Relative Forage Quality: An alternative to relative feed value and quality index. *In*: Proceedings of the 13th Annual Florida Ruminant Nutrition Symposium; University of Florida, Gainsville. p. 16-32.

Moore, J. E., Kunkle, W. E. 1999. Evaluation of equations for estimating voluntary intake of forages and forage-based diets. *Journal of Animal Science.* (Suppl. 1), 204.

Paine, L. K., Undersander, D., Casler, M. D. 1999. Pasture growth, production, and quality under rotational and continuous grazing management. *Journal of Production Agriculture* 12, 569-577.

Paine, L. K., Klemme, R. K., Undersander, D. J., Welsh, M. 2000. Wisconsin's Grazing Networks: History, Structure, and Function. *Journal of Natural Resources and Life Sciences Education* 29, 60-67.

Paine, L., Undersander, D. J., Sample, D. W., Bartelt, G. A., Schatteman, T. A. 1996. Cattle trampling of simulated ground nests in rotationally grazed pastures. *Journal of Range Management* 49, 294-300.

Papadopoulos, Y. A., Kunelius, H. T., Fredeen, A. H. 1993. Factors influencing pasture productivity in Atlantic Canada. *Canadian Journal of Animal Science* 73, 699-713.

Parker, W. J., Muller, L. D., Buckmaster, D. R. 1992. Management and economic implications of intensive grazing on dairy farms in the Northeastern States. *Journal of Dairy Science* 75, 2587-2597.

Phillip, L. E., Goldsmith, P., Bergeron, M., Peterson, P. R. 2001. Optimizing pasture management for cow-calf production: the roles of rotational frequency and stocking rate in the context of system efficiency. *Canadian Journal of Animal Science* 81, 47-56.

Popp, J. D., McCaughey, W. P., Cohen, R. D. H. 1997. Effect of grazing system, stocking rate and season of use on diet quality and herbage availability of alfalfa-grass pastures. *Canadian Journal of Animal Science* 77, 111-118.

Pucheta, E., Bonamici, I., Cabido, M., Diaz, S. 2004. Below-ground biomass and productivity of a grazed site and a neighbouring ungrazed exclosure in a grassland in central Argentina. *Austral Ecology* 29, 201-208.

Reeder, J. D., Franks, C.D., Milchunas, D.G. 2001. Root biomass and microbial processes. *In:* R. F. Follett, J. M. Kimball, and R. Lal (Ed.). The Potential off U. S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect. Boca Raton: Lewis Publishers. pp. 139–166.

Riesterer, J. L., Casler, M. D., Undersander, D. J., Combs, D. K. 2000. Seasonal yield distribution of cool-season grasses following winter defoliation. *Agronomy Journal* 92, 974-980.

Rohweder, D. A., Barnes, R. F., Jorgensen, N. 1978. Proposed hay grading standards based on laboratory analyses for evaluating quality. *Journal of Animal Science* 47, 747-759.

Roxburgh, S. H., Wilson, J. B. 2000. Stability and coexistence in a lawn community: experimental assessment of the stability of the actual community. *Oikos* 88, 409-423.

Schnabel, R. R., Franzluebbers, A. J., Stout, W. L., Sanderson, M. A., Stuedemann, J. A. 2001. The Effects of Pasture Management Practices. *In:* R. F. Follett, J. M. Kimball, and R. Lal (Ed.). The Potential off U. S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect. Boca Raton: Lewis Publishers. p. 442.

Schuman, G. E., Reeder, J. D., Manley, J. T., Hart, R. H., Manley, W. A. 1999. Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. *Ecological Applications* 9, 65-71.

Smart, A. J., Undersander, D. J., Keir, J. R. 1995. Forage growth and steer performance on Kentucky bluegrass vs sequentially grazed Kentucky bluegrass-switchgrass. *Journal of Production Agriculture* 8, 97-101.

Solter, U., Hopkins, A., Sitzia, M., Goby, J. P., Greef, J. M. 2007. Seasonal changes in herbage mass and nutritive value of a range of grazed legume swards under Mediterranean and cool temperate conditions. *Grass and Forage Science* 62, 372-388.

Taylor, J., Foltz, J. 2006. Grazing in the Dairy State: Pasture Use in the Wisconsin Dairy Industry, 1993 – 2003 Center for Integrated Agricultural Systems & Program on Agricultural Technology Studies, University of Wisconsin - Madison: Madison, Wisconsin. Special Reports No. 2.

Taylor, J., Lehmkuhler, J. 2008. The Wisconsin Beef Cattle Industry: A Snapshot. Center for Integrated Agricultural Systems, University of Wisconsin - Madison: Madison, Wisconsin.

Turner, C. L., Seastedt, T. R., Dyer, M. I. 1993. Maximization of aboveground grassland production – the role of defoliation frequency, intensity, and history. *Ecological Applications* 3, 175-186.

Volesky, J. D., Anderson, B. E. 2007. Defoliation Effects on Production and Nutritive Value of Four Irrigated Cool-Season Perennial Grasses. *Agronomy Journal* 99, 494-500.

Vough, L. R. 1990. Grazing Lands in the East. *Rangelands* 12:251-255.

Westoby, M., Walker, B., Noymeir, I. 1989. Range management on the basis of a model which does not seek to establish equilibrium. *Journal of Arid Environments* 17, 235-239.

White, T. A., Barker, D. J., Moore, K. J. 2004. Vegetation diversity, growth, quality and decomposition in managed grasslands. *Agriculture Ecosystems & Environment* 101, 73-84.

Wiens, J. A. 1984. On understanding a non-equilibrium world: myth and reality in community patterns and processes. *In:* D. R. Strong, Simberlong, D., Abele, K. G. and Thistle, A. B. (Ed.). Ecological Communities: Conceptual Issues and the Evidence. Princeton: Princeton University Press. p. 439-457.

Woodis, J. E., Jackson, R.D. 2008a. Subhumid pasture plant communities entrained by management. *Agriculture, Ecosystems and Environment* 129, 83-90.

Woodis, J. E., Jackson, R.D. 2008b. Above- and belowground production of smooth brome and big bluestem under clipping frequencies and heights to mimic rotational and continuous grazing. *Grass and Forage Science* 63, 458-4

Table 1. Mean (SE) percent cover of dominant taxa across last 2 experimental years (2006-2007). Significant differences shown by different superscript letters. Significant differences determined by ANOVA linear mixed effects models, p<0.05.

			Treatment							
Functional group	*Common name*	*Scientific name*	*CONT*		*MIRG*		*HARV*		*NONE*	
Annual grass	Hairy crabgrass	*Digitaria sanguinalis*	0.2	(0.2)						
Cool season	Kentucky bluegrass	*Poa pratensis*	61.6[a]	(10.6)	37.0[b]	(9.0)	43.5[b]	(4.5)	49.9[a]	(4.2)
(C3 grass)	Meadow fescue	*Festuca elatior*	2.5	(3.0)	5.0	(4.9)	1.9	(2.1)	4.1	(5.0)
	Orchardgrass	*Dactylis glomerata*	4.2[a]	(2.4)	24.9[b]	(6.2)	30.9[b]	(6.9)	14.4[b]	(8.1)
	Perennial rye	*Lolium perenne*	2.3	(2.0)	4.2	(2.8)	2.1	(2.7)	0.2	(0.2)
	Quackgrass	*Elymus repens*	0.1	(0.1)	0.1	(0.1)	1.5	(2.1)	3.0	(3.2)
	Redtop	*Agrostis gigantea*			0.3	(0.3)			0.5	(0.6)
	Reed canrygrass	*Phalaris arundinacea*			0.2	(0.2)	0.2	(0.2)	0.7	(0.7)
	Smooth brome	*Bromus inermis*	0.3	(0.2)	2.4	(2.1)	0.6	(0.5)	2.8	(1.5)
	Tall fescue	*Festuca arundinacea*	0.3	(0.4)	0.9	(1.3)	0.9	(1.0)	2.5	(2.5)
	Timothy	*Phleum pratense*	1.5	(1.6)	1.0	(1.3)	2.5	(2.5)	1.9	(2.4)
Perennial	Birdsfoot trefoil	*Lotus corniculatus*	0.6[a]	(0.7)	0.5[a]	(0.5)	7.5[b]	(4.8)	13.7[b]	(7.5)
legumes	Black medic	*Medicago lupulina*	0.8	(0.9)			0.2	(0.4)		
	Red clover	*Trifolium pratense*	0.7	(0.8)	1.4	(0.6)	0.1	(0.1)	0.1	(0.1)
	White clover	*Trifolium repense*	9.1[a]	(4.6)	15.6[a]	(8.1)	0.9[b]	(1.2)		
Forbs	Bull thistle	*Cirsium vulgare*	0.3	(0.3)	0.1	(0.1)	0.1	(0.1)	0.6	(0.8)
	Canada goldenrod	*Solidago canadensis*							0.2	(0.2)
	Canada thistle	*Cirsium arvense*					0.3	(0.5)	0.3	(0.3)
	Carolina horsenettle	*Solanum carolinense*					0.4	(0.4)	0.4	(0.4)
	Dandelion	*Taraxacum officinale*	7.2[a]	(4.7)	3.6[b]	(1.9)	4.4[b]	(2.5)	0.8[b]	(0.8)
	Giantchickweed	*Myosoton aquaticum*	0.3	(0.3)			1.0	(1.0)	0.2	(0.2)
	Rough fleabane	*Erigeron strigosus*	0.1	(0.1)						
	Smartweed	*Polygonum spp.*	0.7	(0.6)						
	Spiny plumeless thistle	*Carduus acanthoides*	0.2	(0.2)			0.2	(0.1)	0.7	(0.4)
	Wild parsnip	*Pastinaca sativa*	0.1	(0.1)					0.3	(0.5)
Sedge	Sedge	*Sedge spp.*	0.2	(0.2)	0.2	(0.2)			2.2	(2.0)
Other			3.1		2.3		0.5		0.3	
Bare ground	Bare		3.6	(2.2)	0.3	(0.3)	0.3	(0.5)	0.2	(0.2)

FIGURES

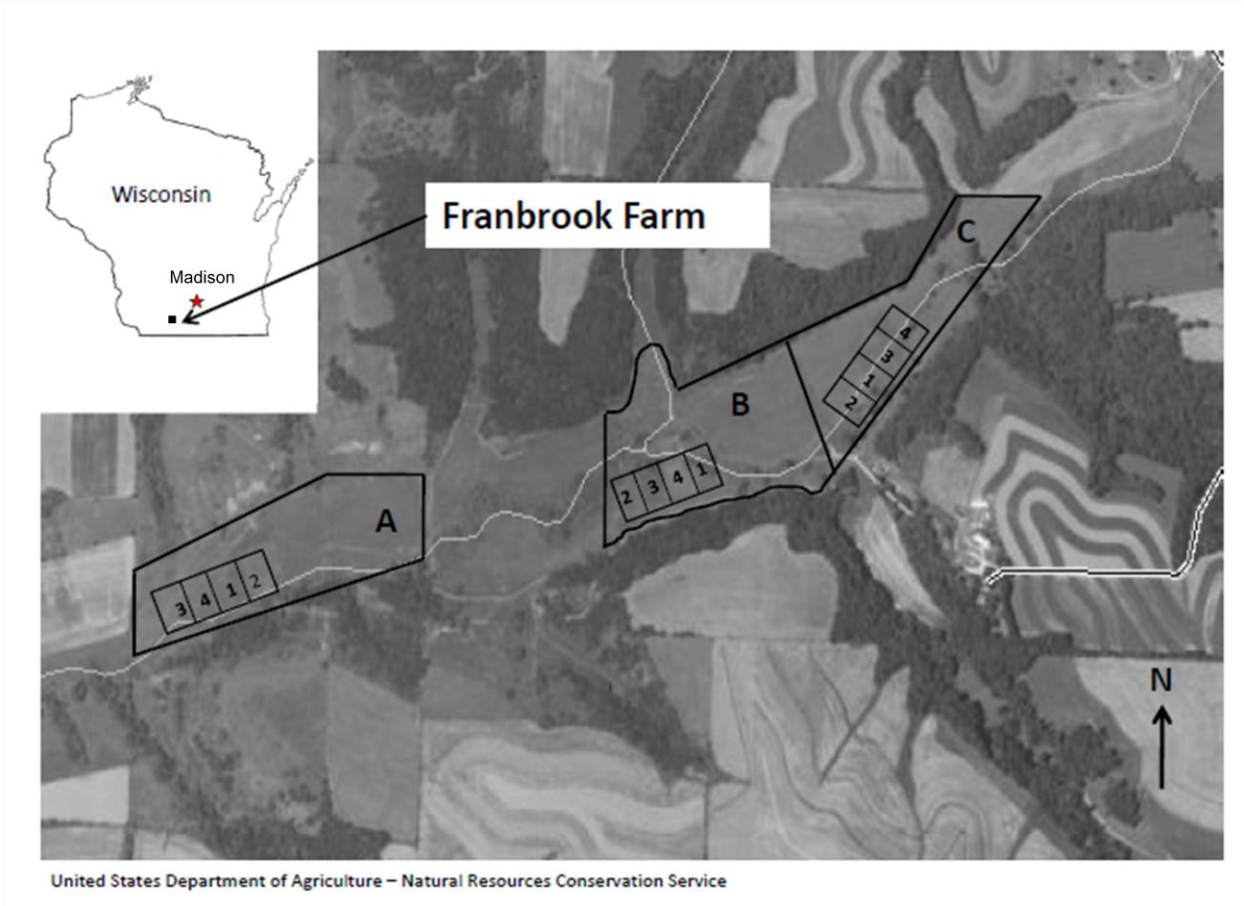

Fig. 1. Layout of Randomized Complete Block Design (RCBD) field experiment with three blocks and four treatment designated by number as: (1) management intensive rotational grazing (MIRG), (2) continuous grazing (CONT), (3) periodic harvesting of pasture forages for hay (HARV), and (4) management removal (NONE).

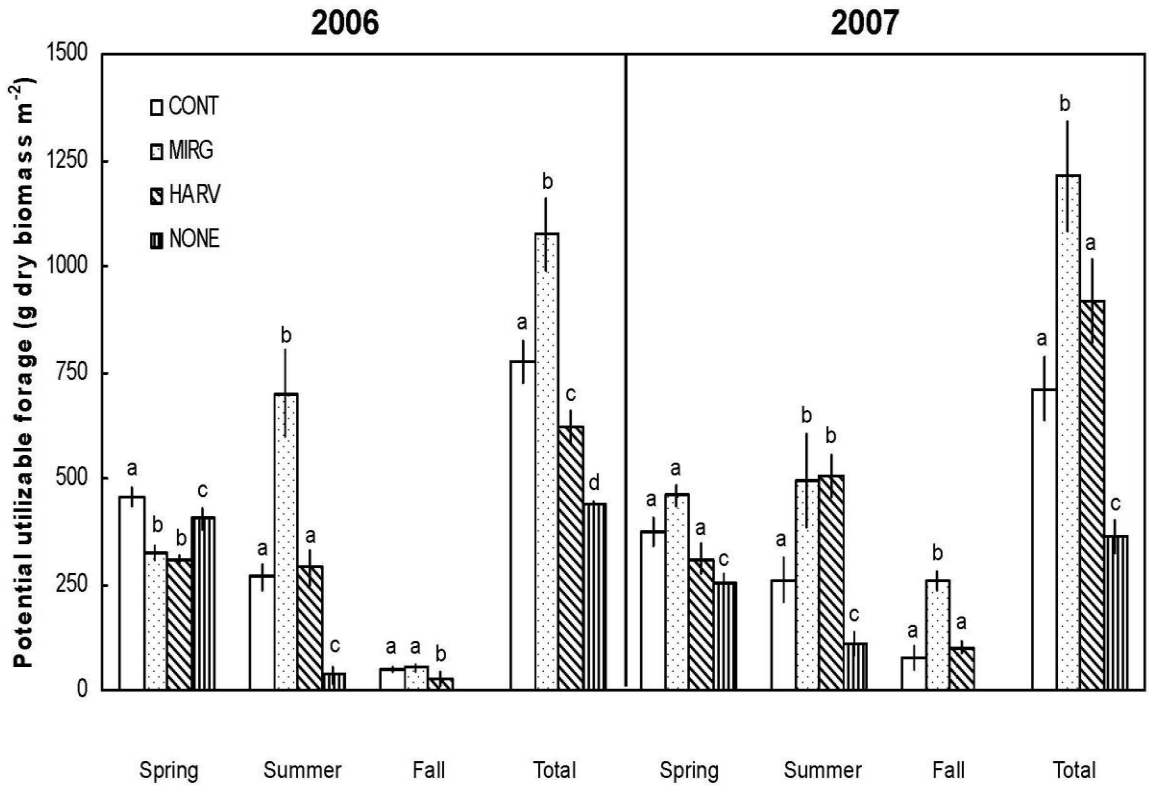

Fig. 2. Potential utilizable forage (PUF) for 2006 and 2007 (Total), and within year by season (spring, summer, and fall). Error bars show ± SE, n = 3. Means of PUF shown with different letters were determined to be significantly different using ANOVA linear mixed-effects model selection, $p<0.05$.

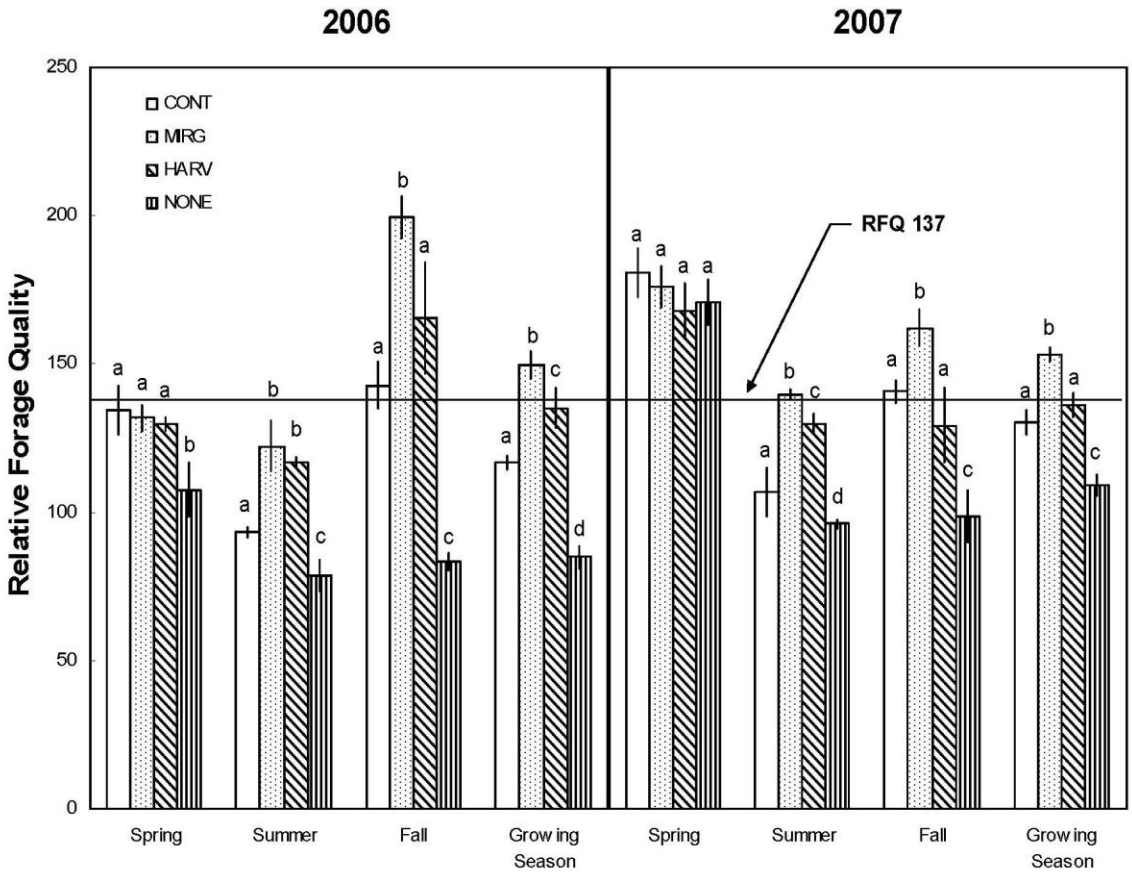

Fig. 3. Relative Forage Quality (RFQ) for 2006 and 2007 (Total), and within year by season (spring, summer, and fall). A RFQ estimate of 137 is the level at which forage is deemed medium quality. At this level growing cattle would gain 0.6 kg d^{-1}, and lactating cows would produce 10 kg milk d^{-1}. Error bars show ± SE, n = 3. Means of RFQ shown with different letters were determined to be significantly different using ANOVA linear mixed-effects model selection, $p<0.05$.

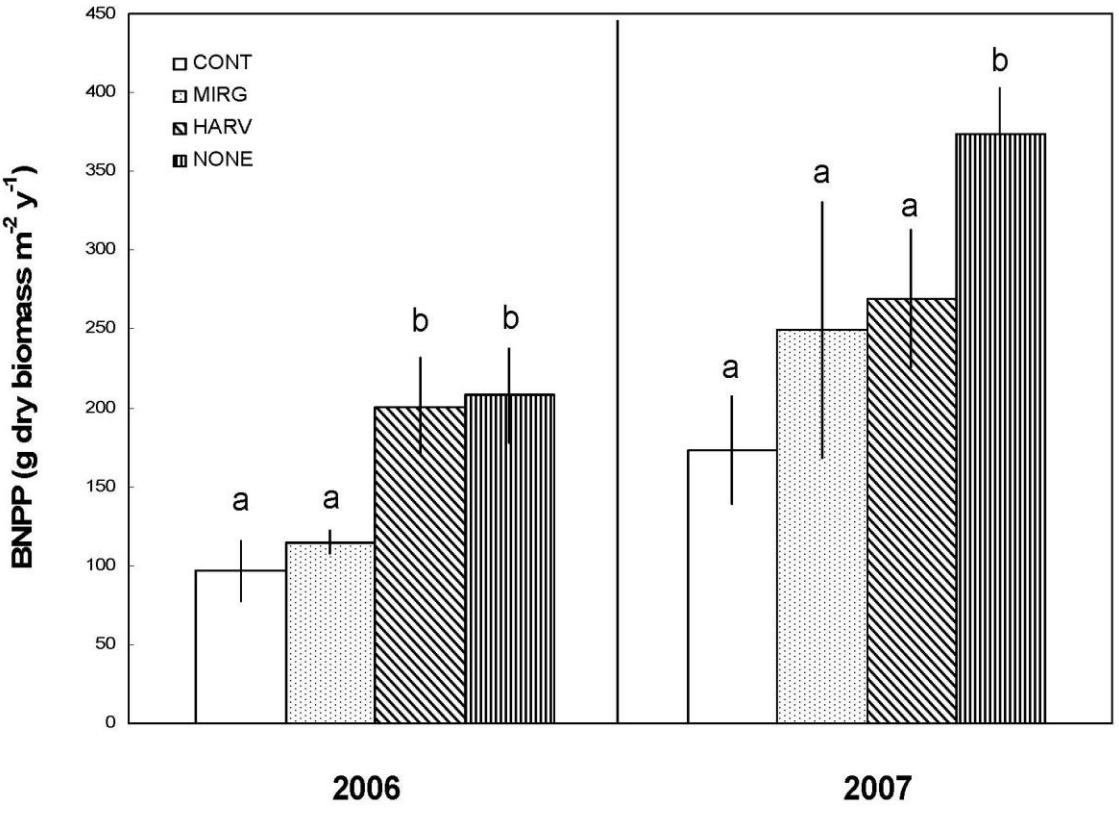

Fig. 4. Belowground net primary production (BNPP) for 2006 and 2007. Error bars show ± SE, n = 3. Means of BNPP shown with different letters above error bars were determined to be significantly different using ANOVA linear mixed-effects model selection, $p<0.05$.

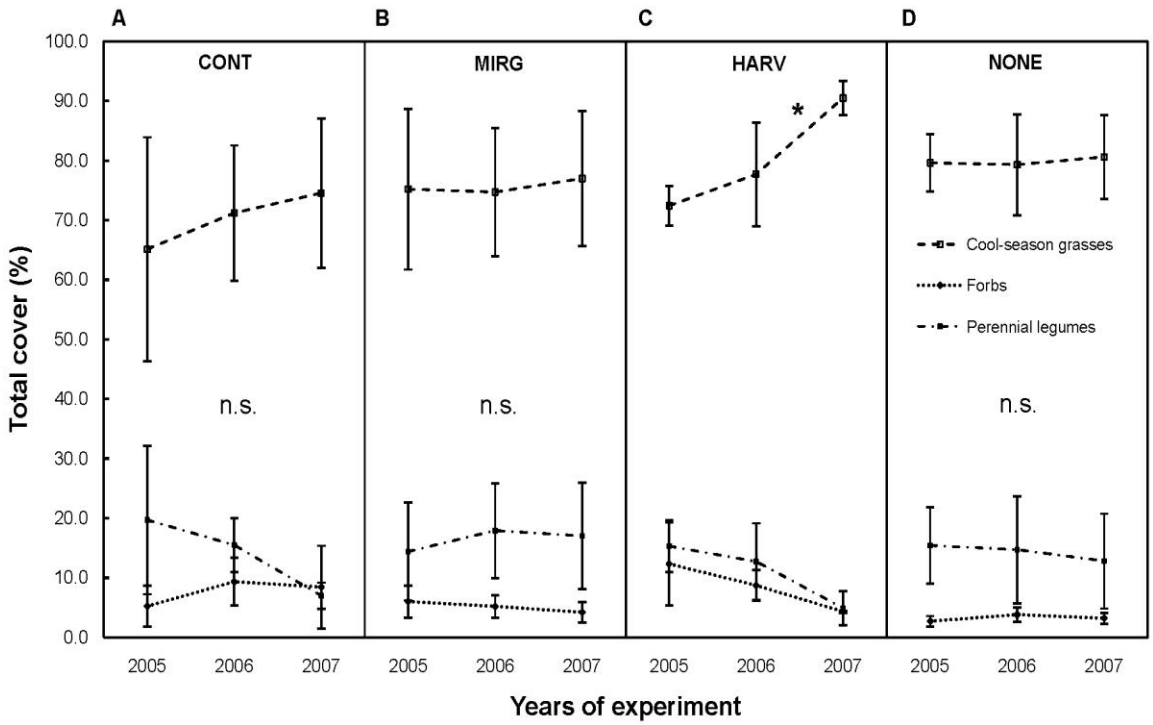

Fig. 5. Mean percent cover by year of C3 cool-season grasses, perennial legumes, and non-leguminous forbs in: (A) continuous grazing, (B) management-intensive rotational grazing, (C) harvest, and (D) no management over the course of 3 experimental years. Significant differences determined with ANOVA linear mixed-effects model selection, $p<0.05$. Error bars show ± SE, n = 3.

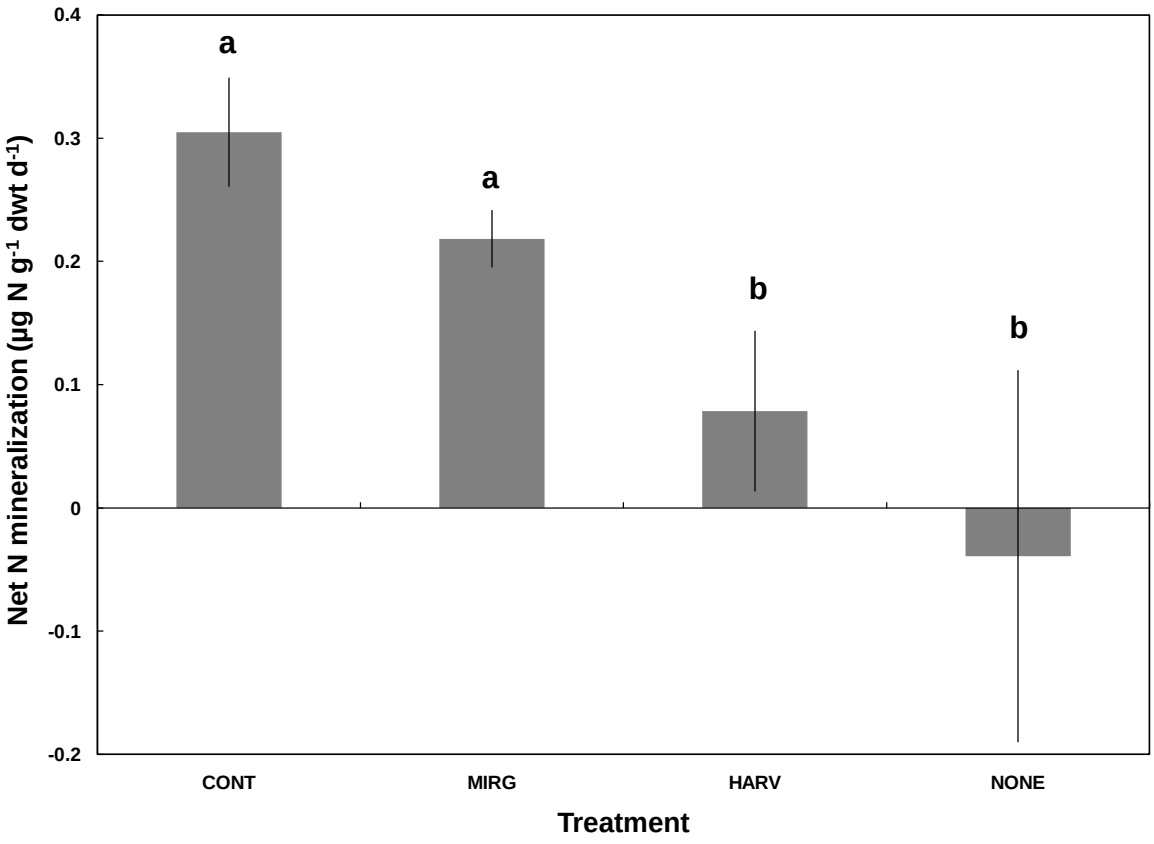

Fig. 6. Average net nitrogen (N) mineralization for 2006 to 2007 growing seasons. Error bars show ± SE, n = 3. Means of N shown with different letters were determined to be significantly different using ANOVA linear mixed-effects model selection, $p<0.05$.

Chapter 2. Livestock management strategy affects net ecosystem carbon balance of subhumid pasture

ABSTRACT

Greenhouse gas offsets traded as carbon (C) credits provide incentives for land managers to use improved land management strategies. As markets for credits develop and expand, accurate estimates of C accumulation or loss are necessary to inform policy. The terrestrial biosphere is a known sink for CO_2, and recognition of additional C sources and sinks has expanded the focus of potential C sequestration to systems such as grasslands. We used a net ecosystem carbon balance approach to quantify the net ecosystem C accumulation or loss in subhumid cool-season grass pastures under four typical livestock management practices in the upper Midwest: management-intensive rotational grazing, continuous grazing, haymaking, and land set aside with no agronomic management. Management-intensive rotational grazing lost significantly less C in 2006 than all other treatments, and in 2007 was the only treatment that was a sink for C. Our net ecosystem carbon balance model resulted in an average C change of -219 ± 31, -3 ± 46, -287 ± 16, and -195 ± 5 g C m^{-2} y^{-1} (mean ± S.E.) for continuous grazing, management-intensive rotational grazing, haymaking, and management removal, respectively. While management-intensive rotational grazing was best with respect to C balance compared to the other treatments, its C balance was positively enhanced with C inputs from outside the pasture boundary. It is important to acknowledge that these C inputs came at a cost to C accumulation elsewhere.

INTRODUCTION

Human activities and land use practices have altered local, regional, and global C cycles (Vitousek et al., 1997). Atmospheric concentrations of CO_2 have increased from 280 ppm pre-industrial concentrations (AD 1000 - 1750 [IPCC 2007]) to 385 ppm in 2008 (NOAA 2009). Most of these increases are from fossil fuel burning and land use change (IPCC 2001). In 2001, the Kyoto Protocol was expanded from recognition of C fluxes related to afforestation, deforestation and reforestation; to include terrestrial C fluxes from land management systems including forest management, crop and production systems, and revegetation (Swift, 2001). The terrestrial biosphere is a known sink for CO_2 (IPCC 2001), but annual C balance is strongly dependent on management.

Recognition of additional sources and sinks of C has expanded the focus of potential C sequestration to systems such as grasslands (Follett et al., 2001). Depending on definition, grassland ecosystems occupy 31 to 43% of the global land area (Gibson, 2009), and store 28 to 37 % of the terrestrial soil organic C pool (Lal, 2004). Improving management of grasslands using practices that increase forage production such as fertilization, irrigation, sowing favorable grasses and forbs, intensive grazing management, and conversion from cultivation to well managed pasture, provides the opportunity to sequester atmospheric C and enhance soil organic matter (SOM) (Conant et al., 2001; Soussana et al., 2004). Conversion from cultivation to permanent pasture has been estimated to increase C storage 50 to 100 g C m^{-2} y^{-1} (IPCC 2001).

The North Central Region of the United States contains ~ 9 M ha of pasture, and within this region Wisconsin contains just less than 1 M ha of highly productive subhumid improved pasture that is used exclusively for livestock grazing and mechanical harvest of forage (Vough,

1990). Improved pasture is land that has been sown with preferred forage and legume species, and is maintained with management inputs including nitrogen fertilization. Management of grazing and harvesting strategies can impact C sequestration by interacting with plant and soil communities (Schnabel et al., 2001). Strategies that increase pasture productivity and improve forage quality are thought to provide opportunities to increase C storage. It is estimated that through improved management pastures may sequester an additional 10 to 130 g C m^{-2} y^{-1} (Follet et al., 2001). Conant et al. (2001) examined 23 studies where cultivated lands were converted to pasture and 31 studies that used improved grazing as a treatment, conversion from cultivation to pasture increased mean annual soil C content by >3%, while changes in grazing management increased mean annual soil C in pasture by 2.9%.

Jackson-Smith et al. (1996) estimated that ~ 15% of all dairy operations in Wisconsin maintained some form of grazing as a management strategy. In 2006, an updated grazing study indicated this estimate had grown to ~ 25% (Taylor and Foltz, 2006). In addition to dairy operations the Wisconsin beef industry, which accounts for ~23% of the state's total cattle population, use grazing as a management tool. Approximately 40% of beef farmers rotate their cattle through pasture, ~33% continuously graze, and the remainder use confinement systems (Taylor and Lehmkuhler, 2008a). While management typically focuses on pasture productivity and forage quality by sowing improved plant species and fertility management, intensively managing grazing has the potential to enhance SOC (Follett et al., 2001). Return of nutrients and plant-derived C in the form of manure, and the quality and distribution of the C inputs can influence C sequestration (Schnabel et al., 2001). Pastures that are hayed or continuously grazed with low stocking densities are thought to have less C sequestration potential, while management-intensive rotational grazing is thought to enhance C sequestration. In a study

comparing intensive rotational grazing to extensive grazing and harvesting for hay in the southeastern United States, total soil C was 22% higher under the intensive treatment (Conant et al., 2003). In contrast, a study in Manitoba, Canada found no differences in soil C between rotationally grazed pastures and pastures grazed continuously regardless of stocking rate (Banerjee et al., 2000), and Weinhold et al. (2001) found that soil C content under moderate grazing in North Dakota pasture was greater and/or comparable to an ungrazed exclosure, while heavy grazing reduced soil C. Comparison of a remnant prairie with a restored prairie in Wisconsin showed that after 70 years the restored site had 37% less stored carbon, and large uncertainties in net ecosystem production argue for quantitative studies based on different management strategies and methodologies (Kucharik et al., 2006). While studies have considered prairie restoration, grazing effects on riparian systems, conversion of cultivated fields to pasture, and grazing comparison studies elsewhere, quantitative studies looking at C balance under intensive rotational grazing systems in sub-humid improved pastures are lacking (Schnabel et al., 2001).

Carbon sequestration provides a climate stabilization service to society that has become a policy and business issue (Chapin et al., 2006; Smith et al., 2007). Carbon markets have developed without significant scientific backing for the amount of C sequestered. Current practices for the assessment of GHG mitigation – specifically, whether C is being sequestered with proposed land management strategies - are limited by methodological gaps in quantifying terrestrial C budgets (Cihlar, 2007; Smith et al., 2007). Published estimates of C exchange have been highly variable depending on ecosystem, and have been shown to be variable between years (Post and Kwon, 2000; Brye et al., 2002). Greenhouse gas offsets traded as carbon credit contracts provide incentives for land managers to enroll in qualifying programs and increase

farm income through incorporation of improved land management. As markets for carbon credits develop and expand, accurate estimates of carbon storage or loss are necessary to inform policy and determine the monetary value of land contracts (Chapin et al., 2006; Smith et al., 2007).

We used an ecosystem carbon balance approach to quantify the net ecosystem C accumulation or loss in subhumid cool-season grass pastures under four typical livestock management practices in the upper Midwest: management-intensive rotational grazing, continuous grazing, haymaking, and land that had been set aside with no agronomic management.

METHODS

Study site

This study was conducted at the Franbrook Farm, a research property in south-central Wisconsin, USA (42°44'16.65"N, 89°45'13.27"W). The climate is continental with warm summers and cold winters. Average temperatures range from ~ -7° C in January to 22 °C in July. For our study period (2006 to 2007) mean temperature range was 0 to 22 and -5 to 21 °C, respectively. Mean annual precipitation is ~ 900 mm, of which ~ 100 mm is from snow. Approximately two-thirds of the annual precipitation falls during the growing season, April through October. Yearly precipitation totals for 2006 and 2007 were 691 and 583 mm, respectively. The research area is ~ 26 ha of valley bottom pasture dominated by cool-season grasses. Soils in this area are ~ 90 cm deep and are classified as Otter silt loam (Cumulic Endauolls), Arenzville silt loam (Typic Udefluvent), and Huntsville silt loam (Cumulic Hapludolls) (USDA, NRCS, 2007). In the top 15-cm of soil; pH, organic carbon, and total

nitrogen are 6.8 (H_2O), 4.2, and 0.31 % respectively. Elevation range at the farm is 265 to 320 m above sea level.

Experimental design

In April 2005, a randomized complete block (RCBD) field experiment with three blocks and four treatments was established. The four treatments mimicked management-intensive rotational grazing (MIRG), continuous grazing (CONT), periodic harvesting of pasture forages for hay (HARV), and management removal (NONE) (Fig. 1). Beginning in May 2005, MIRG paddocks (0.6 ha) were grazed by separate 25 cow-calf pair herds (1 pair = 1.3 animal units [AU]) at high animal stocking rates (i.e., instantaneous stocking rate of 54 AU ha^{-1}) for a brief duration of ~ 2 d (i.e., a stocking rate of 108 AU d $month^{-1}$), and then allowed to rest for 28-29 d (Paine et al., 1999). Continuous pastures (8.1 ha) had lower instantaneous stocking rates (i.e., 4 AU ha^{-1}), but pastures were rested only during the 2 d that the herd was confined to the paddocks, resulting in a comparable stocking rate to MIRG (i.e., a stocking rate of 112 AU d $month^{-1}$). Plant biomass was mechanically harvested and removed in May and mid-August 2006, and May and late July 2007 from the HARV plots (0.3 ha) to mimic the making of hay typical to confinement operations. Finally, we set aside 0.3 ha plots for no agronomic management (NONE) to mimic a conservation reserve program site. To represent standard management practice, granular ammonium phosphate (11-44-0) fertilizer was applied at the University of Wisconsin Extension recommended rate (57 kg N ha^{-1}) to all treatment areas except NONE at the beginning of June in 2005, 2006, and 2007.

Net primary production

Starting in May 2006 and continuing through October 2007, aboveground net primary production (ANPP) was estimated monthly for both MIRG and CONT. In the HARV plots, biomass production was estimated for three growth periods: April through May, June to mid-July in 2006 and June through July in 2007, and from the end of the second growth period through October. Biomass in the NONE treatment was estimated for three growing periods: April through June, July through September, and September through October. Biomass was estimated using Leaf Area Index (LAI), which is defined as the amount of leaf area in a canopy per unit ground area. In 2005, at multiple time points throughout the season, LAI was calculated as a function of intercepted photosynthetically active radiation (IPAR). Intercepted PAR was determined by measuring incoming PAR above and below the leaf canopy in randomly placed 0.1-m^2 quadrats with an AccuPAR LP-80 Ceptometer (Decagon Devices, Inc., Pullman, WA [see (Campbell, 1989)]). Biomass was harvested from these quadrats, dried at 60° C for 48 h, and weighed. An allometric equation was developed from the relationship between LAI measured and biomass (r^2=0.72). We calculated production in the MIRG and HARV paddocks as the difference between pre-grazing/harvest event measurements and post grazing/harvest measurements of the previous grazing/harvest event. For CONT pasture, grazing exclusion cages were randomly located and moved each month. Biomass was estimated inside and outside the cage, and monthly production was calculated as the difference in biomass estimated from inside the cage minus biomass estimated outside the cage.

Live and dead fraction was calculated using a line-point transect at the beginning of the season in all treatments, and the middle and the end of the season in NONE treatment. Five 10-m transects were randomly located in each treatment, and point determinations of live or dead

were made at each decimeter resulting in 100 point determinations in each treatment at each sampling date. Biomass estimates were adjusted by multiplying total biomass by percentage of live biomass to reflect incremental growth of live biomass for the given growth period.

Belowground net primary production (BNPP) was estimated in 2006 and 2007 from root in-growth cores (Fahey, 1999). Five 5-cm diameter × 15-cm deep mesh cores containing a neutral soil medium (75% field soil and 25% sand) were installed within each treatment at the beginning of the growing season (April). The cores were harvested at the end of the season (October), and the roots were washed free of soil over a 1-mm sieve, dried at 60 °C for 48 h, and weighed.

Plant cover was estimated in late July (2005 through 2007) and late September (2006 and 2007). Five 10-m transects were randomly located in a five-die pattern within each treatment plot. At twenty 1-dm intervals along each transect a sharpened point was lowered from above the vegetation and the first plant species intercepting the point was recorded (Heady et al., 1959). Absolute cover was calculated by dividing species intercepts by total intercepts. The cover estimates by species were categorized into the following functional groups, C3 cool-season grasses, perennial legumes, and non-leguminous forbs (Table 1).

Soil moisture, temperature, and bulk density

Soil samples were collected monthly from 28 April through 24 October 2006, and from 14 April through 25 October 2007. Five soil cores (2.5-cm diameter × 15-cm depth) were collected from within each plot. The samples were hand composited, placed into plastic bags, and immediately placed in coolers for transport to University of Wisconsin, Madison. Fifteen g of soil were weighed, oven-dried for 24 h at 105 °C, and then re-weighed for gravimetric soil

moisture determination. Gravimetric moisture content was converted to volumetric water content using soil bulk density from each treatment within block. Daily volumetric moisture contents were interpolated between collection dates beginning (~ 1 April) and through the end of the growing season (~ 30 November) based on soil temperatures above 0 °C. In April 2007, we estimated soil bulk density by carefully excavating 260.24-cm^3 (4.72-cm diameter × 15-cm depth) soil cores that were returned to the laboratory, dried at 105 °C for 48 h, and weighed to determine mass of soil per unit volume soil. Soil bulk density was 1.16 ± 0.08, 1.19 ± 0.06, 1.14 ± 0.07, and 1.11 ± 0.06 (mean ± SE; n = 3) for CONT, MIRG, HARV and NONE respectively. Soil temperature (15-cm depth) was measured monthly at the same time soil respiration measurements were made. Temperature was recorded between 10:00 and 16:00 hours using a temperature probe attached to a portable infrared gas analyzer (IRGA) within ~ 0.5-m of the soil respiration measurement collar. For the period of 1 January 2006 through 31 December 2007, daily maximum, minimum and average soil temperatures (0 to 10-cm) for Arlington, WI were downloaded from the Wisconsin-Minnesota (WI-MN) Cooperative Extension Agricultural Weather webpage (http://www.soils.wisc.edu/wimnext/weather.html). Daily maximum temperatures from WI-MN were correlated with daily temperatures interpolated from monthly on-site measurements (slope and 95% confidence intervals: 0.7 [0.7, 1.85]; r^2 = 0.89) using the OLS bisector method (Isobe et al., 1990) [see method explanation below]). To represent soil temperature over a 24 h period, daily average plot temperatures were then calculated using the WI-MN daily average soil temperature, corrected to plot temperatures by difference between WI-MN daily maximum and the interpolated daily maximum.

Soil respiration

Soil respiration (R_s) was measured using a Li-Cor 6400 portable CO_2 IRGA (Li-Cor Biosciences, Lincoln, NE, USA) equipped with a LI-6400-09 R_s chamber (Norman et al., 1992; Norman et al., 1997). As with soil temperature, the R_s measurements were made monthly between 10:00 and 16:00 hours. Four circular thin-walled (3.2 mm) polyvinyl chloride (PVC) collars (10-cm diameter × 5-cm height) were driven 2.5-cm into the soil surface at random locations within each treatment plot, and were repositioned for each sample date. The measurement protocol produces four estimates of CO_2 flux (each estimate an average of three CO_2 flux determinations) for each treatment plot per sample date.

To assemble annual C budgets, it is necessary to estimate daily R_s to gap fill the data when no direct measurements have been made. Two nonlinear models for predicting R_s were developed by Norman et al. (1992). They compared modeling R_s as 1) a function of soil temperature (T) and volumetric water content (θ), and 2) soil temperature, volumetric water content and LAI. Both models were found to be an acceptable predictor of R_s, although the inclusion of LAI improved the predictive ability of the model. Mowing and grazing can significantly alter R_s by reducing both canopy photosynthesis and C translocation to the rhizosphere (Bremer and Ham, 2002). In systems that are managed with grazing and haying, including LAI in models of R_s can improve the predictive accuracy of gap filling models (Bremer and Ham, 2002). To estimate daily rates of flux, nonlinear least squares regression was used to model R_s between sample dates. Daily rates were then summed to estimate annual R_s, the mass of C per unit area respired. Correlation plots of R_s against temperature showed that R_s behaved quite differently by season. Separate models for spring and fall were fit to 48 observations for

2006, and 2007. For summer, models were fit to both 2006 and 2007 data separately (36 observations for each year). The exponential model was as follows:

$$R_s = a + b \exp (c\,T + d\,\theta + e\,LAI)$$

To validate the predictive ability of each of the R_s models developed, the relationship of the slopes between observed R_s (oRs) and predicted R_s (pRs) were tested using the OLS bisector method (Isobe et al., 1990). An accurate model will result in a slope not significantly different from 1 when regressing oRs against pRs. Since there is uncertainty in both oRs and pRs, it is not clear whether oRs should be regressed on pRs, or vice versa. The OLS bisector method alternatively regresses oRs on pRs and pRs on oRs, which ascertains the slope of the ordinary least squares fit by bisecting the fit from the separate regressions. Confidence intervals (95%) were calculated by bootstrapping (Crawley, 2002) the OLS bisector slope 500 times to determine whether it bounded the 1:1 line.

Net ecosystem carbon balance

Net ecosystem carbon balance (NECB) is defined as the net rate of C accumulation or loss from an explicitly defined ecosystem (Chapin et al., 2006). For this study the ecosystem is delineated by the plant canopy, the rhizosphere, and the pasture boundary fences. Verification of soil C changes may require long-term experiments, while changes over short time periods can be measured over one or more growing seasons using the net balance of inputs and outputs. Net Ecosystem Carbon Balance measured annually for our pasture ecosystem is described by the equation:

$$NECB = NPP + I_h + I_g - R_s - E_h - E_{cb} - F_{ch4} - F_{co2} - DOC, \quad (1)$$

where NPP is equal to the sum of annual ANPP + BNPP and equals total litter inputs to the soil. Grassland intra-annual root turnover is highly variable with reported values ranging from 0.21 to 1.58 (Gill and Jackson, 2000). Root turnover was not measurable with root in-growth cores harvested at season end; therefore NECB was calculated using root production estimates from harvested root ingrowth cores increased 53% to adjust for turnover (Gill and Jackson, 2000). Total root profile was not captured with root ingrowth cores to 15-cm depth, so in addition to the fine root turnover correction we provide estimates of BNPP where 66% of root production was realized in the upper 15-cm (Jackson et al., 1996; Crush et al., 2005). The equation used to calculate BNPP was:

$$\text{BNPP} = F_r \times D_c \times \tau$$

where F_r is live fine root biomass (estimated from root ingrowth cores), D_c is a correction for sampling depth, and τ is turnover rate. Additional inputs to the ecosystem include import of hay (I_h) and grain (I_g) as supplemental feed. An accounting of hay and grain supplementation was kept on a daily basis and summed for the year. Fractional C contents of aboveground and belowground plant biomass for each treatment, and imported hay, were determined by flash combustion on finely ground subsamples of dried plant material (~10 mg) using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). The C fraction of grain was assumed to be 45% (Latshaw and Miller, 1924; Ma and Dwyer, 2001).

Carbon fixed in the ecosystem and lost as a result of heterotrophic respiration was estimated from soil respiration where; R_h is fixed C loss and is soil CO_2 efflux (heterotrophic respiration [R_h] + autotrophic respiration [R_a]) - R_a (adapted from (Hanson et al., 2000). Separating R_h and R_a for quantification is difficult, and investigation of methodologies is beyond

the scope of this study. Results from grasslands have been varied, with reported R_a ranging from 10 to 80% (Hanson et al., 2000). We adopt a value near the middle of this range, 53% from pasture as reported by Robertson et al. (1995). This value for R_a is similar to other findings for pasture and glasshouse experiments from cool-season grasses (Byrne and Kiely, 2006; Chen et al., 2006), and from modeling approaches taken by Bond-Lamberty et al. (2004) and Raich and Schlesinger (1992). Plant shoots were clipped prior to soil CO_2 efflux measurement, so R_s excludes shoot respiration. Our estimates of NECB also included C exported across the pasture boundary. In the HARV treatments hay exports (E_h) were estimated as ANPP produced over two cutting cycles. Hay was harvested for the period of April through mid-May, and mid-May through mid-August in both years. Carbon fraction was determined by NIR. Carbon leaving the pasture as livestock biomass (E_{cb}) was determined as calf body weight × 23% (Emsley, 1989). Brood cows were assumed to be the same weight when they were taken off as when they were put on the pasture.

Following the Food and Agriculture Organization of the United Nations (FAO), and Johnson and Johnson (1995), CH_4 C lost was estimated as 6% of digestible energy (DE):

$$CH_4\ C\ (g\ d^{-1}) = DE \times 0.06,$$

where DE is estimated as 6% of net energy intake (NEI), which in turn is estimated as 62.9% of DMI. To estimate C lost through livestock respiration we use the equation developed by Kirchgessner et al. (1991):

$$CO_2\ C\ (kg\ d^{-1}) = -1.4 + 0.42\ DMI + 0.045\ W,$$

where W is metabolic live weight (kg weight$^{0.75}$). And finally, we assume dissolved organic carbon (DOC) is negligible (Leake et al., 2006; Rasmussen et al., 2007).

Statistical analysis

Averages calculated from sub-samples within each experimental unit (paddock) were used in ANOVA linear mixed-effects (LME) modeling using the maximum likelihood algorithm. All modeling was done using S-Plus 7.0 (Insightful Corp., Seattle, WA, 2005). Saturated models were constructed to analyze response variables (y) as a function of management treatments (T_j), once we accounted for the random effect of block (b).

$$y_{ijk} = \beta_0 + T_j + (b)_k + \varepsilon_{ijk}$$

$i = (1,\ldots, 12), j = (1,\ldots, 4), k = (1, 2, 3)$

$b_k \sim N(0, \sigma_1^2), \varepsilon_{ijk} \sim N(0, \sigma^2)$

To test the significance of the fixed effect, we dropped the treatment term and compared a model with only the intercept term to the model including the treatment term using likelihood ratios (Crawley, 2002). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criterion (AIC) was retained. If not, we selected the simpler model. If treatment was significant, treatment levels were sequentially collapsed and subsequent models were compared with likelihood ratio tests using the same approach of model selection. Separate model selection procedures were run for each year (2006 and 2007) to test for treatment effects on ANPP, BNPP, R_s, and NECB.

RESULTS

Plant community

Estimates of cover by functional group showed no significant directional trends between years for all treatments except for an increase in grass cover in the HARV treatment (Fig. 2).

Modeling CO_2 flux

Separate models by season for spring and fall, and by season and year for summer, allowed us to predict R_s accurately. The slope of the validation lines for spring and fall were shallower and in closer relation to the 1:1 line (panels A and D; Fig.3) than the validation lines for summer of both years (panels B and C; Fig.3), but the 95% confidence intervals for all predictive equations bounded the 1:1 line. Fall had the least scatter around the 1:1 line, followed by spring, summer 2006, and summer 2007 (Fig. 3).

Ecosystem carbon inputs

Treatment effect on ANPP was significant (models 1 and 9; Table C1) with MIRG contributing significantly greater aboveground biomass in both 2006 and 2007 (Fig. 4). Aboveground NPP in CONT was significantly greater than HARV in 2006, but there were no differences between these treatments in 2007 (models 4 and 12; Table C1). The pasture system under management for harvest, be it grazing or hay making, was relatively productive with ANPP estimates ranging from 621 to 1231 g dry matter m^{-2} y^{-1} (Fig. 4). In both years the NONE treatment produced the least biomass, with ANPP totals that were 26 and 22% of MIRG totals for 2006 and 2007, respectively (Fig. 4). Treatment also had a significant effect on BNPP in both years (models 1 and 7; Table C2). Root production in CONT and MIRG was significantly less than HARV and NONE in 2006, and CONT, MIRG, HARV were all significantly less than NONE in 2007 (Fig. 4). Across both years, C contribution (g m^{-2}) to NPP from root biomass in the NONE treatment was ~3.5 times that of the grazed treatments (64 vs. 21 and 17% for CONT and MIRG, respectively), and ~2 times the HARV treatment (33%). Belowground production across both years ranged from 153 to 594 g dry matter m^{-2} y^{-1} (Fig. 4).

Only the grazed treatments received supplemental feed inputs which represent a major import of C into the system. Across both years, CONT and MIRG received C imports in the form of hay and grain that were 27 (CONT), and 20% (MIRG) of the total C inputs from these treatments into their NECB (Table 1).

Ecosystem carbon exports

Heterotrophic respiration represents the major source of C loss from our pasture C budget (Table 1). Treatments resulted in significant differences in R_s for both years (models 1 and 7; Table C3). Carbon lost as R_s was greatest in CONT and MIRG for 2006 (models 3-6; Table C3), but this pattern changed in 2007 with significantly greater C loss from CONT than all other treatments (models 9-14; Table C3).

The second greatest loss of C was realized in the HARV treatment as biomass harvest (Table 1). In 2006, C exported as hay was 36% of total C lost to the NECB for HARV. There was similar loss in 2007, with exported hay resulting in 33% of C detriment to the budget. Carbon losses in both years significantly altered the NECB for the HARV treatment, without biomass export, the value for 2006 would have been less negative and similar to the loss realized in MIRG (-127 and -108 g C m^{-2} y^{-1} for HARV and MIRG, respectively). In 2007, the biomass export was of even greater import as the sign for NECB would have changed from -192 to 65 g C m^{-2} y^{-1} without biomass removal.

Carbon lost through livestock respiration was also a major export pathway. In 2006 livestock respiration produced losses of 207 and 209 g C m^{-2} y^{-1} for CONT and MIRG, respectively. This loss was slightly greater in 2007, with CONT losing 223, and MIRG losing 225 g C m^{-2} y^{-1} (Table 1). Across both years, these losses account for 27% of C detriment to

NECB in both CONT and MIRG. Other pathways for C loss, albeit of less magnitude, were C lost as CH_4 (3% for both CONT and MIRG), and C exported as livestock biomass (< 1% for CONT and MIRG) (Table 1).

Net ecosystem carbon balance

There was a significant effect of treatment and associated C imports and exports on the C balance of our cool-season grass pastures (models 1 and 9; Table C4). Management-intensive rotational grazing lost significantly less C in 2006 than all other treatments, and in 2007 MIRG was the only treatment that was a sink for C (Fig. 5). Our model of NECB resulted in an average C change across both years of -219 ± 31, -3 ± 46, -287 ± 16, and -195 ± 5 g C m^{-2} y^{-1} (mean ± S.E.) for CONT, MIRG, HARV, and NONE, respectively (Fig. 5).

DISCUSSION

The net rate of C exchange across an explicitly defined ecosystem boundary determines whether that ecosystem is a C source or a sink over a given period of time. High spatial variability makes changes in soil C from direct soil measurements difficult to detect over periods less than decadal (Brye et al., 2002). But net ecosystem carbon balance estimates are a good approximation of this C exchange (Chapin et al., 2006). To our knowledge, this study is a unique examination of the C budgets of grazed pastures in the upper Midwest. With one exception (MIRG in 2007) all treatments resulted in net C loss in both years. Others have found grassland ecosystems to be C neutral (Suyker and Verma, 2001; Owensby et al., 2006), net C sinks (Dugas et al., 1999; Frank and Dugas, 2001; Sims and Bradford, 2001; Soussana et al., 2007), or net C sources (Bellamy et al., 2005; Kucharik et al., 2006; Schipper et al., 2007; Chou et al., 2008; Skinner, 2008). These disparate findings point to the high degree of sensitivity of

ecosystem C balance to weather patterns, soil characteristics, and type of management. Therefore, generalization of grassland C balance is not recommended and particular biophysical and management combinations must be considered.

Net primary production drove treatment differences, but this was dominated by greater ANPP. The greater ANPP we observed in the grazed and haying treatments were most likely influenced by 1) maintenance of individual plants in a state of constant regrowth (variability in sward height was almost double in CONT plots when compared to the MIRG plots [data not shown]), 2) relatively little plant community change during the study period, 3) retention preferred productive species (Ch 1), and 4) increased rates of N mineralization (Ch 1). Reduced BNPP in the grazed and hayed treatments were likely the result of accelerated nitrogen cycling rates, which have been shown to reduce root growth (Bardgett et al., 1998; McNaughton et al., 1998).

While ANPP drove our pasture C balance, it is important to note our shoot-to-root ratio was greater than 1. This is opposite of what is typical of prairie in the Midwest where roots of native grasses are generally more productive than their shoots (Wilsey and Polley, 2006). Our results suggest that grasslands dominated by introduced cool-season forage species have a greater flow of energy through aboveground biomass, and less energy stored belowground than native prairie. This has important ramifications for potential C sequestration and caution must be taken in extrapolating our C balance results across all grassland ecosystem types.

Carbon inputs as supplemental feed were critical drivers of the source-sink determination of the grazed treatments. Viewed over the 2 year period, MIRG was neutral with respect to C balance, and the magnitude of C lost in the CONT treatment was reduced. In both cases this was a result of C imported as hay and grain. The next greatest driver of source-sink determination

was metabolic respiration and enteric fermentation of livestock in the CONT and MIRG treatments, and biomass harvest for export in the HARV treatment. These losses were similar in magnitude for respiration and enteric fermentation, and biomass export, respectively. Without changing stocking rate, the losses from respiration and fermentation are unavoidable.

A useful exercise is to determine the pasture C balance with lower stocking rates. Lower stocking rates reduce respiration and enteric fermentation costs, and possibly offset the need for imported C in the form of supplemental feed. For example, in the most productive treatment MIRG, a reduction in stocking rate of 30% would be required to eliminate supplemental feed, assuming a constant and adequate supply of forage through the summer. A concomitant 30% reduction in respiration, enteric fermentation, and livestock biomass export would result in an average yearly C loss of 90 g C m^{-2}. Across the 2 years of this experiment, a reduction from 4 to 2.5 AU ha^{-1} would have been required for the MIRG plots to be C neutral. One possible option for beef and dairy farmers to offset organic C export is to use harvested biomass to summer supplement their livestock. Biomass harvested, if used within the bounds of the pasture system in which it was grown would result in no net C lost to export. If this biomass was used in the CONT or MIRG plots, average change to the C balance would have been a loss of 124 g C m^{-2} y^{-1} in the CONT treatment, and a gain of 93 g C m^{-2} y^{-1} in the MIRG treatment. For the given year in which they were harvested, the C change in HARV plots would reflect C exported to other parts of the pasture system, which could be rotated through the paddock system on a yearly basis.

CONCLUSIONS

The potential for C neutrality in perennial pasture exists, but the C balance of these ecosystems can be affected by management. Perhaps more important, imports and exports of C from pastures should be accounted for at scales broader than the pasture under consideration. When pasture NECB budgets consider all cross boundary transfer of C, management strategies other than those focused on increasing production can also affect where and how C is gained or lost.

LITERATURE CITED

Banerjee, M.R., Burton, D.L., McCaughey, W.P., Grant, C.A. 2000. Influence of pasture management on soil biological quality. *Journal of Range Management* 53, 127-133.

Bardgett, R.D., Wardle, D.A., Yeates, G.W. 1998. Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biology & Biochemistry* 30, 1867-1878.

Bellamy, P.H., Loveland, P.J., Bradley, R.I., Lark, R.M., Kirk, G.J.D. 2005. Carbon losses from all soils across England and Wales 1978-2003. *Nature* 437, 245-248.

Bond-Lamberty, B., Wang, C.K., Gower, S.T. 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? *Global Change Biology* 10, 1756-1766.

Bremer, D.J., Ham, J.M. 2002. Measurement and modeling of soil CO2 flux in a temperate grassland under mowed and burned regimes. *Ecological Applications* 12, 1318-1328.

Brye, K.R., Gower, S.T., Norman, J.M., Bundy, L.G. 2002. Carbon budgets for a prairie and agroecosystems: Effects of land use and interannual variability. *Ecological Applications* 12, 962-979.

Byrne, K.A., Kiely, G. 2006. Partitioning of respiration in an intensively managed grassland. *Plant and Soil* 282, 281-289.

Campbell, G.S., Norman, J. M. 1989. An introduction to environmental biophysics. Springer Verlag, New York.

Chapin, F.S., Woodwell, G.M., Randerson, J.T., Rastetter, E.B., Lovett, G.M., Baldocchi, D.D., Clark, D.A., Harmon, M.E., Schimel, D.S., Valentini, R., Wirth, C., Aber, J.D., Cole, J.J., Goulden, M.L., Harden, J.W., Heimann, M., Howarth, R.W., Matson, P.A., McGuire, A.D., Melillo, J.M., Mooney, H.A., Neff, J.C., Houghton, R.A., Pace, M.L., Ryan, M.G., Running, S.W., Sala, O.E., Schlesinger, W.H., Schulze, E.D. 2006. Reconciling carbon-cycle concepts, terminology, and methods. *Ecosystems* 9, 1041-1050.

Chen, C.R., Condron, L.M., Xu, Z.H., Davis, M.R., Sherlock, R.R. 2006. Root, rhizosphere and root-free respiration in soils under grassland and forest plants. *European Journal of Soil Science* 57, 58-66.

Chou, W.W., Silver, W.L., Jackson, R.D., Thompson, A.W., Allen-Diaz, B. 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. *Global Change Biology* 14, 1382-1394.

Cihlar, J. 2007. Quantification of the regional carbon cycle of the biosphere: Policy, science and land-use decisions. *Journal of Environmental Management* 85, 785-790.

Conant, R.T., Paustian, K., Elliott, E.T. 2001. Grassland management and conversion into grassland: Effects on soil carbon. *Ecological Applications* 11, 343-355.

Conant, R.T., Six, J., Paustian, K. 2003. Land use effects on soil carbon fractions in the southeastern United States. I. Management-intensive versus extensive grazing. *Biology and Fertility of Soils* 38, 386-392.

Crawley, M., 2002. Statistical Computing: An Introduction to Data Analysis Using S-Plus. John Wiley and Sons, West Sussex, England.

Crush, J.R., Waller, J.E., Care, D.A. 2005. Root distribution and nitrate interception in eleven temperate forage grasses. *Grass and Forage Science* 60, 385-392.

Dugas, W.A., Heuer, M.L., Mayeux, H.S. 1999. Carbon dioxide fluxes over bermudagrass, native prairie, and sorghum. *Agricultural and Forest Meteorology* 93, 121-139.

Emsley, J., 1989. The elements. Clarendon Press; Oxford University Press, Oxford; New York.

Fahey, T.J., C. S. Bledsoe, F. P. Day, R. W. Ruess, Smucker, A. J. M. (Ed), 1999. Fine root production and demography. Oxford University Press, New York.

Frank, A.B., Dugas, W.A. 2001. Carbon dioxide fluxes over a northern, semiarid, mixed-grass prairie. *Agricultural and Forest Meteorology* 108, 317-326.

Gill, R.A., Jackson, R.B. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* 147, 13-31.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry* 48, 115-146.

IPCC (Intergovernmental Panel on Climate Change), 2001. The Scientific Basis. In: Houghton, J.T., Ding, Y., Griggs, D.J., et al. (Eds.), Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, 881pp.

IPCC (Intergovernmental Panel on Climate Change, 2007. The Physical Science Basis. In: Solomon, S., Qin, D., Manning, M., et al. (Eds.), Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, 996 pp.

Isobe, T., Feigelson, E.D., Akritas, M.G., Babu, G.J. 1990. Linear-regression in Astronomy. *Astrophysical Journal* 364, 104-113.

Jackson-Smith, D., B.L. Barham, M. Nevius, Klemme, R. 1996. Grazing in Dairyland: the use and performance of Management Intensive Rotational Grazing among Wisconsin dairy farms. Program on Agricultural Technology Studies. Agricultural Technology and Family Farm, University of Wisconsin - Madison, Institute Technical Report No. 5.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108, 389-411.

Johnson, K.A., Johnson, D.E. 1995. Methane emissions from cattle. *Journal of Animal Science* 73, 2483-2492.

Kirchgessner, M., Windisch, W., Muller, H.L., Kreuzer, M. 1991. Release of methane and of carbon-dioxide by dairy cattle. *Agribiological Research* 44, 91-102.

Kucharik, C.J., Fayram, N.J., Cahill, K.N. 2006. A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. *Global Change Biology* 12, 122-139.

Lal, R. 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123, 1-22.

Latshaw, W.L., Miller, E.C. 1924. Elemental composition of the corn plant. *Journal of agricultural research* XXVII, 845-860.

Leake, J.R., Ostle, N.J., Rangel-Castro, J.I., Johnson, D. 2006. Carbon fluxes from plants through soil organisms determined by field (CO2)-C-13 pulse-labelling in an upland grassland. *Applied Soil Ecology* 33, 152-175.

Ma, B.L., Dwyer, L.M. 2001. Maize kernel moisture, carbon and nitrogen concentrations from silking to physiological maturity. *Canadian Journal of Plant Science* 81, 225-232.

McNaughton, S.J., Banyikwa, F.F., McNaughton, M.M. 1998. Root biomass and productivity in a grazing ecosystem: The Serengeti. *Ecology* 79, 587-592.

NOAA/ESRL (National Oceanic and Atmospheric Administration/Earth System Resarch Laboratory), www.esrl.noaa.gov/gmd/ccgg/trends (accessed 31, May 2009; verified 30 Nov. 2009).

Norman, J.M., Garcia, R., Verma, S.B. 1992. Soil surface CO-2 fluxes and the carbon budget of a grassland. *Journal of Geophysical Research-Atmospheres* 97, 18845-18853.

Norman, J.M., Kucharik, C.J., Gower, S.T., Baldocchi, D.D., Crill, P.M., Rayment, M., Savage, K., Striegl, R.G. 1997. A comparison of six methods for measuring soil-surface carbon dioxide fluxes. *Journal of Geophysical Research-Atmospheres* 102, 28771-28777.

Owensby, C.E., Ham, J.M., Auen, L.M. 2006. Fluxes of CO2 from grazed and ungrazed tallgrass prairie. *Rangeland Ecology & Management* 59, 111-127.

Paine, L. K., Undersander, D., Casler, M. D. 1999. Pasture growth, production, and quality under rotational and continuous grazing management. *Journal of Production Agriculture* 12, 569-577.

Post, W.M., Kwon, K.C. 2000. Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology* 6, 317-327.

Raich, J.W., Schlesinger, W.H. 1992. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus Series B-Chemical and Physical Meteorology* 44, 81-99.

Rasmussen, J., Eriksen, J., Jensen, E.S., Esbensen, K.H., Hogh-Jensen, H. 2007. In situ carbon and nitrogen dynamics in ryegrass-clover mixtures: Transfers, deposition and leaching. *Soil Biology & Biochemistry* 39, 804-815.

Robertson, F.A., Myers, R.J.K., Saffigna, P.G. 1995. Respiration from Soil and Litter in a Sown Perennial Grass Pasture. *Australian Journal Soil Research* 33, 167-178.

Schipper, L.A., Baisden, W.T., Parfitt, R.L., Ross, C., Claydon, J.J., Arnold, G. 2007. Large losses of soil C and N from soil profiles under pasture in New Zealand during the past 20 years. *Global Change Biology* 13, 1138-1144.

Schnabel, R.R., Franzluebbers, A.J., Stout, W.L., Sanderson, M.A., Stuedemann, J.A. 2001. The Effects of Pasture Management Practices. *In*: Follett, R.F., J. M. Kimball, and R. Lal (Ed.), The Potential off U. S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect. Lewis Publishers, Boca Raton, pp. 291-322.

Sims, P.L., Bradford, J.A. 2001. Carbon dioxide fluxes in a southern plains prairie. *Agricultural and Forest Meteorology* 109, 117-134.

Skinner, R.H. 2008. High biomass removal limits carbon sequestration potential of mature temperate pastures. *Journal of Environmental Quality* 37, 1319-1326.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S. 2007. Policy and technological constraints to implementation of greenhouse gas mitigation options in agriculture. *Agriculture, Ecosystems & Environment* 118, 6-28.

Soussana, J.F., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., Clifton-Brown, J., Czobel, S., Domingues, R., Flechard, C., Fuhrer, J., Hensen, A., Horvath, L., Jones, M., Kasper, G., Martin, C., Nagy, Z., Neftel, A., Raschi, A., Baronti, S., Rees, R.M., Skiba, U., Stefani, P., Manca, G., Sutton, M., Tubaf, Z., Valentini, R. 2007. Full accounting of the greenhouse gas (CO2, N2O, CH4) budget of nine European grassland sites. *Agriculture Ecosystems & Environment* 121, 121-134.

Soussana, J.F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., Arrouays, D. 2004. Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20, 219-230.

Suyker, A.E., Verma, S.B. 2001. Year-round observations of the net ecosystem exchange of carbon dioxide in a native tallgrass prairie. *Global Change Biology* 7, 279-289.

Swift, R.S. 2001. Sequestration of carbon by soil. *Soil Science* 166, 858-871.

Taylor, J., Foltz, J. 2006. Grazing in the Dairy State: Pasture Use in the Wisconsin Dairy Industry, 1993 – 2003 Center for integrated Agricultural Systems & Program on Agricultural Technology Studies, University of Wisconsin - Madison.

Taylor, J., Lehmkuhler, J. 2008. The Wisconsin Beef Cattle Industry: A Snapshot. Center for Integrated Agricultural Systems, University of Wisconsin - Madison.

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M. 1997. Human domination of Earth's ecosystems. *Science* 277, 494-499.

Vough, L.R. 1990. Grazing Lands in the East. *Rangelands* 12, 251-255.

Wienhold, B.J., Hendrickson, J.R., Karn, J.F. 2001. Pasture management influences on soil properties in the Northern Great Plains. *Journal of Soil and Water Conservation* 56, 27-31.

Wilsey, B.J., Polley, H.W. 2006. Aboveground productivity and root-shoot allocation differ between native and introduced grass species. *Oecologia* 150, 300-309.

Table 1. Net Ecosystem Carbon Balance (NECB) [see Eqn (1)] for all treatments in 2006 and 2007. Mean values with standard errors indicated in parentheses. All units are in g C m^{-2}.

Year	Treatment	NPP	$*I_h$	$*I_g$	R_s	E_h	$*E_{cb}$	F_{ch4}	F_{co2}	NECB
2006	CONT	434 (11)	101	17	549 (7)	0	4	23 (0.8)	207 (7)	-231 (15)
	MIRG	553 (35)	101	17	544 (5)	0	4	22 (0.8)	209 (7)	-108 (23)
	HARV	402 (17)	0	0	529 (0.9)	254 (16)	0	0	0	-381 (11)
	NONE	256 (27)	0	0	506 (2)	0	0	0	0	-250 (28)
2007	CONT	420 (45)	167	31	576 (17)	0	5	26 (0.6)	223 (7)	-212 (56)
	MIRG	698 (72)	167	31	542 (3)	0	5	25 (0.6)	225 (6)	99 (69)
	HARV	573 (31)	0	0	508 (3)	257 (20)	0	0	0	-192 (25)
	NONE	353 (36)	0	0	493 (3)	0	0	0	0	-140 (38)

*Grazing treatments were implemented with similar stocking rates on a yearly basis. Therefore, total hay and grain inputs, and exported livestock biomass, were divided equally between grazing treatments.

Table 2. Mean (SE) percent cover of dominant taxa across last 2 experimental years (2006-2007). Significant differences shown by different superscript letters. Significant differences determined by ANOVA linear mixed effects models, $p<0.05$.

			Treatment							
Functional group	*Common name*	*Scientific name*	*CONT*		*MIRG*		*HARV*		*NONE*	
Annual grass	Hairy crabgrass	*Digitaria sanguinalis*	0.2	(0.2)						
Cool season	Kentucky bluegrass	*Poa pratensis*	61.6[a]	(10.6)	37.0[b]	(9.0)	43.5[b]	(4.5)	49.9[a]	(4.2)
(C3 grass)	Meadow fescue	*Festuca elatior*	2.5	(3.0)	5.0	(4.9)	1.9	(2.1)	4.1	(5.0)
	Orchardgrass	*Dactylis glomerata*	4.2[a]	(2.4)	24.9[b]	(6.2)	30.9[b]	(6.9)	14.4[b]	(8.1)
	Perennial rye	*Lolium perenne*	2.3	(2.0)	4.2	(2.8)	2.1	(2.7)	0.2	(0.2)
	Quackgrass	*Elymus repens*	0.1	(0.1)	0.1	(0.1)	1.5	(2.1)	3.0	(3.2)
	Redtop	*Agrostis gigantea*			0.3	(0.3)			0.5	(0.6)
	Reed canrygrass	*Phalaris arundinacea*			0.2	(0.2)	0.2	(0.2)	0.7	(0.7)
	Smooth brome	*Bromus inermis*	0.3	(0.2)	2.4	(2.1)	0.6	(0.5)	2.8	(1.5)
	Tall fescue	*Festuca arundinacea*	0.3	(0.4)	0.9	(1.3)	0.9	(1.0)	2.5	(2.5)
	Timothy	*Phleum pratense*	1.5	(1.6)	1.0	(1.3)	2.5	(2.5)	1.9	(2.4)
Perennial	Birdsfoot trefoil	*Lotus corniculatus*	0.6[a]	(0.7)	0.5[a]	(0.5)	7.5[b]	(4.8)	13.7[b]	(7.5)
legumes	Black medic	*Medicago lupulina*	0.8	(0.9)			0.2	(0.4)		
	Red clover	*Trifolium pratense*	0.7	(0.8)	1.4	(0.6)	0.1	(0.1)	0.1	(0.1)
	White clover	*Trifolium repense*	9.1[a]	(4.6)	15.6[a]	(8.1)	0.9[b]	(1.2)		
Forbs	Bull thistle	*Cirsium vulgare*	0.3	(0.3)	0.1	(0.1)	0.1	(0.1)	0.6	(0.8)
	Canada goldenrod	*Solidago canadensis*							0.2	(0.2)
	Canada thistle	*Cirsium arvense*					0.3	(0.5)	0.3	(0.3)
	Carolina horsenettle	*Solanum carolinense*					0.4	(0.4)	0.4	(0.4)
	Dandelion	*Taraxacum officinale*	7.2[a]	(4.7)	3.6[b]	(1.9)	4.4[b]	(2.5)	0.8[b]	(0.8)
	Giantchickweed	*Myosoton aquaticum*	0.3	(0.3)			1.0	(1.0)	0.2	(0.2)
	Rough fleabane	*Erigeron strigosus*	0.1	(0.1)						
	Smartweed	*Polygonum spp.*	0.7	(0.6)						
	Spiny plumeless thistle	*Carduus acanthoides*	0.2	(0.2)			0.2	(0.1)	0.7	(0.4)
	Wild parsnip	*Pastinaca sativa*	0.1	(0.1)					0.3	(0.5)
Sedge	Sedge	*Sedge spp.*	0.2	(0.2)	0.2	(0.2)			2.2	(2.0)
Other			3.1		2.3		0.5		0.3	
Bare ground	Bare		3.6	(2.2)	0.3	(0.3)	0.3	(0.5)	0.2	(0.2)

FIGURES

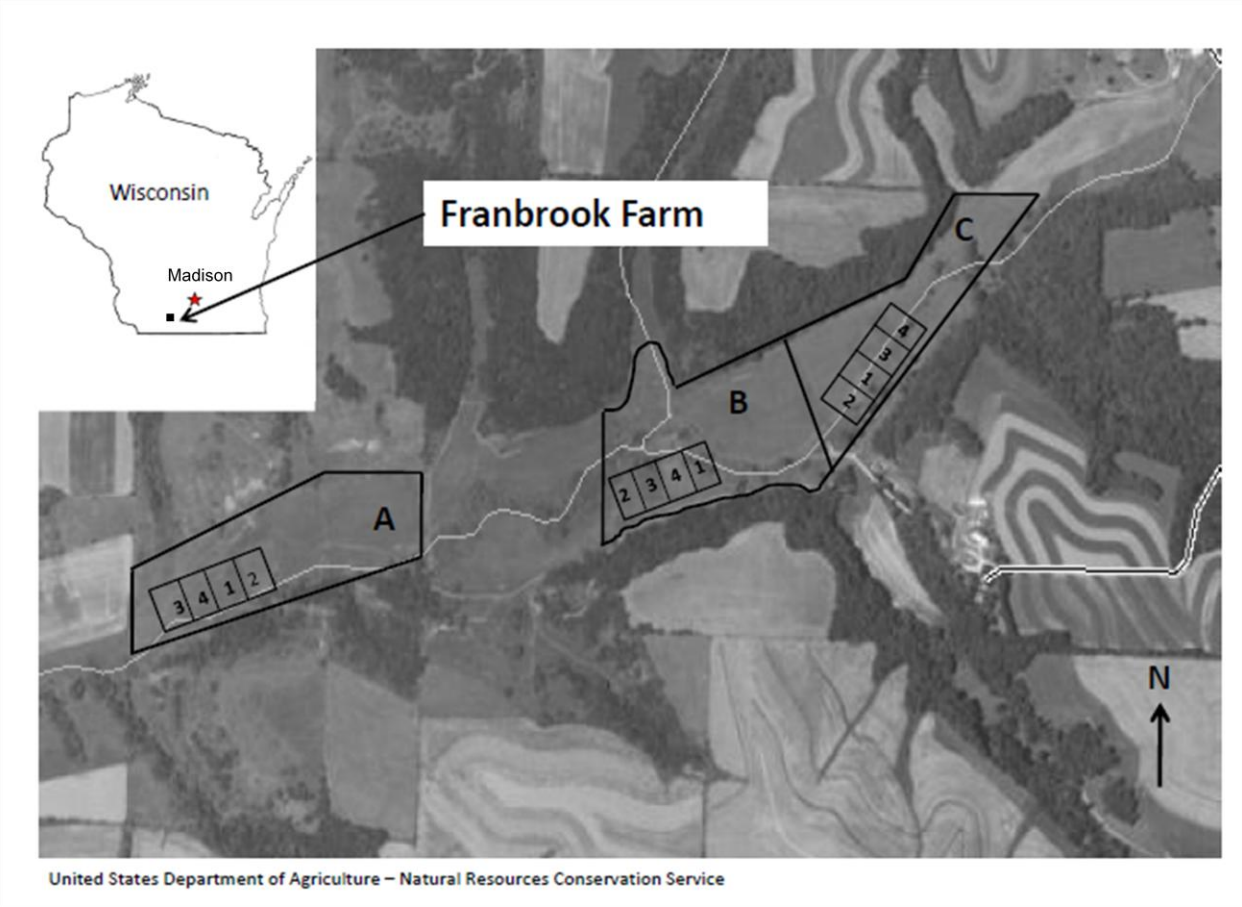

Fig. 1. Layout of Randomized Complete Block Design (RCBD) field experiment with three blocks and four treatment designated by number as: (1) management intensive rotational grazing (MIRG), (2) continuous grazing (CONT), (3) periodic harvesting of pasture forages for hay (HARV), and (4) management removal (NONE).

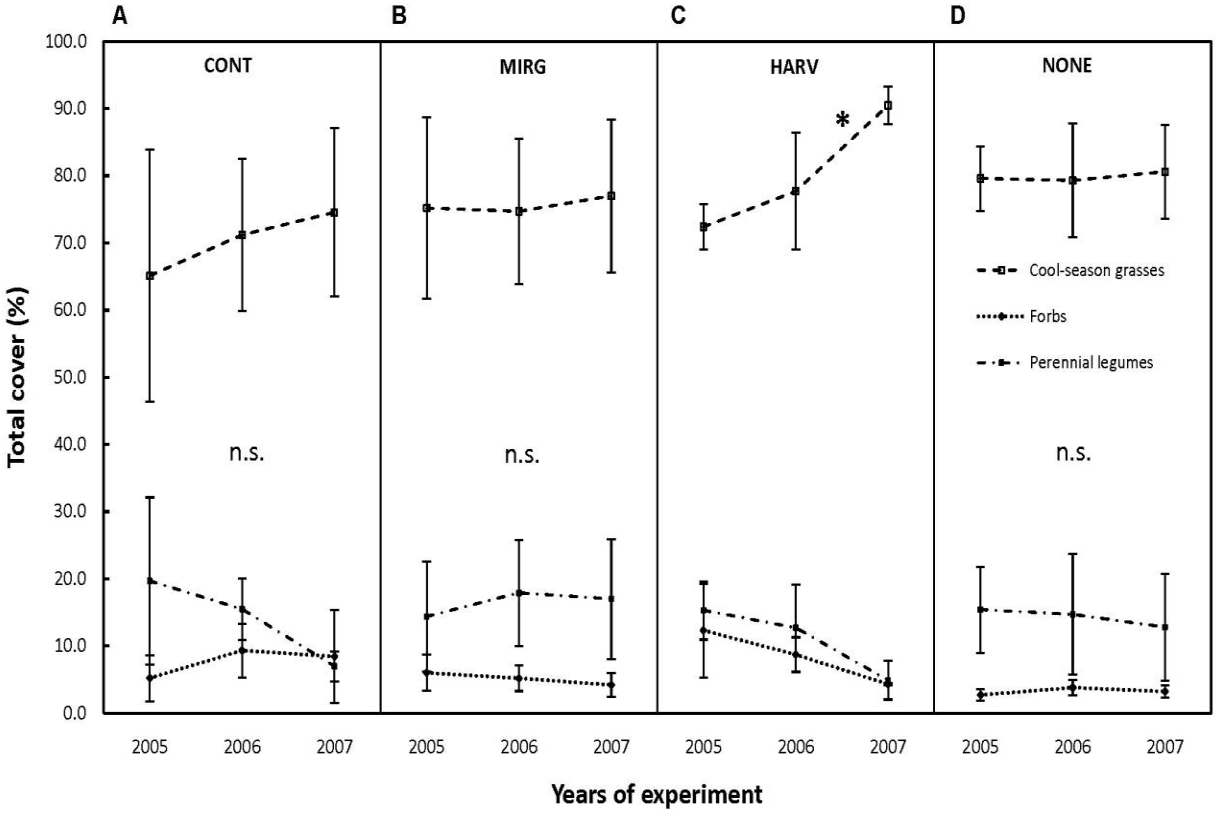

Fig. 2. Mean percent cover by year of C3 cool-season grasses, perennial legumes, and non-leguminous forbs in: (A) continuous grazing, (B) management-intensive rotational grazing, (C) harvest, and (D) no management over the course of 3 experimental years. Significant differences determined with ANOVA linear mixed-effects model selection, $p<0.05$. Error bars show ± SE, n = 3.

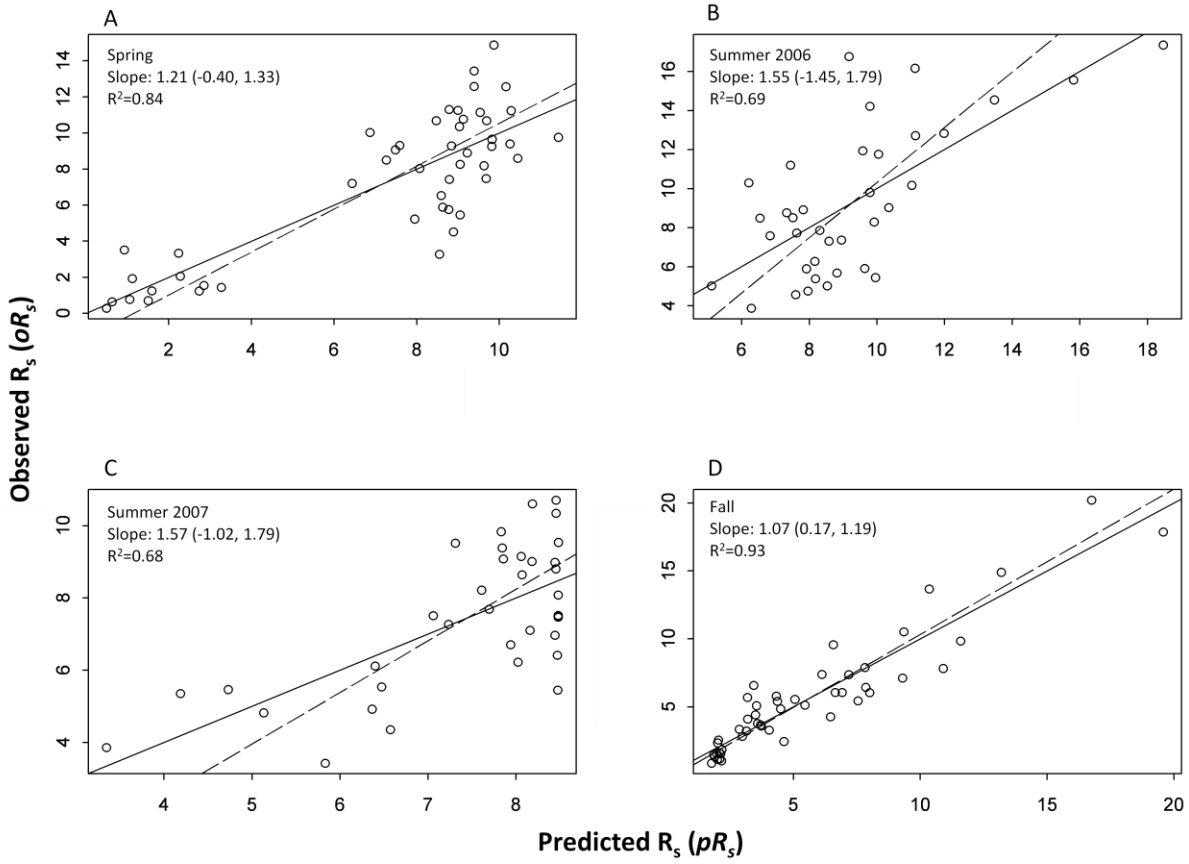

Fig. 3. Scatter plots of actual observed soil respiration (oR_s) and the predicted soil respiration (pR_s) in; (A) spring, (B) summer 2006, (C) summer 2007, and (D) fall using models developed from original data. Solid lines indicate 1:1 and dotted lines the ordinary least squares (OLS) bisector regression line. Ordinary least squares bisector slope estimates, along with their bootstrapped 95% confidence intervals, were used to validate the models.

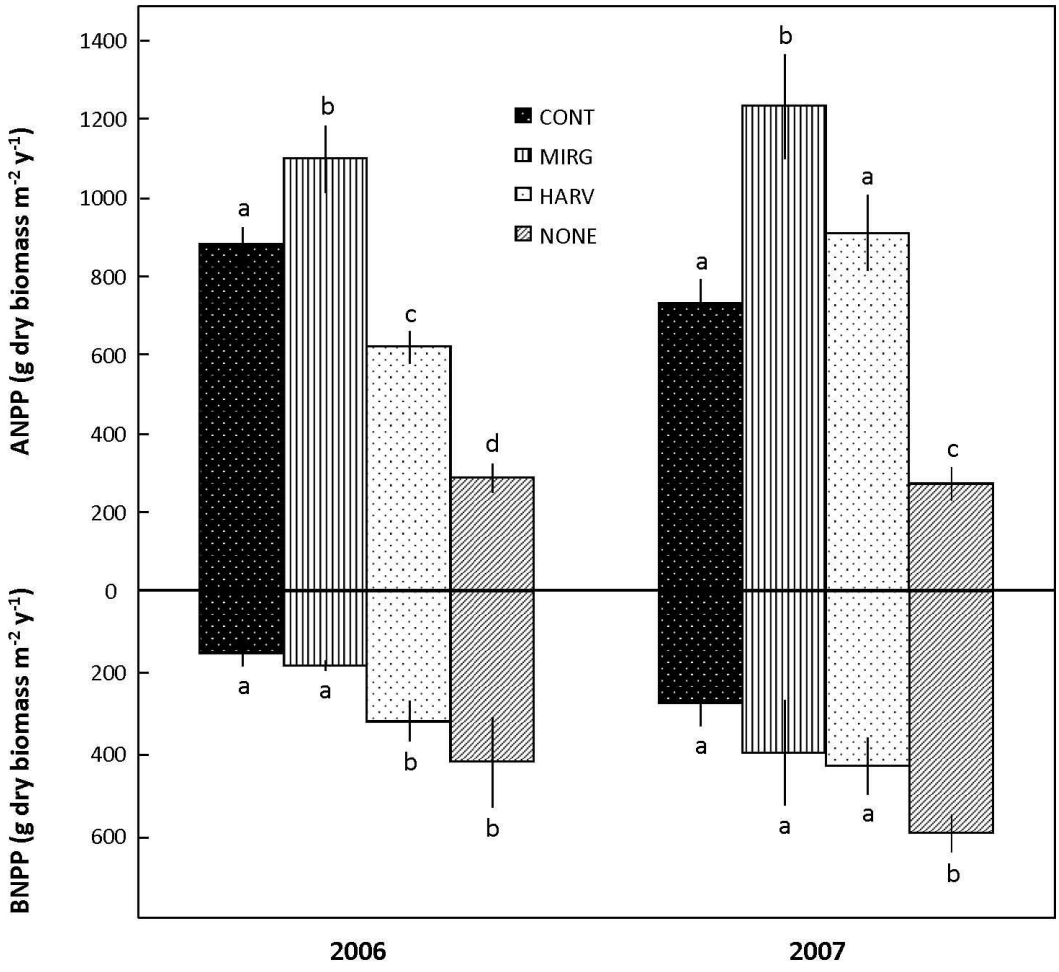

Fig. 4. Aboveground net primary production (ANPP) and belowground net primary production (BNPP) for 2006 and 2007. Error bars show ± SE, n = 3. Means of ANPP and BNPP shown with different letters were determined to be significantly different using ANOVA linear mixed-effects model selection, $p<0.05$.

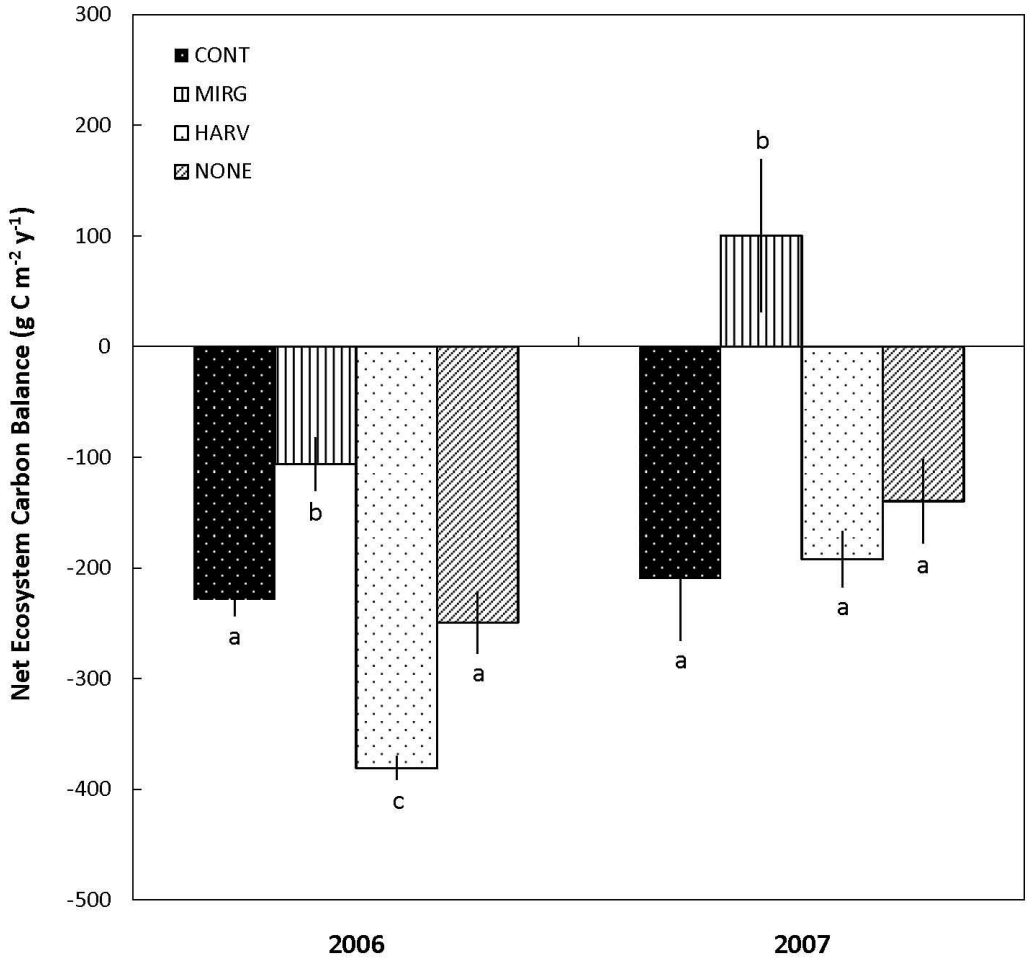

Fig. 5. Net ecosystem carbon balance (NECB) for 2006 and 2007. Error bars show ± SE, n = 3. Means of NECB shown with different letters were determined to be significantly different using ANOVA linear mixed-effects model selection, $p<0.05$.

Chapter 3. Subhumid pasture soil microbial communities affected by grazing, but not grazing management

ABSTRACT

In grasslands managed for livestock production, spatial and temporal patterns of biomass removal, soil disruption, and manure deposition may differentially affect soil microbial biomass and composition leading to altered nutrient cycling rates that feedback to management. Management-intensive rotational grazing has been shown to stimulate net primary production in subhumid pasture compared to extensive grazing strategies. The degree to which this is a direct effect on plant developmental stage and community composition, and/or the indirect effects of grazing on microbial community structure and nutrient feedback to production is an open question. To better understand the effects of pasture management on microbial communities, we used a hybrid phospholipid fatty acid and fatty acid methyl ester analysis to assess microbial biomass, fungal-to-bacterial ratios, and lipid biomarkers in surface soils under four pasture management treatments - management-intensive rotational grazing, continuous grazing, harvesting for hay, and an unmanaged control. We assessed microbial community structure with principal components analysis. Regression trees and linear mixed-effects models were used to explore factors related to microbial community response to management. While there was no effect of pasture management treatments on total microbial biomass, microbial community structure under our two grazed treatments differentiated themselves from ungrazed treatments. Guild comparisons revealed lower fungal-to-bacterial ratios, lower arbuscular mycorrhizal fungi concentrations, and higher actinomycete biomarkers in soil under grazing. Gram positive and anaerobic bacteria biomarkers were significantly greater in treatments that included grazing and grazing removal when compared to the treatment where biomass was harvested mechanically.

There was no evidence of treatment effects on either Gm- bacteria or saprotrophic fungi. Our results provide evidence that there was a general effect of grazing on soil microbial communities, but different grazing management did not affect microbial community composition or biomass. These results indicate that higher production observed with management-intensive rotational grazing likely stems from the direct effects of grazing on plant developmental stage and plant community composition rather than nutrient feedbacks mediated by altered quantity and quality of plant litter.

INTRODUCTION

The effects of grazing management on ecosystem structure and function are highly variable, but an emergent pattern indicates that effects are mediated by the degree of water limitation. In arid and semi-arid regions, grazing intensity - usually quantified as the amount of biomass removed or remaining over some period - appears to be the most important driver of productivity and composition (Barnes et al., 2008; Briske et al., 2008). However, in more mesic climates, management of the timing and spatial distribution of livestock is of paramount importance to maintaining or improving net primary production (NPP) (Fales et al., 1995; Burke et al., 1998). In these environments, grazing may enhance pasture production directly via effects on plant developmental stage and community composition (Fig. 1A and B) and/or indirectly by making nutrients more available to plants from excreta (Fig. 1C), and enhanced litter quality (Fig. 1D) leading to accelerated nutrient mineralization (Frank and Groffman, 1998). In the latter scenario, where nutrient mineralization is accelerated, an open question is whether this effect is related to altered microbial communities.

A tenet of management-intensive rotational grazing is that it maintains sward structure in a state of constant regrowth from defoliation (Voisin, 1959; Gerrish, 2004). The main way this is accomplished is by grazing frequently enough that grasses do not enter their reproductive stage (Turner et al., 1993). A second tenet is that by maintaining a uniform stand of dense regrowing grass, the pasture is resistant to invasion or increase of unpalatable lower productivity species, i.e., a community-level effect (Milchunas and Lauenroth, 1993; Augustine and McNaughton, 1998; Bardgett and Wardle, 2003). In addition to these direct effects, proponents of management-intensive rotational grazing claim increased grazing pressure - more animals for a

given area but for short duration - confers potential benefits to cycling of organic material through plant break down and incorporation into the soil by trampling (Southorn, 2002). Also, more uniform return of manure may reduce spatial variability of inputs, whereas grazing on a rotation schedule may reduce temporal variability of inputs (Bardgett et al., 1997).

Bardgett et al. (1998) suggested that frequent defoliation favors faster rates of nutrient cycling dominated by labile substrates and bacteria, while less frequent defoliation should favor a slower rate of nutrient cycling dominated by more resistant substrates and fungi. Numerous studies have shown that grazing addition, or increasing the intensity of grazing may result in lower fungal-to-bacterial ratios in soil microbial biomass (Bardgett et al., 1996; Bardgett et al., 2001; Grayston et al., 2004). Defoliation has been shown to 1) lower the C:N of litter inputs through increased nutrient content in shoots and roots (Holland and Detling, 1990; Hamilton and Frank, 2001), 2) affect short term carbon (C) allocation belowground and enhance root exudation to the rhizosphere (Mawdsley and Bardgett, 1997; Hamilton et al., 2008), and 3) accelerate litter decomposition rates by returning labile substrates to the soil as excreta (Holland et al., 1992; Frank and Groffman, 1998). As a consequence, intensively grazed pastures tend to have higher C and N mineralization rates (Holland et al., 1992; Bardgett et al., 1997; Frank and Groffman, 1998; Patra et al., 2005; Attard et al., 2008; Klumpp et al., 2009), which ostensibly promote aboveground biomass production (Klumpp et al., 2007). Conversely, cessation or reduction of grazing intensity leads to fungal based decomposer communities with concomitant rate reductions in key ecosystem processes (Bardgett et al., 1996; Bardgett et al., 2001; Grayston et al., 2004).

While the above pathways are well established, they have been mainly studied under constant disturbance, no disturbance, or in the case of grazing management with unequal grazing

pressure, i.e., testing the notion that productive systems under rotational grazing can support greater livestock densities (Bardgett et al., 2001; Wardle et al., 2004; Briske et al., 2008). Here, we test the extent to which four pasture management strategies typical of the upper Midwest affect soil microbial biomass and communities. We then explore potential predictors of community patterns. Finally, we discuss how the observed patterns in microbial parameters relate to key ecosystem processes observed in Chapters 1 and 2.

METHODS

Study site

This study was conducted at the Franbrook Farm, a research property in south-central Wisconsin, USA (42°44'16.65"N, 89°45'13.27"W). The climate is continental with warm summers and cold winters; average daily temperatures range from ~ -7° C in January to 22 °C in July. For our study period (2006 and 2007) mean temperature ranged from 0 to 22 and -5 to 21 °C in January and July, respectively. Mean annual precipitation is ~ 900 mm, of which ~ 100 mm is from snow. Approximately two-thirds of the annual precipitation falls during the growing season, April through October. Yearly precipitation totals for 2006 and 2007 were 691 and 583 mm, respectively. The research area is ~ 26 ha of valley bottom pasture dominated by cool-season grasses. Soils in this area are ~ 90 cm deep and are classified as Otter silt loam (Cumulic Endauolls), Arenzville silt loam (Typic Udefluvent), and Huntsville silt loam (Cumulic Hapludolls) (USDA, NRCS, 2007). In the top 15 cm of soil, pH, organic carbon, and total nitrogen are 6.8 (H_2O), 4.2, and 0.31 % respectively. Elevation range at the farm is 265 to 320 m above sea level.

Experimental design

In April 2005, a randomized complete block (RCBD) field experiment with three blocks and four treatments was established. The four treatments mimicked management-intensive rotational grazing (MIRG), continuous grazing (CONT), periodic harvesting of pasture forages for hay (HARV), and no active management (NONE) (Fig. 2). Beginning in May 2005, MIRG paddocks (0.6 ha) were grazed by separate 25 cow-calf pair herds (1 pair = 1.3 animal units [AU]) at high animal stocking rates (i.e., instantaneous stocking rate of 54 AU ha^{-1}) for ~ 2 d periods and then allowed to rest for 28-29 d (Paine et al., 1999). Continuous pastures (8.1 ha each) had lower instantaneous stocking rates (i.e., 4 AU ha^{-1}), but were rested only during the 2 d that the herd was confined to the MIRG paddocks, resulting in a comparable monthly stocking rate (i.e., a stocking rate of 112 AU d $month^{-1}$ compared to 108 AU d $month^{-1}$ for MIRG). In HARV plots (0.3 ha) plant biomass was mechanically harvested and removed in May and mid-August 2006, and May and late July 2007 typical of haymaking operations. Finally, we set aside 0.3 ha plots for no agronomic management (NONE). To represent standard management practice, granular ammonium phosphate (11-44-0) fertilizer was applied at the University of Wisconsin Extension recommended rate (57 kg N ha^{-1}) to all treatment areas except NONE at the beginning of June in 2005, 2006, and 2007.

Soil sampling for lipid extractions

Five 4.8-cm diameter soil cores were removed from the surface 15-cm in each treatment plot in April, July, and October of 2006, and April and October of 2007. Soils were placed in labeled plastic bags and stored in a cooler for transport to UW-Madison where they were immediately placed in cold storage at 4 °C until processing. Within 48 h of collection, soils were

sieved (2-mm) free of rocks and roots, and hand homogenized. Subsamples (3 g) were weighed out, lyophilized, and stored at -20 °C until used for lipid extraction analysis.

Phospholipid fatty acid analysis

Membrane lipids were extracted from the soil (White, 1998), purified and identified using steps from a modified Bligh and Dyer (1959) method for phospholipid fatty acid extraction (PLFA) which was combined with fatty acid methyl ester (FAME) analysis as described by Microbial ID Inc. (Hayward, CA). Lipids were extracted from 3 g of homogenized freeze-dried soil using a chloroform-methanol extraction with a phosphate buffer. Then, following the procedure for FAME given by Microbial ID Inc., fatty acids were saponified by adding sodium hydroxide, followed by strong acid methanolysis. A 2-*u*l FAME aliquot was analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a flame ionization detector and an Ultra 2 capillary column (Agilent Technologies, Santa Clara, CA). The gas chromatograph method and lipid peak identification were carried out using MIDI peak identification software and bacterial fatty acid standards ("Sherlock microbial identification system", MIDI Inc., Newark, DE). Fatty acids were quantified and converted to nmol lipid g soil^{-1} using peak areas from two internal standards of known concentrations, nonanoic methyl ester (9:0) and nonadecanoic methyl ester (19:0).

Total nmol lipid g soil^{-1} was used as an index of microbial biomass and to calculate fungal-to-bacterial ratio (F:B)(White et al., 1979; Zelles et al., 1992; Balser et al., 2005). The relative contribution of specific lipid biomarkers to the microbial community was determined by calculating the moles of a given lipid/total moles lipid per sample (mol%). We then combined the mol% of chemically similar fatty acids into groups representing segments of the microbial

community, commonly referred to as 'guilds'. Only fatty acids that were identifiable and present in amounts greater than 0.5 mol% were used for analyses (Table 1 shows guild composition of fatty acids used in our analysis).

Soil sampling for environmental and physical data

Soil from cores was also used for collection of ancillary environmental and physical data. Triplicate 10 g subsamples were weighed out, with: one subsample for gravimetric water content (GWC) determination (24 h @ 105 ^{o}C), one subsample for immediate inorganic N extraction in 2 *M* KCl, and one that was aerobically incubated for a period of seven days and then extracted to measure nitrification, ammonification, and net N mineralization following Robertson (1999). Extracts were frozen prior to colorimetric NH_4^+ (method #12-107-06-2-A) and NO_3^- (method #12-107-04-1-B) determination on a Lachat QuikChem flow injection analyzer (Lachat Instruments, Milwaukee, WI). Soil temperature (15-cm depth) was measured and recorded on all sample dates. In April 2007, we estimated soil bulk density by carefully excavating 260.24-cm^3 (4.72-cm diameter × 15-cm depth) soil monoliths that were returned to the lab, dried at 105 ^{o}C for 48 h, and weighed to determine mass of soil per unit volume soil.

Vegetation sampling for environmental data

To estimate quality of aboveground detrital inputs, grab samples of aboveground biomass were harvested monthly from April to October in 2006 and 2007. Five random samples of standing biomass were harvested from each treatment, composited, and then dried at 65 °C for 48 h. Samples were ground through a 1-mm screen in a Model 4 Wiley mill (Thomas Scientific, 19 Swedesboro, NJ), and analyzed at the University of Wisconsin Forage Lab (Madison, WI) by Near Infrared Reflectance Spectroscopy (NIRS) using a Foss NIR Model 6500 (Foss

NIRSystems Inc., Laurel, MD). We used Relative Forage Quality (RFQ), which is a single numeric index that reflects the sum of forage quality attributes measured in a forage sample. For belowground inputs, root biomass was collected from five 4.72-cm diameter × 15-cm deep mesh cores containing a neutral soil medium (75% field soil and 25% sand) installed within each treatment at the beginning of the growing season (April). These cores were harvested at the end of the season (October), and the roots were washed free of soil over a 1-mm sieve, and dried at 60 °C for 48 h. Fractional C and N contents of aboveground and belowground plant biomass were determined by flash combustion on finely ground subsamples of dried plant material (~10 mg) using a Flash EA 1112 16 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy).

Statistical analysis

To assess community structure, multivariate analysis of arcsine transformed mol fractions of individual lipids was performed with principal components analysis (PCA) using JMP 5.0 (SAS Institute Inc., Cary, NC). Also, to assess the variability of fixed and random effects (*date/block/treatment/subsample*) along the first two principal component axes (PC1 and PC2), a linear mixed-effects (LME) model was constructed to fit the variance parameters around the grand mean (intercept). The resulting random effects parameter estimates are the standard deviations of each source of variation. For a given response variable, the variation was standardized by calculating the coefficient of variation (CV%) - dividing the parameter estimate by the grand mean. To relativize variability for a given response variable, CV% was divided by the sum of all CV% for that given component. Principal components 1 and 2 were analyzed using MANOVA to determine whether treatments had an effect on microbial community

composition. Linear mixed-effects models were then constructed to determine specific treatment effects on PC1 and PC2.

Subsamples within each experimental unit (paddock) were used in ANOVA LME modeling using a restricted maximum likelihood (REML) algorithm (Pinheiro and Bates, 2000). All modeling was done using S-Plus 7.0 (Insightful Corp., Seattle, WA, 2005). Models were constructed (Burnam and Anderson, 1998) to analyze response variables as a function of the fixed effects of management (*treatment*), accounting for the random effect of *subsample* nested within *block* nested within *date* (*date/block/subsample)*. Improvements to subsequent models by dropping random effects terms or including a correlation function parameter were assessed with likelihood ratio tests for pairwise model comparison (Crawley, 2002). Once the random effects structure was determined, the significance of fixed effects was determined using Wald-type F-tests while treatment-level differences were inferred from P-values associated with t-tests of significance ($P < 0.05$) for each parameter estimate (Pinheiro and Bates, 2000). If *treatment* was significant, treatment levels were sequentially collapsed and subsequent models were compared using the maximum likelihood algorithm using the model selection approach described above. Separate model selection procedures were run with response variables of total microbial biomass, F:B, and abundances of Gm+ and Gm- bacteria, arbuscular mycorrhizal fingi (AMF), saprotophic fungi, and actinomycetes. Fungal to bacterial ratio was log transformed (log[y+10]), and AMF was square root transformed (sqrt[y]) to normalize the data.

To understand potential mechanisms for the treatment effects on soil microorganisms, average mol% from each experimental unit was used in regression trees to predict which environmental variables explained the most deviance for the response variables in which *treatment* was significant. Regression trees are a method for partitioning and exploring the

structural pattern in data when there are many, potentially interacting, explanatory variables (De'ath and Fabricius, 2000; Crawley, 2002). They explain the deviance of the selected response variable by assessing each explanatory variable in turn and then iteratively splitting the data into subsets using combinations of explanatory variables that best reduce the response variable deviance. This process is repeated until no further reduction in deviance is obtained or there are too few data points to justify further subdivision (Crawley, 2002). Trees can be 'pruned' to reduce nodes based on the significance of subset deviance explained. We pruned the trees by testing whether resulting subsets were significantly different using paired t-tests ($P < 0.05$), retaining only significantly different nodes. The candidate predictor variables included: relative forage quality, shoot C:N, root C:N, soil C:N, net N mineralization, net nitrification, net ammonification, soil NO_3^- pool, soil NH_4^+ pool, soil bulk density, soil gravimetric water content, and soil temperature, in addition to the random effects of date, block and subsample.

Once candidate predictor variables were identified for each response variable, a model was fit either using linear regression or LME depending on whether the random effect of date, block, or subsample was identified as explaining substantial deviance. In the case of linear regression models, simplification was performed by using Akaike Information Criterion (AIC) and log likelihood tests. A summary of the model was performed to determine significance for all predictor variables ($P < 0.05$), the least significant terms were then removed in sequence and subsequent models were tested for an increase in model deviance. The minimally adequate model was retained to quantify significance of the predictor variable using t-tests. For LME models, model simplification was as described above.

RESULTS

Treatment effects on microbial communities

Analysis of total PLFAs extracted showed no significant effect of treatment on microbial biomass (Wald-type $F_{3,102} = 1.32$, $P = 0.27$). Biomass was 399 ± 87, 374 ± 52, 417 ± 96, and 349 ± 75 nmol lipid g soil^{-1} (mean ± SE), for CONT, MIRG, HARV, and NONE, respectively.

Principal components analysis indicated overall soil biota differed between management treatments (Fig. 3). Principal component (PC) 1 explained 44.8% of the total variation, with space (subsample 38%; block 21%) and time (date 16%) explaining greater variability than management (13%). The PLFA concentrations most positively correlated with PC1 were the *antesio* and *iso* Gm+ fatty acids. The biomarkers with the strongest negative correlation were the Gm- fatty acids *cis*16:1ω7 and *cis*18:1ω7, and biomarkers representing both saprotrophic fungi and AMF. Linear mixed-effects models indicated that treatment had no effect on microbial community composition along PC1 (model 1; Table D1).

Principal component 2 accounted for 16.3% of the total variability in microbial composition, with space (subsample 39%; block 33%) and management (23%) having the greatest explanatory power. Treatment was significant along PC2 (model 2; Table D1), with soil microbial community structure differing by whether management included grazing (models 3 - 6; Table D2). Higher concentrations of eight PLFAs were positively correlated with PC2 and associated with HARV and NONE. Most notable were the fungal biomarkers *cis*16:1ω5 and *cis*18:2ω6/aT:18:0, and the Gm- biomarkers *cis*16:1ω9, *cis*17:1ω7, *cis*17:1ω8, and *cis*18:1ω5. The MIRG and CONT treatments associated with ten PLFAs that were negatively correlated with PC2. Of these biomarkers the strongest negative correlation belonged to the Gm+ fatty

acids a15:0, i15:0, i16:0, i17:0, the Gm- biomarker *cis*16:1ω7, the anaerobic cy19:0 biomarker, and the biomarker 10Me18:0, which is indicative of actinomycetes.

Treatment had a significant effect on the ratio of F:B biomarkers isolated from the soil (model 1; Table D2). Fungal-to-bacterial ratio was higher in the treatments that did not include grazing, HARV and NONE, relative to the grazing treatments MIRG and CONT (models 2 - 5; Table D2). Fungal-to-bacterial ratio was 0.52 ± 0.02 for CONT, 0.51 ± 0.03 for MIRG, 0.64 ± 0.03 for HARV, and 0.59 ± 0.04 (mean ± SE) for the NONE treatment.

Pasture management significantly affected Gm+ bacteria (model 1; Table D3), anaerobic bacteria (model 6; Table D3), and actinomycetes (model 11; Table D3), but not Gm- bacteria (model 5; Table D3). Average mol% of lipid biomarkers indicative of the Gm+ bacterial guild was significantly greater in the grazing treatments, CONT and MIRG, and in the no management treatment NONE, compared to the treatment with biomass removal only, HARV (models 2 - 4; Table D3). Anaerobic biomarkers were significantly less abundant in HARV, and actinomycete biomarkers were significantly less abundant in both HARV and NONE treatments (models 7 - 10, 12 - 15; Table D3).

There were no significant treatment effects on saprotrophic fungi (model 16; Table D3), while AMF were affected significantly by treatment (model 17; Table D3). The ungrazed treatments, HARV and NONE, had greater concentrations of AMF when compared to the CONT and MIRG grazing treatments (models 18 - 21; Table D3).

Key predictors of microbial community response

The regression tree identified a split with a mean F:B of 0.82 when root C:N was >40.3 (9 samples) and a mean F:B of 0.55 when <40.3 (51 samples). The low root C:N subset was split by nitrification, with lower F:B associated with higher rates of nitrification; the data were further refined by subsequently splitting the low nitrification subset with root C:N, and the high root C:N subset by NO_3^- pool size. Residual deviance plotted against tree size indicated a tree with five splits (i.e., tree of 6 nodes) explained 74% of the total deviance (Fig. E1). The best predictors of F:B were root C:N, nitrification, and NO_3^- pool ($r^2 = 0.37$, $p < 0.0001$, Fig. 4).

The bacterial guilds were generally predicted by distinct environmental variables, with different C:N ratios being the strongest. The regression tree for Gm+ bacteria contained four splits (5 nodes) and explained 60% of the total deviance (Fig. E2). The first split was identical to the first split for F:B (see above), with a mean mol% of 9.89 at root C:N >40.3 and 12.54 at root C:N < 40.3. The second subset was further split by soil bulk density with higher Gm+ concentrations at lower compaction. The minimal model for Gm+ abundance included only these two terms ($r^2 = 0.31$, $p < 0.0001$, Fig. 5A).

The actinomycete regression tree contained 3 splits (4 nodes), explaining only 37% of deviance (Fig. E3). The first split occurred at root C:N = 37.6 with a mean mol% of 0.73 (12 samples) for the group with a higher root C:N, and a mean of 0.96 (48 samples) for the group with lower root C:N. Soil temperature refined prediction in the second subset, with higher soil temperatures indicating greater concentrations of actinomycetes; in the low-temperature subset greater concentrations of actinomycetes corresponded with higher rates of N mineralization.

Actinomycete abundance was minimally modeled by the interaction of these three terms (r^2 = 0.31, p = 0.006, Fig. 5B).

Anaerobe abundance was modeled by a 5-split tree explaining 70% of the residual deviance (Fig. E4). For soil C:N > 12.1, mean anaerobe mol% was 2.85 (18 samples) compared to 3.56 for soil C:N < 12.1 (42 samples). For the lower soil C:N samples, a larger NO_3^- pool corresponded to a higher mean anaerobe mol%. Because *block* accounted for significant variability in anaerobic bacterial concentrations, we employed a LME model including *block* as a random effect, which identified soil C:N (Wald-type $F_{1,55}$ = 11.79, p =0.001), and NO_3^- pool (Wald-type $F_{1,55}$ = 5.37, p =0.02) as significant predictors of anaerobic bacteria (Fig 5C).

Regression tree analysis identified three splits (4 nodes) that explained 63% of total residual deviance in the AMF community (Fig. E5). The first split was along the same lines as actinomycetes, with mean AMF mol% of 16.1 for root C:N <37.64 (48 samples), and a mean of 26.4 for root C:N >37.64 (12 samples). The low split was again refined by root C:N with the higher root C:N subset again containing greater concentrations of AMF. Interestingly, a further division of this higher AMF abundance subset indicates higher AMF concentration with lower root C:N. AMF abundance was best modeled by root C:N (r^2 = 0.36, p < 0.0001, Fig. 5D) which is not surprising given the symbiotic relationship with the host plant.

DISCUSSION

Grazing removal promoted fungal decomposition pathway

Our results suggest that the rate of flow, but not the amount of energy cycling through the detrital food web was affected by pasture management. The absolute abundance of the fungal proportion of the microbial biomass increased with grazing removal. This was likely the result

of more recalcitrant substrates entering the detrital pool and an increase in root biomass associated with AMF. At the same time, total microbial biomass was not affected by these management changes indicating that a similar amount of energy was available for microbial activity. The positive correlation between root C:N and F:B, and the negative correlation between nitrification and nitrate pool with increased F:B suggests that grazing fundamentally changes the flow of energy within the system. Without grazing, C and N are bound in organic material and the flow of energy is dominated by fungal pathways. Whereas in the presence of grazing, a greater proportion of energy is in the form of more labile substrates leading to a bacterial dominated energy pathway and faster return of nutrients to an inorganic form, and thus plant availability.

Litter quality was the main determinant of the differences in microbial community between grazed and ungrazed treatments. Carbon-to-nitrogen ratio of roots was the best predictor in 4 of the 5 microbial guilds, while soil C:N was the best predictor in the other. Lower root C:N predicted lower F:B, and biomarkers of guilds that were dominant in the grazing treatments, while higher C:N predicted guild biomarkers dominant in the ungrazed treatments. Of secondary importance were soil properties related to N pools and N mineralization, and physical properties such as bulk density, gravimetric water content, and soil temperature. Greater N availability, and physical properties related to grazing, e.g., increased soil compaction (Wienhold et al., 2001), pockets of anaerobicity (Breland and Hansen, 1996), and higher soil temperatures were the overwhelming predictors of the dominant guild biomarkers found under grazing.

Grazing management effects on ecosystem productivity not mediated by microbial communities

While our work has shown that MIRG enhances production (Ch 1 and 2), which benefits C balance (Ch 2), we found no evidence that these effects were mediated by the microbial community. None of the microbial response variables differed between MIRG and CONT. It has been suggested that optimization of forage production and quality may be a function of defoliation timing relative to growth state of the vegetation (see Turner et al., 1993), and the exclusion of less palatable species (Menke and Bradford, 1992; Bardgett et al., 1998), i.e., the direct effects indicated in Fig. 1A and B. We found that indirect effects were important at a relatively coarse level of resolution, i.e., grazed vs. ungrazed. This aligns with Bardgett et al. who found indirect effects are prevalent with changes in grazing intensity. But within our grazed treatments we found no support for the indirect effects of defoliation, leaving direct effects as the most plausible explanation for greater production under MIRG. This emphasizes that when altering the timing and spatial distribution of grazing, but holding grazing intensity constant, productivity response in subhumid pasture is plant mediated.

LITERATURE CITED

Attard, E., Degrange, V., Klumpp, K., Richaume, A., Soussana, J.F., Le Roux, X. 2008. How do grassland management history and bacterial micro-localisation affect the response of bacterial community structure to changes in aboveground grazing regime? *Soil Biology & Biochemistry* 40, 1244-1252.

Augustine, D.J., McNaughton, S.J. 1998. Ungulate effects on the functional species composition of plant communities: Herbivore selectivity and plant tolerance. *Journal of Wildlife Management* 62, 1165-1183.

Balser, T.C., Treseder, K.K., Ekenler, M. 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. *Soil Biology & Biochemistry* 37, 601-604.

Bardgett, R.D., Hobbs, P.J., Frostegard, A. 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils* 22, 261-264.

Bardgett, R.D., Jones, A.C., Jones, D.L., Kemmitt, S.J., Cook, R., Hobbs, P.J. 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. *Soil Biology & Biochemistry* 33, 1653-1664.

Bardgett, R.D., Leemans, D.K., Cook, R., Hobbs, P.J. 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology & Biochemistry* 29, 1285-1294.

Bardgett, R.D., Wardle, D.A. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84, 2258-2268.

Bardgett, R.D., Wardle, D.A., Yeates, G.W. 1998. Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. Soil *Biology & Biochemistry* 30, 1867-1878.

Barnes, M.K., Norton, B.E., Maeno, M., Malechek, J.C. 2008. Paddock size and stocking density affect spatial heterogeneity of grazing. *Rangeland Ecology & Management* 61, 380-388.

Bligh, E.G., Dyer, W. 1959. A rapid method of total lipid extraction and purification. Canadian *Journal of Biochemistry and Physiology*, 911-917.

Breland, T.A., Hansen, S. 1996. Nitrogen mineralization and microbial biomass as affected by soil compaction. *Soil Biology & Biochemistry* 28, 655-663.

Briske, D.D., Derner, J.D., Brown, J.R., Fuhlendorf, S.D., Teague, W.R., Havstad, K.M., Gillen, R.L., Ash, A.J., Willms, W.D. 2008. Rotational grazing on rangelands: Reconciliation of perception and experimental evidence. *Rangeland Ecology & Management* 61, 3-17.

Burke, I.C., Lauenroth, W.K., Vinton, M.A., Hook, P.B., Kelly, R.H., Epstein, H.E., Aguiar, M.R., Robles, M.D., Aguilera, M.O., Murphy, K.L., Gill, R.A. 1998. Plant-soil interactions in temperate grasslands. *Biogeochemistry* 42, 121-143.

Burnam, K.P., Anderson, D. R. 1998. Model selection and inference: a practical information-theoretic approach. Springer, New York.

Crawley, M. 2002. Statistical Computing: An Introduction to Data Analysis Using S-Plus. John Wiley and Sons, West Sussex, England.

De'ath, G., Fabricius, K.E. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology* 81, 3178-3192.

Fales, S.L., Muller, L.D., Ford, S.A., Osullivan, M., Hoover, R.J., Holden, L.A., Lanyon, L.E., Buckmaster, D.R. 1995. Stocking rate affects production and profitability in a rotationally grazed pasture system. *Journal of Production Agriculture* 8, 88-96.

Frank, D.A., Groffman, P.M. 1998. Ungulate vs. landscape control of soil C and N processes in grasslands of Yellowstone National Park. *Ecology* 79, 2229-2241.

Gerrish, J. 2004. Management-Intensive Grazing: The Grassroots of Grass Farming. Green Park Press., Ridgeland, MS.

Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L., Clegg, C.D., Ritz, K., Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J., Millard, P. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. *Applied Soil Ecology* 25, 63-84.

Hamilton, E.W., Frank, D.A. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82, 2397-2402.

Hamilton, E.W., Frank, D.A., Hinchey, P.M., Murray, T.R. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology & Biochemistry* 40, 2865-2873.

Holland, E.A., Detling, J.K. 1990. Plant response to herbivory and belowground nitrogen cycling. *Ecology* 71, 1040-1049.

Holland, E.A., Parton, W.J., Detling, J.K., Coppock, D.L. 1992. Physiological-response of plant-populations to herbivory and their consequences for ecosystem nutrient flow. *American Naturalist* 140, 685-706.

Klumpp, K., Fontaine, S., Attard, E., Le Roux, X., Gleixner, G., Soussana J.F., 2009. Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. *Journal of Ecology* 97, 876-885.

Klumpp, K., Soussana, J.F., Falcimagne, R. 2007. Effects of past and current disturbance on carbon cycling in grassland mesocosms. *Agriculture Ecosystems & Environment* 121, 59-73.

Mawdsley, J.L., Bardgett, R.D. 1997. Continuous defoliation of perennial ryegrass (Lolium perenne) and white clover (Trifolium repens) and associated changes in the composition and activity of the microbial population of an upland grassland soil. *Biology and Fertility of Soils* 24, 52-58.

Milchunas, D.G., Lauenroth, W.K. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63, 327-366.

Paine, L.K., Undersander, D., Casler, M.D. 1999. Pasture growth, production, and quality under rotational and continuous grazing management. *Journal of Production Agriculture* 12, 569-577.

Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, E., Prosser, J.I., Richaume, A., Le Roux, X. 2005. Effects of grazing on microbial functional groups involved in soil N dynamics. *Ecological Monographs* 75, 65-80.

Pinheiro, J.C., Bates, D. M. 2000. Mixed-efects models in S and S-Plus. Springer, New York.

Robertson, G.P., D. C. Coleman, C. S. Bledsoe, Sollins, P. (Ed), 1999. Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York.

Southorn, N.J. 2002. The soil structure component of soil quality under alternate grazing management strategies. *Advances in Geoecology* 35, 163-170

Turner, C.L., Seastedt, T.R., Dyer, M.I. 1993. Maximization of aboveground grassland production – The role of defoliation frequency, intensity, and history. *Ecological Applications* 3, 175-186.

Voisin, A. 1959. Grass Productivity. Philosophical Library Inc., New York.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H. 2004. Ecological linkages between aboveground and belowground biota. *Science* 304, 1629-1633.

White, D.C., Ringelberg, D. B. 1998. Signature lipid biomarker analysis. *In*: Burlage, R.S., R. Atlas. D. Stahl, G. Geesey, and G. Sayler (Ed.), Techniques in Microbial Ecology. Oxford University Press, New York, pp. 255-272.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40, 51-62.

Wienhold, B.J., Hendrickson, J.R., Karn, J.F. 2001. Pasture management influences on soil properties in the Northern Great Plains. *Journal of Soil and Water Conservation* 56, 27-31.

Zelles, L., Bai, Q.Y., Beck, T., Beese, F. 1992. Signature fatty-acids in phospholipids and lipopolysachharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biology & Biochemistry* 24, 317-323.

TABLES

Table 1. Composition of guilds from chemically similar fatty acids used in the analysis. Only fatty acids that were identifiable and present in amounts greater than 0.5 mol% were used.

Guild	Generally indicates	Fatty acids included
Branched	Gram-positive bacteria[a,b]	i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0
	Actinomycetes[c,d]	10Me18:0
Monounsaturated	Gram-negative bacteria[b,e]	*cis*16:1ω7, *cis* 16:1ω9, *cis* 17:1ω7, *cis* 17:1ω8, *cis*18:1ω5, and *cis*18:1ω7
Cyclopropyl	Anaerobic bacteria[e]	cy17:0 and cy19:0
	Mycorrhizal fungi (AMF)[f]	*cis*16:1ω5
	Saprotrophic fungi (SF)[c,f]	*cis*18:1ω9 and *cis*18:2ω6/aT:18:0

Referenced from: a. Zelles et al. (1992); b. Frostegard et al. (1993); c. Federle et al. (1986); d. Vestal and White (1989); e. Wilkinson (1988); f. Balser et al. (2005)

FIGURES

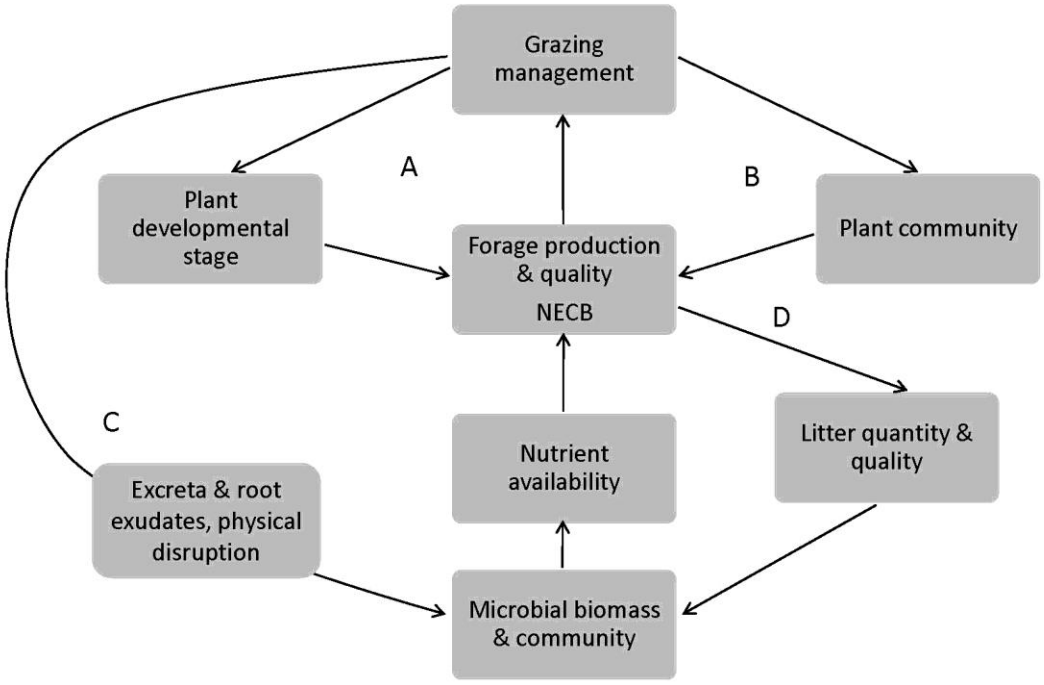

Fig. 1. Relationship between grazing management and the direct effects on A) individual plant development stage, B) plant community composition, and the indirect effects on soil microbes through C) labile substrate return in the form of manure and root exudates, and disruption of soil physical properties, and D) plant litter quantity and quality with theoretical impacts for nutrient availability, forage production and quality, and carbon balance.

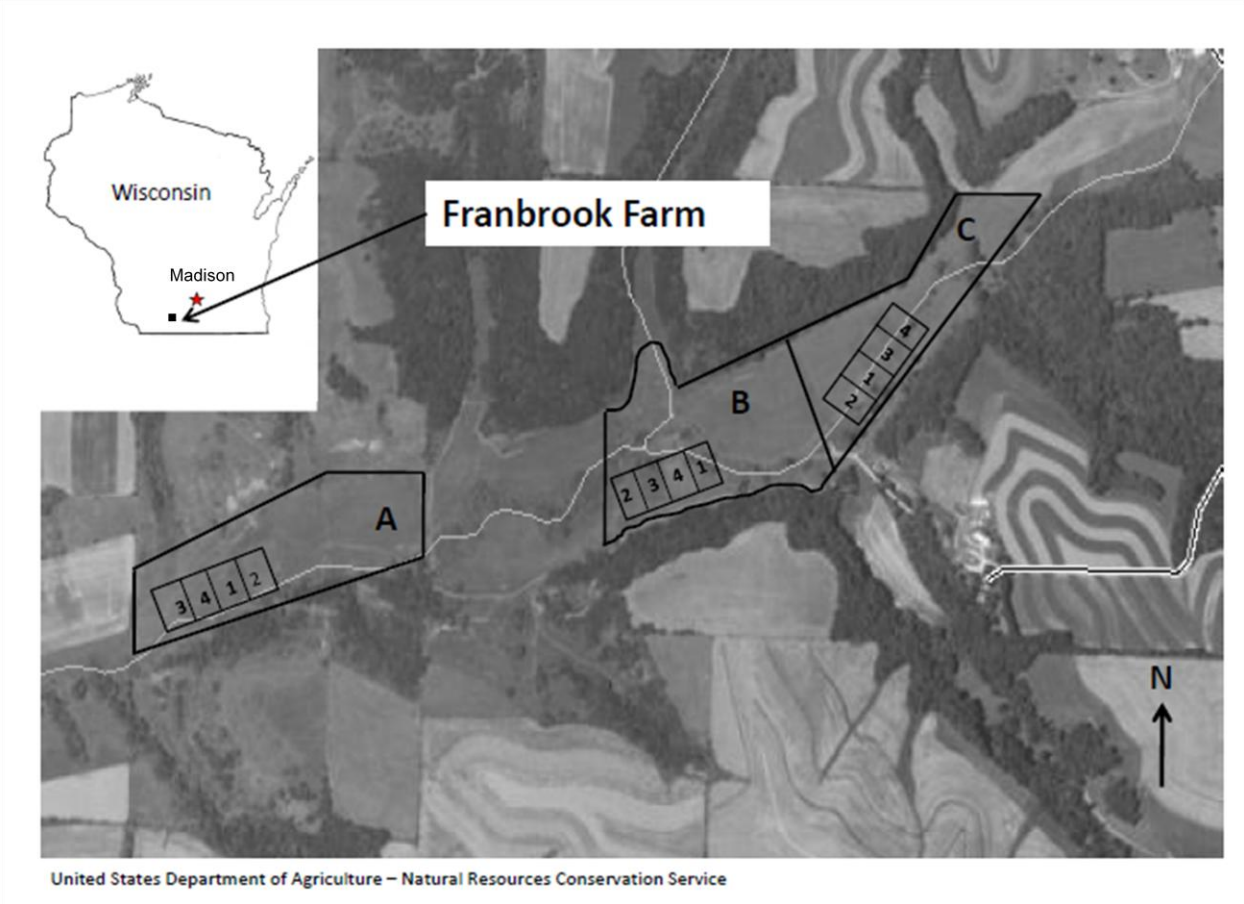

Fig. 2. Layout of Randomized Complete Block Design (RCBD) field experiment with three blocks and four treatment designated by number as: (1) management intensive rotational grazing (MIRG), (2) continuous grazing (CONT), (3) periodic harvesting of pasture forages for hay (HARV), and (4) management removal (NONE).

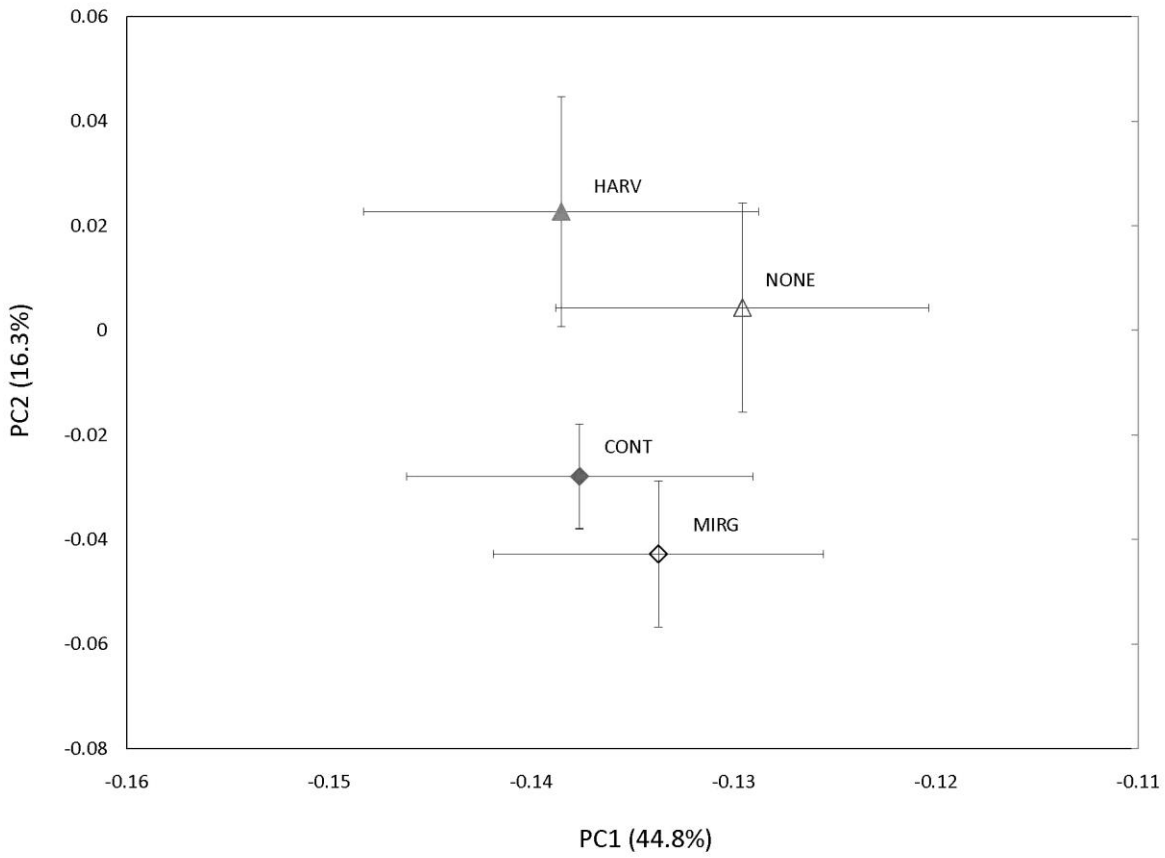

Fig. 3. Plot of PC1 and PC2 from multivariate analysis of microbial lipid means (± SE; n=27) obtained from continuously grazed pasture (◆), management-intensive rotational grazing (◇), biomass harvested by mechanical means (▲), and management removal (△). PLFAs found with a more positive correlation along PC1 were dominated by Gm+ bacteria, while those that were more negative were Gm- bacteria and fungi. Along PC2, PLFAs with the highest positive correlation were fungi and Gm- bacteria, and those negatively correlated were dominated by Gm+ bacteria and actinomycetes. Principal component 1 and 2 accounted for 61.1% of the variability.

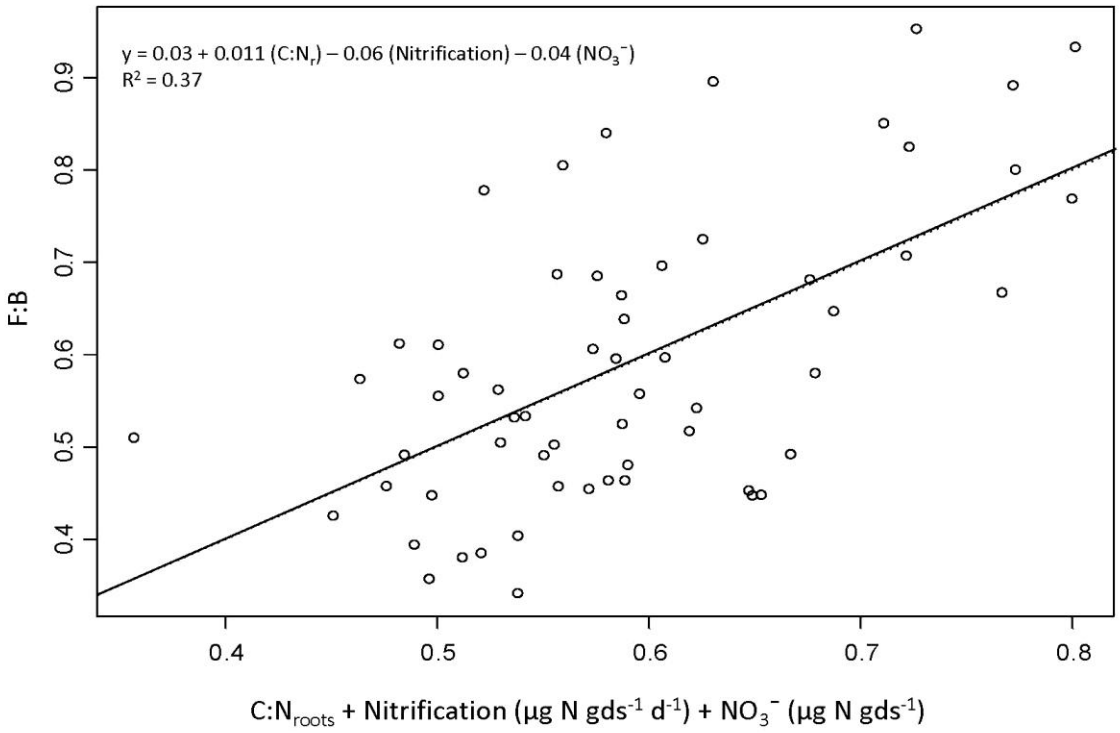

Fig. 4. Relationship between fungal to bacterial ratios (F:B) and the minimally adequate predictive model comprised of root C:N, Nitrification (µg N gds^{-1} d^{-1}), and NO_3^- pool (µg N gds^{-1}).

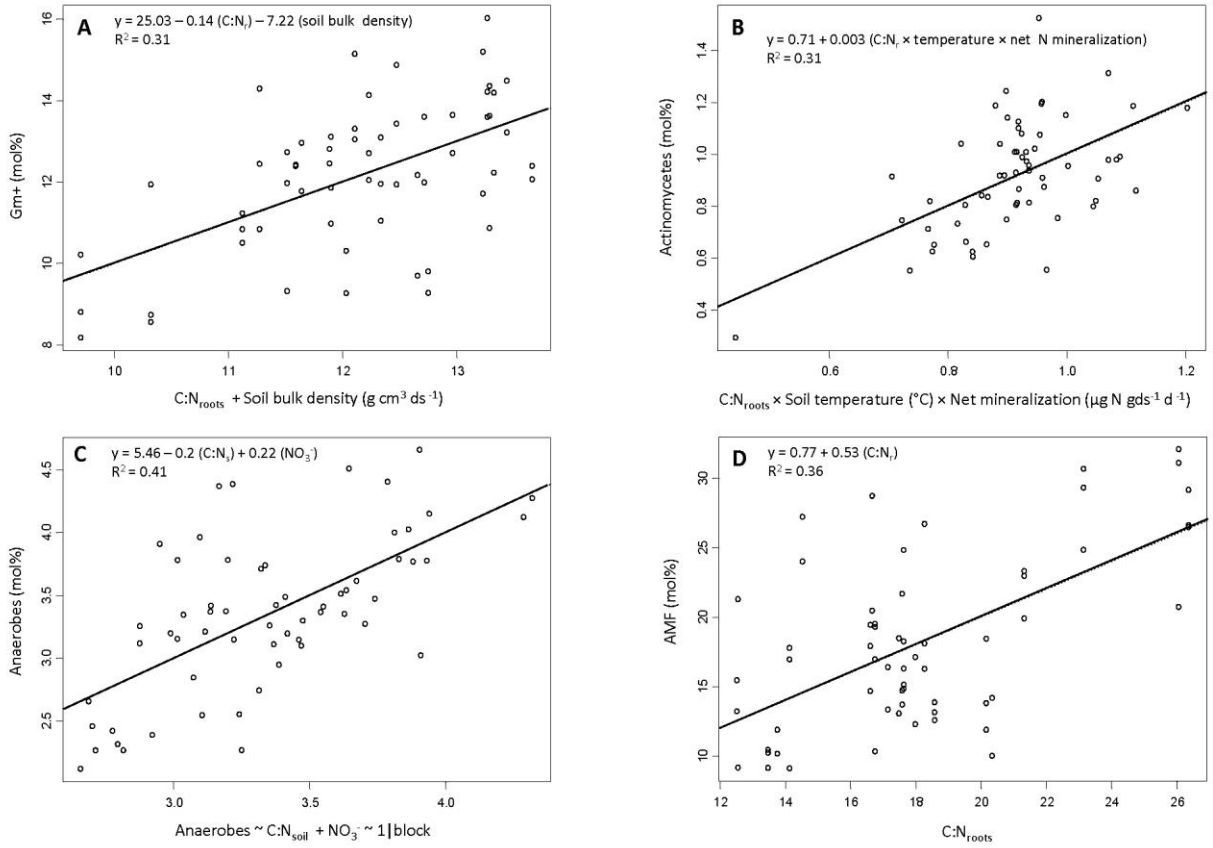

Fig. 5. Relationship between A) gram positive bacteria (Gm+) and root C:N + soil bulk density (g cm^3 ds^{-1}), B) actinomycetes and the interaction between root C:N × Soil temperature (°C) × net N mineralization (µg N gds^{-1} d^{-1}), C) anaerobic bacteria and the model comprised of soil C:N + NO_3^- pool (µg N gds^{-1}) conditioned with the random effect of block, and D) arbuscular mycorrhizal fungi (AMF) and root C:N.

Appendix A. Relative Forage Quality calculation

Relative forage quality is calculated as:
$RFQ = (DMI_{grs} * TDN_{grs})/1.23$

where:

The 1.23 divisor is used to adjust the equation to have a mean and range similar to RFV (Moore and Undersander 2002)

TDN_{grs}=(NFC*.98)+CP*.87)+(FA*.97*2.25)+(NDFn*NDFDp/100)-10 (Moore and Undersander 2002)

where:

TDN_{grs} = total digestible nutrients for mixed cool season grasses
NFC = non fibrous carbohydrate (% of dry matter [DM])
CP = crude protein (% DM)
FA = fatty acids (% DM)
NDFn = nitrogen free neutral detergent fiber (% DM), estimated as NDF*0.93
NDFDp = 48 h in-vitro NDF digestibility (% of NDF), and where NDFDp = 22.7 + 0.664*NDF

DMI_{grs} = -2.318+0.442*CP-0.0100*CP^2-0.0638*TDN+0.000922*TDN^2+0.180*ADF-0.00196*ADF^2-0.00529*CP*ADF (Moore and Kunkle 1999)

where:

DMI_{grs} = dry matter intake of mixed cool season grasses (% of body weight [BW])
CP = as above
TDN – calculated from the above equation
ADF = acid detergent fiber (% DM)

Table B1. Comparison of ANOVA linear mixed effects models for the response variable potential utilizable forage (PUF) in 2006. If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by growing season (April through October), and by spring (April through May), summer (June through August), and fall (September through October).

Model	Fixed effects	Random effects	Variance-covariance	d.f.[1]	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
Growing season 2006										
1	PUF:Treatment[2]	1 | Block[3]	Equal var - Zero cov	6	149.84	-68.92				
2	Grand mean	1 | Block	Equal var - Zero cov	3	172.09	-83.05	1 vs. 2	28.25	< 0.0001	Models sig. diff. - choose lower AIC (model 1)
Separating means										
3	PUF:MIRG-CONT[4]	1 | Block	Equal var - Zero cov	5	160.97	-75.49	1 vs. 3	13.14	0.0003	MIRG sig. diff. than CONT
4	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	168.37	-79.19	1 vs. 4	20.54	<0.0001	MIRG sig. diff. than HARV
5	PUF:MIRG-NONE	1 | Block	Equal var - Zero cov	5	175.29	-82.65	1 vs. 5	27.45	< 0.0001	MIRG sig. diff. than NONE
6	PUF:CONT-HARV	1 | Block	Equal var - Zero cov	5	152.85	-71.44	1 vs. 6	0.01	0.03	CONT sig. diff. than HARV
7	PUF:CONT-NONE	1 | Block	Equal var - Zero cov	5	162.70	-76.35	1 vs. 7	14.86	0.0001	CONT sig. diff. than NONE
8	PUF:HARV-NONE	1 | Block	Equal var - Zero cov	5	154.31	-72.16	1 vs. 8	6.48	0.01	HARV sig. diff. than NONE
Spring										
9	PUF:Treatment	1 | Block	Equal var - Zero cov	6	125.59	-56.80				
10	Grand mean	1 | Block	Equal var - Zero cov	3	141.02	-67.51	9 vs. 10	21.43	0.0001	Models sig. diff.-choose lower AIC (model 9)
Separating means										
11	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	139.92	-64.96	9 vs. 11	16.33	0.0001	MIRG sig. diff. than CONT
12	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	124.46	-57.28	9 vs. 12	0.86	0.35	MIRG NS diff. than HARV
13	PUF:MIRG-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	134.17	-63.09	12 vs. 13	11.72	0.0006	MIRG and HARV sig. diff. than NONE
14	PUF:MIRG-HARV vs. CONT-NONE	1 | Block	Equal var - Zero cov	4	126.87	-59.43	12 vs. 14	4.41	0.04	CONT sig. diff. than NONE
Summer										
15	PUF:Treatment	1 | Block	Equal var - Zero cov	6	151.46	-69.73				
16	Grand mean	1 | Block	Equal var - Zero cov	3	172.75	-83.38	15 vs. 16	27.29	< 0.0001	Models sig. diff.-choose lower AIC (model 15)
Separating means										
17	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	167.65	-78.82	15 vs. 17	18.19	< 0.0001	MIRG sig. diff. than CONT
18	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	166.74	-78.37	15 vs. 18	17.28	< 0.0001	MIRG sig. diff. than HARV
19	PUF:MIRG-NONE	1 | Block	Equal var - Zero cov	5	176.36	-83.18	15 vs. 19	26.90	< 0.0001	MIRG sig. diff. than NONE
20	PUF:CONT-HARV	1 | Block	Equal var - Zero cov	5	149.56	-69.78	15 vs. 20	0.10	0.75	CONT NS diff. than HARV
21	PUF:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	158.53	-75.27	20 vs. 21	10.97	0.0009	CONT and HARV sig. diff. than NONE
Fall										
22	PUF:Treatment	1 | Block	Equal var - Zero cov	6	108.55	-48.28				
23	Grand mean	1 | Block	Equal var - Zero cov	3	117.36	-55.68	22 vs. 23	14.80	0.002	Models sig. diff.-choose lower AIC (model 22)
Separating means										
24	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	106.61	-48.31	22 vs. 24	0.06	0.81	MIRG NS diff. than CONT
25	PUF:MIRG-CONT vs. HARV	1 | Block	Equal var - Zero cov	4	109.65	-50.82	24 vs. 25	5.03	0.02	MIRG and CONT sig. diff. than HARV
26	PUF:MIRG-CONT vs. NONE	1 | Block	Equal var - Zero cov	4	119.19	-55.59	24 vs. 26	14.57	0.0001	MIRG and CONT sig. diff. than NONE
27	PUF:HARV vs. NONE	1 | Block	Equal var - Zero cov	4	109.48	-50.74	24 vs. 27	4.87	0.03	HARV sig. diff. than NONE

[1]d.f., number of parameters estimated

[2]Use of colon (:) indicates specification of interaction terms but no main effects.

[3]Verticle bar (|) separates continuous from categorical random effects in S-plus syntax.

1 specifies random intercepts for each level of block. A '1' to the left of the vertical bar indicates no random slopes should be fitted, only intercepts for those terms to the right of the vertical bar.

[4]Treatment levels collapsed to compare means, e.g. model 3 - MIRG+CONT vs. HARV, NONE.

Table B2. Comparison of ANOVA linear mixed effects models for the response variable potential utilizable forage (PUF) in 2007. If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by growing season (April through October), and by spring (April through May), summer (June through August), and fall (September through October [syntax explained in Table B1 footnotes]).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
Growing season 2007										
1	PUF:Treatment	1 | Block	Equal var - Zero cov	6	162.85	-75.43				
2	Grand mean	1 | Block	Equal var - Zero cov	3	179.63	-86.82	1 vs. 2	22.77	< 0.0001	Models sig. diff.-choose lower AIC (model 1)
Separating means										
3	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	173.47	-81.73	1 vs. 3	12.61	0.0004	MIRG sig. diff. than CONT
4	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	166.81	-78.41	1 vs. 4	5.95	0.01	MIRG sig. diff. than HARV
5	PUF:MIRG-NONE	1 | Block	Equal var - Zero cov	5	183.03	-86.51	1 vs. 5	22.17	< 0.0001	MIRG sig. diff. than NONE
6	PUF:CONT-HARV	1 | Block	Equal var - Zero cov	5	164.15	-77.07	1 vs. 6	3.29	0.07	CONT NS diff. than HARV
7	PUF:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	173.30	-82.65	6 vs. 7	11.14	0.0008	CONT and HARV sig. diff. than NONE
Spring										
8	PUF:Treatment	1 | Block	Equal var - Zero cov	6	135.22	-61.61				
9	Grand mean	1 | Block	Equal var - Zero cov	3	147.19	-70.60	8 vs. 9	17.97	0.0004	Models sig. diff.-choose lower AIC (model 8)
Separating means										
10	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	138.41	-64.20	8 vs. 10	5.19	0.02	MIRG sig. diff. than CONT
11	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	144.84	-67.42	8 vs. 11	11.62	0.0007	MIRG sig. diff. than HARV
12	PUF:MIRG-NONE	1 | Block	Equal var - Zero cov	5	150.29	-70.15	8 vs. 12	17.08	< 0.0001	MIRG sig. diff. than NONE
13	PUF:CONT-HARV	1 | Block	Equal var - Zero cov	5	136.32	-63.16	8 vs. 13	3.09	0.08	CONT NS diff. than HARV
14	PUF:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	140.01	-66.00	13 vs. 14	5.69	0.02	CONT and HARV sig. diff. than NONE
Summer										
15	PUF:Treatment	1 | Block	Equal var - Zero cov	6	155.32	-71.66				
16	Grand mean	1 | Block	Equal var - Zero cov	3	166.24	-80.12	15 vs. 16	16.93	0.0007	Models sig. diff.-choose lower AIC (model 15)
Separating means										
17	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	160.17	-75.09	15 vs. 17	6.86	0.01	MIRG sig. diff. than CONT
18	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	153.33	-71.67	15 vs. 18	0.02	0.90	MIRG NS diff. than HARV
19	PUF:MIRG-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	167.48	-79.74	18 vs. 19	16.15	0.0001	MIRG and HARV sig. diff. than NONE
20	PUF:CONT-NONE	1 | Block	Equal var - Zero cov	4	154.65	-73.33	18 vs. 20	3.32	0.07	CONT NS diff. than NONE
Fall										
21	PUF:Treatment	1 | Block	Equal var - Zero cov	6	124.28	-56.14				
22	Grand mean	1 | Block	Equal var - Zero cov	3	149.77	-71.88	21 vs. 22	31.49	< 0.0001	Models sig. diff.-choose lower AIC (model 21)
Separating means										
23	PUF:MIRG-CONT	1 | Block	Equal var - Zero cov	5	145.59	-67.80	21 vs. 23	23.32	< 0.0001	MIRG sig. diff. than CONT
24	PUF:MIRG-HARV	1 | Block	Equal var - Zero cov	5	142.67	-66.33	21 vs. 24	20.39	< 0.0001	MIRG NS diff. than HARV
25	PUF:MIRG-NONE	1 | Block	Equal var - Zero cov	5	153.15	-71.57	21 vs. 25	30.87	< 0.0001	MIRG sig. diff. than NONE
26	PUF:CONT-HARV	1 | Block	Equal var - Zero cov	5	123.53	-56.76	21 vs. 26	1.25	0.26	CONT NS diff. than HARV
27	PUF:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	133.56	-62.78	26 vs. 27	12.03	0.0005	CONT and HARV sig. diff. than NONE

Table B3. Comparison of ANOVA linear mixed effects models for the response variable relative forage quality (RFQ) in 2006. If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by growing season (April through October), and by spring (April through May), summer (June through August), and fall (September through October [syntax explained in Table B1 footnotes]).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
Growing season 2006										
1	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	91.60	-39.80				
2	Grand mean	1 | Block	Equal var - Zero cov	3	117.40	-55.70	1 vs. 2	31.81	< 0.0001	Models sig. diff.-choose lower AIC (model 1)
Separating means										
3	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	106.40	-48.20	1 vs. 3	16.80	< 0.0001	MIRG sig. diff. than CONT
4	RFQ:MIRG-HARV	1 | Block	Equal var - Zero cov	5	95.34	-42.67	1 vs. 4	5.74	0.02	MIRG sig. diff. than HARV
5	RFQ:MIRG-NONE	1 | Block	Equal var - Zero cov	5	120.17	-55.09	1 vs. 5	30.57	< 0.0001	MIRG sig. diff. than NONE
6	RFQ:CONT-HARV	1 | Block	Equal var - Zero cov	5	97.82	-43.91	1 vs. 6	8.22	0.004	CONT sig. diff. than HARV
7	RFQ:CONT-NONE	1 | Block	Equal var - Zero cov	5	105.88	-47.94	1 vs. 7	16.28	0.0001	CONT sig. diff. than NONE
8	RFQ:HARV-NONE	1 | Block	Equal var - Zero cov	5	114.74	-52.37	1 vs. 8	25.15	< 0.0001	HARV sig. diff. than NONE
Spring										
8	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	99.95	-43.97				
9	Grand mean	1 | Block	Equal var - Zero cov	3	103.86	-48.93	8 vs. 9	9.91	0.02	Models sig. diff.-choose lower AIC (model 8)
Separating means										
10	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	98.07	-44.03	8 vs. 10	0.12	0.73	MIRG NS diff. than CONT
11	RFQ:MIRG-CONT-HARV	1 | Block	Equal var - Zero cov	4	96.32	-44.16	10 vs. 11	0.25	0.62	MIRG and CONT NS diff. than HARV
12	RFQ:MIRG-NONE	1 | Block	Equal var - Zero cov	5	105.32	-47.57	8 vs. 12	7.19	0.007	MIRG sig. diff. than NONE
Summer										
13	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	93.62	-40.81				
14	Grand mean	1 | Block	Equal var - Zero cov	3	110.92	-52.46	13 vs.14	23.29	< 0.0001	Models sig. diff.-choose lower AIC (model 13)
Separating means										
15	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	104.68	-47.34	13 vs. 15	13.06	0.0003	MIRG sig. diff. than CONT
16	RFQ:MIRG-HARV	1 | Block	Equal var - Zero cov	5	92.44	-41.22	13 vs. 16	0.82	0.37	MIRG NS diff. than HARV
17	RFQ:MIRG-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	111.94	-51.97	16 vs. 17	21.50	< 0.0001	MIRG and HARV sig. diff. than NONE
18	RFQ:CONT-NONE	1 | Block	Equal var - Zero cov	4	95.32	-43.66	16 vs. 18	4.88	0.03	CONT sig. diff. than NONE
Fall										
19	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	111.18	-49.59				
20	Grand mean	1 | Block	Equal var - Zero cov	3	131.43	-62.71	19 vs. 20	31.49	< 0.0001	Models sig. diff.-choose lower AIC (model 19)
Separating means										
21	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	121.55	-55.77	19 vs. 21	12.37	0.0004	MIRG sig. diff. than CONT
22	RFQ:MIRG-HARV	1 | Block	Equal var - Zero cov	5	115.41	-52.70	19 vs.22	6.23	0.01	MIRG NS diff. than HARV
23	RFQ:MIRG-NONE	1 | Block	Equal var - Zero cov	5	134.79	-62.40	19 vs. 23	25.61	< 0.0001	MIRG sig. diff. than NONE
24	RFQ:CONT-HARV	1 | Block	Equal var - Zero cov	5	112.46	-51.23	19 vs. 24	3.28	0.07	CONT NS diff. than HARV
25	RFQ:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	126.50	-59.25	24 vs. 25	16.04	0.0001	CONT and HARV sig. diff. than NONE

Table B4. Comparison of ANOVA linear mixed effects models for the response variable relative forage quality (RFQ) in 2007. If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by growing season (April through October), and by spring (April through May), summer (June through August), and fall (September through October [syntax explained in Table B1 footnotes]).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
Growing season 2007										
1	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	84.66	-36.33				
2	Grand mean	1 | Block	Equal var - Zero cov	3	107.33	-50.67	1 vs. 2	28.67	< 0.0001	Models sig. diff.-choose lower AIC (model 1)
Separating means										
3	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	98.24	-44.12	1 vs. 3	15.58	0.0001	MIRG sig. diff. than CONT
4	RFQ:MIRG-HARV	1 | Block	Equal var - Zero cov	5	93.72	-41.86	1 vs. 4	11.06	0.0009	MIRG sig. diff. than HARV
5	RFQ:MIRG-NONE	1 | Block	Equal var - Zero cov	5	111.11	-50.56	1 vs. 5	28.45	< 0.0001	MIRG sig. diff. than NONE
6	RFQ:CONT-HARV	1 | Block	Equal var - Zero cov	5	84.57	-37.28	1 vs. 6	1.91	0.17	CONT NS diff. than HARV
7	RFQ:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	99.90	-45.95	6 vs. 7	17.34	< 0.0001	CONT and HARV sig. diff. than NONE
Spring										
8	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	104.29	-46.15				
9	Grand mean	1 | Block	Equal var - Zero cov	3	100.63	-47.32	8 vs. 9	2.34	0.5	Models NS diff.
Summer										
10	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	88.33	-38.16				
11	Grand mean	1 | Block	Equal var - Zero cov	3	110.31	-52.15	10 vs. 11	27.98	< 0.0001	Models sig. diff.-choose lower AIC (model 10)
Separating means										
12	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	106.32	-48.16	10 vs. 12	19.99	< 0.0001	MIRG sig. diff. than CONT
13	RFQ:MIRG-HARV	1 | Block	Equal var - Zero cov	5	90.94	-40.47	10 vs. 13	4.61	0.03	MIRG sig. diff. than HARV
14	RFQ:MIRG-NONE	1 | Block	Equal var - Zero cov	5	111.69	-50.85	10 vs. 14	25.36	< 0.0001	MIRG sig. diff. than NONE
15	RFQ:CONT-HARV	1 | Block	Equal var - Zero cov	5	100.44	-45.22	10 vs. 15	14.11	0.0002	CONT sig. diff. than HARV
16	RFQ:CONT-NONE	1 | Block	Equal var - Zero cov	5	91.44	-40.72	10 vs. 16	5.11	0.02	CONT sig. diff. than NONE
17	RFQ:HARV-NONE	1 | Block	Equal var - Zero cov	5	106.70	-48.35	10 vs. 17	20.37	< 0.0001	HARV sig. diff. than NONE
Fall										
18	RFQ:Treatment	1 | Block	Equal var - Zero cov	6	105.67	-48.83				
19	Grand mean	1 | Block	Equal var - Zero cov	3	118.19	-56.09	18 vs. 19	18.52	0.0003	Models sig. diff.-choose lower AIC (model 18)
Separating means										
20	RFQ:MIRG-CONT	1 | Block	Equal var - Zero cov	5	107.74	-48.87	18 vs. 20	4.07	0.04	MIRG sig. diff. than CONT
21	RFQ:MIRG-HARV	1 | Block	Equal var - Zero cov	5	111.67	-50.83	18 vs. 21	7.99	0.005	MIRG sig. diff. than HARV
22	RFQ:MIRG-NONE	1 | Block	Equal var - Zero cov	5	121.80	-55.90	18 vs. 22	18.13	< 0.0001	MIRG sig. diff. than NONE
23	RFQ:CONT-HARV	1 | Block	Equal var - Zero cov	5	104.96	-47.48	18 vs. 23	1.30	0.25	CONT NS diff. than HARV
24	RFQ:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	113.38	-52.69	23 vs. 24	10.41	0.001	CONT and HARV sig. diff. than NONE

Table B5. Comparison of ANOVA linear mixed effects models for the response variable belowground net primary production (BNPP) in 2006 and 2007. If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by growing season (April through October [syntax explained in Table B1 footnotes]).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
2006										
1	BNPP:Treatment	1 | Block	Equal var - Zero cov	6	671.33	-329.67				
2	Grand mean	1 | Block	Equal var - Zero cov	3	684.31	-339.15	1 vs. 2	18.97	0.0003	Models sig. diff. - choose lower AIC (model 1)
Separating means										
3	BNPP:MIRG-CONT	1 | Block	Equal var - Zero cov	5	669.61	-329.80	1 vs. 3	0.27	0.60	MIRG NS diff. than CONT
4	BNPP:MIRG-CONT vs. HARV	1 | Block	Equal var - Zero cov	4	680.06	-336.03	3 vs. 4	12.73	0.002	MIRG and CONT sig. diff. than HARV
5	BNPP:MIRG-CONT vs. NONE	1 | Block	Equal var - Zero cov	4	681.80	-336.90	3 vs. 5	14.47	0.0007	MIRG and CONT sig. diff. than NONE
6	BNPP:HARV-NONE	1 | Block	Equal var - Zero cov	4	667.67	-329.83	3 vs. 6	0.33	0.85	HARV sig. diff. than NONE
2007										
7	BNPP:Treatment	1 | Block	Equal var - Zero cov	6	728.37	-358.18				
8	Grand mean	1 | Block	Equal var - Zero cov	3	735.09	-364.55	7 vs. 8	12.72	0.005	Models sig. diff. - choose lower AIC (model 7)
Separating means										
9	BNPP:MIRG-CONT	1 | Block	Equal var - Zero cov	5	728.57	-359.29	7 vs. 9	2.21	0.14	MIRG NS diff. than CONT
10	BNPP:MIRG-CONT vs. HARV	1 | Block	Equal var - Zero cov	4	728.03	-360.02	9 vs. 10	1.46	0.23	MIRG and CONT NS diff. than HARV
11	BNPP:MIRG-CONT vs. NONE	1 | Block	Equal var - Zero cov	4	737.08	-364.54	10 vs. 11	10.50	0.001	MIRG and CONT sig. diff. than NONE

Appendix C. Comparison of ANOVA linear-mixed effects models for Chapter 2

Table C1. Comparison of ANOVA linear mixed effects models for the response variable aboveground net primary production (ANPP). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by year.

Model	Fixed effects	Random effects	Variance-covariance	d.f.[1]	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
2006										
1	ANPP:Treatment[2]	1 | Block[3]	Equal var - Zero cov	6	150.66	-68.33				
2	Grand mean	1 | Block	Equal var - Zero cov	3	177.89	-85.95	1 vs. 2	33.24	<0.0001	Models sig. diff. - choose lower AIC (model 1)
Separating means										
3	ANPP:CONT-MIRG[4]	1 | Block	Equal var - Zero cov	5	156.73	-73.36	1 vs. 3	8.07	0.005	CONT sig. diff. than MIRG
4	ANPP:CONT-HARV	1 | Block	Equal var - Zero cov	5	159.13	-74.57	1 vs. 4	10.47	0.001	CONT sig. diff. than HARV
5	ANPP:CONT-NONE	1 | Block	Equal var - Zero cov	5	173.92	-81.96	1 vs. 5	25.26	<0.0001	CONT sig. diff. than NONE
6	ANPP:MIRG-HARV	1 | Block	Equal var - Zero cov	5	169.47	-79.73	1 vs. 6	20.81	<0.0001	MIRG sig. diff. than HARV
7	ANPP:MIRG-NONE	1 | Block	Equal var - Zero cov	5	180.68	-85.34	1 vs. 7	32.02	<0.0001	MIRG sig. diff. than NONE
8	ANPP:HARV-NONE	1 | Block	Equal var - Zero cov	5	165.27	-76.42	1 vs. 8	14.19	0.0002	HARV sig. diff. than NONE
2007										
9	ANPP:Treatment	1 | Block	Equal var - Zero cov	6	162.25	-75.13				
10	Grand mean	1 | Block	Equal var - Zero cov	3	181.88	-87.95	9 vs. 10	25.64	<0.0001	Models sig. diff.-choose lower AIC (model 9)
Separating means										
11	ANPP:CONT-MIRG	1 | Block	Equal var - Zero cov	5	173.19	-81.59	9 vs. 11	12.93	0.0003	CONT sig. diff. than MIRG
12	ANPP:CONT-HARV	1 | Block	Equal var - Zero cov	5	162.92	-76.46	9 vs. 12	2.67	0.1	CONT NS diff. than HARV
13	ANPP:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	175.96	-83.98	12 vs. 13	15.04	0.0001	CONT and HARV sig. diff. than NONE
14	ANPP:MIRG-NONE	1 | Block	Equal var - Zero cov	5	185.43	-87.71	9 vs. 14	25.18	<0.0001	MIRG sig. diff. than NONE

[1]d.f., number of parameters estimated

[2]Use of colon (:) indicates specification of interaction terms but no main effects.

[3]Verticle bar (|) separates continuous from categorical random effects in S-plus syntax.
1 specifies random intercepts for each level of block. A '1' to the left of the vertical bar indicates no random slopes should be fitted, only intercepts for those terms to the right of the vertical bar.

[4]Treatment levels collapsed to compare means, e.g. model 13 - CONT+MIRG vs. HARV, NONE.

Table C2. Comparison of ANOVA linear mixed effects models for the response variable belowground net primary production (BNPP). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by year (syntax explainedin Table C1 footnotes).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
2006										
1	BNPP:Treatment	1 | Block	Equal var - Zero cov	6	140.35	-64.17				
2	Grand mean	1 | Block	Equal var - Zero cov	3	149.51	-71.75	1 vs. 2	15.16	0.002	Models sig. diff. - choose lower AIC (model 1)
Separating means										
3	BNPP:CONT-MIRG	1 | Block	Equal var - Zero cov	5	138.96	-64.48	1 vs. 3	0.61	0.43	CONT NS diff. than MIRG
4	BNPP:CONT-MIRG vs. HARV	1 | Block	Equal var - Zero cov	4	147.92	-69.96	3 vs. 4	10.96	0.0009	CONT and MIRG sig. diff. than HARV
5	BNPP:CONT-MIRG vs. NONE	1 | Block	Equal var - Zero cov	4	148.93	-70.47	3 vs. 5	11.97	0.0005	CONT and MIRG sig. diff. than NONE
6	BNPP:HARV-NONE	1 | Block	Equal var - Zero cov	5	138.44	-64.22	1 vs. 6	0.09	0.76	HARV NS diff. than NONE
2007										
7	BNPP:Treatment	1 | Block	Equal var - Zero cov	6	159.98	-73.99				
8	Grand mean	1 | Block	Equal var - Zero cov	3	162.16	-78.08	7 vs. 8	12.72	0.04	Models sig. diff. - choose lower AIC (model 7)
Separating means										
9	BNPP:CONT-MIRG	1 | Block	Equal var - Zero cov	5	159.53	-74.77	7 vs. 9	1.55	0.2	CONT NS diff. than MIRG
10	BNPP:CONT-MIRG vs. HARV	1 | Block	Equal var - Zero cov	4	158.62	-75.31	9 vs. 10	1.08	0.3	CONT and MIRG NS diff. than HARV
11	BNPP:CONT-MIRG vs. NONE	1 | Block	Equal var - Zero cov	4	164.15	-78.08	9 vs. 11	6.62	0.01	CONT and MIRG sig. diff. than NONE

Table C3. Comparison of ANOVA linear mixed effects model s for the response variable soil respiration (R_s). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by year (syntax explained in Table C1 footnotes).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
2006										
1	Rs:Treatment	1 | Block	Equal var - Zero cov	6	106.52	-47.26				
2	Grand mean	1 | Block	Equal var - Zero cov	3	127.35	-60.67	1 vs. 2	26.83	< 0.0001	Models sig. diff.-choose lower AIC (model 1)
Separating means										
3	Rs:CONT-MIRG	1 | Block	Equal var - Zero cov	5	105.60	-47.80	1 vs. 3	1.08	0.3	CONT NS diff. than MIRG
4	Rs:CONT-MIRG vs. HARV	1 | Block	Equal var - Zero cov	4	114.80	-53.40	3 vs. 4	11.19	0.0008	CONT and MIRG sig. diff. than HARV
5	Rs:CONT-MIRG vs. NONE	1 | Block	Equal var - Zero cov	4	129.23	-60.61	3 vs. 5	25.62	< 0.0001	CONT and MIRG sig. diff. than NONE
6	Rs:HARV-NONE	1 | Block	Equal var - Zero cov	5	118.04	-54.02	1 vs. 6	13.52	0.0002	HARV sig. diff. than NONE
2007										
7	Rs:Treatment	1 | Block	Equal var - Zero cov	6	126.09	-57.04				
8	Grand mean	1 | Block	Equal var - Zero cov	3	143.27	-68.63	7 vs. 8	23.18	< 0.0001	Models sig. diff.-choose lower AIC (model 7)
Separating means										
9	Rs:CONT-MIRG	1 | Block	Equal var - Zero cov	5	131.19	-60.59	7 vs. 9	7.10	0.008	CONT sig. diff. than MIRG
10	Rs:CONT-HARV	1 | Block	Equal var - Zero cov	5	141.41	-65.70	7 vs. 10	17.32	< 0.0001	CONT sig. diff. than HARV
11	Rs:CONT-NONE	1 | Block	Equal var - Zero cov	5	145.55	-67.78	7 vs. 11	21.46	< 0.0001	CONT sig. diff. than NONE
12	Rs:MIRG-HARV	1 | Block	Equal var - Zero cov	5	131.21	-60.61	7 vs. 12	7.13	0.008	MIRG sig. diff. than HARV
13	Rs:MIRG-NONE	1 | Block	Equal var - Zero cov	5	136.35	-63.18	7 vs. 13	12.26	0.0005	MIRG sig. diff. than NONE
14	Rs:HARV-NONE	1 | Block	Equal var - Zero cov	5	126.15	-58.08	7 vs. 14	2.06	0.15	HARV NS diff. than NONE

Table C4. Comparison of ANOVA linear mixed effects model s for the response variable net ecosystem carbon balance (NECB). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained. Model selection process is shown by year and averageacross both years (syntax explained in Table C1 footnotes).

Model	Fixed effects	Random effects	Variance-covariance	d.f.	AIC	Log L	Model test	L ratio	*P*	Interpretation of model comparison
2006										
1	NECB:Treatment	1 | Block	Equal var - Zero cov	6	127.92	-57.51				
2	Grand mean	1 | Block	Equal var - Zero cov	3	152.43	-72.49	1 vs. 2	29.96	< 0.0001	Models sig. diff. - choose lower AIC (model 1)
Separating means										
3	NECB:CONT-MIRG	1 | Block	Equal var - Zero cov	5	138.98	-64.49	1 vs. 3	13.97	0.0002	CONT sig. diff. than MIRG
4	NECB:CONT-HARV	1 | Block	Equal var - Zero cov	5	142.79	-66.39	1 vs. 4	17.78	<0.0001	CONT sig. diff. than HARV
5	NECB:CONT-NONE	1 | Block	Equal var - Zero cov	5	125.76	-57.88	1 vs. 5	0.75	0.39	CONT NS diff. than NONE
6	NECB:MIRG-HARV	1 | Block	Equal var - Zero cov	5	157.39	-72.45	1 vs. 6	29.89	< 0.0001	MIRG sig. diff. than HARV
2007										
9	NECB:Treatment	1 | Block	Equal var - Zero cov	6	148.25	-68.12				
10	Grand mean	1 | Block	Equal var - Zero cov	3	159.16	-76.58	9 vs. 10	16.92	0.0007	Models sig. diff.-choose lower AIC (model 9)
Separating means										
11	NECB:CONT-MIRG	1 | Block	Equal var - Zero cov	5	160.95	-75.48	9 vs. 11	14.70	0.0001	CONT sig. diff. than MIRG
12	NECB:CONT-HARV	1 | Block	Equal var - Zero cov	5	146.34	-68.17	9 vs. 12	0.09	0.76	CONT NS diff. than HARV
13	NECB:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	145.74	-68.87	12 vs. 13	1.41	0.24	CONT and HARV NS diff. than NONE
Average										
14	NECB:Treatment	1 | Block	Equal var - Zero cov	6	135.16	-61.58				
15	Grand mean	1 | Block	Equal var - Zero cov	3	153.52	-73.76	14 vs. 15	24.36	<0.0001	Models sig. diff.-choose lower AIC (model 14)
Separating means										
16	NECB:CONT-MIRG	1 | Block	Equal var - Zero cov	5	151.17	-70.58	14 vs. 16	18.01	<0.0001	CONT sig. diff. than MIRG
17	NECB:CONT-HARV	1 | Block	Equal var - Zero cov	5	136.67	-63.33	14 vs. 17	3.51	0.06	CONT NS diff. than HARV
18	NECB:CONT-HARV vs. NONE	1 | Block	Equal var - Zero cov	4	137.36	-64.68	17 vs. 18	2.69	0.1	CONT and HARV NS diff. than NONE

Appendix D. Comparison of ANOVA linear-mixed effects models for Chapter 3

Table D1. Comparison of ANOVA linear mixed effects models for the lipid correlations by treatment from principal component axes 1 (PC1) and 2 (PC2). f models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained.

Model	Fixed effects	Random effects	d.f.[1]	AIC	Log L	Model test	F value or L ratio	*p*	Interpretation of model comparison
1	PC1:TREATMENT[2]	1 | Block/Sub[3]	7	-325.42	169.71		$F_{3,90}$= 0.28	0.84	TREATMENT is NS
2	PC2:TREATMENT	1 | Block/Sub	7	-233.60	123.80		$F_{3,90}$= 4.72	0.004	TREATMENT is sig.
Separating means									
3	PC2:CONT-MIRG[4]	1 | Block/Sub	6	-235.00	123.50	2 vs. 3	0.6	0.44	CONT NS diff. than MIRG
4	PC2:CONT-MIRG vs. HARV	1 | Block/Sub	5	-225.49	117.74	3 vs. 4	11.51	< 0.001	CONT and MIRG sig. diff. than HARV
5	PC2:CONT-MIRG vs. NONE	1 | Block/Sub	5	-231.45	120.72	3 vs. 5	5.55	0.02	CONT and MIRG sig. diff. than NONE
6	PC2:HARV-NONE	1 | Block/Sub	6	-234.68	123.34	2 vs. 6	0.92	0.34	HARV NS diff. than NONE

Variance structure for these models = Equal variance - Zero covariance

[1] d.f.. number of parameters estimated
[2] Use of colon (:) indicates specification of interaction terms but no main effects.
[3] Verticle bar (|) separates continuous from categorical random effects in S-plus syntax.
1 specifies random intercepts for each level of block. A '1' to the left of the vertical bar indicates no random slopes should be fitted, only intercepts for those terms to the right of the vertical bar.
[4] Treatment levels collapsed to compare means, e.g. model 3 - CONT+MIRG vs. HARV.

Table D2. Comparison of ANOVA linear mixed effects models or fungal to bacterial ratios (F:B) by treatment. If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained (syntax explained in Table D1 footnotes).

Model	Fixed effects	Random effects	d.f.[1]	AIC	Log L	Model test	F value or L ratio	*p*	Interpretation of model comparison
1	F:B:TREATMENT[2]	1 | Date/Block[3]	7	24.30	-5.15		$F_{3,90}$= 4.85	0.004	TREATMENT is sig.
Separating means									
2	F:B:CONT-MIRG[4]	1 | Date/Block	6	22.62	-5.31	1 vs. 2	0.32	0.57	CONT NS diff. than MIRG
3	F:B:CONT-MIRG vs. HARV	1 | Date/Block	5	33.83	-11.92	2 vs. 3	13.21	< 0.001	CONT and MIRG sig. diff. than HARV
4	F:B:CONT-MIRG vs. NONE	1 | Date/Block	5	24.33	-7.16	2 vs. 4	3.71	0.05	CONT and MIRG sig. diff. than NONE
5	F:B:HARV-NONE	1 | Date/Block	6	24.75	-6.38	1 vs. 5	2.45	0.12	HARV NS diff. than NONE

Variance structure for these models = Equal variance - Zero covariance

Table D3. Comparison of ANOVA linear mixed effects models by treatment for the microbial guilds gram positive (Gm+), and gram negative (Gm-) bacteria, anaerobic bacteria, actinomycetes, saprotrophic fungi, and arbuscular mycorrhizal fungi (AMF). If models were significantly different ($P < 0.05$), the model with the lower Akaike's information criteria (AIC) was retained (syntax explained in Table D1 footnotes).

Model	Fixed effects	Random effects	d.f.[1]	AIC	Log L	Model test	F value or L ratio	*p*	Interpretation of model comparison
Gram positive bacteria									
1	GM+:TREATMENT	1 | Date/Block	7	456.63	-221.32		$F_{3,90}$= 5.12	< 0.01	TREATMENT is sig.
Separating means									
2	GM+:CONT-MIRG[4]	1 | Date/Block	6	454.94	-221.47	1 vs. 2	0.31	0.58	CONT NS diff. than MIRG
3	GM+:CONT-MIRG vs. HARV	1 | Date/Block	5	467.23	-228.62	2 vs. 3	14.29	< 0.001	CONT and MIRG sig. diff. than HARV
4	GM+:CONT-MIRG vs. NONE	1 | Date/Block	5	455.57	-222.79	2 vs. 4	2.63	0.11	CONT and MIRG NS diff. than NONE
Gram negaitive bacteria									
5	GM-:TREATMENT	1 | Block†	8	468.42	-226.21		$F_{3,120}$= *1785*.43	0.39	TREATMENT is NS
Anaerobic bacteria									
6	ANAER:TREATMENT	1 | Block	8	203.88	-93.94		$F_{3,120}$= 2.65	0.05	TREATMENT is sig.
Separating means									
7	ANAER:CONT-MIRG	1 | Block	7	201.91	-93.96	6 vs. 7	0.04	0.85	CONT NS diff. than MIRG
8	ANAER:CONT-MIRG vs. HARV	1 | Block	6	205.92	-96.96	7 vs. 8	6.01	0.01	CONT and MIRG sig. diff. than HARV
9	ANAER:CONT-MIRG vs. NONE	1 | Block	6	199.91	-93.96	7 vs. 9	0.0003	0.99	CONT and MIRG NS diff. than NONE
10	ANAER:HARV-NONE	1 | Block	7	206.76	-96.38	6 vs.10	4.88	0.03	HARV sig. diff. than NONE
Actinomycetes									
11	ACT:TREATMENT	1 | Date/Block	7	10.89	1.55		$F_{3,90}$= 4.46	< 0.01	TREATMENT is sig.
Separating means									
12	ACT:CONT-MIRG	1 | Date/Block	6	12.12	-0.06	11 vs. 12	0.07	0.85	CONT NS diff. than MIRG
13	ACT:CONT-MIRG vs. HARV	1 | Date/Block	5	18.69	-4.34	12 vs. 13	8.56	< 0.01	CONT and MIRG sig. diff. than HARV
14	ACT:CONT-MIRG vs. NONE	1 | Date/Block	5	14.17	-2.09	12 vs. 14	4.05	0.04	CONT and MIRG sig. diff. than NONE
15	ACT:HARV vs. NONE	1 | Date/Block	6	9.60	1.20	11 vs. 15	0.71	0.40	HARV NS diff. than NONE
Saprotrophic funigi									
16	SAPRO:TREATMENT	1|Block‡	7	464.42	-225.21		$F_{3,120}$=*788.47*	0.45	TREATMENT is NS
AMF									
17	AMF:TREATMENT	1 | Date/Block†	7	248.64	-117.32		$F_{3,90}$= 4.46	< 0.01	TREATMENT is sig.
Separating means									
18	AMF:CONT-MIRG	1 | Date/Block	6	247.69	-117.85	17 vs. 18	1.05	0.31	CONT NS diff. than MIRG
19	AMF:CONT-MIRG vs. HARV	1 | Date/Block	5	258.56	-124.28	18 vs. 19	12.87	< 0.001	CONT and MIRG sig. diff. than HARV
20	AMF:CONT-MIRG vs. NONE	1 | Date/Block	5	252.23	-121.12	18 vs. 20	6.54	0.01	CONT and MIRG sig. diff. than NONE
21	AMF:HARV vs. NONE	1 | Date/Block	6	247.56	117.78	17 vs. 21	0.93	0.34	HARV NS diff. than NONE

Variance structure fit for heteroscadasticity in 1) † = the random effects Block term, and 2)‡ = increasing values; no symbol = Equal variance - Zero covariance

Appendix E. Regression trees

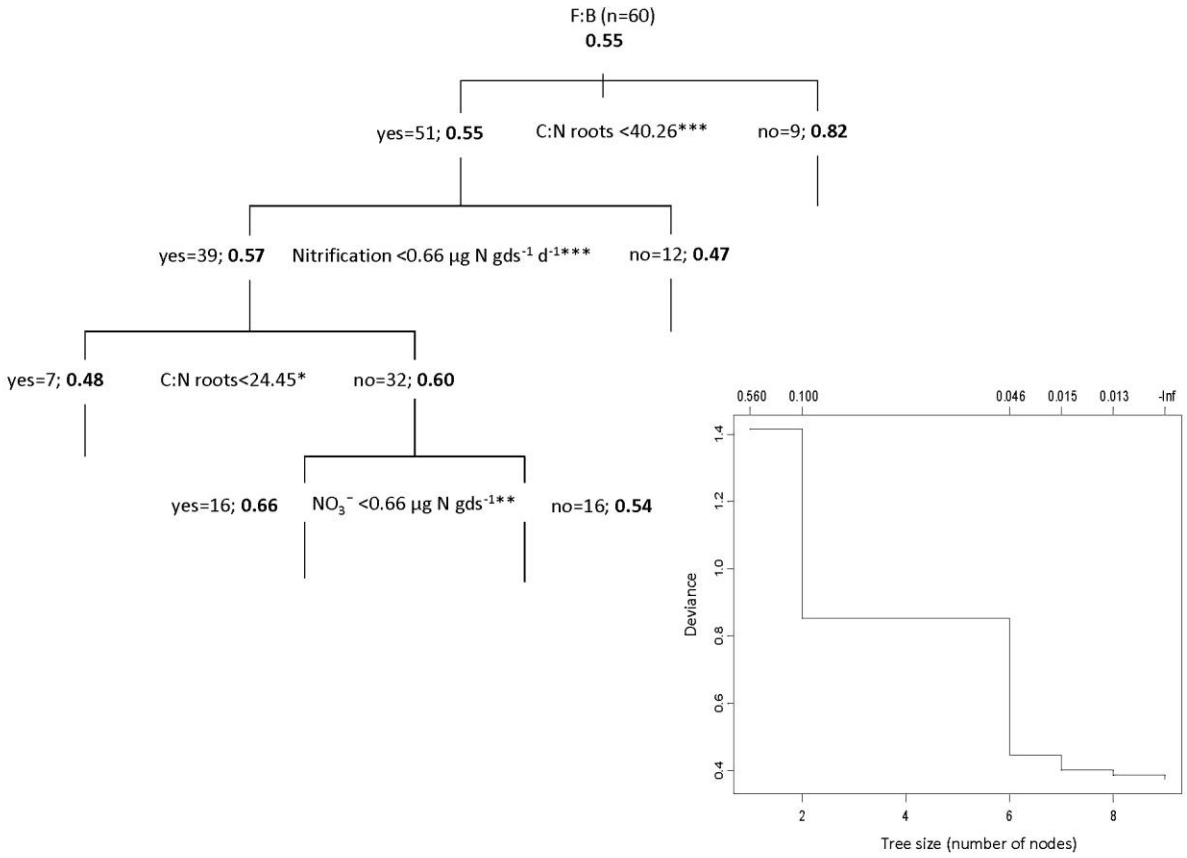

Fig. E1. Classification tree predicting fungal to bacterial ratios (F:B) from 60 soil cores taken to 15-cm in depth. The pool of candidate predictor variables was: date, block, relative forage quality, carbon to nitrogen ratio (C:N) of aboveground shoot C:N, root C:N roots, soil C:N, net N mineralization, net nitrification, net ammonification, soil NO_3^- pool, soil NH_4^+ pool, soil bulk density, soil gravimetric water content, and soil temperature. Inset shows the decrease in residual deviance as a function of the number of data splits and the resulting tree nodes. The tree in this figure has six nodes resulting from five splits. Significance of splits was determined using paired t-tests ($P < 0.05$).

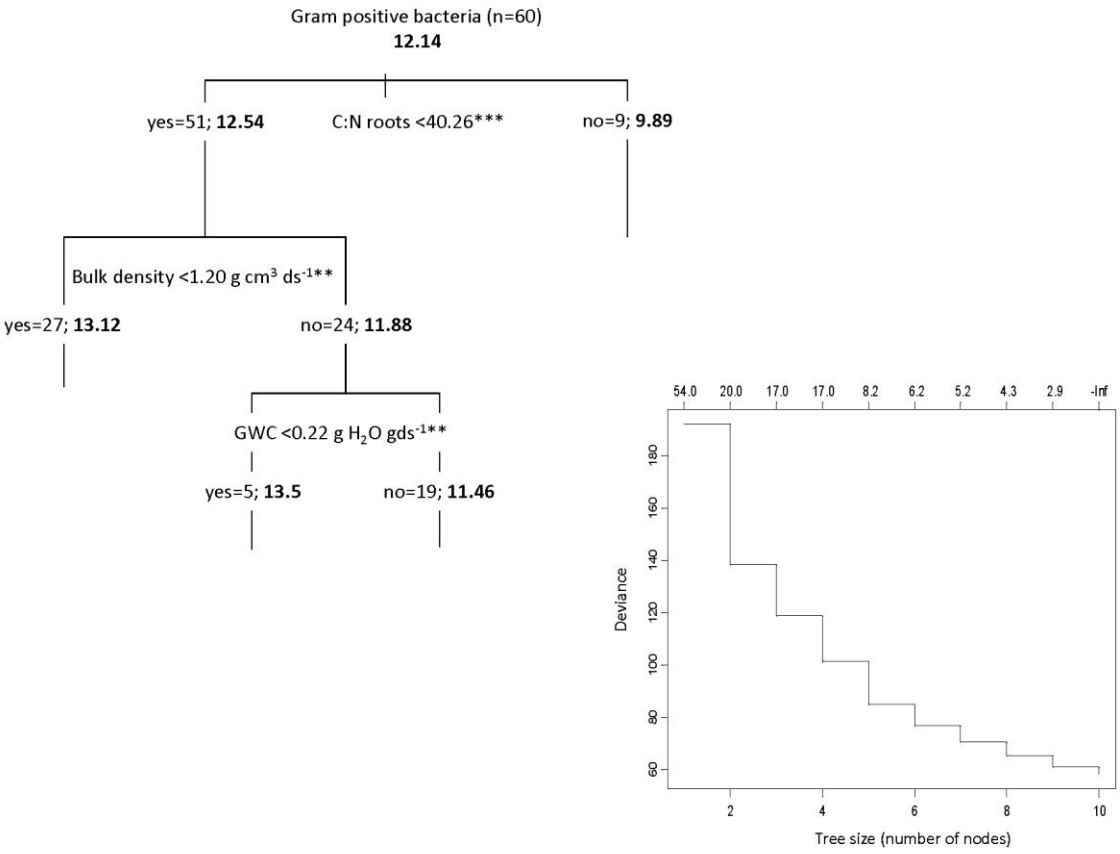

Fig. E2. Classification tree predicting gram positive bacteria (GM+) from 60 soil cores taken to 15-cm in depth (see Fig. C1 caption for list of predictor variables and explanation of the inset). The tree in this figure has five nodes resulting from four splits. Significance of splits was determined using paired t-tests ($P < 0.05$).

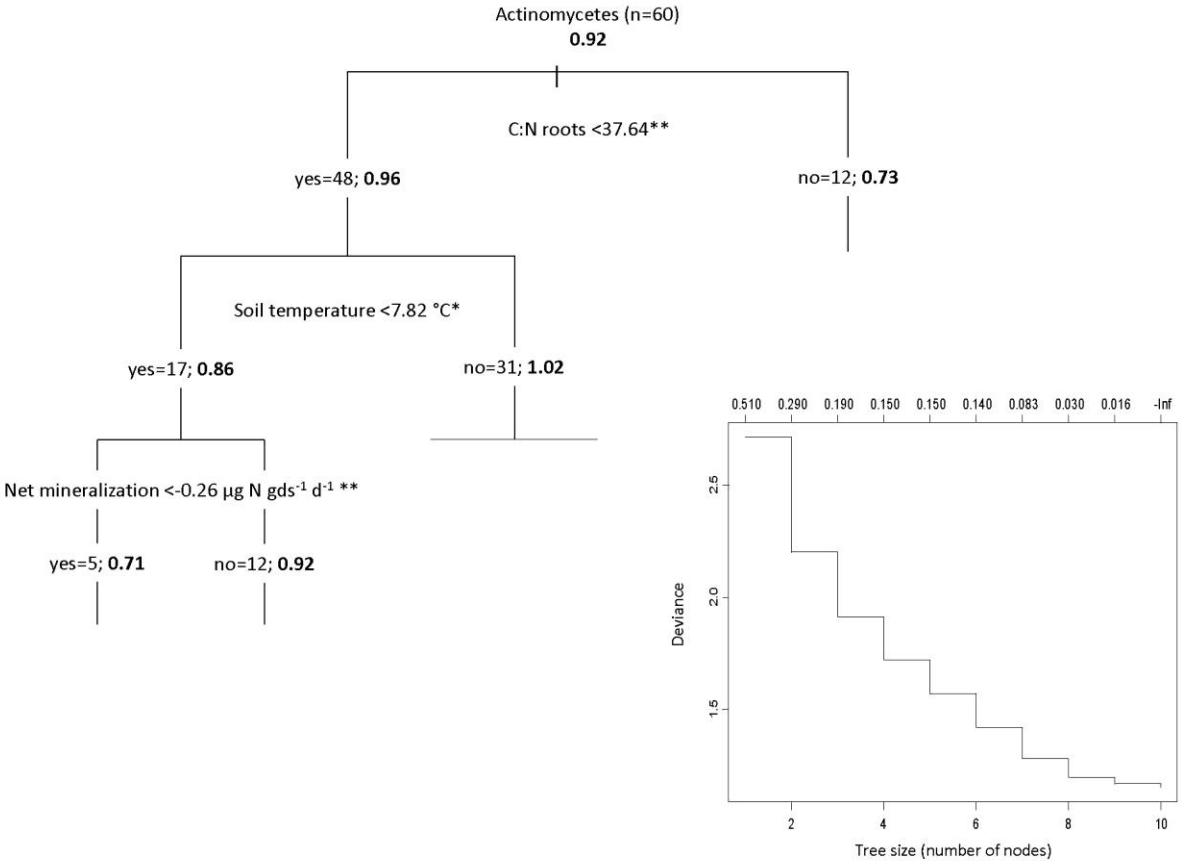

Fig. E3. Classification tree predicting actinomycetes from 60 soil cores taken to 15-cm in depth (see Fig. C1 caption for list of predictor variables and explanation of the inset). The tree in this figure has four nodes resulting from three splits. Significance of splits was determined using paired t-tests ($P < 0.05$).

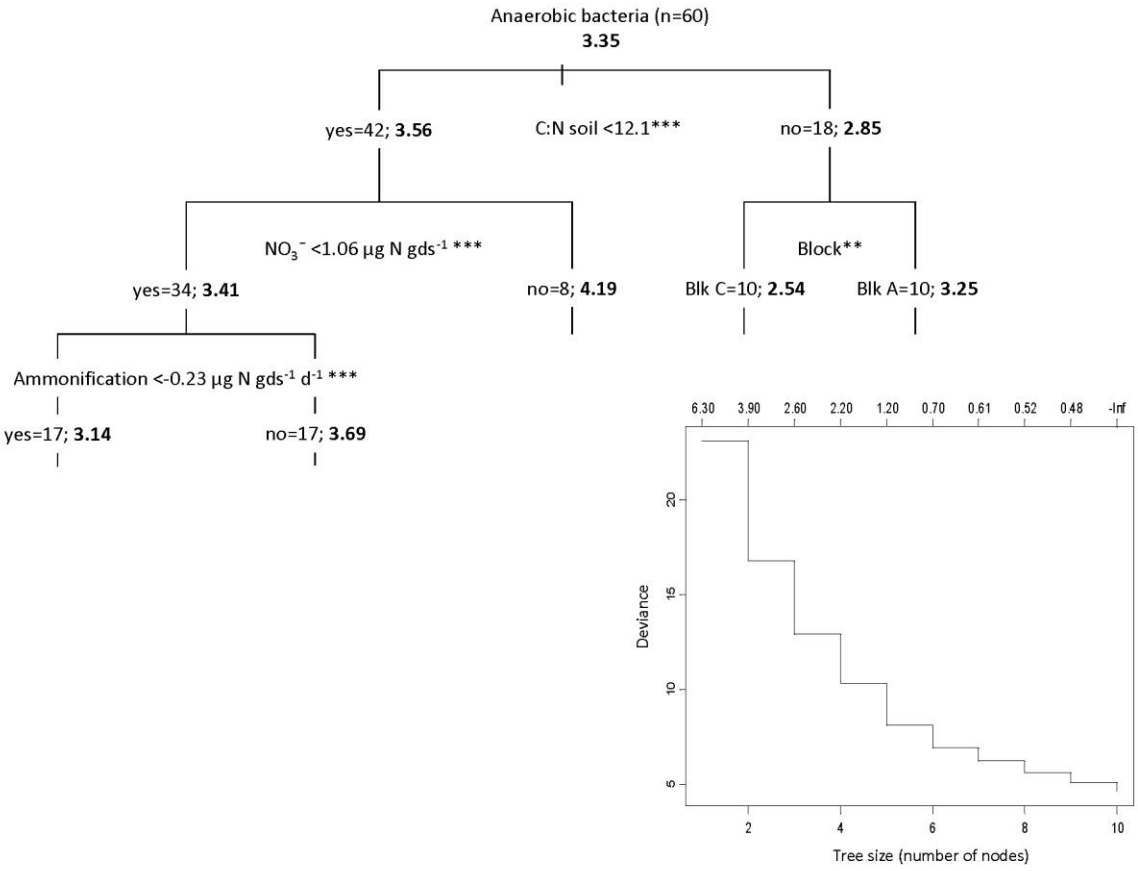

Fig. E4. Classification tree predicting anaerobic bacteria from 60 soil cores taken to 15-cm in depth (see Fig. C1 caption for list of predictor variables and explanation of the inset). The tree in this figure has six nodes resulting from five splits. Significance of splits was determined using paired t-tests ($P < 0.05$).

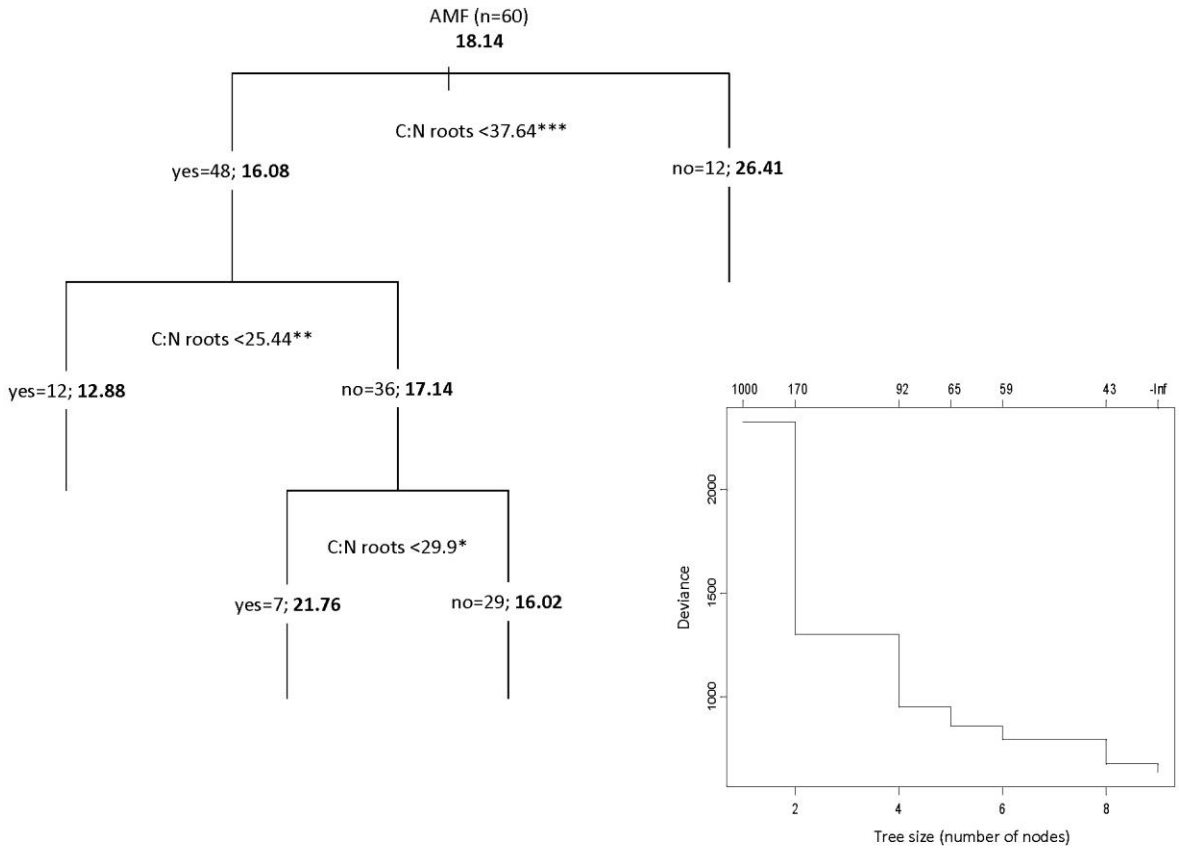

Fig. E5. Classification tree predicting arbuscular mycorrhizal fungi (AMF) from 60 soil cores taken to 15-cm in depth (see Fig. C1 caption for list of predictor variables and explanation of the inset). The tree in this figure has four nodes resulting from three splits. Significance of splits was determined using paired t-tests ($P < 0.05$).