Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

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Project Title: Sustainable Biofuels

Project number: 048-B1

1. **Abstract -** *Summarize the research and its essential qualities including a clear statement on the purpose of the research.*

Our research is designed to provide scientific knowledge needed to better understand, manage and sustain grassland ecosystems in Minnesota. Perennial grassland ecosystems have the potential to provide Minnesota with locally grown energy sources that reduce greenhouse gas emissions, improve water quality, and increase the extent of prairie ecosystems. However, biofuel grasslands might be fertilized and/or irrigated and may experience climate warming in the coming decades. We will study the effects of these factors on biomass yields, carbon sequestration in soils and other aspects of the functioning of alternative biomass crops, including switchgrass and *Miscanthus* monocultures and diverse prairie plantings. Such information is essential if we are to maximize the ability of biofuel crops to simultaneously provide society with sustainable energy and with other important ecosystem services. It will also test if the exotic perennial grass, *Miscanthus*, which has been proposed as a biofuel crop, might be an invasive species of prairie grasslands.

This project will use a warming experiment (consisting of 114 plots) and a fertilization and irrigation experiment (consisting of 96 plots) to determine how irrigation, fertilization, and climate warming impact yields, carbon sequestration, soil nitrogen levels, plant biodiversity, water quality and susceptibility to invasion. It would also experimentally whether *Miscanthus*, an exotic perennial grass species that is considered, by some, as a strong candidate for production of biomass for biofuels, poses a threat to native prairie as an invasive species. Our results will be synthesized to find methods for optimizing biofuel production, carbon storage, and habitat restoration.

2. **Background -** *Provide the basic information and other relevant work that are the context for this research*

Prairie grasslands once covered almost half of Minnesota, and are a core part of the natural heritage of the state. Their management and sustainability would be enhanced by added scientific information on how they function. Such added knowledge is also important because many of the features of prairies might make prairie-like crops, or other perennial grass crops, good sources of biomass for biofuels. An ideal biofuel would provide society multiple goods and services, including significant net energy gains, reduced greenhouse gas emissions and other environmental benefits, low production costs, and minimal competition with food supplies. DOE-supported research has shown switchgrass (*Panicum virgatum*) moncultures to be a viable biofuel crop that could offer multiple benefits to society. Similarly, biofuels derived from low-input high-diversity mixtures of native prairie perennial species have been shown to provide significant net energy gains, greenhouse gas reductions, and much lower agrichemical use per liter of biofuel than corn ethanol or soybean biodiesel (Tilman, Hill and Lehman 2006, Fornara and Tilman 2008, 2009). The potential yield advantage of low-input high-diversity grasslands comes from the ability of the cool-season legumes in the mixture to fix nitrogen that the warm season perennial grasses in the mixture (switchgrass, big bluestem, Indian grass and little bluestem) then convert into high levels of harvestable biomass. The greenhouse gas advantages of low-input high-diversity grasslands come partly from the low energy and fertilizer inputs needed to grow and harvest this biomass, and partly from their high organic soil carbon storage rates when grown on degraded soils (Fornara and Tilman 2008). It is worth noting that soils are the largest storehouse of carbon in Minnesota, and soil carbon sequestration might, at some time, be marketed as part of a carbon cap and trade system. If so, a portion of Minnesota's several million acres of CRP that had been prairie prior to settlement should be evaluated for the benefits that might come from restoring it to prairie, including potential benefits from improved wildlife habitat (Fargione et al. 2009).

However, as a given prairie biomass crop becomes established during the coming decades, changes in key environmental factors could significantly impact the functioning of their grassland ecosystems. In particular, climate change may lead to increased average temperatures (Giorgi et al. 2001; Wuebbles and Hayhoe 2003), greater evapotranspiration, and warmer soils. What might warmer temperatures lead to? Might they increase the decomposition of soil organic matter, and cause increased rates of released of carbon dioxide from the soil to the atmosphere? Might a shift in climate lead to invasions of restored Minnesota grassland ecosystems by exotic plant species? Might such effects be larger or smaller in high diversity mixtures of plant species than in monocultures? Such questions, and many more, are as yet unanswered.

Moreover, in order to increase productivity, farmers may try fertilizing or perhaps even irrigating their prairie biofuel crops. The effects of such management practices on biomass yields, on the net energy gained over the full lifecycle of prairie biomass growth and utilization, on the storage rate of soil carbon, on loss of nitrate to groundwater through leaching and on other aspects of ecosystem functioning are poorly understood for both monoculture energy crops and for high-diversity mixtures of native prairie perennials.

To understand how and why climatic change and management practices could affect the net energy and environmental costs and benefits of prairie grassland biofuel crops, we propose two experimental extensions of the ongoing Biodiversity Experiment at Cedar Creek Ecosystem Science Reserve. The Biodiversity Experiment is a long-term, well-replicated field experiment supported by an NSF LTER grant that manipulates the number and type of perennial prairie plant species that occur within each of its grassland plot. Findings from this experiment were used in the LIHD biofuel studies mentioned above (Tilman, Hill and Lehman 2006).

> **Extension I -- Biodiversity and Climate:** In this experiment, we explicitly manipulate temperature (via infrared heat lamps; warming treatments of 1.5 ^{o}C and 3.0 ^{o}C above ambient air temperature) and have unmanipulated controls within replicated plots planted to have one of four levels of plant diversity (monocultures, 4 species, 16 species, 32 species). Methods are described below.
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> **Extension II – Management:** The other extension would explicitly manipulate water availability, via weekly irrigation, and fertilization, as well as having unmanipulated control plots, in a full factorial well-replicated field experiment using both monoculture and 32 species plots. Methods are described below.
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> We would measure biomass productivity, plant species compositions, soil carbon and nitrogen stores, rates of soil respiration (CO2 release to the atmosphere), leaching of

nitrate into groundwater, and other relevant variables in the plots of these two experiments.

Previous studies using the Biodiversity Experiment have shown that greater local plant diversity leads to greater productivity and greater year-to-year temporal stability of ecosystem annual above ground biomass production (Tilman et al. 2001; Tilman, Reich and Knops 2006, Fornara and Tilman 2009). Increased species diversity has also been linked to large increases in soil carbon and nitrogen accumulation rates (Fornara and Tilman 2008), and thus the production of more fertile soils (Dybzinski et al. 2008, Fornara and Tilman 2009). Species composition (the functional types of species present) also affects these attributes. The most promising low-input high-diversity biofuel crop mixtures gained their greatest benefits from their combination of several legume species with several warm-season grass species (Tilman et al. 2001, Fornara and Tilman 2008, 2009). Increased plant diversity also led to greater resistance to invasion by plant species (Knops et al. 1999, Fargione and Tilman 2005). Finally, greater plant diversity led to greater insect diversity, and shifted dominance from leaf-eating (herbivorous) insects to parasitoid and predator insects that server as biocontrol agents (Haddad et al. 2009).

To understand how and why climate change, fertilization and irrigation affect ecosystems and their sustainability we must understand how organisms, ecosystem processes, and their interrelationships and feedbacks are affected by shifts in temperature, water availability and nitrogen supply. There are many plausible alternative hypotheses for how projected changes in climate and resource supply might impact Minnesota grassland communities and ecosystems. Given current knowledge, it is difficult to know which species and which life history stages of those species will be major drivers of community and ecosystem changes. For example:

- Warming might affect the composition of plant communities mainly via effects of soil temperature and/or soil moisture on establishment of different species. For instance, a 2006 field experiment at Cedar Creek showed that germination and seedling survival were highly dependent on soil water availability, plant species identity and their interactions.

- Alternatively, changes in community composition might be determined by the temperature-dependence of interspecific competition for nitrogen among adult plants, as hypothesized in models in which maximal growth rates are a Gaussian function of temperature and in which species differ in their optimal temperatures (e.g., Tilman 1999, Lehman and Tilman 2000).

- Greatly increased availability of biologically active nitrogen via fertilization might favor plants that are better competitors for light or that are more tolerant of a deep layer of soil-surface plant litter (Tilman 1993). Other work suggests that nitrogen fertilization might lead to losses of native species while favoring exotic weedy species such as quack grass (Clark and Tilman 2008). However it is unclear if the annual autumn harvesting of prairie biofuel crops might reduce or eliminate the effects of nitrogen fertilization on plant diversity and abundances of exotic plant species.

- Climate warming might impact ecological communities mainly though direct and indirect affects on soil carbon and nitrogen dynamics. For example, elevated soil temperatures might increase decomposition and nitrogen mineralization rates (Wan et al., 2005), but longer periods of soil moisture deficit might inhibit plant nutrient uptake in summer and early autumn. Resultant shifts in the seasonality of nitrogen availability might then favor cool-season (C3) plant species and deep-rooted species (generally perennial non-legume forbs) over warm-season (C4) and shallow-rooted species.

- Both irrigation and nitrogen fertilization are likely to increase yields, but it is unclear (a) which might lead to the greater increase, (b) if addition of both nitrogen and water together might lead to a more than additive increase in yield, and (c) if the yield increase would be greater in single-species plots (monocultures) or in high-diversity mixtures of prairie species. It is also (d) unclear how these two treatments, and plant diversity (monocultures versus high-diversity mixtures) might interact to determine the rate of nutrient leaching and effects of biofuel crops and management inputs on groundwater quality.

- Warmer (and consequently drier) growing season conditions could cause water and nitrogen to become co-limiting, or even cause water to replace nitrogen as the major limiting soil resource. In either case, interspecific competition would favor species with markedly different traits than had been favored when nitrogen, rather than water, was limiting. Put differently, warming might cause plant community composition to shift dramatically through invasions or through favoring one type of established plant species (e.g., C3 versus C4 physiologies) over another (Knapp, Briggs and Koelliker 2001).

3. **Hypothesis -** *State the premise or propositions set forth to explain and achieve the described outcome of the research.*

The overarching hypothesis of our proposed experiments is that climate change and management practices such as nitrogen fertilization and irrigation have both direct and indirect effects on the ecosystem services that prairie-like biofuel crops can provide, including biomass yields, soil carbon and nitrogen storage, and the quality of groundwater. Moreover, we hypothesize that climate and management inputs will interact with prairie plant diversity and species composition such that plant diversity will be an important determinant of functioning of biofuel agro-ecosystems, especially for those growing on more nutrient-poor soils. This general framework, which builds on results of our Biodiversity Experiment (reviewed above) and on related mathematical theory (e.g., Tilman, Lehman and Thompson 1997, Lehman and Tilman 2000) leads to three major specific sets of hypotheses to test:

a) *The Effects of Warming and Agricultural Inputs on* ***Biomass Production*** *Depend on Diversity*: We will test the possibility that the net effects of experimental warming will be decreased biomass production through its indirect effects on soil moisture and nitrogen availability. We will determine if decreases in biomass from warming are smaller in higher diversity plots, following the supposition that higher diversity plots utilize resources more efficiently and have higher productivity overall. Although it seems highly likely that both nitrogen fertilization and irrigation will increase biomass production in all crops, we will test the hypothesis that the increase from nitrogen is greater in monocultures than in high diversity treatments, but that the increase from irrigation is larger in high diversity plots since a bulwark of legumes provides them with a reliable source of nitrogen, thus making water relatively more limiting.

b) *Potential for* ***Species Invasions*** *is Increased by Warming*: Because of its potential importance to prairie conservation and sustainability, we will test the hypothesis that warming increases the potential for invasion of Minnesota prairies by species more typical of shortgrass western/southerm prairie by adding seed of 30 such species to a 30 cm x 30 cm area within each of the 114 plots, and then following seedling germination, survival and growth for each of these species throughout all three growing seasons of this experiment.

c) *Effects of Warming and Agricultural Inputs on* ***Soil Carbon Storage and on Soil Nitrogen Levels***: Because soil carbon storage depends on the balance between inputs of carbon to the soil through plant productivity and losses from decomposition, we suspect that warming will lead to lower soil carbon storage overall as a function of both decreased productivity and increased decomposition. We hypothesize that both fertilization and irrigation would lead to higher soil carbon storage as a consequence of increased productivity, but that this effect will be somewhat countered by the increased decomposability of the plant species favored by these higher inputs. We hypothesize that increases in soil total nitrogen, and thus soil fertility, would depend on a positive interaction between C4 grasses and legumes, and be decreased by warming because warming likely decreases legume abundance.

In total, the research that we propose should elucidate the effects of climate warming, irrigation and nitrogen fertilization on numerous aspects of the functioning of prairie-like biofuel ecosystems (e.g., biomass yields and their stability; carbon storage in or release from soils; nutrient leaching to groundwater). Our work is designed to contribute to basic scientific knowledge at the same time that it provides information of central importance to better understanding how to manage such perennial ecosystems in the face of climate change. Such information is essential if we are to maximize the ability of biofuel crops to simultaneously provide society with a sustainable and renewable energy source, with greenhouse gas benefits, and with other societally important ecosystem services. It will also test if the exotic perennial grass, *Miscanthus*, which has been proposed as a biofuel crop, might be an invasive species of high-diversity prairie, and the potential dependence of such invasive ability on soil moisture and soil fertility, as determined by comparing invasiveness for all factorial combinations of irrigation or no irrigation and no fertilization, low N fertilization and high N fertilization.

4. **Methodology -** *Describe the methodology to be employed to carry out the proposed research. Including descriptions of the sample design(s), if applicable.*

Biodiversity and Climate Experiment: This field experiment consists of 114 plots designed to determine the effects of climate warming, plant diversity, and plant community functional composition on the functioning of prairie-like ecosystems. The experiment uses a nested design in which three temperature treatments (ambient temperature, low warming and high warming) are nested within each of 38 biodiversity treatment plots. The 38 biodiversity plots (each one being 9 meters x 9 meters in size) consist of 14 monoculture plots, nine plots planted with four species, nine plots planted with 16 species, and six plots planted with 32 species. The compositions of the experimental plots focus on 14 perennial grassland plant species that are in one of four major functional groups: warm season (C4) grasses (*Andropogon geradri, Panicum virgatum, Schizachyrium scoparium and Sorghastrum nutans)*, cool season (C3) grasses (*Koeleria cristata, Poa pratensis*) , nitrogen-fixing legumes (*Lespedeza capitata, Lupinus perennis, Petalostemum purpureum*) and non-legume forbs (*Achillea millefolium, Asclepius tuberosa, Liatris aspera, Monarda fistulosa* and *Solidago rigida*). All 14 species are in monoculture plots, the four-species plots are random combinations of these 14 species, and all 14 species are in the 16 species plots and in the 32 species plots. The two highest diversity plots also contain other perennial prairie plant species (see Tilman et al. 1997). The three temperature treatment subplots nested within each experimental plot are each 3 meters x 2.5 meters in size. The "high" subplot is warmed by +3.0ºC using a 1500 watt Kal-Glo® infrared heat lamp, the "low" subplot is warmed by +1.5ºC using an 800 watt Kal-Glo® infrared heat lamp, while the control plot is unwarmed but has a sham (empty) "heat lamp" erected over it. A fourth subplot was also established within each of the 38 plots that was also un-warmed but that lacked any structure so that the non-warming effects, if any, of the structure and heat lamp housing can be determined.

As detailed in Tilman et al. (2001), the location of each replicate of each diversity treatment was randomly assigned, as was the species composition of each replicate. Moreover, these 38 plots represent a randomized subset of the replicate plots for these diversity levels from within our larger Biodiversity Experiment. This randomization and replication allow rigorous statistical analysis of the effects of plant diversity and plant functional group composition on ecosystem functioning. The nesting of the three temperature treatments within each of the 38 diversity plots allows analyses to also rigorously determine the effects of temperature.

Please note that items A – F in the Biodiversity and Climate portion of the Timetable (below) describe in detail the sampling to be done each year. Sampling methods are all well-established Cedar Creek protocols that meet peer-review standards of major scientific journals and of the National Science Foundation. That material is not repeated here for brevity.

Management Experiment: This experiment consists of 104 plots, each one being 9 meters x 9 meters in size. The experiment is designed to determine how irrigation and nitrogen fertilization would impact the yields and ecosystem functioning of four potential cellulosic biofuel crops. The four biofuel crops are:

> Switchgrass monocultures: In the spring of 2010, twenty-eight plots will be planted with a commercial switchgrass variety recommended for biofuel biomass production, using pre-planting and post-planting methods designed to assure optimal establishment and yields of switchgrass.
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> *Miscanthus* monocultures: Similarly, twenty-eight abandoned plots will be planted in spring of 2010 with rhizomes of the sterile hybrid variety of *Miscanthus (called Miscanthus x giganteus)*, which is an exotic perennial that has been proposed as a potential biomass crop for biofuels. Pre-planting methods and post-planting methods will be those known to optimize establishment and yields.
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> Prairie polycultures: Twenty-four pre-treated plots planted to contain three warm-season grass species (switchgrass, big bluestem and Indian grass) and three legume species (lupine, purple prairie clover and bush clover).
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> Higher-diversity prairie polycultures: Twenty four pre-treated plots planted to contain 16 prairie perennials, as in the existing Biodiversity Experiment.

These 104 plots will be located within the same experimental grid that contains the Biodiversity Experiment and the Biodiversity and Climate Experiment. This grid consists of 342 plots, of which 168 are in our original Biodiversity Experiment and 35 are in our LCCMR/ USGS supported study of the abilities of different vegetation types to intercept various agrichemicals and thus help ameliorate agricultural effects on groundwater pollution. One hundred four of the remaining plots will be randomly assigned to one of the four biofuel crops listed above.

This experiment employs a full-factorial design. The treatments are three levels of nitrogen addition (none, 7 $g/m^2/yr$, 14 $g/m^2/yr$) and two irrigation treatments (no irrigation or addition of 2.4 cm/week of water every week from mid-May through August), for a total of 6 different combinations. There will be four replicates of each management treatment combination applied to each of the four biofuel crops. This then gives a total of 96 plots, since 3 nitrogen treatments x 2 irrigation treatments x 4 replicates per biofuel crop types x 4 biofuel crops = 96 total. In addition, the experiment will also include four more switchgrass monocultures and four more

Miscanthus monocultures that will receive treatments that have been observed, in other studies, to optimize their biomass yields. In total, these 104 plots will allow side-by-side comparisons of biomass yields as well as of soil carbon sequestration and other ecosystem services of these different biofuel crops.

This replicated full factorial design will provide the experimental power to determine the direct and interactive effects of management treatments and biofuel crop type on yields as determined by an annual mowing of plot biomass each autumn (late September), the year-to-year stability and reliability of these yields, on rates of storage of carbon in soil, on changes in soil total organic nitrogen and resultant soil fertility, and on leaching losses of nitrate and nitrite to the groundwater.

***Miscanthus* Invasiveness:** We will do additional work on *Miscanthus (Miscanthus x giganteus)* to test if it might be an invasive species of prairie grasslands. Although this hybrid clone has never been reported to produce viable seeds, it is reported to be a strong competitor in established high-biomass monocultures. Thus, there is the possibility that it might be able to invade native prairie via vegetative spread and displace native species. If this were so, its use as a biofuel crop could threaten the native prairie that remains in Minnesota. To test this possibility, the 24 Management Experiment *Miscanthus* plots (within the full-factorial set of 96 plots) will be laid out in a unique manner. *Miscanthus* will be planted in a 5 meter x 9 meter corridor through the center of the 9 meter x 9 meter plot, bounded on the edges by two 2 meter x 9 meter strips of undisturbed high-diversity prairie. In one strip, Strip A (randomly chosen from the two at the start of the experiment), we will visually sample and map the locations of any *Miscanthus* that enters the prairie vegetation via vegetational spread at four times throughout the year. This will allow us to quantify any *Miscanthus* movement into the prairie vegetation. We will also make a complementary visual sampling and mapping of all native species in Strip A, so we can document any displacement of native prairie species caused by *Miscanthus*, should it spread. In the other prairie strip, Strip B, we will plant a row of seven rhizome plugs of *Miscanthus*, one meter apart and one meter from the edges. Strip B will also be sampled by mapping the spread of the planted *Miscanthus* plugs and the locations and changes in native prairie species as well as by recording any mortality experienced by any species.

Because these 24 *Miscanthus* plots will receive the same six irrigation and nitrogen treatments as all other biofuel crops in the 96-plot full-factorial experiment, we will also be able to determine if either soil water availability (irrigated or not) or soil fertility (two levels of nitrogen fertilization or not fertilized) or their interactions influence the *Miscanthus* invasiveness.

Soil and Nutrient Analyses: In both the Biodiversity and Climate Experiment, and in the Management Experiment, we will annually analyze soils cores for total soil carbon and nitrogen. The soils cores will be taken from each of the 114 plots of the BAC Experiment and the 96 plots of the Management Experiment at depth intervals of 0-20 cm, 20-40 cm, 40-60 cm and 60-100 cm. We will measure extractable soil nitrate and soil nitrite from similarly collected fresh soil cores three times each growing season (late-June, early-August and mid-September), and used these data to estimate leaching rates based on methods we have developed in our collaborative work with the USGS.

Please note that items A – E in the Management Experiment portion of the Timetable (below) describe in detail the sampling to be done each year. Sampling methods are all well-established Cedar Creek protocols that meet peer-review standards of major scientific journals and of the National Science Foundation. That material is not repeated here for brevity.

5. **Results and Deliverables -** Describe in detail the expected outcomes of each of the results and deliverables.

Our project has four specific aims. For each of four potential biofuel crops, we will determine the effects of warming and agricultural inputs on: i) biomass production and sustainability, ii) invasions by exotic species (including testing if *Miscanthus* might be invasive), and iii) soil process, especially carbon storage, changes in soil nitrogen content, and leaching of soil nitrate or nitrite into groundwater. Our fourth aim is to synthesize the results from all of the proposed experiments to form a general picture of how we can best optimize biofuel production, carbon storage and habitat restoration in Minnesota, given the potential for climate change. We detail the expected outcomes and deliverables for each of these aims below.

> *Result 1: Effects of Warming and Agricultural Inputs on Biomass Production and Sustainability.* This study will lead to two scientific papers, on one the effects of simulated temperature change on biomass yields and another on how agricultural inputs affect yields of monocultures and high diversity prairies. In addition to reporting how warming and agricultural impacts affect biomass yields, our experimental design allows us to determine the mechanisms responsible for these effects (e.g. altered soil moisture or nitrogen, shifts in species composition as a function of treatments, etc).
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> *Result 2: Warming and Input Effects on Invasions by Exotic Species.* In the scientific literature, there is currently no general framework or conceptual model for projecting how elevated temperatures or agricultural inputs might affect an ecosystem's susceptibility to invasion by exotic species. Thus, our experimental results will fill a large gap. We will likely publish two separate papers describing the specific results on how warming and agricultural inputs affect the potential for ecosystems that differ in species composition to be invaded by exotic species. Our data will also indicate whether *Miscanthus* has the potential to invade other grassland ecosystems if it is grown as a biofuel, as we will report in a separate study. Last, we will synthesize results from all of our work on plant invasion and publish a paper that proposes a general model of how environmental change, resource availability, and the traits or characteristics of the invading species interact to promote or attenuate plant invasion. Thus, we anticipate that four scientific papers will result from this study.
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> Result 3: *Effects of Warming and Agricultural Inputs on Soil Carbon, Soil Nitrogen and Nitrate Leaching.* As soils are the largest storage reservoir for carbon in Minnesota ecosystems, it is critical to understand how both climate change and management may affect this large carbon pool, and how this varies between single plant species-crops versus multi-species mixtures. We will publish one synthetic paper on how both warming and agricultural inputs affect the levels of carbon stored in the top 1 meter of soil. If possible to do so, we will also include our results on treatment and biofuel crop on soil stores of nitrogen, and on rates of leaching loss of nitrate and nitrite.
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> Result 4: *Sustainable Restoration Practices.* Perhaps the most important result that this project will deliver is a synthetic integration of all of our findings. We will write a scientific paper that discusses how climate change, agricultural inputs and biodiversity affect biofuel production, carbon storage and habitat restoration in Minnesota.

6. **Timetable -** Layout the proposed times for completing the proposed research including proposed dates for individual results and deliverables.

Timetable

Summer 2010:

1. Biodiversity and Climate Experiment:

A. Assure, on a day-by-day basis, that warming treatments are operating, that soil temperature sensors are operating and data is regularly downloaded, that total soil moisture data are regularly collected; that total soil respiration (carbon dioxide release rates) data are regularly collected; that data quality is verified at time of data collection.

B. Collect soil samples from four depths in each plot for (1) analysis of total soil carbon and nitrogen; sieve, dry, archive all soils and for (2) analysis of extractable soil nitrate and nitrite (late-June, early-August and mid-September); do nitrate and nitrite analyses on fresh extracts.

C. Sample vegetation in each of the 114 subplots in August by clipping a subsample strip, sorting it to each plant species, drying and then weighing each sample. Immediate quality assurance with re-sampling, if needed. Also clip biomass in late September, dry and weigh (biomass to be unsorted).

D. On a bi-weekly basis, visually census all plant seedlings, including seedlings in the permanent 30 cm x 30 cm sub-sub-plot in each of the 114 subplots. These seed addition sub-sub-plots were established in summer 2009 to determine how warming, plant diversity, and plant functional group composition would impact the ability of native prairie plants from drier south/western to invade.

E. Twice each summer sample insect communities in each of the 114 subplots to determine how treatments influence insect community composition.

F. Collect all other data needed to test hypotheses.

2. Management Experiment (Irrigation and Nitrogen manipulations)

A. Install pipe and sprinklers to the 48 plots that are to be irrigated (early in growing season); apply prescribed amounts of nitrogen fertilizer to the 64 appropriate plots

B. In mid month of May, June, July and August, survey and map all plant species in the Miscanthus invasion strips of the 24 Miscanthus plots

C. Collect soil samples from four depths in each of the 96 plots for (1) analysis of total soil carbon and nitrogen; sieve, dry, archive all soils and for (2) analysis of extractable soil nitrate and nitrite (late-June, early-August and mid-September ; do nitrate and nitrite analyses on fresh extracts).

D. Sample vegetation in each of the 96 plots in August by clipping a subsample strip, sorting it to each plant species, drying and then weighing each sample. Immediate quality assurance with re-sampling, if needed.

E. Mow each of the 97 plots for collection of biomass in later September, weigh biomass both as harvested ("wet" weight) and after drying (dry weight) to determine biomass yields.

F. Do all other sampling needed to test our hypotheses.

Fall 2010 and Winter 2011:

A. Establish data bases for each experiment, with quality checks and off-site data backups and archives

B. Perform analyses of total soil C and N for all relevant samples of both experiments
C. Graduate students and principal investigators analyze all collected data, testing our numerous hypotheses, generating new hypotheses and preparing papers for publication, as appropriate given the results to date.

Summary 2011; and the same for Summer 2012

1. Biodiversity and Climate Experiment:

The same six sets of tasks listed for Summer 2010 will be performed each subsequent summer

2. Irrigation and Nitrogen Management Experiment

The same sets of tasks listed for Summer 2010 will be performed each subsequent summer

Fall 2001 and Winter 2012; and the same for Fall 2012 and Winter 2013

A. Merge new data into established data bases for each experiment, with quality checks and off-site data backups and archives
B. Perform analyses of total soil C and N for all relevant samples of both experiments
C. Graduate students and principal investigators will continue to analyze all collected data, testing our numerous hypotheses, generating new hypotheses and preparing papers for publication, as appropriate given the results to date. Analyses will increasingly focus and synthesis and relevance of results to Minnesota biofuels and land conservation issues as more results are accumulated. Papers will be prepared for publication, as detailed in our proposal, and we will actively communicate our findings with government officials and agencies, farmers, businesses, organizations interested in climate change, biofuels, conservation and related topics, and the general public.

7. Budget

Budget Item	Amount
Personnel: 2 full-time graduate student research assistants per summer to conduct research under the guidance of PIs. Projected expenses for graduate student will average $8125 ($6500 salary plus $1625 fringe benefits) per student per summer.	$49,000
Personnel: 8 full-time undergraduate summer research interns per summer to collect soil samples, harvest and sort plant biomass, maintain experiment, etc. Projected expenses for interns will average $5005 ($4580 salary plus $425 fringe benefits) per intern per summer.	$120,000
Personnel: 1 adjunct faculty member (Clarence Lehman) to participate on a part-time basis. Expenses for the research associate are projected to average $9260 ($7000 salary plus $2260 fringe benefits) per year.	$28,000

Additional Budget Items: Soil C:N analyses- $6720 Plant C:N analyses- $5040 Soil NO3-NH4 analyses- $9510 Soil nitrogen mineralization analyses- $2730	$24,000
TOTAL PROJECT BUDGET REQUEST TO LCCMR	$221,000

8. Credentials

Dr. David Tilman is Regents' Professor and McKnight Presidential Chair in Ecology at the University of Minnesota, and is Director of Cedar Creek Ecosystem Science Reserve. His applied research explores how to sustainably meet human needs for energy, food and ecosystem services, while his theoretical work has become foundational to understanding biodiversity and resource competition. He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and a J. S. Guggenheim Fellow. He is the recipient of the Ecological Society of America's Cooper Award and its MacArthur Award, and the Botanical Society of America's Centennial Award and the Princeton Environmental Prize. In 2008, he received the International Prize for Biology. He has written two books, edited three more, and published more than 200 scientific papers, including more than 40 in *Science, Nature* and the *Proceedings of the National Academy of Sciences USA*. For the past 18 years, the Institute for Scientific Information has ranked him as the world's most highly cited environmental scientist.

Selected publications:

Searchinger, T., S. Hamburg, J. Melillo, W. Chameides, P. Havlik, D. Kammen, G. Likens, R. Lubowski, M. Obersteiner, M. Oppenheimer, P. Robertson, W. Schlesinger, D. Tilman. 2009. Climate change: Fixing a critical climate accounting error. Science 326:527-528.

Haddad, N., G. Crutsinger, K. Gross, J. Haarstad, J. Knops and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters 12:1028-1038

Tilman, D., R. Socolow, J.A. Foley, J. Hill, E. Larson, L. Lynd, S. Pacala, J. Reilly, T. Searchinger, C. Somerville and R. Williams. 2009. Beneficial Biofuels—The Food, Energy, and Environment Trilemma. Science 325:270-271.

Cadotte, M.W., J. Cavender-Bares, G.D. Tilman and T.H. Oakley. 2009. Using evolutionary, functional and trait diversity to understand patterns of plant community productivity. PLoS 4:e5695.

Fargione, J.E., T.R. Cooper, D.J. Flaspohler, J. Hill, C. Lehman, T. McCoy, S. McLeod, E.J. Nelson, K.S. Oberhauser, and D. Tilman. 2009. Bioenergy and Wildlife: Threats and Opportunities for Grassland Conservation. BioScience 59:767-777.

Clark, C.M., S.E. Hobbie, R. Venterea and D. Tilman. 2009. Long-lasting effects on nitrogen cycling 12 years after treatments cease despite minimal long-term nitrogen retention. Global Change Biology 15:1755-1766.

HillRisLambers, J., W.S. Harpole, S. Schnitzer, D. Tilman and P.B. Reich. 2009. CO2, nitrogen and diversity differentially affect seed production of prairie grasses. Ecology 90:1810-1820.

Fornara, D.A., and D. Tilman. 2009. Ecological mechanisms associated with the positive diversity-productivity relationship: evidence from a long-term, N-limited grassland experiment. Ecology 90:408-418.

Hill, J., S. Polasky, E. Nelson, D. Tilman, H. Huo, L. Ludwig, J. Neumann, H. Zheng, and D. Bonta. 2009. Climate change and health costs of air emissions from biofuels and gasoline. PNAS 106: 2077-2082.

Cleland, E.E., C. Clark, S. Collins, J. Fargione, L. Gough, K. Gross, D. Milchunas, S. Pennings, W. Bowman, I. Burke, W. Lauenroth, G.P. Robertson, J. Simpson, D. Tilman and K. Suding. 2008. Species responses to nitrogen fertilization in herbaceous plant communities, and associated species traits. Ecology 89:1175.

Fornara, D.A., D. Tilman and S.E. Hobbie. 2008. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. Journal of Ecology 97:48-56.

Dybzinski, R., J.E. Fargione, D.R. Zak, D. Fornara and D. Tilman. 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. Oecologia 158:85-93.

Fargione, J., J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Biofuels: Effects on land and fire- Response. Science 321:199-200.

Fargione, J., J. Hill, D. Tilman, S. Polasky and P. Hawthorne. 2008. Land clearing and the biofuel carbon debt. Science 319: 1235-1238.

Clark, C.M. and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451: 712-715.

Dybzinski, R. and D. Tilman. 2009. Mechanisms of plant competition and coexistence. Pages 186-195 in S. A. Levin, Ed., Princeton Guide to Ecology, Princeton University Press, Princeton, NJ.

Fornara, D. A. and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96: 314–322.

Dybzinski, R. and D. Tilman. 2007. Resource use patterns predict long-term outcomes of plant competition for nutrients and light. The American Naturalist: 170:305-318.

Fargione, J., D. Tilman, R. Dybzinski, J. Hille Ris Lambers, C. Clark, W. S. Harpole, J. M. H. Knops, P. B. Reich and M. Loreau. 2007. From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proceedings of The Royal Society B 274:871-876.

Fridley, J. D., J. J. Stachowicz, S. Naeem, D. F. Sax, E. W. Seabloom, M. D. Smith, T. J. Stohlgren, D. Tilman and B. Von Holle. 2007. The invasion paradox: reconciling pattern and process in species invasions. Ecology 88:3-17.

Harpole, W. S. and D. Tilman. 2007. Grassland species loss resulting from reduced niche dimension. Nature 446:791-793.

Tilman, D., J. Hill and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314:1598-1600.

Tilman, D., P. B. Reich and J. M. H. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629-632.

Díaz, S., J. Fargione, F. S. Chapin III. and D. Tilman. 2006. Biodiversity loss threatens human well-being. PLoS Biology 4:1300-1305.

Fargione, J. and D. Tilman. 2006. Plant species traits and capacity for resource reduction predict yield and abundance under competition in nitrogen-limited grassland. Functional Ecology 20:533-540.

Harpole, S. and D. Tilman. 2006. Non-neutral patterns of species abundance and species traits. Ecology Letters 9:15-23.

Hill, J., E. Nelson, D. Tilman, S. Polasky and D. Tiffany. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proceedings of the National Academy of Sciences 103:11206-11210.

Reich, P. B., S. E. Hobbie, T. Lee, D. S. Ellsworth, J. B. West, D. Tilman, J. M. H. Knops, S. Naeem and J. Trost. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature* 440:922-925.

Waldrop, M. P., D. R. Zak, C. B. Blackwood, C. D. Curtis and D. Tilman. 2006. Resource availability controls fungal diversity across a plant diversity gradient. Ecology Letters 9:1127-1135.

Wright, J. P., S. Naeem, A. Hector, C. Lehman, P. B. Reich, B. Schmid and D. Tilman. 2006. Conventional functional classification schemes underestimate the relationship with ecosystem functioning. Ecology Letters 9:111-120.

Tilman, D. 2005. Biodiversity and ecosystem services: Does biodiversity loss matter? Pages 95-102 in, J.-P. Le Duc, Editor, *Proceedings of the International Conference: Biodiversity: Science and Governance,* January 24-28, 2005, Paris, France.

Tilman, D. and S. Polasky. 2005. Ecosystem goods and services and their limits: The roles of biological diversity and management practices. Pages 78-97 in, R. D. Simpson, M. A. Toman and R. U. Ayres, eds., *Scarcity and Growth Revisited: Natural Resources and the Environment in the New Millennium*. RFF Press, Resources for the Future, Washington, DC.

Tilman, D., S. Polasky and C. Lehman. 2005. Diversity, productivity and temporal stability in the economies of humans and nature. Journal of Environmental Economics and Management 49:405-426.

Chase, J. M., P. Amarasekare, K. Cottenie, A. Gonzalez, R. D. Holt, M. Holyoak, M. F. Hoopes, M. A. Leibold, M. Loreau, N. Mouquet, J. B. Shurin and D. Tilman. Competing theories for competitive metacommunities. 2005. Pages 335-354 in, M. Holyoak, M. A. Leibold and R. D.

Holt, Eds., *Metacommunities: Spatial Dynamics and Ecological Communities*. The University of Chicago Press, Chicago and London.

Diaz, S., D. Tilman, J. Fargione, F. S. Chapin III, R. Dirzo, T. Kitzberger, B. Gemmill, M. Zobel, M. Vilà, C. Mitchell, A. Wilby, G. C. Daily, M. Galetti, W. F. Laurance, J. Pretty, R. Naylor, A. Power, D. Harvell, S. Potts, C. Kremen, T. Griswold and C. Eardley. 2005. Biodiversity regulation of ecosystem services. Pages 297-329 in, R. Hassan, R. Scholes and N. Ash, eds. *Ecosystems And Human Well-Being: Current State And Trends, Volume 1*. Findings of the Condition and Trends Working Group of the Millennium Ecosystem Assessment Series. Island Press, Washington, D.C.

Fargione, J. and D. Tilman. 2005. Niche differences in phenology and rooting depth promote coexistence with a dominant C_4 bunchgrass. Oecologia 143:598-606.

Fargione, J. and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. Ecology Letters 8:604-611.

Loreau, M., J. Roy and D. Tilman. 2005. Linking ecosystem and parasite ecology. Pages 13-21 in, F. Thomas, F. Renaud and J.-F. Guégan, eds., *Parasitism & Ecosystems*. Oxford University Press, Oxford.

Stachowicz, J. J. and D. Tilman. 2005. Species invasions and the relationships between species diversity, community saturation, and ecosystem functioning. Pages 41-64 in, D. F. Sax, J. J. Stachowicz and S. D. Gaines, eds., *Species Invasions: Insights into Ecology, Evolution, and Biogeography*. Sinauer Associates Inc., Massachusetts.

Tjoelker, M. G., J. M. Craine, D. Wedin, P. B. Reich and D. Tilman. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist 167:493-508.

Dr. Jennifer Powers studies the effects of anthropogenic environmental changes including global warming and land-use change on ecosystem processes and the biogeochemical cycle of carbon. She received her PhD in Biology from Duke University in 2001 and joined the faculty of the University of Minnesota in 2006, where she has a split appointment in the Departments of Ecology, Evolution and Behavior and Plant Biology. Since then she has been the recipient of a NASA New Investigator Award and a McKnight Land-Grant Professorship (the highest honor that UMN bestows upon junior faculty), and is also a Resident Fellow at the Institute on the Environment at UMN.

Selected publications (out of 20 total):

Powers, J.S., J.M. Becknell, J. Irving, and D. Perez-Aviles. 2009. Diversity and structure of regenerating tropical dry forests in Costa Rica: environmental drivers and geographic patterns. Forest Ecology and Management 258: 959-970.

Powers, J.S., R.A. Montgomery, E.C. Adair, F.Q. Brearley, S.J. DeWalt, C.T. Castanho, J. Chave, E. Deinert, J.U. Ganzhorn, M.E. Gilbert, J. Antonio-Gonzalez, S. Bunyavejchewin, H.R. Grau, K.E. Harms, A. Hiremath, S. Iriarte-Vivar, E. Manzane, A.A. de Oliveira, L. Poorter, J.B. Ramanamanjato, C. Salk, A.Varela, G.D. Weiblen and M.T. Lerdau. Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient. 2009. Journal of Ecology 97: 801-811.

Sayer, E.J., J.S. Powers, and E.V.J. Tanner. 2007. Increased litterfall in tropical forests boosts the transfer of soil CO_2 to the atmosphere. PLoS ONE 2(12): e1299. doi10.1371/journal.pone.0001299.

Powers, J.S., and E. Veldkamp. 2005. Regional variation in soil carbon and $\delta^{13}C$ in paired forests and pasture of Northeastern Costa Rica. Biogeochemistry 72: 315-336.

Powers, J.S., K.K. Treseder and M.T. Lerdau. 2005. Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four Neotropical rain forests: patterns across large geographic distances. New Phytologist 165: 913-921.

Powers, J.S., M.H. Kalicin, and M.E. Newman. 2004. Tree species do not influence local soil chemistry in a species-rich Costa Rican rain forest. Journal of Tropical Ecology 20: 587-90.

Powers, J.S. 2004. Soil carbon and nitrogen storage following contrasting land-use transitions in Northeastern Costa Rica. Ecosystems 7: 134-146.

Powers, J.S., J.M. Read, J.S. Denslow, and S.M. Guzman. 2004. Estimating soil carbon fluxes following land-cover change: a test of some critical assumptions for a region in Costa Rica. Global Change Biology 10: 170-181.

Powers, J.S. 2004. New perspectives in comparative ecology of Neotropical rain forests: Reflections on past, present and future. Biotropica 36: 2-6.

Powers, J.S., P. Sollins, M.E. Harmon, and J.A. Jones. 1999. Plant-pest interactions in time and space: a Douglas-fir bark beetle outbreak as a case study. Landscape Ecology 14: 105-120.

Dr. Clarence Lehman is an adjunct faculty member of in the Department of Ecology, Evolution and Behavior at the University of Minnesota, and served for six years as Associate Director of Cedar Creek Ecosystem Science Reserve. Dr. Lehman's research covers theoretical, experimental, and computational ecology, renewable biofuel energy and the planet's future temperature trajectory, biodiversity and its ecosystem properties, connections between ecology and economics, and restoration of natural habitats. He has restored several areas of native prairies, savannas and wetlands in northwestern Minnesota and maintains them through specialized experiments for adaptive management.

Selected publications:

Lehman, C. 2008. Biofuels—The next great source of energy? Encyclopaedia Britannica Special Report 2008:106.

Gorham, E., C. Lehman, A. Dyke, J. Janssens and L. Dyke. 2007. Temporal and spatial aspects of peatland initiation following deglaciation in North America. Quaternary Science Reviews 26:300–311.

Lehman, C. L. 2001. The concept of stability. Pages 467–479 in, S. A. Levin, Editor-in-Chief, Encyclopedia of Biodiversity, Vol. 5. Academic Press, San Diego, CA.

Tilman, D. and C. Lehman. 2001. Human-caused environmental change: Impacts on plant diversity and evolution. Proceedings of the National Academy of Science 98:5433–5440.

Lehman, C. and D. Tilman. 2000. Biodiversity, stability, and productivity in competitive communities. The American Naturalist 156:534–552.

Lehman, C., and D. Tilman. 1997. Competition in spatial habitats. Pages 185–203 in D. Tilman and P. Kareiva, eds., Spatial Ecology: The Role of Space in Population Dynamics and Interspecific Interactions. Princeton University Press, New Jersey.

Tilman, D., R. May, C. Lehman and M. Nowak. 1994. Habitat destruction and the extinction debt. Nature 371:65–66.

9. Dissemination and Use – Describe how the findings of the research will be disseminated and describe the expected audience and potential use.

As detailed in **Results and Deliverables**, we plan to report our results in at least eight scientific papers published in high-impact peer-reviewed journals.

Data collected with the support of this LCCMR grant will be included in the online database supported by the Cedar Creek LTER grant and managed and distributed in accordance with LTER requirements. All such LTER data is required to be published within four years of its collection on Cedar Creek's publically accessible website:
http://www.lter.umn.edu/research/data

In addition, because these results are likely to be of great interest to general audiences concerned with biofuel production in the Midwest, we will make it a priority to communicate our findings in public presentations and through direct contact with government bodies, agricultural organizations, and researchers in the field.

References

Clark, C.M. and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712-715.

Dybzinski, R., J. Fargione, D.R. Zak, D. Fornara and D. Tilman. 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. Oecologia 158:85-93.

Fargione, J. and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementary effects. Ecology Letters 8:604-611.

Fargione, J. Hill, D. Tilman, S. Polasky and P. Hawthorne. 2008. Land clearing and the biofuel carbon debt. 2008. Science 319:1235-1238.

Fargione, J., T.R. Cooper, D.J. Flaspohler, J. Hill, C. Lehman, T. McCoy, S. McLeod, E.J. Nelson, K.S. Oberhauser and D. Tilman. 2009. Bioenergy and wildlife: Threats and opportunities for grassland conservation. BioScience 59:767-777.

Fornara, D. and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96:314-322.

Fornara, D. and D. Tilman. 2009. Ecological mechanisms associated with the positive diversity-productivity relationship: evidence from a long-term, N-limited grassland experiment. Ecology 90:408-418.

Giorgi, F., B.C. Hewitson, J.H. Christensen, M. Hulme, H. vonStorch, P.H. Whetton, R.G. Jones, L.O. Mearns and C. Fu. 2001. Regional climate information—evaluation and projections. In: J.T. Houghton et al. (Eds), Climate Change 2001: The Scientific Basis. 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, NY USA, pp. 881.

Haddad, N., G. Crutsinger, K. Gross, J. Haarstad, J. Knops and D. Tilman. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters 12:1028-1038.

Knapp, A.K., J.M. Briggs and J.K. Koelliker. 2001. Frequency and extent of water limitation to primary production in a mesic temperate grassland. Ecosystems 4:19-28.

Knops, J.M.H., D. Tilman, N.M. Haddad, S. Naeem, C.E. Mitchell, J. Haarstad, M.E. Ritchie, K.M. Howe, P.B. Reich, E. Siemann and J. Groth. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters 2:286-293.

Lehman, C. and D. Tilman. 2000. Biodiversity, stability and productivity in competitive grasslands. American Naturalist 156:534-552.

Tilman, D. 1993. Species richness of experimental productivity gradients: How important is colonization limitation? Ecology74:2179-2191.

Tilman, D., C. Lehman and K.T. Thompson. 1997. Plant diversity and ecosystem productivity: Theoretical considerations. Proceedings of the National Academies of Science 94:1857-1861.

Tilman, D. J. Knops, D. Wedin, P. Reich, M. Ritchie and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. Science 277:1300-1302.

Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. The Robert H. MacArthur Award Lecture. Ecology 80:1455-1474.

Tilman, D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314:1598-1600.

Tilman, D., P.B. Reich, J. Knops, D. Wedin, T. Mielke and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science 294:843-845.

Tilman, D., P.B. Reich and J.M.H. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629-632.

Wuebbles, D.J. and K. Hayhoe. 2003. Climate change projections for the United States Midwest. Mitigation and Adaptation Strategies for Global Change 9:335-363.

Wan, S., D. Hui, L. Wallace and Y. Luo. 2005. Direct and indirect effects of experimental warming on ecosystem carbon processes in tallgrass prairie. Global Biogeochemical Cycles 19:GB2014