2010 Project Abstract
For the Period Ending June 30, 2012

PROJECT TITLE: Proposal #048-B1 – Sustainable Biofuels
PROJECT MANAGER: G. David Tilman
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: ML 2010, Chap. [362], Sec. [2], Subd. 7(b)

APPROPRIATION AMOUNT: $221,000

Overall Project Outcome and Results

Minnesota's perennial grasslands produce considerable biomass that could become a valuable resource for producing renewable energy. How might Minnesota's capacity to produce biomass for biofuels be impacted by climate change and anticipated mitigation practices? We explored the impacts of warming, fertilization, and irrigation on biomass production at the Cedar Creek Ecosystem Science Reserve.

Our major overall finding is that high diversity mixtures of prairie perennials provided the best combination of biomass production, invasion resistance, carbon storage in soil, and response to climate warming of all the biomass crops we tested.

Specific findings from the Climate Experiment include:

1. Compared to low diversity mixtures of prairie plant species, high diversity mixtures produced much more biomass when experiencing normal weather, were more resilient to the stress of warming, and had their biomass production increase the most from warming.
2. High diversity mixes enhanced ecosystem services more than low diversity mixes by sequestering more carbon in soils and being less prone to invasion by non-native species.
3. Warming inhibited seed establishment. This could reduce invasions by non-native species, but might threaten establishment of native prairie restorations.

The Fertilization & Irrigation Experiment found:

1. Fertilization had similar impacts across all species mixtures.
2. Moderate fertilization and irrigation increased productivity, with the largest effects in the Panicum, Panicum+Grasses, and High Diversity plots.

Overall findings on plant invasion showed:

1. Invasion is inhibited by higher diversity species mixtures.
2. A potential biofuel crop, Miscanthus (as a sterile hybrid), was ineffective at producing biomass in central Minnesota, at least on sandy, drier soils. It had detectable, but moderate invasion into native prairies.

This research has been documented in one publication. Two manuscripts have been submitted and are either in review or under revision. Another manuscript is in preparation. We anticipate additional publications will follow. In 2012, the education programming Cedar Creek reached 6,619 users, including K-12 students, teachers, and the general public.

Project Results Use and Dissemination

The data from these studies will be included in Cedar Creek's database and made publicly available on the Cedar Creek website. Researchers around the world access and use the data on this site for diverse ecological analyses in many research areas including, among others, biodiversity, invasion, and climate change studies.

The results of these studies are integrated into the educational programming and outreach at Cedar Creek. In 2012, 1,777 K-12 students participated in on-site programs. 1,062 K-12 students participated in off-site programs. Furthermore, 120 K-12 teachers participated in professional development opportunities at Cedar Creek and in their schools. At the university level, 845 students and faculty have made use of Cedar Creek programs, courses, meetings, and workshops both on and off-site. There have been 1,070 visitors to the experimental sites where this study took place.

One journal article that documents findings from this study has been published. See:
Isbell, F., 2013, *Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity*, PNAS, **110:** 29.
A second publication by Heather Whittington is under revision in *Oecologia* and a third has been submitted to *Functional Biology*. Jane Cowles has a fourth article in preparation. We anticipate additional publications will result from this work.

Environment and Natural Resources Trust Fund (ENRTF)
2010 Work Program Final Report

Date of Report: September 9, 2013
Final Report:
Date of Work Program Approval: June 9, 2010
Project Completion Date: July 1, 2013

I. PROJECT TITLE: Proposal #048-B1 – Sustainable Biofuels

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Location: *Cedar Creek Ecosystem Science Reserve*
2660 Fawn Lake Drive Northeast
East Bethel, MN 55005 (Anoka/Isanti Counties)

Total ENRTF Project Budget:	**ENRTF Appropriation**	**$ 221,000**
	Minus Amount Spent:	**$ 221,000**
	Equal Balance:	**$ 0**

Legal Citation: ML 2010, Chap. [362], Sec. [2], Subd. 7(b)

Appropriation Language: $221,000 is from the trust fund to the Board of Regents of the University of Minnesota to determine how fertilization and irrigation impact yields of grass monoculture and high diversity prairie biofuel crops, their storage of soil carbon, and susceptibility to invasion by exotic species. The appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

II. FINAL PROJECT SUMMARY:

Minnesota's perennial grasslands produce considerable biomass that could become a valuable resource for producing renewable energy. How might Minnesota's capacity to produce biomass for biofuels be impacted by climate change and anticipated mitigation practices? We explored the impacts of warming, fertilization, and irrigation on biomass production at the Cedar Creek Ecosystem Science Reserve.

Our major overall finding is that high diversity mixtures of prairie perennials provided the best combination of biomass production, invasion resistance, carbon storage in soil, and response to climate warming of all the biomass crops we tested.

Specific findings from the Climate Experiment include:

1. Compared to low diversity mixtures of prairie plant species, high diversity mixtures produced much more biomass when experiencing normal weather, were more resilient to the stress of warming, and had their biomass production increase the most from warming.
2. High diversity mixes enhanced ecosystem services more than low diversity mixes by sequestering more carbon in soils and being less prone to invasion by non-native species.
3. Warming inhibited seed establishment. This could reduce invasions by non-native species, but might threaten establishment of native prairie restorations.

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2. A potential biofuel crop, Miscanthus (as a sterile hybrid), was ineffective at producing biomass in central Minnesota, at least on sandy, drier soils. It had detectable, but moderate invasion into native prairies.

This research has been documented in one publication. Two manuscripts have been submitted and are either in review or under revision. Another manuscript is in preparation. We anticipate additional publications will follow. In 2012, the education programming Cedar Creek reached 6,619 users, including K-12 students, teachers, and the general public.

III. PROGRESS SUMMARY AS OF ***[6/27/2011]*****:** Results 1-3 are proceeding on schedule. The Fertilization-Irrigation and Invasion Experiment plots (Results 1 & 2) were cleared and planted in 2010, and continue to be maintained (weeded, watered and/or fertilized) in 2011. The Climate experiment was maintained and sampled in 2010 (and 2011), and the 2010 data were analyzed together with the results from 2009. One of the graduate students (who will be supported by this grant as of 2011) oversaw soil carbon and nitrogen sampling (Result 3) in 2010, and sent the collected samples out for chemical analysis. Result 4 has been delayed until more data from these experiments are available for synthesis.

Amendment Request (6/27/2011):
2010 graduate student budgets were redirected to hire additional undergraduate interns critically necessary for establishing the experiments, as we were fortunate the graduate students working on this project had obtained other funds. The 2010-2011 salary budgeted for Dr. Clarence Lehman for Result 4 was similarly redirected because sufficient data were not yet ready for the analysis and synthesis that he will perform.

Amendment Request (9/1/2011):
As suggested by the LCCMR staff, progress report due dates are shifted to synchronize with the annual research cycle. Fall reporting dates will be at the end of October, when

the field season is complete, and spring reporting dates will be at the end of April, when data gathering and chemical analyses have been completed and analyzed.

Amendment Request (4/24/2013):
We request that $6,600 be moved from result 4 to result 3. This would make result 3 budget $69,900 and result 4 total budget $17,800.
We are also request that $24,000 for chemical analysis and $9,800 for graduate student support be shifted to fund interns needed to achieve result 3. In the middle of the 2012 field season it became clear that we could not achieve our goals without a major increase in intern effort since this field work for result 3 was more complex and much more time-consuming than we had anticipated. This we did in the summer of 2012, at which time we were able to use other funds to support the graduate students working on this project and to pay for much of the chemical analyses. We also request that $9,400 for Clarence Lehman's salary in result 4 be shifted the to result 3 and to interns in result 4. Clarence Lehman's salary has been and will be covered from other college sources. The increase of intern money from $15,000 to $17,800 is needed to help with sample analyses. **Amendment Approved: 5/13/2013**

PROGRESS SUMMARY AS OF (4/26/2013): Results 1-3 proceeded on schedule. The Fertilization-Irrigation Experiment and Invasion Experiment were maintained (weeded, watered and fertilized) in 2012. Along with the maintenance we harvested aboveground plant biomass (as would be done for biofuel production) in these two experiments in the summer of 2012, and quantified the abundances of all plant species in this biomass. Samples were retained for analysis of tissue chemistry relevant to biofuel production. The climate experiment was maintained and sampled as well in 2012. Maintenance included weeding the plots and monitoring of the heat lamps. Sampling included above and below ground biomass harvests, quantification of the abundances of all plant species in this biomass, and soil sampling for soil carbon storage, for soil fertility (total soil nitrogen, and for efficiency of plant use of soil nitrate. Samples have been processed and/or sent out for analyses that are now complete (nitrate) or in progress. Much of the data from 2012 have been synthesized with previously collected data and are being used in the final analysis this spring (results 4).

IV. OUTLINE OF PROJECT RESULTS:

RESULT 1: Effects of Agricultural Inputs and Warming on Biomass Production and Sustainability

Description:
<u>Fertilization-Irrigation Experiment</u>
The Fertilization-Irrigation Experiment consists of sixty-four 9x9 meter plots, and is designed to determine how irrigation and fertilization would impact the yields and ecosystem functioning of four potential cellulosic biofuel crops. The four crops are 1) four varieties of switchgrass (Summer, Sunburst, Pathfinder and Blackwell); 2) the four varieties of switchgrass plus red clover and alfalfa; 3) the four varieties of switchgrass plus big bluestem, Indian grass and little bluestem; and 4) the four varieties of switchgrass plus big bluestem, Indian grass, little bluestem, red clover and alfalfa.

These Fertilization-Irrigation Experiment plots will be located within the same grid of plots that contains the thirty-eight Climate Experiment plots. This grid as a whole contains a total of 342 plots, of which 168 are in the Biodiversity Experiment (which provides control plots for the experiments discussed here) and thirty-five are in an LCCMR/ USGS supported study of the abilities of different vegetation types to prevent various agrochemicals from entering and polluting the groundwater.

The Fertilization-Irrigation Experiment employs a full-factorial design, with each plot receiving one of two levels of nitrogen addition (none and14 $g/m^2/yr$) and one of 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 four possible treatment combinations. There will be four replicates of each irrigation/fertilization treatment combination applied to each of the four biofuel crops. Thus:

2 nitrogen treatments (using a split-plot design) x 2 irrigation treatments x 4 replicates per biofuel crop type x 4 biofuel crops = 64 plots total.

Climate Experiment
The Climate Experiment consists of thirty-eight 9x9 meter plots designed to determine the effects of climate warming, plant diversity, and plant community functional composition on the functioning of prairie-like ecosystems. The Climate Experiment also has a full factorial design, with each 9x9 meter plot being maintained at one of the four levels of diversity (1, 4, 16 and 32 species), and all three warming treatments (ambient temperature, low warming and high warming) nested as smaller subplots within. 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 to control for non-warming effects, if any, such as shading. An additional fourth subplot was established, also un-warmed, and lacking any heat lamp structure.

Sampling
By weighing vegetation samples clipped from our Climate Experiment and Fertilization-Irrigation Experiment plots at peak biomass, we will determine how biomass yield (dry weight of above-ground plant matter) depends on plant diversity (the number of species the samples contained), plant composition (which species were in the plot and in what proportion), soil temperature, nitrogen fertilization and irrigation. We will discover if yields of specific plant communities are harmed or helped by warming, and how diversity is impacted by fertilization and irrigation. Statistical analysis of this data will entail a combination of regression and analysis of variance (the generalized linear models approach), taking our experimentally imposed treatments as independent variables for which we will examine both direct and interactive effects.

Summary Budget Information for Result 1: **ENRTF Budget:** **$ 67,500**
Amount Spent: **$ 67,500**
Balance: **$ 0**

Deliverable	Completion Date	Budget

1. Summer 2010: Interns (3.5 undergraduates) establish the Fertilization-Irrigation Experiment. After first clearing and seeding its new plots, all 96 plots are regularly watered and fertilized. Interns maintain the 114 Climate Experiment plots as well, weeding and collecting temperature data. At the end of the summer, interns clip, sort and weigh aboveground biomass samples from the Climate plots.	8/31/10	$16,800
2. Summer 2011: Interns (7 undergraduates) maintain all Fertilization-Irrigation Experiment and Climate Experiment plots: Fertilization-Irrigation plots are watered and/or fertilized and Climate plots are weeded and have their temperature data collected. At the end of the summer, interns clip, sort and weigh aboveground biomass samples from the Fertilization-Irrigation and Climate plots.	8/31/11	$32,500
3. Summer 2012: Interns (3.5 undergraduates) maintain all Fertilization-Irrigation Experiment and Climate Experiment plots: Fertilization-Irrigation plots are watered and/or fertilized and Climate plots are weeded and have their temperature data collected. At the end of the summer, interns clip, sort and weigh aboveground biomass samples from the Fertilization-Irrigation and Climate plots.	8/31/12	$18,200

Result Completion Date: August 31, 2012

Result Status as of (June 27, 2011)**:**

In the summer of 2010, interns prepared new areas for planting the 8 new 21x19m blocks of fertilization-irrigation plots by clearing the existing vegetation, marking out the new plots, tilling the soil, setting up irrigation lines, planting the switchgrass varieties and starting irrigation and nitrogen treatments. The interns have continued the irrigation-nitrogen treatments this summer, and maintained the species composition of the plots through weeding.

In the summers of 2010 and 2011, the interns also continued the maintenance of the climate treatment plots, sampling each in late summer of 2010.

Result Status as of (October 31, 2011)**:**

Result Status as of (April 30, 2012)**:**

Result Status as of (April 26, 2013)

In the summer of 2012 the **fertilization-irrigation plots** were maintained and harvested. The experiment was set up in 8 blocks, with 8 subplots. Four seed treatments were: 1. A switch grass (Panicum virgatum) variety mix containing the cultivars deemed best for central Minnesota, 2. Switch grass - clover/ alfalfa mix, 3. Switch grass variety and other native warm season grasses mix, and 4. Switch grass, native warm season grasses, and clover/alfalfa mix. Each seed treatment was fertilized or not and irrigated or not, making a 64 plot factorial design. Each plot was harvested, by clipping four strips, each 10cm wide by 6m long. Three of the clip strips were bagged, dried and weighed for total biomass productivity. The fourth clip strip was taken back to the lab, where it was sorted to plant species, and each plant species was then dried and weighed. All of these data are being analyzed at the present time. Results of these analyses will be in our final report.

In the summer of 2012, the **climate experiment** plots were maintained, harvested and otherwise sampled. Sampling of soil and air temperatures, air humidity, soil moisture in surface soils and at depth, and remotely-sensed ("NDVI") plant biomass was done on a minute to minute basis (soil temperatures), hourly basis (humidity), weekly basis (remotely-sensed plant biomass) or an every ~3 week basis (soil moisture) throughout the growing season. An immense amount of labor, much more than we had originally anticipated, was needed for these important tasks. We also sampled aboveground biomass in June and in August, sorting the biomass from each plot to its component species, and then drying and weighing the biomass of each species in each plot. We also took root samples (belowground biomass), sorted each sample into course and fine roots, then dried and weighed each sample. Finally, we collected soil samples from each plot.

We also collected data on an estimate of biomass throughout the season on the 114 climate plots using "NDVI" measurements, which detect the amount of "green" in each plot. The NDVI method is the technique that was originally developed and tested for satellite measurement of the productivity of plants in ecosystems. The reason for the intense biomass sampling was to determine how warming impacts the seasonal pattern of plant demand for soil water and nitrate, and how this temperature-dependence demand then influences biomass production.

Final Report Summary (9/9/2013)**:**
Deliverable 1: Determine the effects of warming on biofuel yields.

Based on the **climate experiment**, we can report the following deliverables related to the impact of warming on biofuel yields for biomass harvested:

- *High diversity plots were more productive than low diversity plots throughout the growing season.* This shows that higher diversity offers a biofuel productivity advantage, which is consistent with other studies demonstrating greater productivity in high diversity ecosystems.

- *High diversity plots were even more productive when exposed to experimental warming.* This is a unique, important and never-before-reported discovery. That higher biodiversity mixtures enhance plant performance and harvested biofuel biomass in response to warming reinforces other findings that higher plant biodiversity helps ecosystems when exposed to novel environmental conditions, such as elevated atmospheric deposition of nitrogen and elevated atmospheric carbon dioxide.

- *Plant biomass had the greatest increase in the warmest plots, but this difference was larger in the spring than in late summer when biomass is harvested for biofuel.* This may indicate that temperature is not the most limiting factor for biomass production across the full growing season. Further studies are required to determine what the other limiting factors may be. Soil nitrogen availability and water availability are the most likely drivers.

From the **fertilization and irrigation experiment**, we report the following deliverables regarding the impact sustainability and plant diversity in highly diverse, restored prairies used for biofuel biomass production:

- *Warming lowers seed establishment in native prairie plants.* This potentially poses significant threats to future native prairie restoration efforts. This effect is reminiscent of patterns observed in the Short Grass Prairie of western Colorado, where a major native perennial grass, Blue Gramma, mainly establishes from seed in native prairie during unusually cool and wet springs and summers. Our results suggest that a higher seeding rate (more seed per hectare) may be needed to achieve the desired establishment of prairie. For instance, results shown in Figure 4 suggest that a seeding rate 2 to 3 times the rates currently used in prairie restoration in Minnesota would likely overcome the effects of a 3^{o} C warming. Alternatively, seeding earlier in the growing season, and timing major restoration to coincide with springs predicted by long-range forecasts to have favorable weather, might overcome the seedling survival issues posed by warming.

- *With respect to biofuel sustainability in Minnesota, we have found that high-diversity mixtures of native prairie plant species offer many advantages in addition to the ones that our earlier work had identified.* Our results suggest that switchgrass may be about as viable as high-diversity mixtures when it comes to production of biomass for biofuels on un-amended soils, but that high-diversity mixtures offer many important additional benefits. The added benefits include a greater ability to withstand and take advantage of a warming climate, the ability to provide a more stable source of biomass, the ability to increase groundwater quality and the ability to remove carbon dioxide from the atmosphere and store it as soil organic matter (humus). Moreover, under conditions of moderate fertilization and moderate irrigation, high-diversity mixtures of native prairie plant species produced the most biomass of all five types of biomass plantings that we tested, although the statistical significance of these differences was low.

Plant biomass, as estimated by remote sensing methods (NDVI), was greater in 16-Species Mixtures than in 4-Species Mixtures or 1-Species Monocultures (Figure 1) throughout the full growing season. In addition, warming led to a marked increase in biomass relative to that of un-warmed plots in the spring, but this effect declined during the summer (Figure 2). The decline in the impact of warming suggests that biomass production is ultimately limited more by a factor other than temperature than by temperature. Further work will be needed to determine what this factor is; the most likely candidates are soil nitrogen availability and water availability.

Our direct measures of plant biomass in all plots in June and August (Figure 2) show that 16-Species Mixtures were able to take advantage of warming more than were lower diversity treatments. In particular, in August, which is when biomass likely would be harvested for biofuels, the 4-Species and the 16-Species Mixtures were not only much more productive than the 1-Species Monocultures, but also had an even greater biomass advantage when experiencing warmed conditions (Figure 2). These results demonstrate that use of higher plant diversity offers a biofuel productivity advantage under conditions of climate warming. The ability of high-diversity mixtures to have the greater enhancement in their performance in response to warming is a never-before-reported discovery that has come from this research. It reinforces other findings that higher plant biodiversity enhances the ability of ecosystems to respond to novel environmental conditions.

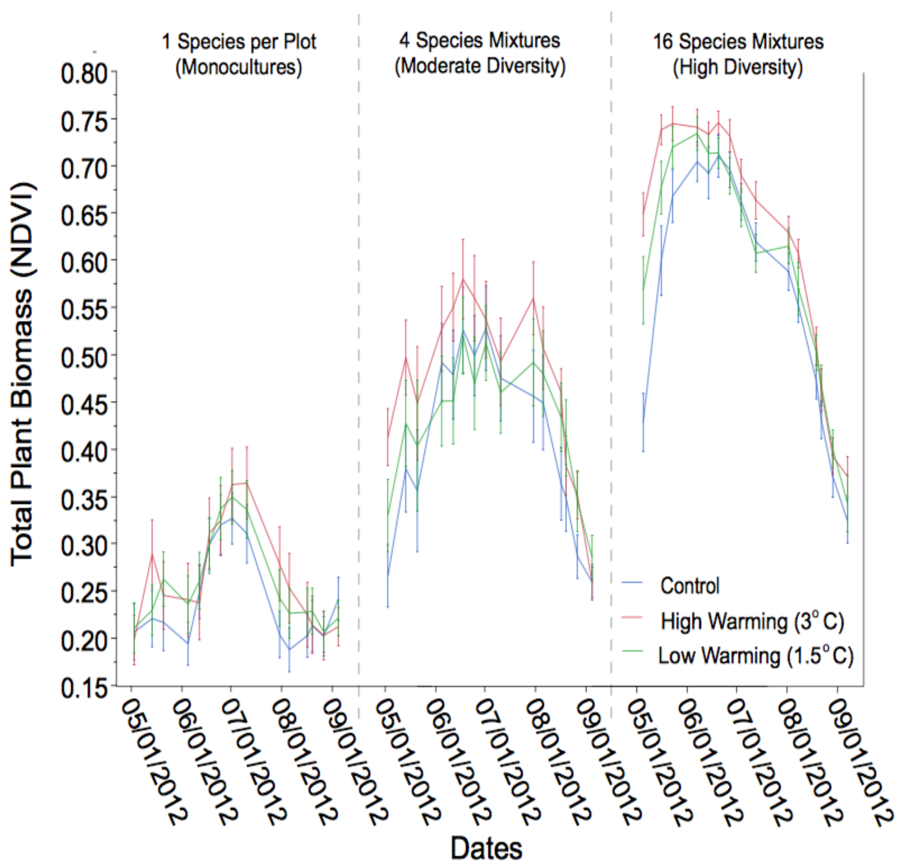

Figure 1 (above). The dependence of biomass, as measured by NDVI, on plant diversity and on warming treatments.

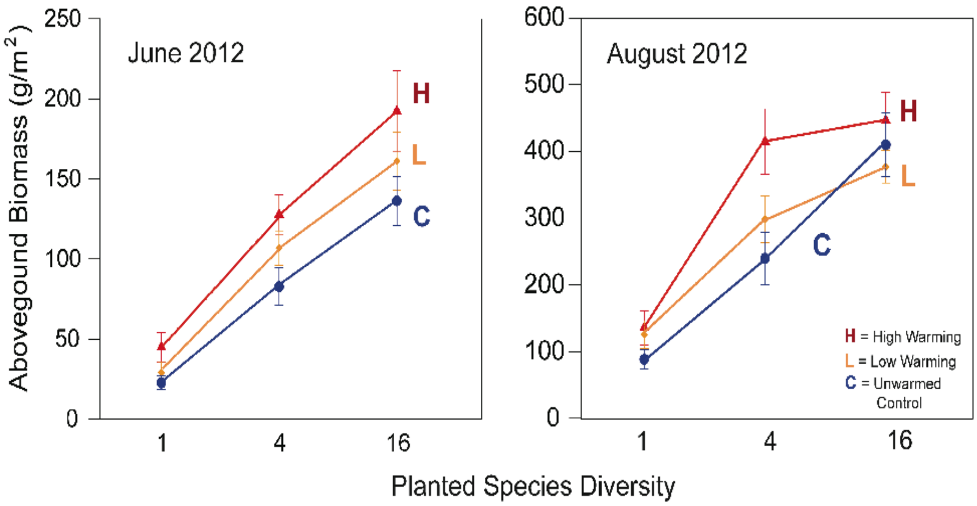

Figure 2 (above). The dependence of harvested plant biomass on diversity and warming in both June and August of 2012.

Deliverable 2: Measure of how fertilization and irrigation impact sustainability and plant diversity in high-diversity restored prairie used for biofuel biomass production.

We examined five different potential biofuel biomass crops and how their productivities responded to fertilization and/or irrigation. Four of these biofuel crops included varieties of switchgrass (*Panicum virgatum*) that have been bred to be biofuel crops We will refer to these four potential crops, as shown in Figure 3 below, as (1) Panicum; (2) Panicum & Legumes; (3) Panicum & Grasses; and (4) Panicum& Legumes & Grasses. In addition, because the **fertilization-irrigation experiment** was performed in the same field as a similar and already established study of the effects of fertilization or no fertilization and of irrigation or no irrigation on biomass productivity of highly-diverse mixtures of native prairie plant species (planted with 32 species), we also report results for this fifth biomass crop, which we refer to as High Diversity Mixtures.

The major results of the fertilization-irrigation experiment are summarized in Figure 3.

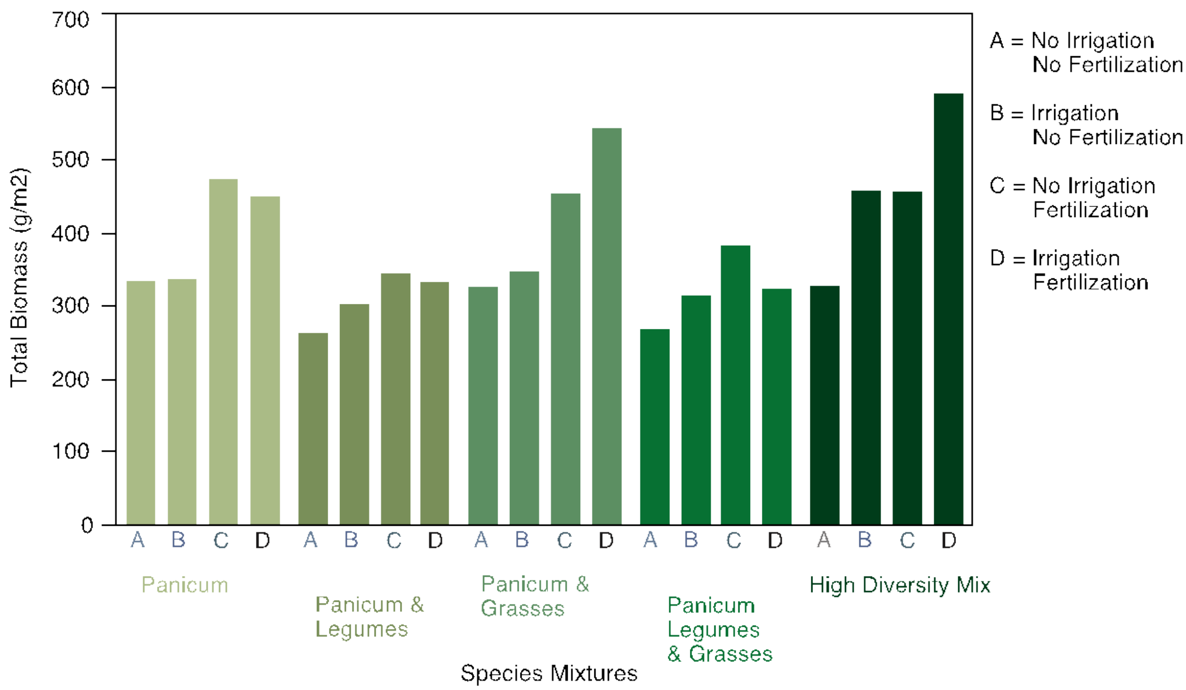

Figure 3 (above). Biomass productivity of five potential biofuel biomass crops, tested for each of four different types of growing conditions (A=no irrigation or fertilization; B=irrigated but not fertilized; C=moderately fertilized but not irrigated; D= irrigated and moderately fertilized). Primary findings from this experiment include:

- *Fertilization and nitrogen treatments had similar impacts across all species mixtures.* All five biofuel biomass crops were fairly similar in their biomass productivities when experiencing the same treatment.

- *Moderate fertilization with nitrogen significantly increased productivity, with the largest effects in the Panicum, Panicum+Grasses, and High Diversity Mixtures.*

- *The greatest overall biomass productivities occurred in the High Diversity Mixtures and in the Panicum+Grasses Mixtures when each was receiving both moderate N fertilization and irrigation.* This might be expected for the sandy, low-nitrogen soils of our research site. The combination of moderate nitrogen fertilization and irrigation led to about an 80% increase in yield.

The net benefits to a farmer and to society of biomass crops can only be determined by life-cycle analyses that consider many factors other than just yield. For instance, fertilization and irrigation can represent major expenses for a biomass farmer, and can greatly increase the amount of time that a farmer must dedicate to a biomass crop. An earlier study of production of prairie hay suggested that unfertilized and un-irrigated production was, for the case of Woodson County, Kansas, more profitable.

As to environmental costs, irrigation is energy intensive, and thus releases greenhouse gasses. Nitrogen fertilization also has environmental costs. The manufacturing of nitrogen fertilizer is extremely energy-intensive and thus releases much greenhouse gas to the atmosphere. Moreover, fertilization with nitrogen leads to emissions of nitrous oxide, a greenhouse gas that is about 300-times more potent than carbon dioxide. Since a major factor motivating societal demand for biomass fuels is lower greenhouse gas emissions than result from fossil fuels, it is plausible that fertilization and/or irrigation could greatly reduce or eliminate the greenhouse gas benefits that biomass from perennial grasses might otherwise provide. Life cycle analyses will be needed to evaluate these possibilities.

It is well established in the scientific literature that high-diversity mixtures of perennial plant species offer many ecosystem service benefits compared to low-diversity plantings. Indeed, earlier studies of plots in this same field have shown that greater plant diversity leads to greater stability of productivity (i.e., greater year-to-year reliability of biomass production), to lower incidence of plant diseases, and to greater accumulation and storage of carbon in soils. As discussed in our synthesis at the end of this report, these ecosystem service benefits can be of great importance when evaluating the net environmental benefits of alternative biofuel biomass crops.

RESULT 2: Agricultural Input and Warming Effects on Invasions by Exotic Species

Description: By carefully mapping the spatial locations of the plants in the Invasion Experiment plots (a subset of plots nested within the Fertilization-Irrigation Experiment), we will be able to observe which species invade our experimental plots each year and determine how warming, plant diversity, plant composition, fertilization and irrigation impact invasion susceptibility. Each Invasion Experiment plot will explicitly test the invasion potential of *Miscanthus* by vegetative spread, by planting a wide strip of *Miscanthus* down the center of the each plot, and a series of *Miscanthus* rhizome plugs down one of the side strips (which contain the regular prairie mixtures or monocultures). In addition, we will study the invasion potential of specific non-native invasive plant species, including *Miscanthus*, in Climate Experiment plots by adding seed (or rhizome plugs in the case of *Miscanthus*) of those species to a specific area of a subset of the plots, and measuring their survival, growth and spread.

Summary Budget Information for Result 2: **ENRTF Budget: $ $65,800**
Amount Spent: $ $65,800
Balance: $ 0

Deliverable	Completion Date	Budget
1. Summer 2010: Interns (2.5 undergraduates) work with the Result 1 interns to establish the Invasion Experiment within the Management Experiment, and make initial maps and surveys of the Invasion Experiment plots. May also assist with trait and variable measurement of invasion studies within the Climate Experiment.	8/31/10	$13,600
2. Summer 2011: Interns (5 undergraduates) survey and map the Invasion Experiment plots multiple times throughout the summer. May	8/31/11	$25,000

assist with trait and variable measurement of invasion studies within the Climate Experiment.		
3. Summer 2011: Graduate student continues to analyze invasion data and provide guidance to the interns working on the experiments. Begins research	8/31/11	$8,100
4. Summer 2012: Interns (2 undergraduates) survey and map Invasion Experiment plots multiple times throughout the summer. May assist with trait and variable measurement of invasion studies within the Climate Experiment.	8/31/12	$10,400
5. Summer 2012: Graduate student continues to analyze invasion data and provide guidance to the interns working on the experiments.	8/31/12	$8,700

Result Completion Date: July 1, 2013

Result Status as of *(*June 27, 2011*)***:** In the summer of 2010, the interns prepared the new experiment site, clearing existing vegetation, marking plot boundaries, tilling, setting up irrigation lines, and transplanting the *Miscanthus* plugs into the plots. We used a sterile hybrid Miscanthus variety because it was reported to be among the better varieties for Minnesota, and was obtained from a source that grew it in Minnesota. Two Ph. D. students, Heather Whittington and Peter Wragg, did research that contributed to our work on Results 2, but both had other means of support. Nitrogen and irrigation treatments have been continued in the summer of 2011, with the species composition of the plots being maintained by weeding.

In the summer of 2011, U of M a Ph. D. student, Jane Cowles, began studying the warming experiment and researching closely related issues concerning competition among different grass species, coexistence and the factors controlling invasion.

Result Status as of *(*October 31, 2011*)***:**

Result Status as of *(*April 30, 2012*)***:**

Result Status as of *(*October 31, 2012*)***:**

Result Status as of *(*April 26, 2013*)***:** Miscanthus
In the summer of 2012 the Miscanthus experiment was maintained, harvested and otherwise sampled. Nested within the irrigation-nitrogen experiment, 16 plots of high-diversity prairie were split in half to form a “Miscanthus half” and a “prairie half.” In 2010 the Miscanthus half of each of the 16 plots was tilled and 102 Miscanthus plants were planted. The prairie half of each plot was left as undisturbed diverse prairie. In 2012 we harvested the experiment by clipping a 6 meter by 50 cm in size vegetation strip within each half of each plot. The clip strip in the Miscanthus half was adjacent to the plot mid-line, allowing us to know how Miscanthus grew where it had been planted and how productive it had been. The clip strip in the prairie half was also adjacent to the plot mid-line, allowing us to measure Miscanthus invasion of prairie, and the impact

Miscanthus had on native prairie plant species. We sorted, dried and weighed the clipped biomass.

Result Status as of *(*April 26, 2013*)***:** Warming and Plant Invasion into Prairie

In the summer of 2011 and 2012, another plant invasion experiment was nested inside the climate experiment. Peter Wragg, a Ph. D. student, studied the effects of our experimentally-imposed climate change on the abilities of 25 different native and non-native plant species to invade prairie. Peter mapped the location of each germinated seedling and followed its survival and growth through two growing seasons. He is currently analyzing the data.

Final Report Summary (9/9/2013): Warming and Plant Invasion into Prairie
Deliverable 1: Find out the effects of warming on invasion, and its dependence on diversity and species composition.

In 2009-2011, we compared the germination, survival and growth of 25 exotic and native species that were seeded into each plot of the climate experiment. On average, across all added species, we found that germination, survival and growth of seedlings were significantly inhibited by warming and by plant diversity. For most species, this resulted in substantially fewer of the additional species becoming established in the warmed and diverse plots than in the ambient-temperature and low-diversity plots (Figure 4).

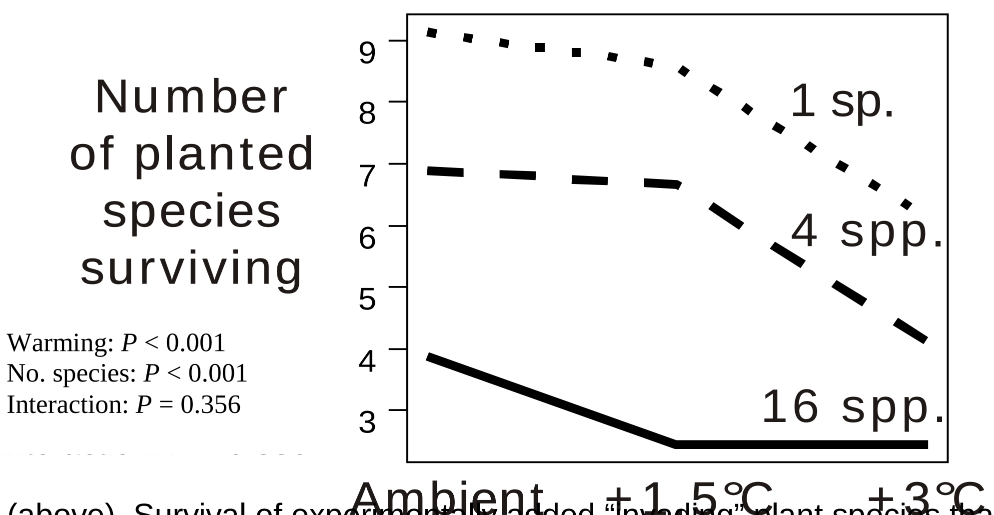

Figure 4 (above). Survival of experimentally added “invading” plant species that were seeded into the control and warmed plots of the climate experiment for all combinations of warming (control, low warming (1.5C) and high warming (3C)) and plant diversity (1, 4 and 16 established prairie plant species). Note the marked decrease in invader plant success under high warming.

In 2012, we added seeds of an additional seven native species that showed the same overall trends in their responses to the warming and diversity treatments as did the 25 seeded species not already in the invasion experiment. A few species responded differently from this overall trend. A native prairie legume, Baptisia, had higher survival under warmed conditions, especially in higher-diversity plots (Figure 5). A native goldenrod, Solidago speciosa, was strongly inhibited by high warming at all levels of diversity (Figure 5), and a western perennial grass, Bouteloua, was not detectably impacted by the warming treatments (Figure 5).

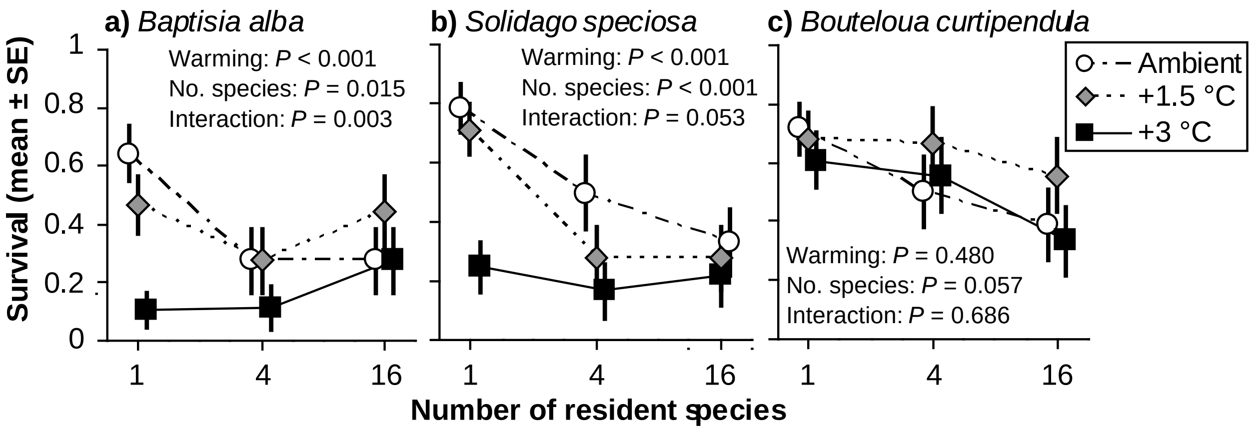

Figure 5. Resident species that deviated from the overall response to warming were inconsistent with general trend of inhibition with warming.

Our major results are as follows:

- *Plant reproduction and establishment of seedlings are inhibited by warming in native prairie.* Our results are the first to show that warming significantly decreases the chance that an adult plant of a given species will be able to replace itself before it dies. The net effect is that warming may well lead to lower plant diversity in native prairie.

- *Invasion is inhibited by warming.* The greater trend that warming decreases seedling germination, survival, and growth also applies to exotic species. This suggests that warming may not lead to increased abundances of exotic plant species in native or restored prairie.

- *Invasion is inhibited by higher diversity species mixtures.* This result is consistent with much of the established scientific literature.

Deliverable 2: Determine how low- versus high-input biofuel production methods influence the dynamics of invasive species.

Research in the highly fertile and moist soils of Illinois have suggested that a hybrid Asian grass, *Miscanthus*, might be a productive crop for biofuel. However, many concerns have been raised about the potential for *Miscanthus* to invade and negatively impact native prairie. Using 16 previously established plots of high diversity prairie, half of each plot was tilled and planted with Miscanthus. This allowed the “Miscanthus-half” to directly abut with the “Prairie-half” and better indicate the extent to which Miscanthus invaded the adjacent prairie.

Our plots of Miscanthus suffered from partial winter-kill in the winter of 2011/12. This suggests that at least the variety of sterile hybrid Miscanthus that we used (and we had chosen it because of its reported winter hardiness and productivity) may not have sufficient hardiness to be a viable crop in Minnesota. Sampling in 2012 showed the

Miscanthus-half of each plot produced only about 40% of the biomass as the high-diversity, prairie-half.

Our results show the following:

- *The sterile hybrid Miscanthus variety we tested appears to be ineffective at producing biomass in the climate of central Minnesota, at least on sandy, drier soils such as occur at Cedar Creek Ecosystem Science Reserve.* Since Miscanthus grows best in warmer climates and on rich and wet soils (such as in lowlands of Illinois), the poor performance of Miscanthus may not seem surprising in retrospect. However, much had been written asserting that Miscanthus could be a much better producer of biomass than native high diversity prairie. Our results do not support that assertion.

- *Additional observation is needed to determine if Miscanthus is an invasive threat to native prairies.* As part of this experiment, we had planted Miscanthus plants on the immediate edge of the native high diversity prairie half of each of these plots. Our measurements in 2012 showed that there had been detectable, but moderate, invasion into the native, high diversity prairie by Miscanthus. Only further observation over the next several years will determine if this invasion threatens the native prairie.

RESULT 3: Effects of Agricultural Inputs and Warming on Soil Carbon

Description: The amount of soil carbon stored in or lost from soils depends on the balance between the carbon sequestered through plant growth and the carbon lost through microbial decomposition in the soil. Chemical analyses of plant biomass and soil samples can be used to characterize these dynamics for a given locale. Carbon content of soil and of above- and below-ground plant biomass provides an estimate of the total carbon within the current system. The changes in these quantities indicate if the soil is releasing or sequestering carbon and nitrogen. Prairie grasslands tend to be limited in their growth by nitrogen, so the concentration of biologically available nitrogen (nitrate and ammonium) in the soil indicates the potential for plant growth. The nitrogen mineralization analyses indicate how quickly microbes are able to convert dead or dying plant material into biologically available nitrogen, which has feed-back effects on plant growth. In addition, we will directly monitor the amount of carbon released from the soil by decomposition (referred to as soil respiration). By compiling these analyses with the data collected for Result 1, we will be able to determine the reasons why warming, fertilization or irrigation may impact the carbon balance.

Summary Budget Information for Result 3: ENRTF Budget: $ 69,900
Amount Spent: $ 69,900
Balance: $ 0

Deliverable	**Completion Date**	**Budget**
1. Summer 2010: Interns (2 undergraduates) collect and prepare 3186 soil/root samples for analysis and measure soil respiration in the field	8/31/10	$7,200

monthly.		
2. Winter 2010: Chemical analysis of soil and below- and above-ground biomass samples (3396 samples total - aboveground biomass samples collected as part of Result 1).	2/31/11	$9,000
3. Summer 2011: Interns (2 undergraduates) collect and prepare 2346 soil/root samples for analysis and measure soil respiration in the field monthly.	8/31/11	$7,500
4. Summer 2011: Graduate student continues analyses with the 2010 biomass and soil carbon and nitrogen data.	8/31/11	$8,100
5. Winter 2011: Chemical analysis of soil and below- and above-ground biomass samples (2556 samples total – aboveground biomass samples collected as part of Result 1).	2/31/12	$6,000
6. Summer 2012: Interns (2 undergraduates) collect and prepare 3186 soil/root samples for analysis and measure soil respiration in the field monthly..	8/31/12	$7,800
7. Summer 2012: Graduate student continues analyses with the 2011 biomass and soil carbon and nitrogen data.	8/31/12	$8,700
8. Winter 2012: Chemical analysis of soil and below- and above-ground biomass samples (3396 samples total – aboveground biomass samples collected as part of Result 1).	2/31/13	$9,000

Result Completion Date: July 1, 2013

Result Status as of *(*June 27, 2011*)***:** Soil cores were taken in 2010 by interns from the nitrogen-irrigation, climate and *Miscanthus* invasion experiments to provide initial values. These samples will be processed and analyzed once the 2011 samples are ready. U of M Ph. D. graduate student Heather Whittington is pursuing Result 3 for the project and is overseeing the sampling and analyses in 2011. She made similar contributions in 2010 while supported on other funds.

Result Status as of *(*October 31, 2011*)***:**

Result Status as of *(*April 30, 2012*)***:**

Result Status as of *(*October 31, 2012*)***:**

Result Status as of *(*April 26, 2013*)***:** In the summer of 2012 interns helped us collect data on many soil properties within the climate experiment. Two Ph. D. students, Peter Wragg and Jane Cowles, were deeply involved in this research on a day-by-day basis all summer.

We sampled available soil nitrate and ammonium at six different times during the growing season in all 114 plots. We sent all samples out for analysis. Preliminary analyses suggest that both warming and diversity treatments affect the quantity of ammonium ions in the soil matrix. Nitrate is harder to interpret, but it appears nitrate concentrations increase from warming during potions of the growing season. Throughout the growing season we also measured surface soil moisture weekly and deeper soil moisture every 3 weeks.

Ph. D. student Heather Whittington has analyzed other results of the warming experiment and found that:

- Warming decreased soil moisture and increased soil temperature, but had no effect on plant available total mineral nitrogen (as measured by net N mineralization) in 2009 or in 2010.
- Warming increased aboveground biomass of two legumes species (*Lupinus* in 2009 and 2010 and *Dalea (Petalostemum)* in 2010).
- Warming decreased the concentration of nitrogen found in the stems and leaves of four legumes species in 2010, but there was no evidence that warming affected nitrogen fixation in these legumes.
- Legumes growing with greater numbers of other species (higher species diversity) relied more on nitrogen fixation but had lower nitrogen concentrations than legumes growing at lower species diversity.
- Net nitrogen mineralization, legume nitrogen concentration, and nitrogen fixation were greater in the wet year (2010) than the dry year (2009). Differences between these years were often greater than differences between warming treatments, suggesting that future changes in precipitation may have greater impact than changes to air temperature.
- Warming accelerated the flowering of four species (*Amorpha*, *Dalea*, *Andropogon*, and *Schizachyrium*). In 2011, warming also accelerated spring green-up but had no effect on the timing of senescence.

More results will be in the final report.

Final Report Summary: (9/9/2013)

Deliverable 1: Quantify how warming, fertilization and irrigation interact with various single plant species crops or with multispecies mixtures to control carbon soil storage. Communicate these results and their relevance to Minnesota biofuels.

Soil bacteria and fungi decompose soil organic matter (humus), and in so doing release nutrients (nitrate, ammonium, phosphate) into the soil and also release carbon dioxide into the atmosphere. Our analyses, to date, of carbon lost from soils as carbon dioxide have revealed little differences attributable to the warming treatments. However, further analyses are underway, and no conclusions can yet be validly drawn about the effects of warming on soil carbon. Similarly, analyses of measurements to date of soil total carbon content reveal that soil carbon levels are changing slowly. Given the well-know sampling noise associated with the spatial heterogeneity of soils, it is clear that further measurements this summer and in the coming years will be needed to determine the magnitude of changes in soil carbon stores that might result from warming.

Figure 6 (above) shows the experimental set up in the field. The heat lamps are suspended by scaffolding and warm patches of prairie plants that vary in number of species (1, 4, 16, or 32 species). High warming results in ~2.5 °C increase in surface soil temperature, while the low warming treatment elevates surface soil temperature by ~ 1 °C.

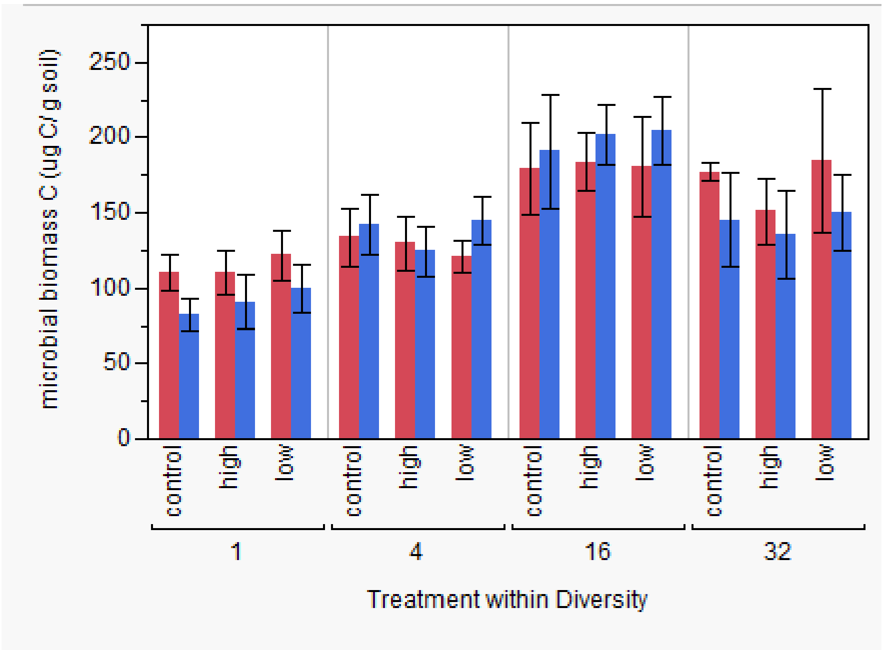

Figure 7 (above) shows microbial biomass carbon (C) measured in 2009 at the start of the experiment (red bars) and in 2012 after three years of experimental warming treatments (blue bars). In both years, microbial biomass C was significantly higher in plots with high diversity. However, there were no significant warming treatment effects in either year. The difference between years is likely driven by differences in soil moisture at the time of sampling.

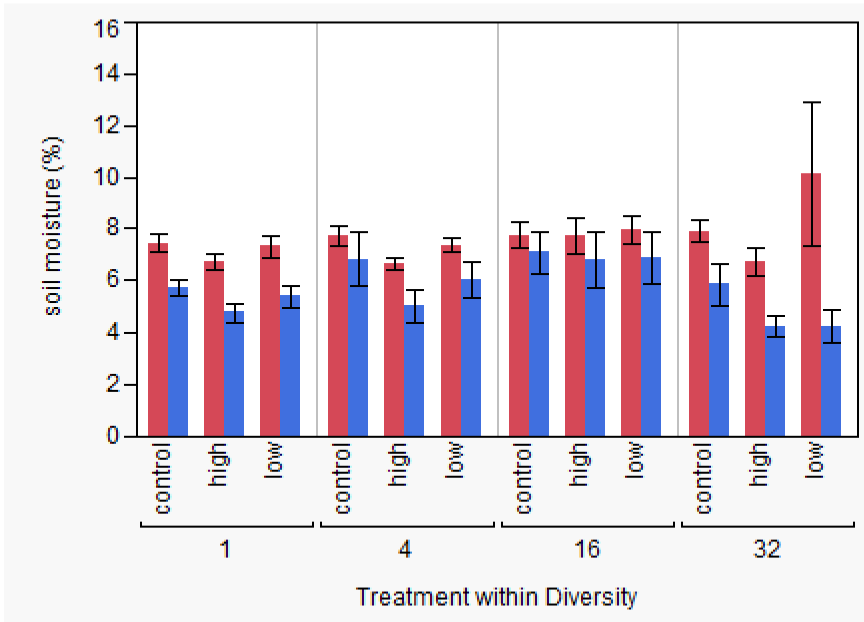

Figure 8 (above) shows soil moisture measured in 2009 at the start of the experiment (red bars) and in 2012 after three years of experimental warming treatments (blue bars). Soil moisture was much lower during the 2012 sampling.

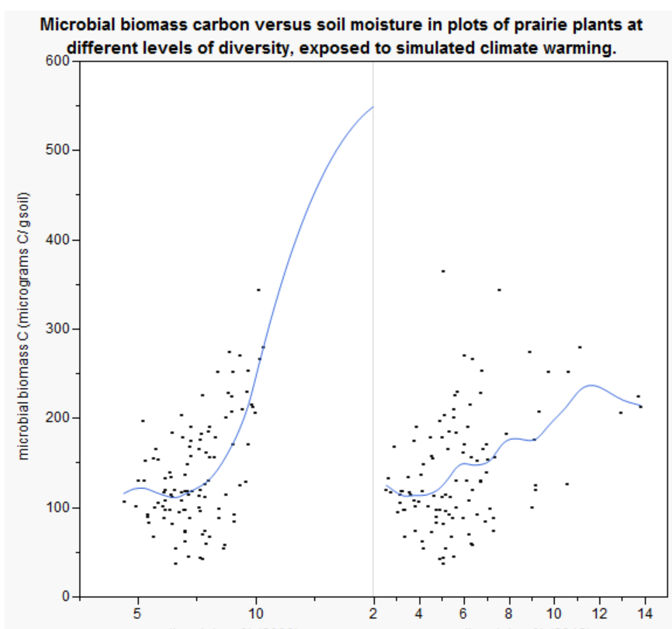

Figure 9 (above) shows strong correlations between microbial biomass C and soil moisture for both sampling years.

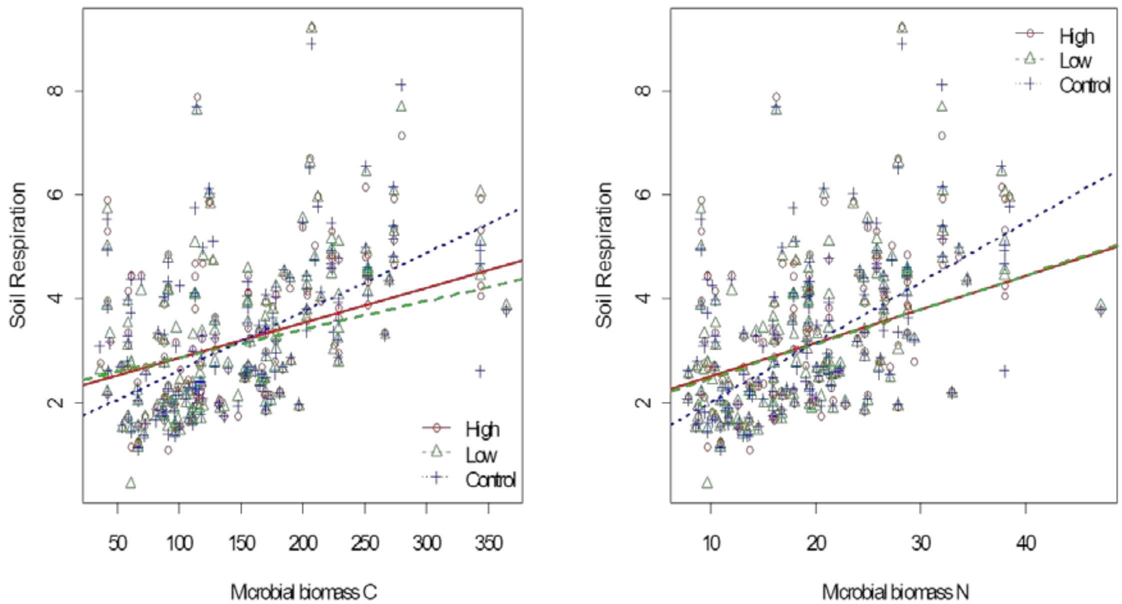

Figure 10 (above) shows strong correlations between microbial biomass C and soil respiration. Initially, we hypothesized that warming would decrease soil C stocks, as warmer temperatures would lead to increased microbial respiration. However, we found no warming effect on microbial respiration or microbial biomass C. There are several potential explanations for the lack of warming treatment effects on microbial carbon stocks or soil respiration (which both are strongly correlated with total carbon stocks). First, these sandy soils have very low total soil carbon stocks, which may constrain potential responses to elevated temperatures. Second, increasing soil temperature led to decreases in soil moisture. It is possible that microbial processes and hence belowground carbon dynamics are more constrained by soil moisture then temperature.

Using other (non-LCCMR) funds, we are currently analyzing soil samples collected in 2012 for total carbon and nitrogen, which we strongly suspect will parallel the differences in microbial carbon and nitrogen. These will be compared to the 2009 data. In sum, in this sandy, prairie soil, we found strong effects of plant species richness on belowground carbon pools and fluxes. However, there were no statistically significant effects of three years of simulated climate warming on belowground carbon processes. Our major findings to date include:

- There were no warming effects on microbial respiration or microbial biomass C.
- In both years, microbial biomass C was significantly higher in plots with high diversity.

RESULT 4: Sustainable Restoration Practices
Description: Results 1-3 will be synthesized to report the optimal methods for combining biofuel production, carbon storage, and habitat restoration.

Summary Budget Information for Result 4: **ENRTF Budget: $17,800**
Amount Spent: $17,800
Balance: $ 0

Deliverable	Completion Date	Budget
<u>Summer</u> 2010: Research assistant (1 undergraduate) works with Dr. Lehman, assisting with research and data analysis	2/31/11	$4,800
<u>Summer</u> 2011: Research assistant (1 undergraduate) works with Dr. Lehman, assisting with research and data analysis	2/31/12	$5,000
Winter 2012: Dr. Lehman continues synthesis of biofuel restoration sustainability	2/31/13	$9,400
Winter 2012: Research assistant (1 undergraduate) works with Dr. Lehman, assisting with research and data analysis	2/31/13	$5,200

Result Completion Date: July 1, 2013

Result Status as of *(*June 27, 2011*)***:** Because sufficient data were not yet ready for the analysis and synthesis that Dr. Lehman will perform, he has not yet participated for pay in this project, but is fully engaged in and aware of its progress. His research assistant worked with the other undergraduate interns at Cedar Creek in establishing the experiments. Dr. Lehman plans to assist with analyzing and publishing our findings during the final year of the project.

Result Status as of *(*October 31, 2011*)***:**

Result Status as of *(*April 30, 2012*)***:**

Result Status as of *(*October 31, 2012*)***:**

Result Status as of *(*April 26, 2013*)***:** Most data were collected in the summer of 2012. Samples have been recently processed, quality-checked and analyzed. All three Ph. D. students and I (David Tilman) are now writing scientific papers based on these results.

Two papers are already in review already from this work:

Whittington H.R., D. Tilman, and S.E. Powers (2013) Consequences of elevated temperatures on legume biomass and nitrogen cycling in a field warming and biodiversity experiment in a North American prairie. *Functional Plant Biology*. In review.

Whittington H.R., D. Tilman, and S.E. Powers (2013) Phenological responses of prairie plants to three years of elevated temperatures in a field experiment in Minnesota. *Oecologia*. In review.

Final Report Summary:

Deliverable 1: All of these results will be synthesized to find the optimal ways to combine biofuel production, carbon storage, and habitat restoration.

Summary points of our results:

- High diversity plots were more productive than low diversity plots throughout the growing season.
- High diversity plots were even more productive when exposed to additional warming.
- Plant biomass had the greatest increase in the warmest plots, but this difference became less prominent over the course of the growing season.
- Warming lowered seed establishment in native prairie plants, and thus may lead to lower diversity in native prairie.
- With respect to biofuel sustainability in Minnesota, we have found that high-diversity mixtures of native prairie plant species offer many advantages in addition to the ones that our earlier work had identified.
- Fertilization and nitrogen treatments had similar impacts across all of the prairie biofuel crops that we tested.
- Moderate fertilization with nitrogen significantly increased productivity, with the largest effects in the Panicum, Panicum+Grasses, and High Diversity Mixtures.
- The greatest overall biomass productivities occurred in the High Diversity Mixtures and in the Panicum+Grasses Mixtures when each was receiving both moderate N fertilization and irrigation.
- Plant reproduction and seedling establishment are inhibited by warming in native prairie.
- Invasion is inhibited by warming.
- Invasion is inhibited by higher diversity species mixtures. This result is consistent with much of the established scientific literature.
- The sterile hybrid Miscanthus variety we tested appears to be ineffective at producing biomass in the climate of central Minnesota, at least on sandy, drier soils such as occur at Cedar Creek Ecosystem Science Reserve.
- Additional observation is needed to determine if Miscanthus is an invasive threat to native prairies.
- There were no warming effects on microbial respiration or microbial biomass C.
- In both years, microbial biomass C was significantly higher in plots with high diversity.

In total, our results suggest that optimizing biofuel production, carbon storage, and habitat restoration in Minnesota will likely require the use high-diversity mixtures of native prairie perennial plant species. Our results indicate high diversity plant mixtures are the most productive in terms of biomass and confer additional ecological benefits to restoration efforts including, more efficient use of nitrogen inputs, higher microbial biomass carbon, and lower susceptibility to invasion.

Initial plantings may benefit from higher seed additions and strategically timing seeding to minimize the effects of warming on seed germination, survival and growth.

Moderate fertilization and irrigation will likely increase biomass production, but additional work will be needed to quantify the total environmental impacts of such inputs. It is plausible that nitrogen fertilization and/or irrigation may negate the greenhouse biofuel gas benefits that high-diversity mixtures of prairie plants could otherwise provide.

The sterile hybrid Miscanthus variety we used is unlikely to be a viable option for biofuel production in areas of Minnesota with sandy, dry soils. Miscanthus does not appear to be sufficiently winter-hardy, despite considerable documentation to the contrary.

V. TOTAL ENRTF PROJECT BUDGET: ***$221,000***

Personnel: $197,000
Additional Budget Items: $0
Please see Amendment Request (4/24/2013). Funds for chemical analyses were transferred to personnel costs. Non-LCCMR funds were used to pay for chemical analyses.

Breakdown of Additional Budget Items: Chemical Analyses				
Analyses *	**Plant Carbon-Nitrogen Content**	**Soil Carbon-Nitrogen Content**	**Soil Active Nitrogen Content**	**Soil Nitrogen Mineralization Rate**
Experiment(s)	*Climate & Management*	*Climate & Management*	*Climate & Management*	*Climate only*
Plots	0	0	0	0
Samples required per analysis	0	0	0	0
Number of depths analyzed	**0	0	0	0
Replicates per plot	0	0	0	0
Measurements per year	0	0	0	***0
Number of years measured	0	0	0	0
Total samples	0	0	0	0
Estimated price per sample	$0	$0	$0	$0
Subtotals	$0	$0	$0	$0
Total				****$0

* Analyses will be performed by the Ecosystem Analysis Lab at the University of Nebraska-Lincoln. The Cedar Creek LTER has a long-standing partnership with the EAL to obtain laboratory services unavailable at the University of Minnesota.

** Analyses of above- and below-ground plant biomass

*** Samples taken in spring and fall

**** To balance the budget, only 1080 Soil Nitrogen Mineralization analyses will be paid for by this LCCMR grant ($2160); the remaining 288 ($576) will be covered by other funds (see Section VI.C).

VI. PROJECT STRATEGY:

A. Project Partners:

Dr. David Tilman (Regents' Professor and Director of Cedar Creek Ecosystem Science Reserve, U of M) will lead the research on sustainability and on effects of warming and inputs on invasion by exotic plant species (time donated in kind). Dr. Jennifer Powers (Assistant Professor, U of M) will lead work on soil carbon and nitrogen dynamics and will supervise graduate students and interns (time donated in kind). Dr. Clarence Lehman (Adjunct Faculty, U of M) will lead the synthesis of biofuel and restoration sustainability ($9400).

B. Project Impact and Long-term Strategy:

The funds requested here are essential to allow the study of the sustainability, carbon and nitrogen dynamics, and susceptibility to invasion of the grassland biofuel ecosystems established at Cedar Creek Ecosystem Science Reserve. The scale of the prairie research field experiments located at Cedar Creek are unparalleled, both in replication and long-term research capability. These attributes are essential to gaining meaningful insights into the potential impacts of prairie biofuel agriculture and, in doing so, setting standards of analysis for other biofuel industries. Such research at Cedar Creek has been supported for the past 20 years by Dr. Tilman's continuing Long Term Ecological Research (LTER) grants from the National Science Foundation, grants provided to support basic ecological research on ecosystem functioning. Funds from LCCMR and earlier funds from the University of Minnesota's Initiative for Renewable Energy and the Environment (now fully expended) are allowing us to apply the fundamental advances in ecological science we have achieved to societally relevant issues, particularly the production of biofuels from ecosystems that offer society multiple, simultaneous energetic and environmental benefits. It is thus our strategy to continue to combine support from NSF for basic research with support from LCCMR for more applied research to develop new ways of producing biofuels that help restore prairie grassland ecosystems in Minnesota, that use soils as a site for significant sequestration of carbon, that help improve water quality in agricultural ecosystems, and that provide a significant and sustainable new source of energy for our society.

C. Other Funds Proposed to be Spent during the Project Period:

Each year the National Science Foundation provides Cedar Creek researchers with about $800,000 in funding for our long-term ecological research projects. We used these NSF funds to establish our large Biodiversity Experiment and our Climate Experiment, both of which are the basis for the work we propose to LCCMR. NSF will continue to pay the majority of the costs associated with the operation and maintenance of these experiments, however LCCMR funds allow us to gather additional data, and to do some additional experiments (such as the watering and fertilization experiment and the invasion experiment) that would not be possible with our NSF funds. Thus, in essence, LCCMR support is providing the marginal, additional expenses needed to address the major but more applied issues articulated in our proposal, and does so by building on the expensive infrastructure that is already supported by the National Science Foundation.

D. Spending HIstory:

At present, the LTER grant alone provides approximately $800,000 annually for its research projects based at Cedar Creek, approximately $300,000 of which are allocated to Drs. Tilman and Powers. The infrastructure of the warming experiment, including buried wiring, circuit boxes, and heat lamps, was established in 2008 and 2009 with about $120,000 in LTER funds. Sampling of the 150 control plots for this research has been supported for the past 15 years by the same grant.

VII. DISSEMINATION:

As detailed by our deliverables, we plan to report our results with at least eight scientific papers published in high-impact peer-review journals.

Data collected with the support of this LCCMR grant will be included in the 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 Minnesota and nationally, we will make it a priority to communicate our findings in public presentations and through direct contact with legislators, other government bodies, agricultural organizations, researchers in the field, farmers, energy businesses and the general public.

Progress as of *[6/27/2011]*:
Numerous public and private groups have visited Cedar Creek and learned about the experiments as part of educational tours. Dr. Tilman also serves on an carbon sequestration and offsets advisory committee to the President's Committee of Advisors on Science and Technology for which these experiments are of direct relevance.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than October 2011, April 2012, October 2012, April 2013

IX. RESEARCH PROJECTS:

(See Peer Review Addendum)

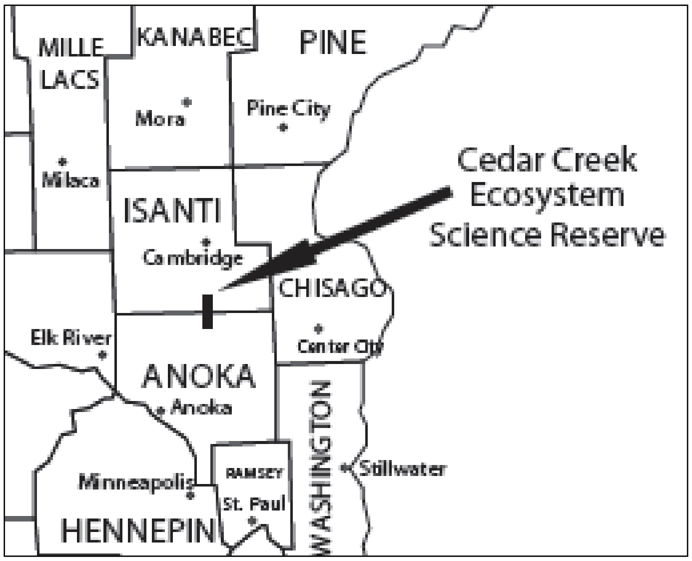

Figure 4. Map of research location

Final Attachment A: Budget Detail for 2010 Projects - Summary and a Budget page for each partner (if applicable)

Project Title: Proposal # 048-B1 - Sustainable Biofuels: Impacts of Climate Change and Management

Project Manager Name: *G. David Tilman*

Trust Fund Appropriation: $ *221,000*

2010 Trust Fund Budget	Result 1 Budget *(6/24/11)*	Amount Spent *(4/24/2013)*	Balance *(4/24/2013)*	Result 2 Budget (6/24/11)	Amount Spent *(4/24/2013)*	Balance *(4/24/2013)*	Results 3 Budget (4/24/13)	Amount Spent *(4/24/2013)*	Balance *(4/24/2013)*	Results 4 Budget (4/24/13)	Amount Spent *(9/9/2013)*	Balance *(9/9/2013)*	TOTAL BUDGET (6/24/11)	TOTAL BALANCE (9/9/13)
	Effects of Warming and Agricultural Inputs on Biomass Production and Sustainability	*Effects of Warming and Agricultural Inputs on Biomass Production and Sustainability*		*Warming and Input Effects on Invasions by Exotic Species*			*Effects of Warming and Agricultural Inputs on Soil Carbon*			*Sustainable Restoration Practices*				
PERSONNEL: Undergraduate Interns (8/summer) Average $5005 ($4580 salary + $425 fringe) per intern per summer	67,500	67,500	0	49,000	49,000	0	62,900	62,900	0	17,800	17,800	0	197,200	0
PERSONNEL: Graduate Students (2/summer) Average $8235 ($6500 salaray + $1625 fringe) per student per summer	0	0	0	16,800	16,800	0	7,000	7,000	0	0	0	0	23,800	0
PERSONNEL: Clarence Lehman (part-time) $9260 ($7000 salary + $2260 fringe) per year	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTHER: Chemical Analyses (no. of samples) Plant Carbon-Nitrogen Content (1260) - $5040 Soil Carbon-Nitrogen Content (1680) - $6720 Soil Active Nitrogen Content (5040) - $10080 Soil Nitrogen Mineralization (1080) - $2160	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COLUMN TOTAL	**$67,500**	**$67,500**	**$0**	**$65,800**	**$65,800**	**$0**	**$69,900**	**$69,900**	**$0**	**$17,800**	**$17,800**	**$0**	**$221,000**	**$0**