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dissertation entitled

CHALLENGES OF DEVELOPING SUSTAINABLE
NITROGEN SOURCES IN AGRICULTURE: COVER CROPS,
NITROGEN FIXATION AND ECOLOGICAL PRINCIPLES

presented by

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has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Crop and Soil Sciences

Major Professor's Signature

19 August, 2010

Date

CHALLENGES OF DEVELOPING SUSTAINABLE NITROGEN SOURCES IN AGRICULTURE: COVER CROPS, NITROGEN FIXATION AND ECOLOGICAL PRINCIPLES

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Crop and Soil Sciences

2010

ABSTRACT

CHALLENGES OF DEVELOPING SUSTAINABLE NITROGEN SOURCES IN AGRICULTURE: COVER CROPS, NITROGEN FIXATION AND ECOLOGICAL PRINCIPLES

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Substantial increases in nitrogen additions to agricultural ecosystems over the past century have increased crop yields, but have also led to ecological damage due to nitrogen losses to water and the atmosphere. Traditional inputs of nitrogen were in the form of organic material such as manure or legumes, which led to longer retention of nitrogen in the agroecosystem compared to synthetic fertilizers. These methods remain a viable option for contemporary farmers, but are rarely used instead of inorganic nitrogen fertilizers. I examined methods to improve integration of organic nitrogen sources, particularly legume winter cover crops, into cropping systems. Specifically, I tested the effect of planting date, temperature, varieties and mixtures on the ability of hairy vetch (*Vicia villosa*) to meet nitrogen demands of common cash crops (Chapters 1, 2). In addition, I examined the relative rates of nitrogen fixation by another legume cover crop, red clover (*Trifolium pratense*), across a gradient of management intensity (Chapter 3).

Variation existed among hairy vetch varieties in growth, morphology and phenology, and this variation translated into differences in relative success within different cropping systems. Yet, planting date and accumulated growing degree days after planting outweighed the variability among varieties in determining the amount of biomass produced by hairy vetch. In two long-term cropping experiments, the rate of nitrogen fixation by red clover differed across

environments, with a trend towards higher rates of nitrogen fixation in systems managed with chemical sources of nitrogen fertilizer.

Together, these results indicate that legume winter cover crop performance is influenced by genetic information and environmental characteristics. Species and varieties offer different opportunities within different cropping systems, and management history can influence the rate of nitrogen fixation of legume cover crops. In addition, red clover may act as a self regulating fertilizer source over time by fixing lower rates of nitrogen from the atmosphere when growing in more fertile soils, thus reducing the total amount of nitrogen entering the system and reducing long term costs for farmers and to the environment.

The final chapter of this dissertation is a review of the impacts of agricultural management on greenhouse gas concentrations in the atmosphere, and is written in a format for use in educational settings, particularly in college classrooms.

ACKNOWLEDGEMENTS

First and foremost, I thank God for leading me in this direction and giving me the strength to continue through the really difficult times.

I also have many people and organizations to thank for assisting in the completion of this dissertation; it truly would not have been possible without them. I thank my wife Emily for supporting my pursuit and loving me along the way, and helping with work when needed, even while preparing to give birth to our first child. I thank my kids, Charlie and Wendell, for humbling me, giving me time to work on this dissertation and prioritize my life, even though they didn't know what I was doing. I thank my parents, in laws and family members for supporting me. In particular, I thank my mother in law, Lil Darany, for watching the kids when I needed to work or leave town, and my father, Kurt Wilke, for participating in some experimental trials as well as for providing guidance with farming details. Thanks to Matt Wiley for also participating in research trials.

I thank all of the people on my graduate committee, at Michigan State University and at the W.K. Kellogg Biological Station (KBS) who have helped along the way. I am grateful for my advisor, Dr. Sieglinde Snapp for her guidance and leadership through this process. I thank my remaining committee members, Dr. Phil Robertson, Dr. Jennifer Lau, and Dr. Michael Russelle for taking time to teach and guide me. I thank Dr. Andy Anderson for teaching me so much about education, for employing me during the final years of graduate school, and for taking a forefront in my professional development. Thanks to Laurel Hartley and the participants in the KBS K-12 Partnership, particularly the teachers at Martin Public Schools who taught me about education and befriended me, particularly John Edgerton, Rob Robrahn, Colleen Vader and Christy Wonderly. I thank the entire faculty at KBS, specifically Dr. Dale Mutch for leadership in sustainable agriculture and Kay Gross for directing KBS. I am indebted to Greg Parker, Joe Simmons and the staff at the KBS Farm Systems Center for all of the field work they helped accomplish. Thanks to all of the staff, post docs and graduate students at KBS, EEBB and Crop and Soil Science for support and friendship. I thank the past and current members of the Snapp Lab for field and lab help and intellectual guidance, particularly Kathleen O'Neil, Lowell Gentry, Nikhil Jaikumar, Steve Culman, Jennifer Herbert, Bryan Wallace, John Green, and Christy Wonderly. I thank Rita House of Crop and Soil Sciences and Pat Reslar of EEBB for their assistance. Thanks to the KBS Long Term Ecological Research (LTER) experiment database, all those managing and working in the KBS LTER, and also in the LTER network who aided in my development during graduate school.

I acknowledge my funding sources, the Michigan State University Plant Science Fellowship, the KBS GK12 Program and the Environmental Literacy Project in the Michigan State University Department of Teacher Education for providing stipends and benefits. Thanks to The Land Institute, The North Central Region Sustainable Agriculture Research and Education (NCR-SARE), The W.K. Kellogg Biological Station and The Department of Crop and Soil Science at Michigan State University for financially supporting my research. Thanks to the Au Sable Institute Graduate Fellows Program for stipends, but more importantly for the opportunity to gather with others interested in Christian Environmental Stewardship. Finally, thanks to all those unmentioned that lent support also.

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INTRODUCTION

Agricultural production has substantially altered the global nitrogen cycle, nearly doubling the amount of plant available nitrogen entering terrestrial ecosystems (Vitousek *et al.*, 1997). In addition, non point source pollution of nitrogen to aquatic systems has resulted in several cases of coastal eutrophication, including the well documented hypoxic area in the Northern Gulf of Mexico (Robertson and Vitousek, 2009). Much of the rise in aquatic nitrogen levels has been attributed to excess nitrogen fertilizer applied to agricultural fields, which leaches down to the groundwater or runs off with surface water flow or sediment erosion. For example, because nitrogen use efficiency in field corn (*Zea mays*) averages about 40% (Cassman, 1999), there is excess nitrogen in the soil after harvest, which can leave the soil to the atmosphere, groundwater or surface water. Likewise, contemporary agriculture is a significant contributor of greenhouse gases to the atmosphere, primarily carbon dioxide, methane and nitrous oxide (Duxbury, 1994; Robertson, 2004).

In theory, substituting ecological methods (e.g. biological nitrogen sources) for conventional practices (e.g. chemical nitrogen sources) can reduce agriculture's impact on soil fertility, water quality and the atmosphere. Integrated pest management, cover cropping, perennialization and diversification are a few such methods. However, few farmers have adopted these ecological based practices, which may not be practical for farmers. Is it possible to sustain adequate grain crop productivity while simultaneously limiting nitrogen losses and reducing greenhouse gas emissions? What are the farming methods and plant traits that are needed to accomplish these goals?

Natural systems provide excellent models for low input agriculture. Complete mimicry of complex natural systems is impractical for agricultural purposes (Swift *et al.*, 2004), but the importance of certain natural processes like functional diversity must not be overlooked. Approximately two-thirds of the global terrestrial landscape is covered by diverse mixtures of perennial plants. These communities have tighter nutrient cycles and store more carbon than annual dominated communities (Cox *et al.*, 2006). In agriculture, annual communities lose 30-50 times the amount of nitrogen compared to perennial cropping strategies (Randall and Mulla, 2001). Perennial dominated ecosystems, both managed and unmanaged, sequester greenhouse gases but annual agricultural ecosystems emit greenhouse gases (Robertson *et al.*, 2000).

Cover cropping is one technique proposed to tighten nitrogen cycling, add soil organic matter and enhance soil quality in annual grain cropping systems (Drinkwater *et al.*, 1998), but it is rarely practiced by farmers in the USA. Winter cover crops can supply additional inputs of organic matter to the soil. Retention of soil carbon and nitrogen in cover crop residues is a service to both the ecosystem and the farmer (Drinkwater *et al.*, 1998). Soil organic matter is essential for maintenance of fertility, structure and biological activity (Watson *et al.*, 2002). Substantial losses in soil organic matter facilitate other consequences such as erosion, water-holding capacity and nutrient leaching (Matson *et al.*, 1997).

Likewise, cover crops also may aid in synchronization of available nitrogen with crop demand (AbdulBaki *et al.*, 1996; Agustin *et al.*, 1999; Cline and Silvernail, 2002) and provide weed suppression and disrupt pest and disease cycles. Soil erosion is reduced and nutrient recycling enhanced through aboveground and belowground vegetation

(Snapp *et al.*, 2005). Nitrate leaching is reduced by up to 70% and biologically available carbon is retained in soil for longer periods of time, which appears to be a key driver of more sustainable production systems (Tonitto *et al.*, 2006).

Finally, cover crops provide a gateway for incorporation of diverse plant assemblages into agro-ecosystems. Long term studies have shown that diverse cropping systems maintain higher soil organic matter levels than monocultures while supplying nitrogen to crops more evenly throughout the growing season (Sanchez *et al.*, 2001). For example, cover crops and compost additions in the Living Field Laboratory at the Kellogg Biological Station have increased soil organic matter, decreasing the amount of nitrogen additions needed to sustain crop productivity (Gentry, pers. comm. 2008).

Cover crops were used frequently prior to widespread use of inorganic fertilizers. Yet, much of the information about cover crop species, varieties and management has since been lost. Present-day farmers struggle to succeed when trying cover crops, and when low cost fertilizer options are available, there's less incentive for farmers to try cover crops. Fertilizer prices are rising, and agroecosystems managed with conventional practices are showing no signs of improvement (Mulvaney *et al.*, 2009).

In the first chapter of this dissertation, I report on the morphological and phonological diversity of hairy vetch varieties available commercially and in seed banks and suggest specific avenues for integration of these different varieties (Chapter One). Next, I used a subset of the commercially available varieties to examine their performance in temperate grain cropping ecosystems (Chapter Two). In chapter three, I address the impact of management history on nitrogen fixation by a common legume cover crop species (red clover). Together, these studies illustrate that substantial variation exists between species and varieties of legume cover crops, and that this variation can be utilized as a resource to improve cover crop performance across a range of cropping systems. In addition, environmental characteristics can influence rates of nitrogen fixation, potentially leading to internally regulated nitrogen cycles via reduced rates of nitrogen fixation from the atmosphere in high fertility conditions.

Chapter Four is a literature review of the effects of agricultural management on greenhouse gases in the atmosphere, packaged for use in educational situations, particularly college and high school classrooms. Much of the environmental degradation resulting from conventional agriculture stems from the public desire for cheap, uniform food products. Education is crucial towards reversing this trend. In particular, highlighting the hidden costs (e.g. greenhouse gas emissions) of managing farmland for cheap food at the grocery store may help shift consumer demand towards agricultural products that have less negative environmental impact. Simultaneously, I include information about agricultural practices (e.g. growing more perennials) that reduce greenhouse gas emissions.

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CHAPTER ONE

Wilke, B.J., Snapp, S.S., 2008. Winter cover crops for local ecosystems: linking plant traits and ecosystem function. Journal of the Science of Food and Agriculture 88, 551-557.

CHAPTER ONE

Winter Cover Crops for Local Ecosystems: Linking Plant Traits and Ecosystem Function

Abstract: Winter cover crops are capable of supplying multiple economic and environmental benefits in temperate environments of North America, but the lack of adapted populations for specific environmental and agricultural contexts has resulted in cover crops that are unreliable and perform ecosystem functions unevenly. To maximize the benefits provided by winter cover crops, we argue for trait selection by crop scientists that is cognizant of desired ecosystem functions, with the goal of providing commercially available populations that have variable functions. We illustrate this approach through a case study of a promising winter annual legume cover crop, hairy vetch (*Vicia villosa*). Six key traits and associated functions are considered within specific agroecological contexts. We discuss tradeoffs that may occur among desired plant traits and illustrate how over-selection for a particular trait could negatively affect performance and overall benefits from a cover crop. Intraspecific combinations of complementary cover crops are suggested as means to achieve multiple agroecosystem functions.

***Key Words*:** cover crops, hairy vetch, functional traits, agroecosystem, local adaptation

Introduction

Winter cover crops in temperate environments can play important roles in soil protection and environmental amelioration. As cash crops are grown during the warm seasons but not cool seasons, the use of winter cover crops is an economically-feasible alternative to bare fallow. Filling the winter niche with cover crops extends the presence of living roots and shoots, acting as sinks for nitrogen across the season, (Robertson, 1997; Crews and Peoples, 2005) and the resulting organic inputs buffer against pulses of nitrogen during fertilization or rain events. The opportunity cost of replacing a cash crop in the rotation is not incurred during the winter, and farmers from Europe, North America and other temperate regions are experimenting with integrating cover crops into cropping systems (Thorup-Kristensen *et al.*, 2003).

Agroecosystems that are not planted to winter covers remain bare and unproductive for up to eight months of the year. Perennial and cover cropped ecosystems, by way of contrast, have the presence of living roots and green cover throughout the year. There are costs associated with the lack of cover crop use, including lost time for nitrogen (N) and carbon (C) fixation and storage (Drinkwater *et al.*, 1998), excess nutrient losses to the environment (Robertson, 1997; Tonitto *et al.*, 2006) and lack of weed control (Mutch *et al.*, 2003). Catch crops are used world-wide, particularly in Northern Europe, Australia and Africa to reduce nitrate loss to groundwater (Thorup-Kristensen *et al.*, 2003; Tonitto *et al.*, 2006; Drinkwater and Snapp, 2007). Further, Brady and Weil (2002) indicate soil organic matter accumulation occurs between 0-25 °C when living plants are present, as plant synthesis rates are greater than decomposition by aerobic microbes.

However, if plants are not growing when temperatures are above 10°C, active mineralization causes a net loss of soil carbon.

Rising fertilizer prices are an on-going challenge to farm managers, and are expected to escalate with fossil fuel prices. This has enhanced demand for alternative nitrogen sources in crop production. There are successful examples of cover crop integration into row crop systems that supply biologically fixed N to cash crops, particularly for rotations that include small grains such as wheat. Red clover (*Trifolium pratense*) is one such cover crop (Figure 1) and capable of producing enough nitrogen to maintain cash crop yields in temperate environments (Drinkwater *et al.*, 1998).

Figure 1. Left: Red clover is the green plant that can be seen between the rows of golden wheat. Red clover is one of the most widely used cover crops in North America and can be effectively established in small grain crops. Right: Hairy vetch can be seeded in the fall and still fix significant amounts of nitrogen prior to cash crop planting in May. Hairy vetch is often grown in mixture with cereal rye to obtain maximum benefits from the cover crop.

Crop rotations that do not include small grains, and that are dominated by long season crops such as corn are becoming the major agricultural land use in many regions of the world (>90% of the USA Midwest). Corn and soybean systems lack reliable cover crop options for the winter niche. Corn is harvested late in the fall and only the most winter hardy annuals can successfully establish when planted into the harsh environment of late fall fields, with plunging temperatures and an inhospitable seed bed. Hairy vetch

(*Vicia villosa*) is one of few annual legumes capable of winter survival, after planting late in the fall season. Hairy vetch has been shown to produce large amounts of biomass over short time periods. For example, in Freeville, NY, Teasdale *et al.* (2004) established four cultivars of hairy vetch in September, which on average produced between 2,500 – 3,000 kg ha^{-1} of biomass and 85 – 118 kg N ha^{-1} before incorporation in May of the following year. Termination date had substantial influence on biomass accumulation; early June termination resulted in more than twice as much biomass compared to mid-May termination. Other studies conducted in warmer regions of North America have shown hairy vetch cover crops can release 90-132 kg N ha^{-1} to subsequent corn crops after spring incorporation (Ebelhar *et al.*, 1984; Ranells and Wagger, 1996).

Although there are many benefits of winter covers, but there are often drawbacks that accompany the use of annual and biennial legumes in certain regions. Disadvantages include modification of the environment by cover crop presence, and subsequent delayed development of a cash crop planted into the cover crop-amended soil. Soil moisture depletion that reduces water availability to subsequent cash crops, delayed soil warming in cool regions and delayed N mineralization for cash crop use are potential drawbacks (Snapp and Borden, 2005). Winter legume cover crops are often unreliable in terms of establishment, weed competition, nitrogen production and ease of termination. All of these factors pose a risk to managers who invest seed and labor, and who look for reliable cover crop impact that reduces rather than increases cropping system risks. These drawbacks frequently limit adoption by farmers (Snapp *et al.*, 2005).

A barrier to farmer adoption of winter cover crops is the lack of adapted varieties. Delorit and Ahlgren (1967) state in their seminal book, "The use of unadapted seed is a common cause of failure to obtain good stands of red clover. As a rule, most satisfactory results are obtained when seed that is produced locally is used," (pg. 269). Supporting this point, a recent study in southwest Michigan showed that a Michigan derived population of red clover produced more biomass as a cover crop than a Canadian derived population (Mutch *et al.*, 2003). Likewise, hairy vetch populations grown as cover crops have not been systematically assessed or traits selected, resulting in few populations that are suited to specific regions.

We use hairy vetch here as a model system to illustrate the importance of investigating trait variation, and developing criteria for selecting superior cover crop types. As a species, hairy vetch shows considerable promise as a winter annual cover crop, being one of very few annual legumes with traits that encompass cold tolerance, fast growth and high N fixation capacity. Due to substantial variation within the hairy vetch germplasm, quantitative assessment of plant traits and selection by breeders is possible for traits that convey favorable functions within specific contexts. We identify six scenarios where trait selection would enhance the value for farmers, and the general ecosystem benefits from integrating the cover crop. The scenarios chosen are specific to hairy vetch in a temperate ecosystem, but the general approach can be applied for all cover crops.

1. THE FUNCTIONAL IMPORTANCE OF PLANT TRAITS

It may seem obvious that locally adapted winter cover crops would provide benefits beyond those achievable from cover crops adapted to other regions. However, high performance from locally produced cover crop populations is not always observed. Rather, the presence of specific, adapted traits may be critical to cover crop performance and derived ecosystem benefits. The realized biological niche for each individual plant becomes an important driver for trait selection criteria (McGill *et al.*, 2006). There is growing interest in ecology and agriculture regarding the impact of specific traits and suites of traits for system properties such as nutrient cycling, soil water dynamics and management intensity.

Lavorel and Garnier (2002) identified two trait categories, response and effect, to consider when assessing linkages between traits and ecosystem function. *Response* traits are those that govern how the plant responds to the environmental factors and *effect* traits determine the effect the plants have on ecosystem function. The boundary between these two categories is not distinct, as many traits have both response and effect functions. For example, a leguminous cover crop may respond to favorable environmental conditions by exhibiting high biomass production, but subsequent decomposition of the large quantities of biomass in soil may increase available nitrogen in the ecosystem.

2. HAIRY VETCH: A PROMISING WINTER LEGUME COVER CROP

In northern regions of the Unites States, research and practice have shown that hairy vetch is an outstanding legume species for use as a winter annual cover crop. Hairy vetch is one of few legumes that can be planted late in the fall and still fix substantial amounts of atmospheric nitrogen prior to planting a cash crop the following spring. Farmers interested in planting hairy vetch in Midwestern states can usually obtain seed from regional merchants, which is labeled 'common', where the cultivar is not specified. However, a limited amount of hairy vetch seed is produced in the Midwest for commercial sale. The hairy vetch seed that is marketed is usually labeled as common or

'Variety Not Stated (VNS)' and is produced on forage seed production farms in Pacific Coast states for distribution across the United States.

Prior to farm reliance on inorganic nitrogen fertilizer, as occurred in the mid 20th century, two populations of hairy vetch were cultivated in North America. Smooth leaved hairy vetch was primarily grown in Oregon and southern states, and was a population thought to have moderate winter hardiness. The cultivar 'Madison,' which was developed in Nebraska and cultivated in Midwestern States, was more pubescent and was thought to be highly cold tolerant and winter hardy. The origin of the common hairy vetch sold throughout much of the USA today is unknown (Jannink *et al.*, 1997).

Within the past 15 years, researchers at Auburn University have released three cultivars of hairy vetch selected for their adaptation to southern U.S. regions (Mosjidis *et al.*, 1995; Surrency *et al.*, 1995; Mosjidis, 2002), but Teasdale *et al.* (2004) found that common hairy vetch exhibited equal or higher growth as a winter cover crop in Maryland and New York compared to the three cultivars developed at Auburn. Several populations are also being cultivated and sold in specific regions of the Midwest including Minnesota and Nebraska, but little is known about the characteristics of these populations in relation to common and other wild and domesticated hairy vetch populations. We compared nine populations of hairy vetch in the greenhouse and found significant morphological and phenological variation between the populations (for methods, see Appendix). In our study, considering all traits together, common hairy vetch did not appear to specifically match any of the other populations from Nebraska, Minnesota, Alabama, California, Argentina, Turkey or Germany (Table 1).

Table 1. Morphological and phenological data from greenhouse comparisons of hairy vetch populations show high amounts of variation between the nine populations studied. Mean (± 1 SE) values are shown for each trait measured.

Population	% Survival at -6°C	SLA[f] ($cm^2\ g^{-1}$)	% Flowering at 104 Days	Root:Shoot[g]	# Secondary Stems	Pubescence (Rachis Hairs mm^{-1})
Turkey[a]	0.0 (0.0)	940.1 (100.3)	83.3	0.28 (0.02)	30.0 (4.5)	50.8 (12.7)
Germany	NA	654.0 (64.9)	0.0	0.36 (0.06)	37.0 (4.0)	67.7 (26.8)
Argentina	NA	878.5 (70.4)	100.0	0.41 (0.08)	29.2 (4.6)	57.4 (13.5)
Americus[b]	0.0 (0.0)	883.4 (53.5)	16.7	0.31 (0.05)	35.2 (4.1)	24.7 (2.0)
Early Cover	16.7 (9.6)	823.9 (65.6)	100.0	0.12 (0.03)	24.4 (1.0)	5.8 (1.9)
Lana	0.0 (0.0)	794.2 (68.2)	100.0	0.25 (0.03)	39.6 (5.4)	58.2 (16.5)
Common[c]	33.3 (13.6)	901.9 (69.9)	16.7	0.30 (0.03)	34.7 (2.2)	37.5 (5.9)
Minnesota[d]	NA	959.0 (79.7)	0.0	0.37 (0.05)	20.5 (3.8)	83.8 (14.9)
Nebraska[e]	25.0 (16.0)	974.0 (81.8)	0.0	0.41 (0.04)	26.8 (4.0)	81.3 (16.4)

[a] The first three populations are landraces obtained from the USDA-ARS Western Regional PI Station and are listed by origin. Accession numbers for each population are: Turkey – PI 206492 W6 11613, Germany – PI 251679 W6 11686, Argentina – PI 628321 Ica 76

[b] The italicized populations are registered cultivars and were obtained from the USDA-ARS Western Regional PI Station. Accession numbers are: Americus – PI 383803, Early Cover – PI 575701, Lana - 595756

[c] Common population seed was obtained from Michigan State Seed (Grand Ledge, MI) and was originally produced in Oregon.

[d] The Minnesota population was an organically produced landrace grown in Minnesota and obtained from Albert Lea Seed House (Albert Lea, MN)

[e] The Nebraska population was a landrace grown in Nebraska and obtained from Kaup Forage and Turf (Norfolk, NE)

[f] Specific leaf area (SLA) is defined as the ratio of leaf surface area (cm^2) divided by leaf dry mass (g)

[g] Root:shoot was calculated using plants grown in cylindrical plastic pots (22.86 cm diameter, 25.4 cm tall) in the greenhouse, and may not accurately reflect root:shoot from field grown plants. Roots and shoots were harvested from individual plants after initiation of flowering, which occurred on day 84 for early flowering individuals and day 104 for later flowering individuals. Non-flowering plants were also harvested after 104 days. At the time of harvest, all individuals contained roots that had completely explored the soil volume in the pots.

3. SELECTION FOR TRAITS THAT CONVEY A SPECIFIC AGROECOSYSTEM FUNCTION

The central question for cover crop improvement efforts is; what function are we looking for in a specific context? The particular ecosystem functions vary in relationship to environmental and agronomic circumstances, and desired plant traits will vary accordingly. The challenge remains that these desired functions may differ not only between regions, but from farm to farm depending on the soil, climate and goals of each individual farm manager. We have identified six agroecosystem functions that will be particularly important when selecting for improved hairy vetch populations (Table 2). For each cover crop function, we have identified one important plant trait and specific environmental conditions where the plant trait may be of particular interest.

Table 2. Six different functions that winter cover crops provide and the corresponding plant traits that could be used as selection criteria in a population improvement program

Agroecosystem Function	Trait of Interest
Winter Survival	Degree of Cold Tolerance
Reduced Soil Water Depletion	Low Specific Leaf Area
Determinacy for Organic No-Till Systems	Flowering Phenology
Residual Nitrogen Uptake	High Root : Shoot Ratio
Re-growth Following Grazing	Secondary Stem Production
Temperature and Drought Stress Resistance	High Leaf Pubescence

3.1 Winter survival

In temperate environments, cold tolerance, which conveys the capability of winter survival, is quite arguably the most important response trait determining the northern range for species and crop planting zones.

Winter covers must be somewhat cold tolerant in the upper Midwest, but the Great Lakes provide moderation in temperature extremes for much of the upper Midwest. In order for a winter cover crop to be effective, it must survive the winter cold season, yet there may be tradeoffs between cold tolerance and other beneficial traits such as net primary production. Therefore, simply choosing a population from an extreme northern region, or breeding for a universal cold tolerant population could have negative consequences, such as low growth potential in regions that experience less severe winter conditions. Brandsaeter *et al.* (2002) found hairy vetch populations in Norway exhibited different levels of cold tolerance, indicating variability among populations available from commercial sources and gene banks. Among nine week old plants, the cultivar 'Welta' exhibited 50% higher survival at lower temperatures than the commercially available cultivar 'AU Early Cover' (-9.1°C vs. -3.3 °C respectively).

Three hairy vetch populations we studied, 'AU Early Cover,' common, and a landrace from Nebraska, exhibited partial survival when five week old plants were subjected to low temperatures in a controlled environment chamber. The cold treatment of -6°C reduced survival to 16.7% in 'AU Early Cover,' 33.3% in common hairy vetch and 25% in the Nebraska population, while at 4°C, there was no reduction in survival (Table 1). Three other populations exhibited no survival when exposed to the -6°C cold treatment. The difference in survival illustrates to some degree that hairy vetch populations vary in cold tolerance, as measured by survival in a harsh environment. Controlled environment studies like these convey information about population response, but they lack important field environment characteristics such as snow cover and precipitation that may alter survival in cold weather.

One way to mitigate risk of a bare soil in a harsh winter would be to grow a mixture of hairy vetch cultivars, or hairy vetch and a highly cold tolerant species such as a winter cereal. The growth stage at the time of a cold temperature event has a strong impact on winter hardiness, and genotypes vary in which growth stage is the most sensitive to cold (Brandsaeter *et al.*, 2002; Teasdale *et al.*, 2004). Thus combining different cultivars or species would reduce the risk that all plants would be killed in extreme weather. There is also the possibility that complimentary growth habits of different genotypes might improve the efficiency of resource use, as more determinant types would grow fast initially but may be less deep-rooted, and thus leave resources deep in the soil profile for slower growing genotypes that invest greater resources belowground.

3.2 Low soil water depletion

Specific leaf area (SLA) is both a response and effect trait. This trait has received considerable attention in ecology, but little notice in agricultural research. Ecosystems low in nitrogen and water, yet high in light availability tend to have plants with low SLA. Subsequently, plants with high SLA use more water resources, are more productive, have higher leaf turnover rates and may be less resilient to environmental fluctuations and decomposition.

Particular attention should be given to SLA in regions with low precipitation and soil fertility, where populations with low SLA, such as the 'Lana' hairy vetch cultivar may be more resilient and conserve more soil moisture than populations with higher SLA (Table 1). For example, farmers in semi-arid regions of North America are dependent on soil moisture regeneration during fallow summer and winter periods (McGuire *et al.*, 1998; Unger and Vigil, 1998). Choosing a low SLA population could reduce soil moisture depletion by the cover crop while maintaining the benefits of soil cover, carbon and nitrogen fixation.

Alternatively, we expect that cover crops with high SLA may be better adapted to low light conditions. Red clover is well known to be able to tolerate low light, which has led to its use as an under-seeded cover crop in small grain canopies and even understory to corn. The growth of plants under forest canopies is highly dependent on light; SLA has been shown to decrease as light increases (Wang *et al.*, 1994). Farmers that are inter-seeding cover crops into standing cash crops may want to select a hairy vetch population with high specific leaf area such as Minnesota and Nebraska landraces. There is almost no agroecosystem research to date that has addressed this cover crop trait.

3.3 Determinacy for organic agroecosystems using no-till practices

Recent research at the Kellogg Biological Station (Mutch, DR, pers. comm.) has shown the possibility of using no-till practices in organic corn and soybean production. These systems utilize machines that roll down and crimp the leaves and stems of winter cover crops, killing and conditioning the residues to be used as mulch during the growing season for weed and moisture management. Crop plants are no-till planted into the rolled and crimped residues. Hairy vetch phenology is a particularly important effect trait in this system, as winter annuals must be in anthesis to be killed by this process (Ashford and Reeves, 2003).

Corn and soybean planting on U.S. Midwest organic farms ranges from late April through mid June depending on the particular farm strategy. Breeding for hairy vetch populations that mature slightly before corn or soybean planting time will optimize their usefulness in specific regions, but will require selection under local conditions to maximize relevance to specific climatic conditions and planting dates. Although greatly influenced by lighting and temperature effects in the greenhouse environment, which makes results not directly applicable to growth under field environment, hairy vetch populations have been shown to exhibit a wide range of floral phenology patterns (Table 1). Many organic farmers delay corn planting until June to permit soil temperatures to warm and to allow weeds to germinate so they can be killed mechanically. While growing, hairy vetch cover crops compete with spring germinating weeds, and roller/crimping provides a mulch layer that reduces weed growth after crop planting. Uniform flowering time would benefit the farmer, so that all hairy vetch individuals can be terminated during one management event. At least two breeding programs have selected for earlier flowering populations of hairy vetch. Auburn University scientists released the cultivar 'AU Early Cover' in 1995 (Mosjidis *et al.*, 1995) and scientists at the Agriculture Research Service in Beltsville, Maryland have released 'Purple Bounty,' which may exhibit better winter survival in colder regions than 'AU Early Cover' (2007).

3.4 Residual nitrogen uptake

Inorganic N resides in the soil profile at the end of the growing season. Residual inorganic N levels are particularly high in years when environmental conditions support high N mineralization and poor plant growth. This inorganic N pool is highly susceptible to loss through leaching, volatilization and denitrification processes. Belowground productivity is an important effect trait allowing a plant to scavenge large amounts of residual N from the soil. Inorganic N is preferentially acquired as it is energetically favorable compared to the energy-expensive process of symbiotically fixing N from the atmosphere (Russelle *et al.*, 2001). Hairy vetch, as observed for other legumes, has an effective buffering system. That is, N fixation activity will be moderate in fields where soil inorganic N is high, and high where soil inorganic N is low. Based on our greenhouse assessment, hairy vetch populations differ significantly in root:shoot ratio, as earlier maturing populations have lower root:shoot ratios (Table 1). Breeding for later maturing populations that allocate energy to early root growth will facilitate recovery of soil inorganic N in the fall and subsequent N release the following year during periods of rapid cash crop growth.

3.5 Re-growth following grazing

Many farmers utilize their crop fields as winter grazing lands, where farm animals are allowed to forage on remaining grain and stover. Cover crop biomass can substantially increase the forage quantity and quality available, particularly for niches where cover crops have sufficient time to develop after seeding and before livestock are introduced. For example, cover crops will accumulate more biomass if they are seeded early in the fall after small grain harvest or inter-seeded with standing crops earlier in the year (e.g., frost-seeded red clover into wheat).

To be adapted to grazing, plant growth patterns must be able to tolerate, or even compensate for, the effects of herbivory. Plant growth type may be a key response trait for this adaptation. For example, secondary stem producing populations are expected to be better able to resist herbivory by grazing animals than plants producing a single stem. Within hairy vetch populations studied, early flowering plants produced the most secondary axial stems whereas later flowering plants produced more secondary basal stems than those flowering early (Wilke BJ, unpublished). Breeding and farmer selection of grazing tolerant populations may improve cover crop performance on farms with livestock.

3.6 Temperature and drought stress resistance

Leaf pubescence amplifies the boundary layer between the leaf and environment, thus decreasing heat transfer from the leaf to the environment (Meinzer and Goldstein, 1985). Pubescence is recognized as an important response trait for water retention in dry, warm environments through reflectance of photosynthetically active radiation, thus moderating leaf temperature and reducing the requirement for evapotranspiration (Ehleringer and Mooney, 1978; Sandquist and Ehleringer, 1997; Save *et al.*, 2000). Baruch and Smith (1979) report equal photosynthetic rates across wet and dry seasons for a pubescent plant species while a glabrous plant species exhibited significantly decreased photosynthesis during the dry season.

The few studies that have investigated correlations between cold tolerance and pubescence concluded that there are positive correlations between the two parameters (Miller, 1986; Geeske *et al.*, 1994; Maes *et al.*, 2001). The ability to tolerate cold temperatures subsequently increases the probability of a plant surviving the winter. However, leaf hairs are energetically costly to produce and this could lead to reduced carbon assimilation (Ehleringer and Bjorkman, 1978). The tradeoff between cold tolerance and carbon assimilation indicates that plant traits associated with superior cover crop performance in specific regions are not necessarily beneficial in all regions. In our greenhouse study, hairy vetch populations from Nebraska and Minnesota had leaves with the highest levels of pubescence, while 'AU Early Cover' and 'Americus' populations had much lower levels of pubescence (Table 1). Contrary to earlier observations from the literature, (Miller, 1986; Geeske *et al.*, 1994; Maes *et al.*, 2001) we did not observe any association between pubescence and cold tolerance. Further investigation of relationships between pubescence and response to environmental conditions will aid in the selection of populations for local environmental conditions. Easily observable traits such as pubescence would aid local selection being carried out by farmers, as well as improvement efforts by researchers.

Conclusion

We've identified six specific scenarios where breeding and careful farmer selection can improve the agroecosystem function of hairy vetch as a winter cover crop. Similar approaches are needed for other promising cover crop species, to improve value for farmers. Breeding for specific functions will enhance cover crop performance and acceptability to farm managers, helping to eliminate some of the drawbacks that prevent farmers from growing winter legume cover crops. Quantifying the benefits of breeding for specific traits and agroecosystems would provide a significant incentive for plant improvement, but presents scientific challenges and would require a sustained effort with considerable resources.

Overall, farmers in temperate environments require cover crop genotypes that fix atmospheric nitrogen while depleting minimal soil moisture and that provide a range of benefits such as fodder for grazing animals, as well as soil protection. Adaptation to winter climate extremes and highly variable fall weather conditions will further improve cover crop reliability. Agricultural systems are facing increased environmental stress from weather variability associated with climate change, which poses a new challenge for cover crop integration within row crop agroecosystems.

Many plant traits vary independently and cannot be captured by functional classification schemes (Eviner and Chapin, 2003). Breeding winter cover crops for specific functions has many advantages, but tradeoffs occur among desired plant traits such that over-selection for a particular trait may negatively affect the overall value of the cover crop. For example, there are energetic costs associated with some plant traits such as pubescence that may confer adaptive advantage within extreme climates, but may reduce performance in more moderate climates. Cold tolerance is an extremely important trait in cold regions, but may come at a cost of rapid growth, which is an important cover crop trait (Ehleringer and Bjorkman, 1978). Traits which confer environmental benefits such as carbon sequestration may be at odds with those traits associated with economic benefits such as nitrogen fixation due to energetic costs of hosting nitrogen fixing soil organisms (Crews, 1999; Vitousek and Field, 1999). Due to these and many other tradeoffs between traits, diverse plantings of complementary intraspecific populations may prove to be the best way to provide multiple services at one time and increase the dependability of cover crop performance within row crop systems in temperate environments.

Acknowledgements

This work was supported by a Michigan State University Plant Science Fellowship, the North Central Region Sustainable Agriculture Research, and Education and the W.K. Kellogg Biological Station. We thank Kathleen O'Neil, Rich Price, Bill Quackenbush, Tracy Beedy and the Michigan State University Plant Science Greenhouse staff for their assistance. We thank three anonymous reviewers for their detailed comments. This is the W.K. Kellogg Biological Station contribution # 1451.

Appendix: Methods for morphological and phenological data from greenhouse comparisons of nine hairy vetch populations.

Nine hairy vetch populations obtained from commercial sources and USDA seedbanks. Specifically, The first three populations in Table 1 were landraces obtained from the USDA-ARS Western Regional PI Station and are listed by origin. Accession numbers for each population are: Turkey – PI 206492 W6 11613, Germany – PI 251679 W6 11686, Argentina – PI 628321 Ica 76. The italicized populations were registered cultivars and were obtained from the USDA-ARS Western Regional PI Station. Accession numbers are: Americus – PI 383803, Early Cover – PI 575701, Lana – 595756. Common population seed was obtained from Michigan State Seed (Grand Ledge, MI) and was originally produced in Oregon. The Minnesota population was an organically produced landrace grown in Minnesota and obtained from Albert Lea Seed House (Albert Lea, MN). The Nebraska population was a landrace grown in Nebraska and obtained from Kaup Forage and Turf (Norfolk, NE).

Temperature was monitored throughout the growing space to identify any temperature gradients in the greenhouse. Plants were grown in cylindrical plastic pots (22.86 cm diameter, 25.4 cm tall) in the greenhouse in a complete randomized block design, with 12 total replicates (pots) arranged across three different tables (block). Four replicates for each hairy vetch population were placed on each table. Pots were filled with a custom potting mix consisting of 75% play sand and 25% composted dairy manure. The potting mixture was sterilized using a steaming technique prior to planting hairy vetch seeds. Two seeds of the same population were planted on November 22, 2005 in each pot at 1.5 cm depth. Plants were inoculated with the appropriate type of *Rhizobium* bacteria

(*Rhizobium leguminosarum* biovar *viceae*) using a peat based inoculum. After emergence, the less vigorous seedling was removed by cutting the stem at the base of the plant to reduce the population to one plant per pot. Pots were watered with automatically three times per day for 10 minutes at each interval, and each pot was fertilized twice with 100 mL of a nutrient solution (19-4-23 with calcium and magnesium). One bamboo stake was anchored in each pot and the plants were tied to the stake as they grew to promote upright growth. Six plants of each population were destructively harvested on February 14, 2006, and the remaining six plants were destructively harvested on March 6, 2006.

Specific leaf area (SLA) is defined as the ratio of leaf surface area (cm^2) divided by leaf dry mass (g), and was calculated using four leaves on each plant at each of the two destructive harvests. Fresh leaves were scanned into a digital image and then analyzed for area using WinRHIZO software. Leaves were dried at 60°C to constant mass. Prior to drying, all rachis hairs were counted on each leaf to generate the values for pubescence. Percent flowering was calculated by counting each plant in bloom relative to the 12 total plants. Roots and shoots were harvested from individual plants during both destructive harvests. All plant biomass was dried at 60°C to constant mass. At the time of harvest, all individuals contained roots that had completely explored the soil volume in the pots. All secondary stems were counted at the time of harvest.

Six populations (Turkey, Americas, Early Cover, Lana, Common, and Nebraska) were chosen for the cold tolerance study. Three treatments (4°C, -6°C, -12°C) included four replicates for each population. In each pot (12 cm square), five seeds of a single population were inoculated with *Rhizobium* bacteria (*Rhizobium leguminosarum* biovar *viceae*) using a peat based inoculums, and were planted 1.5 cm deep. Seedlings were thinned to three plants per pot after emergence. Plants were grown in the greenhouse for three weeks, and then moved to a cold chamber at 4°C for two weeks to harden the plants, while keeping lights on for 12 hours per day. Plants in the freezing treatment were then moved to two independent freezing chambers for one week and held at constant freezing temperatures (-6°C, -12°C) for one week, with lights on for 12 hours per day. Following the freezing treatment, all plants were moved back to the greenhouse for three more weeks. The percent of surviving plants was calculated for each pot, and then pots were used as the replicate to generate percent survival shown in Table 1.

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CHAPTER 2

Hairy vetch cover crops in Upper Midwest Cropping Systems: Evaluation of Varieties and Mixtures.

Abstract: Legume cover crops have the potential to improve sustainability of row crop ecosystems, through supplying a renewable source of nitrogen and building soil resources. Yet, achieving consistent growth and associated nitrogen fixation by legumes is challenging in temperate climates. There is limited knowledge about cover crop species, varieties and their performance in varying environmental conditions. We examined three hairy vetch varieties and performance within two grain production systems: October planting after a soybean crop and July planting after a wheat crop. . We evaluated the response of a subsequent corn crop to cover crop traits such as biomass production and impact of hairy vetch genotypes versus farmer systems of rye cover crop and bare fallow, with and without fertilization. In addition, we quantified the effect of mixing hairy vetch varieties within a single planting. Growing degree days was the most important predictor of biomass production by hairy vetch; July-planted hairy vetch produced an average of 469 g m^{-2} more biomass than October-planted hairy vetch. AU Early Cover was the only variety to flower prior to incorporation, while a landrace from Nebraska exhibited the highest winter survival rates and early spring biomass production. Corn biomass and grain yield were not influenced by hairy vetch cover crops relative to fertilized and unfertilized bare plots, although a rye cover crop was associated with corn suppression. We hypothesize that overall water, not nitrogen, may have been the limiting factor for corn growth due to drought periods during corn growth. Variety mixtures did not influence productivity by hairy vetch cover crops or the stability of biomass

production across replicates and planting systems. Results indicate that cover crop varieties have the potential to occupy different roles in grain cropping ecosystems. Utilizing varietal mixtures are not likely to improve biomass production, although it may improve cover crop reliability in the event of individual variety failures.

Key Words: Cover Crops, Hairy Vetch, *Vicia villosa*, Variety Mixtures, Planting Date, Growing Degree Days

Introduction

Legume cover crops provide significant benefits for agroecosystems (Drinkwater *et al.*, 1998; Dabney *et al.*, 2001; Tonitto *et al.*, 2005), but in order to be economically viable, a cover crop must provide multiple services (Cherr *et al.*, 2006) such as nitrogen fixation, nutrient retention, erosion control and carbon sequestration. Legume cover crops are specifically valued for their ability to provide biologically fixed nitrogen to subsequent cash crops. Yet, cover crop productivity and effect on the agroecosystem are often unpredictable and do not always meet the needs of managers, particularly in dry and cool climates (Snapp *et al.*, 2005). As a result, obtaining nitrogen from fixation by cover crops comes with a higher risk, and often a net economic cost, compared to using chemically produced fertilizers.

Choosing varieties that perform the necessary functions is one method to improve cover crop performance (Wilke and Snapp, 2008). Local adaptation experiments have identified traits that are selected by particular environmental dynamics (Kawecki and Ebert, 2004). In a classic reciprocal transplant experiment, Clausen *et al.* (1940) showed that ecotypes of sticky cinquefoil (*Potentilla glandulosa*) found at three elevations exhibited greater fitness than other ecotypes at the elevation they naturally occurred.

Additional experiments have documented correlations between traits and environment. For instance, leaf pubescence increased with increasing elevation within the Hawaiian plant species *Metrosideros polymorpha* (Geeske *et al.*, 1994). Del Pozo *et al.* (2002) found a positive correlation between onset of flowering and the ratio of mean annual precipitation/potential evapotranspiration (PP/ETP) and a negative correlation between days from first flower to pod ripening and PP/ETP in 69 populations of

Medicago polymorpha. In a similar field study, annual legumes originating from cold climates in the Middle East are generally more frost tolerant than those from mild environments (Cocks and Ehrman, 1987).

The benefits of diversity in agroecosystems has been discussed in detail (Altieri, 1999), and intraspecific diversity has been shown to influence yield, disease resistance and weed suppression (Kiær *et al.*, 2009). Extension of spatial and temporal resource use may augment ecosystem goods and services essential for long term sustainability (Hooper *et al.*, 2005). Plant species and their interactions have pronounced impacts on fertility, especially for nitrogen cycling (Hobbie, 1992; Eviner and Chapin, 2003). Enhanced resource use throughout the season by diverse rotations and mixtures that more nearly mimic natural communities increases invasion resistance and nutrient efficiency, which are major concerns of ecologists (Jackson and Jackson, 1999; Hooper *et al.*, 2005).

Diverse varieties already identified within *Vicia villosa* (hairy vetch) (Wilke and Snapp 2008) may complement each other to provide maximum nitrogen cycling by buffering against environmental conditions and increase resource use that might otherwise limit single genotype plantings. For example, fast growing varieties can capture resources in the fall, while cold tolerant varieties may have higher winter survival and therefore may exhibit extended resource use and superior spring biomass production. As a result, combinations of these varieties may lead to more biomass production and nitrogen fixation compared to monocultures. Similarly, early and late maturing varieties can be combined to provide temporal partitioning of resources, as was shown in species mixtures by Hooper (1998). Variation in aboveground growth characteristics (e.g. specific leaf area, determinacy) may provide complementary light use between two contrasting varieties. Complementary varietal combinations may be able to increase total cover crop NPP above individual genotype plantings, particularly in variable environments. Within hairy vetch, early maturing varieties may grow quickly in the fall, but may exhibit lower winter survival, whereas later maturing genotypes should better survive the winter and produce more biomass in the spring. Another mechanism for increased stability of variety mixtures may be the sampling effect, where mixtures are more likely to contain the most productive variety than monocultures. However, the opposite may also be true, where variety mixtures may be more likely to contain a variety that depletes soil moisture or has large allelopathic effects, thus causing variety mixtures to be less beneficial than monocultures.

In this study, we investigated hairy vetch, which is one of the most cold tolerant winter annual cover crop legume (Madson, 1951; SARE, 1998; Brandsaeter *et al.*, 2002). Hairy vetch can be grown in regions with minimum winter temperatures as low as -30°C. When grown with annual grass species such as cereal rye (*Secale cereale*), high amounts of spring biomass and nitrogen fixation can be produced by the mixture (SARE, 1998).

Recommended planting dates for hairy vetch range from mid August to late September, depending on climate and geographic location (Jannink *et al.*, 1997; SARE, 1998), but little information is available regarding the outcomes of variable planting dates (Teasdale *et al.* 2004). In North American temperate regions, corn (*Zea mays*) and soybeans (*Glycine max*) are generally harvested in October, which is already past the latest recommended planting date. Thus, the ability to plant hairy vetch later in the fall after most cash crops are harvested would facilitate integration into cropping systems (Jannink *et al.*, 1997).

Most studies have generally used one variety of hairy vetch, yet varieties vary considerably in morphology and phenology (Wilke & Snapp 2008). Most farmers in the United States North Central Region (NCR) have easy access to common varieties, which are often grown in the western part of the USA and may not be suitable for winter survival and/or vigorous growth during cold weather common to the NCR. Little information is available about commercially available varieties in terms of winter hardiness and productivity.

Results from initial characterization of hairy vetch genotypes have indicated the need for further characterization and selection for specific winter hardy varieties for the northern U.S. (Jannink *et al.*, 1997; Yeater *et al.*, 2004). Brandsaeter *et al.* (2002) found hairy vetch varieties exhibited variable tolerance to freezing temperatures in growth chamber experiments, and Teasdale *et al.* (2004) showed Common varieties of hairy vetch in Maryland and New York produced equal or more biomass than three varieties developed for use in the southern U.S. Wilke and Snapp (2008) characterized nine hairy vetch varieties in a greenhouse settings, three of which survived -6^{o} C temperatures in a growth chamber, and suggested that properly matching varieties with desired agroecosystem function would facilitate cover crop adoption. Harbur *et al.* (2009) found that local ecotypes of hairy vetch exhibited greater winter hardiness in Minnesota than ecotypes from other states.

The goals of this study are to evaluate hairy vetch cover crop performance, primarily biomass production, at two common planting windows (after small grain harvest and after soybean harvest) in Upper Midwest USA cropping systems, and to investigate two potential strategies to stabilize and increase legume cover crop productivity and benefit to subsequent crop growth. First, we tested the effects of planting three different cover crop varieties originating from distinct geographic and climatic regions on biomass production and subsequent corn crop yields. Second, we tested the performances of planting variety mixtures in comparison to single varieties. We predicted that varieties would produce more biomass and lead to more cash crop growth within their region of adaptation. Nitrogen accumulation by legume cover crops is related to net primary productivity, so biomass measurements are a good indicator of nitrogen fixation amounts (Wagger 1989). We also predicted that mixtures of early and late maturing varieties would buffer against adverse environmental conditions, thereby increasing the productivity and stability of winter legume cover crops.

Materials and Methods

Study System

We conducted this study at the W.K. Kellogg Biological Station (KBS), which is located in southwest Michigan, 50 km east of Lake Michigan (42^{o} 24'N, 85^{o} 24'W, elevation 288 m). Soils are of the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) series. The site receives approximately 90 cm of precipitation each year, half of which occurs as snow. The mean annual temperature is 14.6^{o}C. The specific fields used for this study were adjacent to the KBS Long Term Ecological Research experiment (LTER), for which detailed site and soils descriptions are available at http://lter.kbs.msu.edu. Precipitation and temperature data were taken from the KBS LTER weather database, which is accessible at http://lter.kbs.msu.edu/datatables.

We chose three hairy vetch varieties to evaluate productivity and complementarity: two landraces from Nebraska and Oregon respectively, and one variety from Alabama (AU Early Cover from Auburn University (Mosjidis *et al.*, 1995). All three varieties differ in origin, phenology and morphology (Wilke and Snapp, 2008). The Nebraska landrace is highly pubescent and has higher specific leaf area (SLA) than most other varieties tested. This population matures later than other varieties, has high root to shoot ratios, produces numerous basal secondary stems, and exhibited 25% survival when exposed to -6°C temperatures in a growth chamber (Wilke and Snapp, 2008). The Oregon variety (hereafter "Common") is intermediate compared to other varieties in terms of pubescence, specific leaf area, flowering time and secondary stems. Surprisingly, our earlier experiments showed that Common exhibited the highest survival rates (33%) when exposed to -6°C temperatures. AU Early Cover was obtained from a producer in Oregon and is the least pubescent of the three varieties and has intermediate SLA. In our previous work, AU Early Cover accumulated more biomass in the first 23 days of growth than any other variety tested, but flowered very early and had the lowest relative growth rate between 23 days and 104 days after planting. The root to shoot ratio was the lowest of all three varieties and it produced very few basal secondary stems, which is expected of this determinant variety. About 17% of the plants survived the -6°C freezing treatment (Wilke and Snapp, 2008).

Experimental Design and Management

We conducted one study with three independent field experiments to evaluate hairy vetch varieties and variety mixtures compared to cereal rye. Two experiments were planted following soybean harvest (October 7, 2006 and October 9, 2007), and one following wheat harvest (July 27, 2007) (Table 3). Field experiments were randomized complete block designs, with eight total treatments (Table 3). The July-planted experiment was broadcast seeded by hand onto recently tilled soil, and the plot sizes were 6.1 x 3.1 m. The October-planted experiments were seeded with a John Deere no-till drill on plot sizes of 9.1 x 6.1 meters.

Table 3. Experimental design including main treatments during the cover crop and cash crop growing periods.

Cover Crop Treatment (Fall - Spring)	Cash Crop Treatment (Summer)
Hairy Vetch - Common	Corn
Hairy Vetch - Nebraska	Corn
Hairy Vetch – AU Early Cover	Corn
Hairy Vetch Mixture (Common + AU Early Cover)	Corn
Hairy Vetch Mixture (Nebraska + AU Early Cover)	Corn
Cereal Rye	Corn
Bare	Corn
Bare	Corn + 120 kg ha^{-1} N Fertilizer

Prior to cover crop planting, varieties were inoculated with a peat basted inoculant containing the appropriate strain of *Rhizobium* bacteria (*Rhizobium leguminosarum biovar viceae*). Germination rates were measured in the lab and seed weights were calculated in order to obtain consistent plant populations of approximately 100 plants m^{-2}. The treatments, which were consistent across experiments, included three variety monocultures and two variety mixtures (Nebraska with AU Early Cover and Common with AU Early Cover). Mixtures were planted at a total of 100 plants m^{-2}, with one-half of the germinable seed coming from each variety. These mixtures were chosen based on the differences in phenology between the combined varieties. Two control treatments were not seeded with cover crops, and were subsequently used as fertilized and unfertilized control treatments during following cash crop season. The unfertilized plot is hereafter referred to as "bare" and the fertilized plot is referred to as "bare fertilized." A cereal rye cover crop treatment was also included and planted at 200 plants m^{-2}. The rye cover crop failed within the experiment planted on July 27, 2007 due to poor germination, and was not included in analysis for that experiment.

The vetch and cereal rye cover crops were killed mechanically with a tractor mounted roto-tiller and incorporated to a depth of approximately 15 cm on May 25, 2007and May 18, 2008. On the same day, corn (Blue River Hybrids (51B31) was planted over the entire experimental area at 62,000 plants ha^{-1}. No fertilizer was applied to the corn crop, except for the fertilized control plot which received 30 kg N ha^{-1} fertilizer N in the form of urea at the time of planting, and 90 kg N ha^{-1} in the form of ammonium nitrate granules at the V6 growth stage. Weeds were managed with between-row cultivation, and no pest infestation was observed.

Plant Performance

Plant density and winter survival were calculated for hairy vetch in each plot by counting plants in 50 x 50 cm quadrats (two per plot, randomly placed) on November 18, 2006 and November 26, 2007 in respective years. Spring counts were conducted in the same locations on April 2, 2007 and April 16, 2008 in respective years. Aboveground cover crop biomass was measured in April and May (April 30, and May 18, 2007; April 16, April 30, and May 17, 2008) by collecting all aboveground plant material in two 50 x 50 cm quadrats. The number of flowering stalks within each quadrat sample was counted at each sampling date. For the October planted experiments, two subsamples were taken from each plot and bulked together, and one random sample was taken from each of the smaller plots in the July-planted experiment to allow repeated sampling. Aboveground biomass was separated into weeds and cover crops.

Belowground biomass and root:shoot ratio were estimated by randomly selecting four plants in each plot for measurement. A shovel was used to remove a 20 cm deep and 20 cm diameter cylinder of soil (approximately 6.3 L) surrounding the roots of each plant. Loose soil was gently shaken off of each root in the field. Whole plants were then sealed in plastic bags until the remaining soil could be removed by washing with tap water over a 6 mm wire mesh screen. After washing, roots and shoots of individual plants were separated and all plant material was dried at 65°C for ≥ 72 hours. Samples from each variety were combined to generate a root:shoot ratio for each experimental unit. Root:shoot ratios were subsequently used to estimate belowground biomass for individual plots.

To investigate in-season N status of corn, a Minolta SPAD 502 chlorophyll meter (Spectrum Technologies, Plainfield, IL) was used two (2008) to three (2007) times (V7, R1 and R3 growth stages). Twenty subsamples from each plot were averaged to provide each data point. The last fully extended leaf was sampled at the V6 stage, and the leaf immediately above the ear was sampled at the R1 and R3 growth stages.

TDR soil moisture measurements from 0-16 cm deep were collected several times throughout the growing season to gauge moisture in the top soil layer. Soil inorganic nitrogen was calculated using the KCl extraction method at the corn V6 growth stage. A composite sample of eight cores were collected from the 0-25 cm depth of each plot. Samples were stored at 4°C and were processed within 24 hours. Soils were sieved to ≤ 4 mm and homogenized before two 10-g subsamples were weighed. For the initial sample extracted immediately, 100 mL of 1 N KCl was added, shaken for 1 minute, and allowed to settle overnight. The next day, the samples were shaken again, allowed to settle for one hour, filtered through Whatman #1 filter paper into scintillation vials, and then frozen until colorimetric analysis. The second soil sample were weighed for gravimetric water content, then were placed into a drying oven held at 60°C, dried to constant mass (>48 hours), and weighed again to determine water content and dry weight for the soil N analyses.

Data Analyses

To evaluate whether Common hairy vetch contributes substantial nitrogen benefits to temperate cropping systems, we used two-way analysis of variance (ANOVA), with experiment and cover crop species as the main factors, was used to analyze plant density, winter survival and biomass production by common hairy vetch and cereal rye. Because of significant interactions between experiment and species, these data were analyzed separately for each experiment using one way ANOVA. Growing degree days (Base 4°C) were calculated and used in regression models to predict biomass production by hairy vetch. Using literature estimates of 3.6% for average nitrogen concentration in common hairy vetch (Teasdale *et al.* 2004), the amount of nitrogen in hairy vetch biomass was estimated on a kg ha^{-1} basis. We also estimated the amount of nitrogen in cereal rye at 1.5% using data from an adjacent long-term experiment (Gentry unpublished data, 2010).

The corn crop following hairy vetch incorporation, including aboveground biomass, grain biomass, harvest index, leaf chlorophyll and soil moisture was utilized as a bioassay to analyze the effect of hairy vetch and rye cover crops relative to fertilized and unfertilized corn crops without a prior cover crop, which are referred to as bare plots in the results. One way ANOVA was used to analyze soil inorganic nitrogen and corn crop characteristics within each experiment, while repeated measures ANOVA was used to analyze corn leaf chlorophyll and soil moisture levels for which compound symmetry was utilized as the variance-covariance structure. Linear regression analysis was used to assess relationships between cover crop biomass and growing degree days. Statistical analyses were conducted using PROC MIXED and PROC REG in SAS (SAS Institute 2004).

To evaluate whether cover crop variety influences performance, three hairy vetch varieties and cereal rye were compared across the three experiments to identify differences in winter survival, flowering, biomass production and influence on subsequent corn crops. Relative growth rate differences between the three hairy vetch varieties were estimated by examining the relationship between aboveground biomass and growing degree days for each experiment independently, and examining biomass production at each sampling date. Winter survival was estimated only in the two October planted experiments, and treatments were compared using two-way ANOVA with cover crop variety and experiment as main factors. Total cover crop biomass production at the time of incorporation was analyzed across experiments using a two way ANOVA. Treatment means were evaluated within specific experiments when interactions between main factors were marginally significant ($P \leq 0.10$). One way ANOVA was used to evaluate aboveground biomass within individual experiments during different time periods, and aboveground biomass was plotted against growing degree days for the July and October plantings separately to analyze growth rates for different varieties across growing degree days. In all analyses, plot was included in the model as a random factor and experiment was included as a fixed factor. One way ANOVA was used to analyze soil inorganic nitrogen and corn crop characteristics within each experiment, while repeated measures ANOVA was used to analyze corn leaf chlorophyll and soil moisture levels for which compound symmetry was utilized as the variance-covariance structure.

To evaluate whether variety mixtures increase and/or stabilize cover crop performance, aboveground biomass for variety mixtures was compared to respective monocultures by examining them using the net biodiversity effect analysis (Lehman and Tilman, 2000; Loreau and Hector, 2001; Polley *et al.*, 2007). This method utilizes the average parameter value of the monocultures and subtracts that value from the mean value of the mixture to produce the net biodiversity effect. This method allows estimates of overyielding; a mixture overyielded if the net biodiversity effect is statistically greater than 0, meaning that the mean value of the mixture was statistically higher than the average of the two monocultures (Tilman, 1999). Statistical analyses were conducted using PROC MIXED in SAS (SAS, 2004), utilizing treatment and experiment as main factors. Overyielding and underyielding were confirmed by positive or negative least square mean values ($P \leq 0.05$).

Two methods were utilized to compare monocultures and variety mixtures for stability of biomass production. First, the coefficient of variation was calculated for each of the variety mixtures and relevant monocultures by using replications within each experiment to generate means and standard deviations. ANOVA analysis was then utilized to compare mixtures to monocultures utilizing individual treatments within each experiment as replicates. Statistical analyses were conducted using PROC MIXED in

SAS (SAS Institute 2004). For this analysis, lower values indicate more stable treatments. Second, temporal stability was estimated by calculating the mean to standard deviation ratio across the three experiments, similar to Tilman *et al.* (2006). Statistical analyses were not performed for these ratios because only two different variety mixtures and three different monocultures were studied (i.e., n=2 and n=3 respectively). Higher values indicate more stable treatments across experiments. However, temporal stability is confounded with the fact that the July planting date is expected to have higher aboveground biomass than the October plantings, thus inflating standard deviation.

Results

Environmental Conditions

Rainfall and average temperatures over the three years in which the experiments were conducted are reported in Table 4. The October 2006 planted experiment accumulated 878 growing degree days by the time of incorporation in May, while the October 2007 experiment accumulated 782 growing degree days. This difference was largely due to cooler temperatures in May of 2008 relative to 2007. The July planted experiment accumulated 2,083 growing degree days by late May of 2008.

Table 4. Precipitation and temperature records for the three years of the study. Precipitation values are bolded in months when corn crops were growing, and temperature values are bolded in months when cover crops were growing.

	Total Precipitation (mm)				Mean Temp (°C)			
Month	**2006**	**2007**	**2008**	**20 Year Average**	**2006**	**2007**	**2008**	**20 Year Average**
January	111	75	107	45	1.1	**-2.2**	**-3.4**	-0.1
February	42	20	43	33	-2.7	**-7.6**	**-5.3**	1.4
March	94	118	46	55	2.3	**4.6**	**0.3**	7.6
April	62	79	70	74	10.8	**7.2**	**9.9**	14.9
May	140	**65**	**54**	96	14.1	**16.8**	**13.5**	20.8
June	51	**46**	**153**	79	19.6	21.1	20.3	26.1
July	79	**19**	**131**	86	23.0	**21.6**	21.6	27.8
August	149	**171**	**15**	102	21.2	**21.9**	20.5	26.9
September	100	**47**	**354**	86	15.2	**18.1**	17.4	23.1
October	127	147	70	85	**8.5**	**14.0**	9.5	15.9
November	103	54	29	89	**5.2**	**3.1**	3.3	8.6
December	94	60	80	43	**1.6**	**-2.3**	-4.0	1.8
Totals	1156	906	1156	874	10.0	9.7	8.6	14.6

Can common hairy vetch contribute substantial nitrogen benefits to temperate cropping systems?

Cover crop species and experiment influenced the amount of biomass produced by cover crops (Table 5, Figure 2). When planted in October, common hairy vetch produced significantly less total biomass than cereal rye in both years, while the July planted hairy vetch produced more total biomass than October-planted rye or hairy vetch (Figure 2). Above and belowground biomass analyzed separately exhibited similar patterns to total biomass. Aboveground weed biomass was also substantial in bare plots averaging 194 g m^{-2} in the October 2006 experiment, 77 g m^{-2} in the October 2007 experiment and 121 g m^{-2} in the July 2007 experiment.

Winter survival of hairy vetch was comparable to cereal rye in the 2006-planted trial, but was lower than cereal rye in the 2007-planted trial. We were unable to obtain winter survival estimates for the July 2007-planted experiment due to the inability to distinguish individual plants without substantial disruption of the plots.

Table 5. Common hairy vetch biomass production relative to cereal rye biomass at the time of incorporation (May 17th or 18th) across planting dates. Means in a column followed by same lower case letter are not statistically significant at $P = 0.05$.

Planting Date	Cover Crop	Plant Density (# m^{-2})	Winter Survival (%)	Above Ground Biomass (g m^{-2})[a]	Below Ground Biomass (g m^{-2})	Total Biomass (g m^{-2})	Estimated Nitrogen Content (kg ha^{-1})[b]
October 2006	Common Hairy Vetch	144 (16) a	98 (4.8) a	108 (10) c	6 (0.5) d	114 (11) c	41
October 2007	Common Hairy Vetch	96 (5) b	52 (8.0) c	25 (5) d	8 (1.6) d	32 (7) d	12
July 2007	Common Hairy Vetch	NA	NA	435 (37) a	107 (9.2) a	542 (47) a	195
October 2006	Cereal Rye	160 (10) a	86 (3.4) ab	280 (22) b	39 (3.0) c	319 (25) b	48
October 2007	Cereal Rye	172 (7) a	87 (0.6) b	231 (19) b	80 (6.6) b	311 (26) b	47

[a] Harvest Dates:

[b] Nitrogen content estimated based on assumption of 3.6% nitrogen in hairy vetch biomass and 1.5% nitrogen in cereal rye biomass.

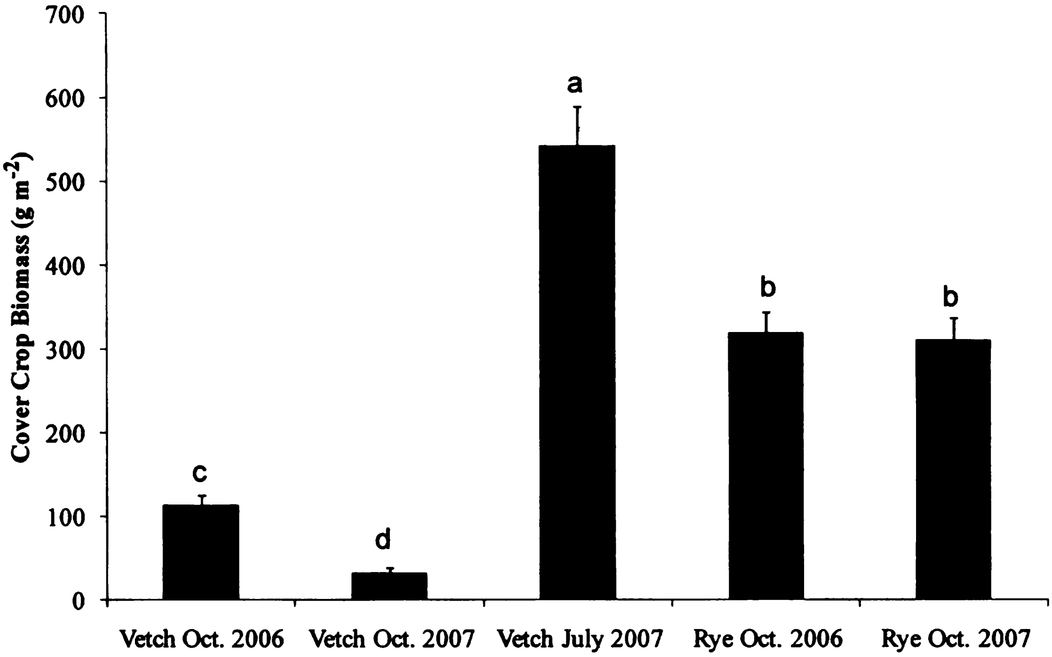

Figure 2. Total biomass production by hairy vetch and cereal rye across experiments. Bars followed by the same lower case letter are not statistically different at P = 0.05.

Aboveground biomass production by Common hairy vetch across planting and harvest dates was positively related to growing degree days ($F_{1,34} = 123.97$, $P < 0.0001$). Aboveground biomass increased exponentially with growing degree days in both cropping systems, after soybean (October planted) and after wheat (July planted) (October; $F_{1.22} = 62.99$ $P < 0.0001$, July; $F_{1.10} = 64.02$ $P < 0.0001$). Overall, aboveground biomass production per growing degree day was less in this experiment compared to data reported by Teasdale *et al.* (2004) (Figure 3).

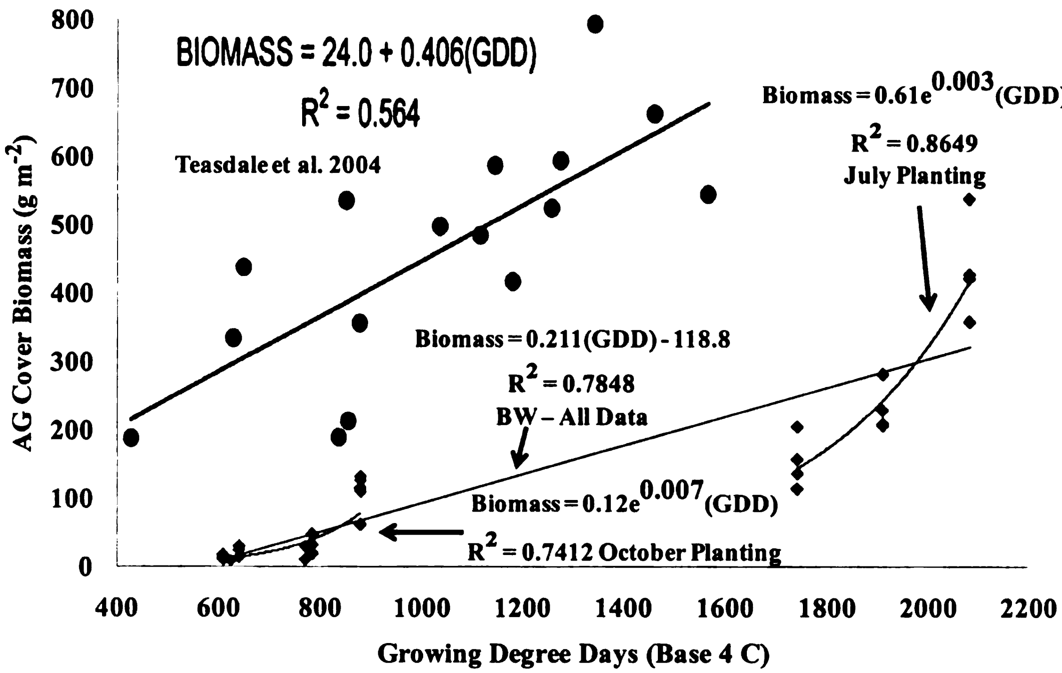

Figure 3. Common hairy vetch aboveground biomass regressed against growing degree days (Base 4°C). October-planted data points are indicated with squares, while July planted data points are indicated with triangles. Best fit trendlines are included for all data from this experiment as well as October and July-planted experiments separately. Comparison is made to data collected by Teasdale *et al.* (2004) in Maryland and New York in 1999 and 2000, which are circular data points.

Total aboveground corn biomass and grain biomass were lower in the rye treatment relative to common hairy vetch and the two bare plots. No differences existed between common hairy vetch and bare plots (Figure 4). Harvest index did not differ between treatments for the 2006 planting, but common hairy vetch plots exhibited higher harvest index values relative to rye plots in the October 2007 planting ($t_{36} = 2.98$, $P = 0.005$; Table 6). Corn grain yields in the bare fertilized plots ranged from approximately 2,900 kg ha^{-1} to 4,500 kg ha^{-1} (Figure 4), whereas average corn grain yields in the adjacent Long Term Ecological Research Experiment are just over 5,800 kg ha^{-1} (lter.kbs.msu.edu, 2010). For the July 2007 planting, the bare fertilized treatment exhibited lower harvest index values compared to common hairy vetch ($t_{36} = 4.33$, $P = 0.0001$) and the bare treatment ($t_{36} = 3.12$, $P = 0.0036$; Table 6).

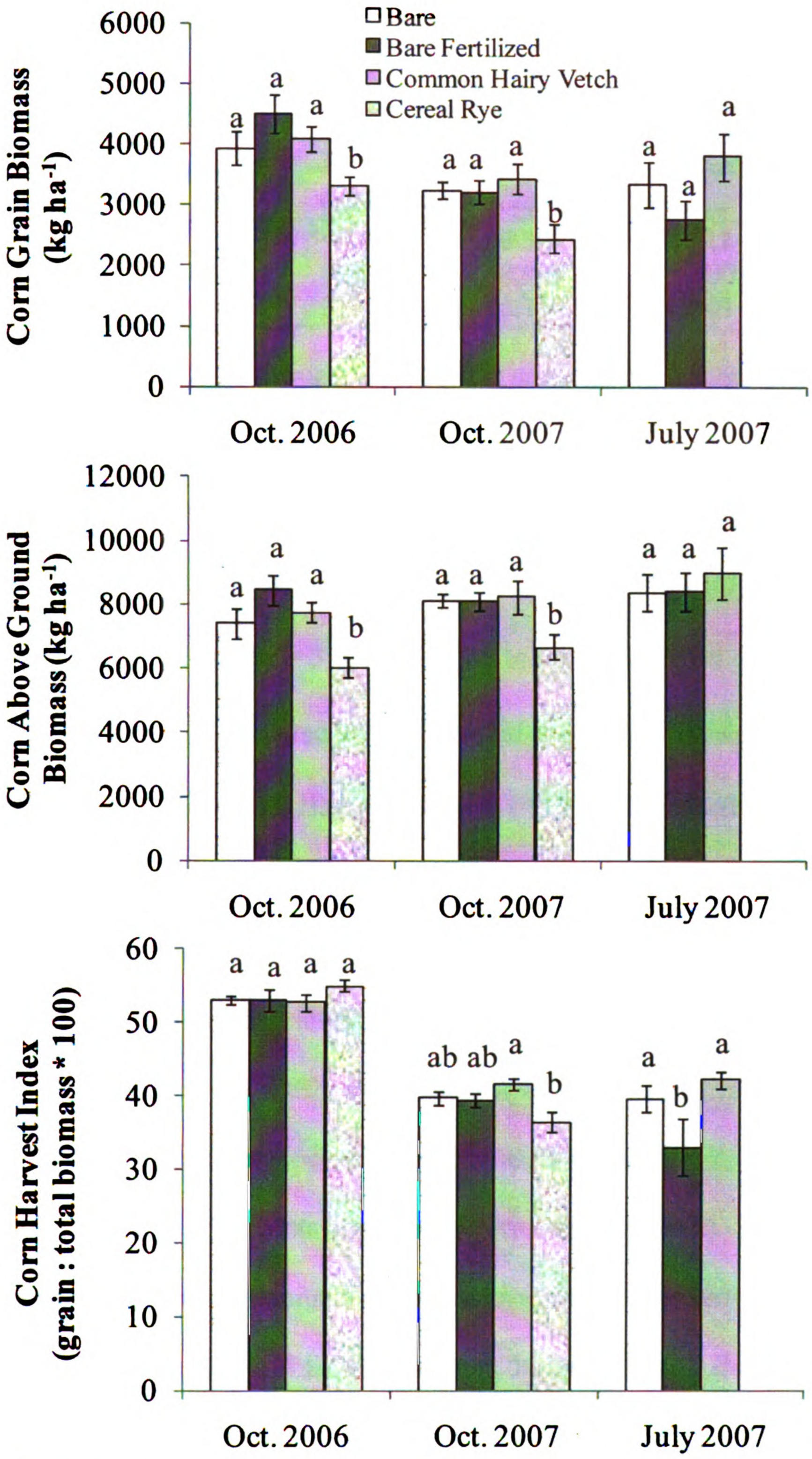

Figure 4. Biomass characteristics including grain biomass, aboveground biomass and harvest index for the corn crop following cover crop treatments are shown for each experiment. Significant differences ($P \leq 0.05$) between treatments for specific experiments are indicated by different letters on top of bars.

Table 6. Two way ANOVA results for corn biomass characteristics. Significant *F* tests ($P \leq 0.05$) are bolded.

Source of Variation	DF Num	DF Den	Corn Biomass (kg ha^{-1})	Grain Biomass (kg ha^{-1})	Harvest Index (Grain:Total Biomass)
			Probability of significant *F* test		
Cover Treatment	3	36	**<0.001**	**0.001**	**0.012**
Experiment	2	13	0.193	**0.001**	<0.001
Treatment*Date	5	36	0.663	0.149	**0.003**

In both of the October planted experiments, corn leaf chlorophyll levels were lower in cereal rye plots relative to the other three treatments (Table 5). No differences existed among the other three treatments across the season, except that the fertilized bare treatment was 8% higher than the unfertilized bare treatment at the R3 growth phase in the 2006 planted experiment ($t_{55} = 3.0$, $P = 0.004$). In the July planted experiment, Common hairy vetch plots exhibited higher corn leaf chlorophyll levels than both bare plots (Bare: $t_{15} = 2.23$, $P = 0.042$; Bare Fertilized $t_{15} = 2.18$, $P = 0.046$). Soil moisture of the top soil during corn crop growth did not differ between Common hairy vetch and bare plots across all three experiments and sampling dates. However, cereal rye plots exhibited significantly higher soil moisture levels compared to the other three treatments for the October 2006 experiment, but not for the October 2007 experiment (Table 7).

Soil inorganic nitrogen concentrations in mid June were similar in fertilized bare plots and common hairy vetch plots across all experiments (Figure 5). In the October 2006-planted experiment, soil inorganic nitrogen levels in the bare fertilized plots were 20% higher than the rye plots and 14% higher than the bare plots. In the October 2007-planted experiment, inorganic nitrogen levels in the common hairy vetch plot were 22% higher than levels in the cereal rye plots. The inorganic nitrogen levels were 68% higher within the October 2006 planted experiment compared to the 2007 planted experiments.

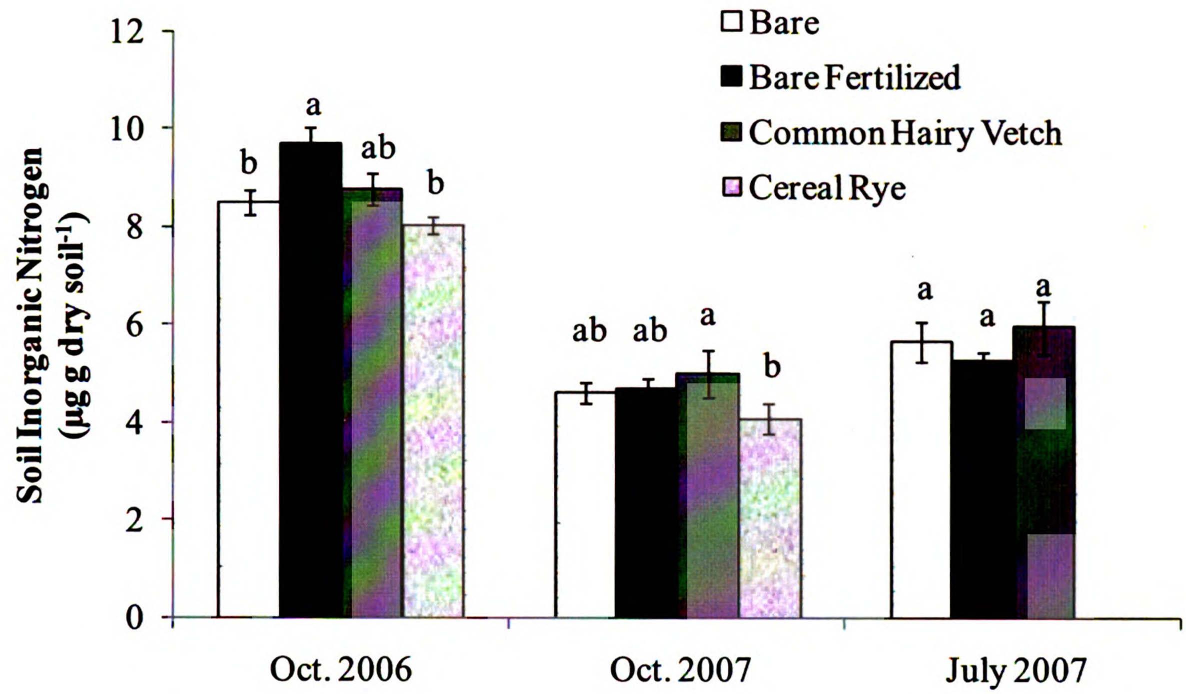

Figure 5. Top soil inorganic nitrogen levels (nitrate + nitrite + ammonium) at the V7 corn growth stage, which occurred in mid June. Significant differences ($P \leq 0.05$) between treatments for specific experiments are indicated by different letters on top of bars.

Table 7. Corn leaf chlorophyll and soil moisture are shown following cover crop treatments across planting dates. Data are presented as means followed by standard errors. For each planting date, means in individual columns followed by same lower case letter are not statistically significant at $P \leq 0.05$ for each sampling time. Differences between average means across sampling times are indicated by different letters in the column to the right of the data for each planting date and variable.

Planting Date	Treatment	Chlorophyll Reading				Soil Moisture			
		V7	R1	R3		Early June	Late June	Late July	
October 2006	Common Hairy Vetch	49.4 ± 0.8 a	39.3 ± 0.6 a	49.1 ± 0.4 ab	a	21.3 ± 0.9	12.4 ± 1.7	8.3 ± 0.9	b
	Cereal Rye	43.5 ± 1.6 b	39.5 ± 0.6 a	48.2 ± 1.2 b	b	25.2 ± 1.3	15.2 ± 0.9	8.0 ± 0.4	a
	Bare	48.3 ± 1.3 a	41.0 ± 0.5 a	47.0 ± 0.7 b	a	20.4 ± 1.6	12.5 ± 2.8	6.9 ± 0.6	b
	Bare Fertilized	48.1 ± 1.1 a	41.0 ± 1.2 a	50.9 ± 0.4 a	a	22.1 ± 0.9	11.4 ± 0.8	6.5 ± 0.5	b
October 2007	Common Hairy Vetch	38.0 ± 0.7	46.5 ± 1.4	NA	a	13.1 ± 0.7	11.7 ± 0.3	NA	NS
	Cereal Rye	36.6 ± 1.0	41.8 ± 1.9	NA	b	14.1 ± 0.9	12.1 ± 0.4	NA	NS
	Bare	38.7 ± 0.6	46.8 ± 0.9	NA	a	13.0 ± 0.6	11.6 ± 0.8	NA	NS
	Bare Fertilized	38.7 ± 1.0	46.0 ± 1.0	NA	a	12.8 ± 0.4	11.2 ± 0.3	NA	NS
July 2007	Common Hairy Vetch	42.8 ± 0.7	50.7 ± 1.2	NA	a	13.0 ± 0.9	12.4 ± 0.6	NA	NS
	Bare	39.5 ± 1.3	50.5 ± 1.9	NA	b	13.1 ± 1.0	12.3 ± 0.3	NA	NS
	Bare Fertilized	38.7 ± 1.0	51.4 ± 1.0	NA	b	13.7 ± 1.4	12.8 ± 0.3	NA	NS

Does variety influence cover crop (hairy vetch) performance?

For the October planted experiments, total cover crop biomass production at the time of incorporation (late May) averaged 3.6 times higher in cereal rye plots relative to hairy vetch varieties, and no difference was detected among hairy vetch varieties (Figure 6). Common hairy vetch produced the most biomass in the July planted experiment, 46% more than Nebraska hairy vetch and 192% more than AU Early Cover.

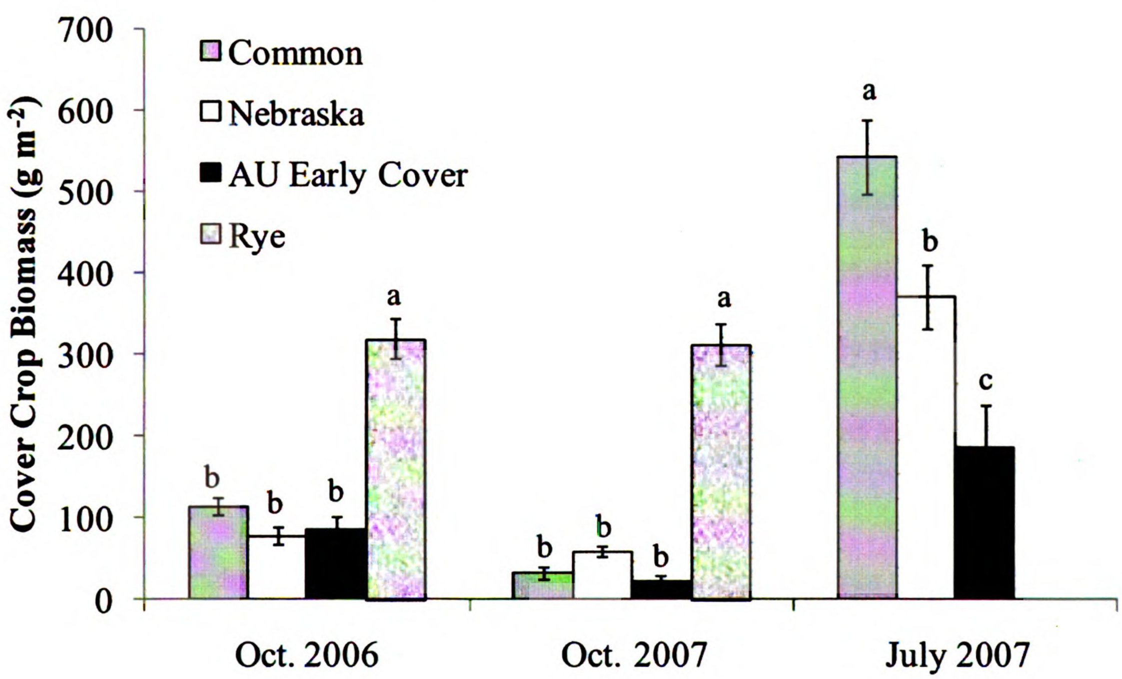

Figure 6. Total cover crop biomass at the time of incorporation (late May sampling date) for three hairy vetch varieties and rye across the experiments. Significant differences (P ≤ 0.05) between treatments for specific experiments are indicated by different letters on top of bars.

Nebraska hairy vetch exhibited higher winter survival than the other two hairy vetch varieties across both October experiments (Table 8). All three hairy vetch varieties had lower survival in the 2007 planted experiment relative to 2006, but cereal rye maintained similar survival percentages across years.

Table 8. Winter survival of hairy vetch varieties and cereal rye across the two October planted experiments. For each experiment, treatment means followed by same lower case

letter are not statistically significant at P = 0.05. Values for Nebraska and AU Early Cover were greater than 100% in the 2006 planted experiment because some seedlings did not emerge until after the winter period.

Experiment	Treatment	%Winter Survival	
October 2006	Common	98 ± 5	bc
	Nebraska	129 ± 7	a
	AU Early Cover	102 ± 6	b
	Cereal Rye	86 ± 3	c
October 2007	Common	63 ± 5	b
	Nebraska	87 ± 4	a
	AU Early Cover	53 ± 2	b
	Cereal Rye	87 ± 1	a

AU Early Cover was the only hairy vetch variety to flower, and these plants flowered only in the October 2006 planted experiment, averaging 35 flowering plants m^{-2}. No flowers were produced prior to incorporation by any variety in either of the other two experiments. All cereal rye plants were in anthesis at the time of incorporation in both October-planted experiments.

Hairy vetch varieties exhibited different relationships between aboveground biomass and growing degree days. When planted in July, AU Early Cover exhibited lower aboveground biomass relative to the other two varieties across all three spring sampling dates. Nebraska hairy vetch produced more biomass than Common at the early May sampling date (t_6 = -3.20, P = 0.019), but Common had more aboveground biomass at the late May sampling date (t_6 = 1.95, P = 0.10).

We detected little evidence that hairy vetch varieties influenced subsequent corn growth or properties, as corn biomass, grain mass and harvest index were all equivalent among the three hairy vetch variety treatments across all experiments (Table 9). In comparison, cereal rye plots exhibited lower corn grain and corn biomass relative to hairy vetch in the two October-planted experiments. In addition, harvest index of corn planted subsequent to rye was lower than hairy vetch for the October 2007 planted experiment (Table 9). Corn leaf chlorophyll and soil moisture levels presented in Table 7 reflect these differences between cereal rye and hairy vetch treatments.

Table 9. Corn bioassay response to hairy vetch variety and cereal rye treatments, mean plus or minus standard error shown. For each experiment, treatment means followed by same lower case letter are not statistically significant at P = 0.05.

Experiment	Treatment	Corn Above-ground Biomass (kg ha^{-1})	Corn Grain Biomass (kg ha^{-1})	Harvest Index (grain : total biomass * 100)
October 2006	Common	7740 ± 320 a	4090 ± 200 a	52.7 ± 1.1 a
	Nebraska	7820 ± 560 a	4180 ± 390 a	53.1 ± 2.4 a
	AU Early Cover	7770 ± 360 a	4120 ± 180 a	53.1 ± 0.5 a
	Cereal Rye	6050 ± 310 b	3330 ± 150 b	55.1 ± 0.8 a
October 2007	Common	8230 ± 530 a	3440 ± 260 a	41.6 ± 0.7 a
	Nebraska	7910 ± 410 a	3260 ± 200 a	41.2 ± 1.0 a
	AU Early Cover	7670 ± 390 a	3090 ± 160 a	40.4 ± 0.9 a
	Cereal Rye	6660 ± 400 b	2450 ± 230 b	36.5 ± 1.3 b
July 2007	Common	8990 ± 820 a	3810 ± 380 a	42.3 ± 1.1 a
	Nebraska	9110 ± 840 a	3740 ± 470 a	40.7 ± 1.4 a
	AU Early Cover	8800 ± 1130 a	3600 ± 590 a	40.3 ± 1.7 a

Do variety mixtures increase and/or stabilize cover crop performance?

Overall, the net biodiversity effect on aboveground biomass averaged -13 g m^{-2} for the Common / AU Early Cover mixture, and -8 g m^{-2} for the Nebraska / AU Early Cover mixture, indicating that mixtures performed more poorly than monocultures, yet neither of the values were statistically different from 0. However, a significant interaction was found between treatment and experiment indicating that the net effect of the mixtures was not consistent across experiments (Figure 7). The Common / AU Early Cover mixture significantly underyielded the monospecific hairy vetch treatments in the July 2007-planted experiment (t_{12} = 3.30, P = 0.006), but no other mixtures significantly overyielded or underyielded across the three experiments.

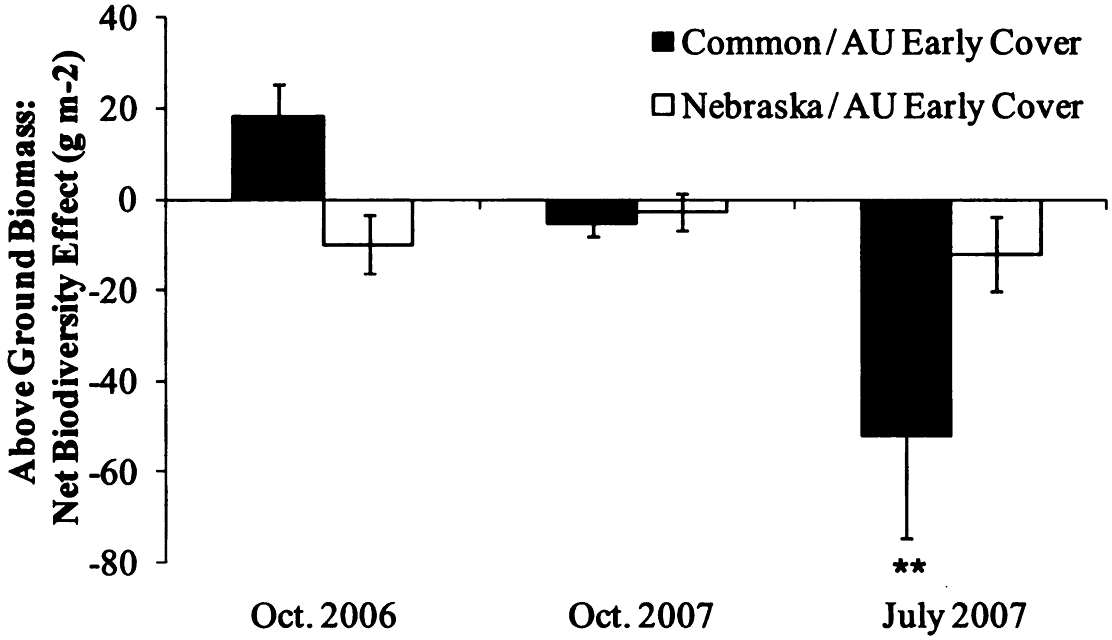

Figure 7. Net biodiversity effect for variety mixtures relative to monocultures of the varieties in the mixture. Positive values indicate overyielding, and negative values indicate underyielding. Error bars represent standard errors. Stars indicate level of statistical difference from zero for each mixture at each experiment (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

The coefficient of variation for treatment means within planting dates did not differ among variety mixtures (n=6) and monocultures (n=9) across all experiments (Variety Mixture = 0.36 ± 0.08, Monocultures = 0.39 ± 0.06). Similarly, temporal stability values were similar across treatments, as were variety mixture values for respective monocultures in each mixture (Table 10).

Table 10. Temporal stability (ratio of mean to standard deviation across experiments) for three monocultures and two variety mixtures included in the experiment.

Treatment	Temporal Stability (μ/σ)
AU Early Cover	1.2
Common	0.9
Nebraska	1.0
Common * AU Early Cover	1.1
Nebraska * AU Early Cover	1.0

Discussion

The cropping system substantially influenced Common hairy vetch biomass, as aboveground biomass in the July planted experiment after wheat was 327 g m^{-2} higher than the 2006 October-planted experiment after soybean and 410 g m^{-2} higher than the 2007 October-planted experiment. These results are similar to other studies reported in the literature (Table 11), together suggesting that late fall-planted hairy vetch produces substantially less biomass than summer and early fall plantings. Teasdale *et al.* (2004) suggested a target of 400 g m^{-2} of aboveground biomass to supply an adequate amount of nitrogen (approximately 140 kg ha^{-1} N in aboveground biomass) for subsequent cash crops such as corn. Across all studies, only the July planting in Michigan and August planting in New York produced this amount of biomass; all other plantings would theoretically need to be supplemented with additional nitrogen fertilizer to cash crops.

Table 11. Comparison of common hairy vetch aboveground biomass in this experiment to other published reports.

Study	Location	Planting Date	Aboveground Biomass (g m^{-2})
Present	Hickory Corners, MI	October 7, 2006	108
Present	Hickory Corners, MI	October 9, 2007	25
Present	Hickory Corners, MI	July 27, 2007	435
(Jannink *et al.*, 1997)	Stillwater, ME	August 14, 1990	181
(Jannink *et al.*, 1997)	Stillwater, ME	September, 4, 1990	31
(Teasdale *et al.*, 2004)	Freeville, NY	August 25, 1998	430
(Teasdale *et al.*, 2004)	Freeville, NY	September 14, 1998	307
(Miguez and Bollero, 2006)	Urbana, IL	October 31, 2001	12
(Miguez and Bollero, 2006)	Urbana, IL	September 25, 2002	220

Growing degree days were a good predictor of aboveground biomass by Common hairy vetch (Figure 3), but the trend did not match the results from Teasdale *et al.* (2004) as more growing degree days were required in this study to obtain similar biomass production relative to Teasdale *et al.* (2004). Most of the data points from Teasdale *et al.* (2004) were collected in Maryland, a warmer climate than SW Michigan where this study was conducted. We hypothesize that across climates, growing degree days for winter annual plants may interact with soil temperature to determine biomass production, as cold winter temperatures likely resulted in a slower warming of the soil in this study relative to those in Maryland. Soil type and other environmental factors may have also contributed to the differences observed among studies.

Corn has been shown to be highly responsive to nitrogen inputs, and the literature indicates its usefulness as a bioassay of nitrogen availability (Carpenter-Boggs *et al.*, 2000; Gentry *et al.*, 2001; McSwiney et al., 2010;). In southwest Michigan, continuously planted maize yield increases linearly with increasing nitrogen fertilization up to approximately 120 kg N ha^{-1} (McSwiney and Robertson, 2005). Yet, unfertilized corn, which was used as a bioassay in this study, did not reflect the differences in hairy vetch biomass in our study. Nitrogen inputs were somewhat reflected by chlorophyll monitoring, which indicated higher plant tissue chlorophyll content in corn grown subsequent to hairy vetch compared to bare fallow. However, corn biomass, grain yield and soil nitrogen levels were equivalent among the Common hairy vetch and bare plots. We speculate that water was the primary limiting factor for corn growth in both years because both corn growing seasons were marked by dry months (July 2007, August 2008; Table 4) and corn yields in fertilized plots (3,490 kg ha^{-1}) were 40% lower than average long term yields in the adjacent LTER experiment (5,827 kg ha^{-1}).

The hypothesis that nitrogen was not the limiting factor for corn crop growth was supported by chlorophyll meter readings on corn leaves, as leaf chlorophyll levels were similar between hairy vetch and rye plots in October planted experiments. The chlorophyll meter measures leaf greenness, which is highly correlated with leaf chlorophyll levels (Blackmer *et al.*, 1993; Markwell *et al.*, 1995) and consequently, leaf nitrogen concentration (Schepers *et al.*, 1992). Corn leaf chlorophyll levels in the July-planted experiment were slightly higher in the hairy vetch plots relative to bare plots at the V7 growth stage, but not at R1.

Corn grown subsequent to hairy vetch, however, did exhibit higher biomass and grain yield than plots planted with cereal rye prior to the corn crop. Chlorophyll meter readings indicated that nitrogen availability was greater in the hairy vetch and bare plots relative to cereal rye plots, indicating that cereal rye biomass, which has a higher carbon to nitrogen ratio than hairy vetch, might have immobilized available nitrogen in the soil (Rosecrance *et al.*, 2000).

That we did not detect a reduction in surface soil moisture content within cover cropped plots during the cash crop growing period is noteworthy. In fact, cereal rye plots maintained higher soil moisture levels than other treatments in the 2006 planted experiment, which was likely a combined result of decreased evapotranspiration due to decreased corn growth and more soil organic matter and water holding capacity. During dry summers, the additional organic matter from cover crops may help to retain soil moisture and boost crop yields over time. Soil moisture and nitrogen availability have been shown to strongly interact (Birch, 1958; Paul *et al.*, 2003), and this may explain the limited corn response observed here to a wide range of biomass in hairy vetch and bare treatments.

Although cropping system was the most important factor governing hairy vetch biomass, and degree days appeared to explain much of the growth response, variety also influenced biomass production, winter survival and relative growth rate. These results support the idea that varieties may be optimal for different functions within cropping systems, similar to the suggestion made by Wilke and Snapp (2008). Specific attention should be given to the growing degree days available prior to termination and incorporation. The Nebraska variety, which has very pubescent leaves and may stem from the old 'Madison' variety (Duke, 1981), should be chosen for optimal winter survival and early spring growth where few growing degree days are available. In situations where the cover crop can be left in the field for longer periods of time in the spring, the common variety grown in Oregon is likely to outperform the other varieties.

Farmers using organic no-till management might consider AU Early Cover for its early flowering characteristic, yet also expect that this variety may produce less biomass than the other two varieties.

The potential benefits of intraspecific diversity in agroecosystems can occur via two distinct mechanisms. The first is through complementary growth; each variety has different resource needs, thus reducing competition between the two varieties. This may be determined by evaluating individual plant mass in mixtures and monocultures. The second mechanism is the sampling effect; planting multiple varieties increases the likelihood of including one that is productive.

Variety mixtures did not influence productivity across the experiments in this experiment compared to average monoculture values, indicating that complementary resource use was not achieved during the growth periods of hairy vetch in our study. We suggest two possible reasons for this outcome. First, it is possible that differences in morphology and phenology between varieties are not great enough to cause differential resource capture. Second, plants were not grown to maturity, and competition between plants for resources may increase as the plants grow and age. Similarly, no differences in stability were detected between mixtures and monocultures when measured as the variation in productivity across experiments. The variety mixtures were intermediate in terms of stability, but were not statistically different.

However, given the variability in growth among varieties, variety mixtures still may be a wise choice for farmers given the unpredictable outcome of individual varieties in different environments. Thus, planting variety mixtures reduces the risk when initially trying cover crop plantings, in case one variety underperforms.

Conclusion

When seeded in late July, Common hairy vetch produced sufficient aboveground biomass as a winter cover crop to meet the nitrogen demands of a subsequent corn crop, but produced inadequate amounts of biomass when seeded in October. Other varieties of hairy vetch (Nebraska, AU Early Cover) did not increase biomass production, but exhibited distinctive traits that could allow them to be used in specific roles within cropping systems. Nebraska exhibited high winter survival and early spring biomass accumulation while AU Early Cover flowered earlier than the other varieties. Finally, variety mixtures assumed to provide complementary qualities did not necessarily increase cover crop productivity or stability.

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CHAPTER THREE

Temperate cropping system management influences nitrogen fixation by a red clover cover crop

Abstract: We examined the influence of agroecosystem management on nitrogen fixation by red clover (*Trifolium pretense*) in two long term grain cropping experiments (LTER and LFL) in Southwest Michigan, USA. We hypothesized that systems managed with continual organic inputs will exhibit increased soil fertility and decreased percent of nitrogen from fixation by legumes due to sanctions placed on *Rhizobia.* This hypothesis was supported in the LFL; the Compost system managed with organic nitrogen sources (compost + legume cover crops) exhibited 22% lower percent of nitrogen from fixation by red clover compared to the Conventional system managed solely with inorganic fertilizers. In contrast, this hypothesis was not supported in the LTER; the Zero Input system, which was managed with organic nitrogen sources (legume cover crops) was not different from the Conventional system in terms of the percent of nitrogen from fixation. Interestingly, the percent of nitrogen from fixation was 23% higher in the Zero Input system compared to the Low Input system, which was managed with a mixture of nitrogen from legume cover crops and inorganic fertilizers plus herbicides to control weeds, which may indicate that herbicides influence the rates of nitrogen fixation. Differences in the percent of nitrogen from fixation did not translate to differences in total amounts of nitrogen derived from the atmosphere. Combined, these results support the hypothesis that the fraction of nitrogen derived from the atmosphere is negatively related to soil fertility, but that other factors, such as the use of herbicides, may also influence nitrogen fixation.

***Key Words*:** Red Clover, Nitrogen Fixation, Rhizobia, Cropping Systems, LTER

Introduction

A central research question in ecology examines how changing resource gradients affect symbiotic relationships in nature (Johnson *et al.*, 1997; Hoeksema and Bruna, 2000; West *et al.*, 2002). More specifically, better knowledge of the legume – *Rhizobium* relationship would aid in predicting spatial and temporal fluctuations in legume abundance in grazed ecosystems through time (Schwinning and Parsons, 1996) as well as improving productivity and resilience of cropping systems.

Agricultural ecosystems, where plant species are controlled by management activities, provide simplified scenarios to examine the legume - *Rhizobium* relationship. Systems managed for increases in soil nitrogen supply through time should result in decreased system demand for external nitrogen inputs. As soil nitrogen supply increases, *Rhizobium* bacteria may become parasitic on legume roots instead of benefiting the legume through symbiosis (Kiers *et al.*, 2003), but whether that results in lower legume biomass accumulation or not depends on whether nitrogen availability from all sources constrains net photosynthesis.

The degree of interaction between mutualists can change across a resource gradient (Ledgard and Steele, 1992). For example, alfalfa (*Medicago sativa*) continues to fix atmospheric nitrogen with high soil nitrogen supply, but at lower rates than with lower soil nitrogen supply (Lamb *et al.*, 1995; Blumenthal and Russelle, 1996). Soybean (*Glycine max*) grown in greenhouse settings exhibits the same pattern (Wanek and Arndt, 2002). Therefore, legume cover crops may have the ability to selectively exclude *Rhizobium* from infecting roots and decrease resource allocation to nodules during periods of high soil nitrogen supply, thus reducing rates of nitrogen fixation (Kiers *et al.*, 2006). This pattern is common in experimental systems where large pulses of inorganic nitrogen are present, but less studied is whether rates of nitrogen fixation will be reduced in systems with relatively high soil organic matter and subsequent high rates of inorganic nitrogen release from the soil.

Nitrogen fixing plants contribute to the accumulation of soil organic nitrogen through time, thus increasing plant available nitrogen in the soil (Crocker and Major, 1955; Knops and Tilman, 2000). In this study, we estimated nitrogen fixation by red clover (*Trifolium pratense*) in several agroecosystems that varied from chemical to ecological management. We hypothesized that the accumulation of soil nitrogen over time in organically managed systems would feed back to negatively affect the rates of nitrogen fixation by red clover and associative *Rhizobium* bacteria. In agricultural ecosystems where biological sources of nitrogen (e.g. cover crops, compost, livestock or poultry manure) are utilized as sources of fertility, we expect the fraction of nitrogen derived from the atmosphere (fNdfa) by legumes to be lower than in systems maintained without biological sources of nitrogen due to more soil nitrogen mineralization in the biologically managed system.

Alternatively, the slow change in plant available soil nitrogen over time in agricultural ecosystems may not be substantial enough to induce a shift in the interaction between legumes and *Rhizobium*. Schipanski *et al.*, (2010) reported that soil inorganic nitrogen was a weak predictor of the variation in rates of nitrogen fixation by soybean across management systems, but that soil texture and site characteristics were better predictors. Finally, it could be hypothesized that legumes facilitate their own growth by positively affecting other soil parameters such as available phosphorus or moisture, which may result in equal or higher levels of the fraction of nitrogen derived from the atmosphere (fNdfa) through time.

Total nitrogen fixation may show a different pattern than the percentage of nitrogen fixed from the atmosphere. Areas of poor soil, corresponding to low soil N and other necessary minerals and nutrients, may reduce growth during early plant development, or regrowth following winter senescence or biomass harvest, and reduce the potential for abundant nitrogen fixation due to poor plant growth. High soil N may reduce nodulation and nitrogen fixed from the atmosphere as hypothesized above. Thus, maximum total nitrogen fixation may be achieved when soils exhibit medium supplies of nitrogen (Figure 8).

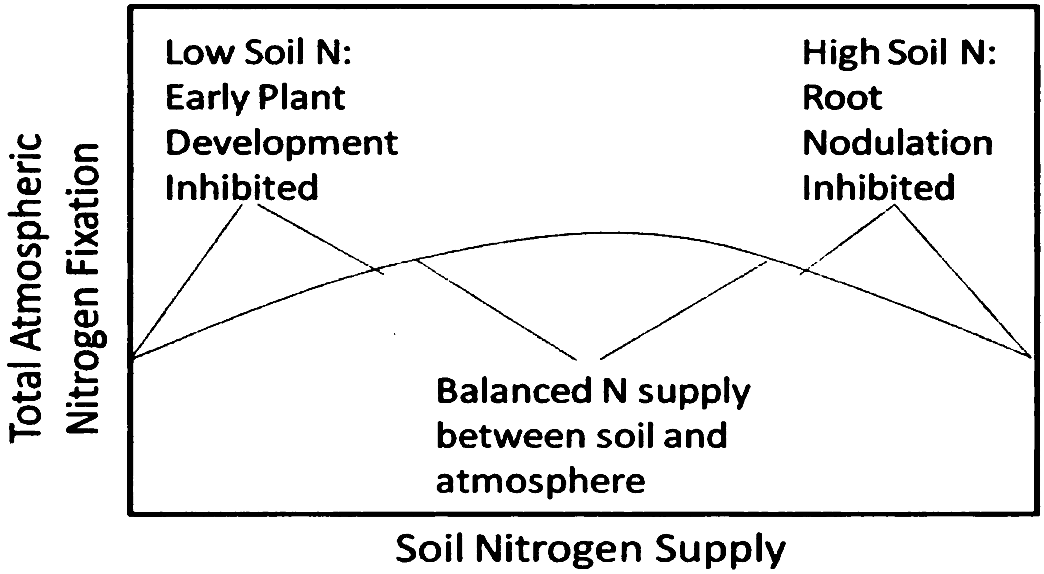

Figure 8. Conceptual hypothesis about relationship between soil nitrogen supply and total nitrogen fixation.

Materials and Methods

Study System

We used two ongoing long term experiments at the W.K. Kellogg Biological Station to test the above hypotheses: the Long Term Ecological Research experiment

(LTER) established in 1989 and the companion Living Field Laboratory (LFL) established in 1993. Both experiments include an array of grain cropping ecosystems that range from chemical based management to ecological based management (Table 12). Three treatments of the LTER Main Cropping System Experiment were included in the study: Conventional, Low Input and Zero Input. All three treatments have the same basic crop rotation; corn (*Zea mays*), soybeans and wheat (*Triticum aestivum*), with the same crop present across all three treatments in a given year. Mechanical tillage tools are used in all three treatments to control weeds and prepare seedbeds. The Conventional cropping system relies on synthetic chemicals for fertility and weed control, without cover crops. The other two ecosystems (Low Input & Zero Input) utilize red clover and cereal rye (*Secale cereale*) as green manure cover crops. The Low Input treatment receives 1/3 of the synthetic chemicals compared to the Conventional system, whereas the Zero Input treatment receives no synthetic chemicals. The experiment is arranged in a randomized complete block design with six 1-hectare replications per treatment. More information about this experiment can be found on the internet at: http://lter.kbs.msu.edu/.

Table 12. Cropping system treatments utilized for this study in the LTER and LFL with standard management information and average soil organic matter levels. LTER values were calculated from dataset KBS024 available at lter.kbs.msu.edu. LFL values were contributed by Gentry *et al.* (2009 unpublished data).

Long Term Ecological Research (LTER) – Initiated in 1989

Treatment	Nitrogen Source	Weed Control	% Soil Organic Matter (4/4/2001)
Conventional	Inorganic Fertilizer – Full Rate	Tillage + Herbicide	1.54
Low Input	Inorganic Fertilizer (1/3 Full Rate) + Legume Cover Crops	Tillage + Reduced Herbicide Rates	1.89
Zero Input	Legume Cover Crops	Tillage	1.95

Living Field Laboratory (LFL) – Initiated in 1993

Treatment	Nitrogen Source	Weed Control	% Soil Organic Matter (4/2/2008)
Conventional	Inorganic Fertilizer – Full Rate	Tillage + Herbicide	1.26
Low Input	Inorganic Fertilizer (Reduced based on Soil Tests) + Legume Cover Crops	Tillage + Herbicide	1.60
Compost	Composted Dairy Manure + Legume Cover Crops	Tillage + Herbicide	1.99

Three treatments in the Living Field Laboratory (LFL), Conventional, Integrated Fertilizer and Integrated Compost were also used as an independent experiment to test the hypotheses presented in the introduction. From 1993-2005, plots were planted in a four year crop rotation: corn, corn, soybeans, wheat. In 2006, the rotation was shortened to a three year cycle: corn, soybeans, wheat. Mechanical tillage tools and synthetic pesticides were utilized in each experiment as needed to prepare seedbeds and control weeds. The Conventional treatment relied on chemical fertilizers at pre-determined levels based on best management practices, and no cover crops were used. The Integrated Fertilizer treatment was comparable to the Low Input system in the LTER, receiving chemical fertilizers at reduced rates based on plant nutrition needs as well as red clover and cereal rye as cover crops. From this point forward, we refer to the Integrated Fertilizer treatment as the LFL "Low Input" treatment to allow easy comparisons with the LTER Low Input system. The Integrated Compost treatment received composted dairy manure as a fertilizer source as well as the same cover crop management as the Low Input. We refer to the Integrated Compost treatment as "Compost" from this point forward. The experiment was arranged in a randomized complete block design with four replications and each replicate split into three sub-plots to allow for each phase of the crop rotation to be present each year. Sub plots were 21.3 x 9.1 meters in size. Cover crops were planted on half of each sub-plot (21.3 x 4.55 m) while the other half of each plot remained fallow when cash crops were not growing. More information about this experiment can be found on the internet at:

http://lter.kbs.msu.edu/research/long_term_experiments/living_field_lab.php.

Field Plots

The experiment was conducted in the LTER for one red clover growing season (2007-2008). Two adjacent microplots, each measuring 10m x 5m, were established along the north side of all six replications for each of the three treatments. One randomly selected 10m x 5m microplot was broadcast sown with red clover (*Trifolium pretense*) seed and the other with perennial rye (*Lolium perenne*) seed.

In the LFL, two successive growing seasons (2006-2007 and 2007-2008) were included in the experiment. Different sub-plots within each replicate were used each year, which was enabled by the split plot design of the experiment. All methods referenced in this paper were conducted within the cover cropped half of each plot. Red clover was broadcast sown across the entire cover cropped half of the plot.

Cover crops were seeded in March (Table 13) at 13.5 kg ha^{-1} onto frozen ground directly into the existing winter wheat crops, effectively acting as an intercrop with winter wheat until the wheat was harvested.

Table 13. Planting and harvest dates in respective experiments

Experiment	Planting Date	Summer Harvest Date (Wheat)	Fall Harvest Date (Clover, Ryegrass)	Spring Harvest Date (Clover, Ryegrass)
LTER	March 20, 2007	July 2, 2007	Dec. 1, 2007	May 5, 2008
LFL 1	March 20, 2006	July 13, 2006	NA	May 3, 2007
LFL 2	March 20, 2007	July 11, 2007	NA	May 6, 2008

Data Collection

Aboveground winter wheat biomass samples were sampled in early July from two 0.25 m^2 quadrats within each of the respective plots. Wheat grain was separated from the rest of the plant and the two subsamples were combined for analyses. Aboveground biomass from the red clover and perennial ryegrass microplots was sampled in early December in the LTER and in early May in both experiments, prior to incorporation of the cover crop. Aboveground biomass was clipped from two separate 0.25m^2 square quadrats within each microplot, and subsamples were bulked for analysis. All biomass samples were oven dried at 65° C for at least 72 hours and ground to pass through a 1 mm sieve. Subsamples were analyzed for percent nitrogen and $\delta^{15}N$ (sample ^{15}N:^{14}N – atmosphere ^{15}N:^{14}N) at the UC Davis Stable Isotope Facility using a continuous flow Isotope Ratio Mass Spectrometer.

Soil samples were taken to measure inorganic nitrogen and nitrogen mineralization potential on April 7, 2008 during the period of cover crop growth. A composite sample of eight cores from the 0-25 cm depth were collected from each red clover subplot and stored at 4°C until they were processed within 24 hours. Soils were sieved to 4 mm and homogenized before two 10-g subsamples and a sample for soil moisture were weighed. For the initial sample extracted immediately, 100 mL of 1 N KCl was added, shaken for 1 minute, and allowed to settle overnight. The next day, the samples were shaken again, allowed to settle for one hour, filtered through Whatman #1 filter paper into scintillation vials, and then frozen until analysis. Soil samples weighed for gravimetric water content were placed into a drying oven held at 60°C, dried for 48 hours, and weighed again to determine water content and dry weight for the soil N analyses.

The second 10-g subsample was corrected to 19% soil moisture, placed in an incubator held at 25°C for 30 days, and then extracted in 1 N KCl as described for the initial sample. Nitrate-N and ammonium-N were determined for soil extracts using a SmartChem 140 discrete analyzer (Westco Scientific, Danbury CT). The hydrazine reduction method and azo dye was used to measure NO_3^- and the phenolate method for NH_4^+. Nitrogen mineralization potential was calculated as the difference between total mineral N (NO_3^- + NH_4^+ g dry soil^{-1}) from the incubated soil and the total mineral N from the soil that was extracted immediately.

Data Analyses: Do Cropping Systems influence Nitrogen Fixation by Red Clover?

The ^{15}N natural abundance technique was used to estimate biological nitrogen fixation (BNF) by red clover in each of the experimental ecosystems. To calculate BNF, differences in $\delta^{15}N$ values between red clover cover crops and two non-fixing reference plants, grain from intercropped winter wheat plants and perennial ryegrass. Specifically, the following equation was used to calculate the fraction of nitrogen derived from the atmosphere (fNdfa) for each sampling date:

$$\text{fNdfa} = (\delta^{15}N_{ref} - \delta^{15}N_{fix}) / (\delta^{15}N_{ref} - \delta^{15}N_{b})$$

where 'ref' are non-fixing and 'fix' are nitrogen fixing plants grown under the same conditions, and 'b' is the fixing plant grown with N_2 as the sole external nitrogen source. The b value was estimated as an apparent b value (*b*app) based on the lowest $\delta^{15}N$ value of *T. pratense*, which represents 100% fNdfa (Eriksen and Hogh-Jensen, 1998; Hansen and Vinther, 2001; Hansen *et al.*, 2002; Huss-Danell and Chaia, 2005). The lowest $\delta^{15}N$ value we measured was -1.84, which was used as the *b*app value in both experiments. Three out of 48 fNdfa values from the LTER were calculated as negative, which is an irrational result and were treated as missing values in analysis. All three of these negative values were calculated from the spring sampling, two from Zero Input replicates and one from a Low Input replicate.

Statistical analyses were conducted using PROC MIXED in SAS (2004). LTER biomass, percent nitrogen and nitrogen fixation data (fNdfa and BNF) were analyzed using repeated measures analysis of variance (ANOVA) with a compound symmetry model as the variance-covariance structure, which was chosen based on lowest AIC values relative to other models tested. Replication was considered a random factor. Years were analyzed separately if there was a significant interaction between year and treatment ($P \leq 0.10$). Soil nitrogen properties were analyzed using one way ANOVAs including replication as a random factor.

Biomass, percent nitrogen and nitrogen fixation data (fNdfa and BNF) in the LFL experiment were analyzed using two-way ANOVAs with treatment and year as the main factors, and replication as a random factor. No significant interactions were detected between year and treatment, so treatments were analyzed across years in the analysis. Soil nitrogen properties were analyzed using one-way ANOVAs including replication as a random factor.

Data Analyses: Is Nitrogen Fixation related to Soil Nitrogen Properties?

Soil nitrogen data were analyzed as one way ANOVA in both experiments because data were collected only in one year. Multiple regression analyses were conducted in SAS using PROC REG to assess relationships between soil nitrogen properties and nitrogen fixation by red clover. For each study system (LTER and LFL), two multiple regressions were conducted to evaluate the relative contribution of soil inorganic nitrogen and 30 day nitrogen mineralization potential (independent variables) on two different dependent nitrogen fixation variables (fNdfa and Total BNF). One data point for nitrogen mineralization potential in the LFL was determined to be an outlier for the purposes of the regression analyses and was discarded.

Results

Cover Crop Growth and Nitrogen Content

Red clover aboveground biomass at the spring harvest date averaged 1,610 kg ha^{-1} in the LTER, while the LFL averaged 1,868 kg ha^{-1} and 1,445 kg ha^{-1} in 2007 and

2008, respectively. No statistical differences were detected between treatments within either experiment (Table 14, 15). Similarly, no statistical differences between treatments were detected for total nitrogen in the aboveground biomass, which ranged from 47 kg ha^{-1} to 88 kg ha^{-1} (Table 14, 15). Percent nitrogen in the samples during spring sampling dates ranged from 3.41% to 4.76%.

Table 14. Red clover above ground biomass, percent nitrogen and total nitrogen across the two sampling periods in the LTER. Means are presented plus or minus standard errors. Means in a column followed by same lower case letter are not statistically significant at P = 0.05.

LTER	Above Ground Biomass (kg/ha)		% N in Biomass		Above Ground N (kg/ha)	
Treatment	**Fall 2007**	**Spring 2008**	**Fall 2007**	**Spring 2008**	**Fall 2007**	**Spring 2008**
Conventional	927 ±226	1296 ±253	2.87 ±0.04	3.85 ±0.11 a	27 ±6.5	49 ±9.4
Low Input	1093 ±240	1857 ±137	3.00 ±0.14	3.60 ±0.10 ab	32 ±5.9	67 ±6.4
Zero Input	1171 ±221	1678 ±137	3.20 ±0.15	3.41 ±0.20 b	36 ±6.7	59 ±11.2
Treatment Average	1071 ±126	1610 ±136	3.04 ±0.08	3.62 ±0.09	32 ±3.6	59 ±5.3

Table 15. Red clover above ground biomass, percent nitrogen and total nitrogen across the two years in the LFL. Means are presented plus or minus standard errors. Means in a column followed by same lower case letter are not statistically significant at P = 0.05.

LFL	Above Ground Biomass (kg/ha)		% N in Biomass		Above Ground N (kg/ha)	
Treatment	**Spring 2007**	**Spring 2008**	**Spring 2007**	**Spring 2008**	**Spring 2007**	**Spring 2008**
Conventional	1946 ±261	1359 ±76	4.17 ±0.08 b	3.49 ±0.08 b	81 ±10.2	47.28 ±2.42
Low Input	1810 ±90	1636 ±198	4.63 ±0.12 a	3.94 ±0.13 a	84 ±2.9	64.91 ±9.56
Compost	1850 ±145	1339 ±66	4.76 ±0.14 a	3.59 ±0.29 ab	88 ±7.7	48.54 ±6.08
Treatment Average	1868 ±96	1445 ±79	4.52 ±0.10	3.67 ±0.11	84 ±4.1	53.58 ±4.25

In the LTER, aboveground biomass, percent nitrogen and aboveground nitrogen were significantly higher during the spring sampling date relative to the fall sampling date (Table 14, Appendix: Table 16). A significant interaction was detected between treatment and sampling date for percent nitrogen in the LTER ($F_{2,13} = 3.60$, $P = 0.057$), so we investigated each time point independently. During the spring sample, percent nitrogen in the conventional samples was 13% higher than in zero input samples ($t_{13} = 2.33$, $P = 0.037$).

In the LFL, aboveground biomass, percent nitrogen and aboveground nitrogen were all significantly higher in 2007 compared to 2008 (Table 15, Appendix: Table 17). Percent nitrogen in Low Input samples were on average 12% higher than Conventional samples averaged across the two years ($t_{18} = 2.91$, $P = 0.009$), while Compost samples were on average 9% higher than Conventional samples ($t_{18} = 2.21$, $P = 0.040$) (Table 15).

Do Cropping Systems influence Nitrogen Fixation by Red Clover?

The fraction of nitrogen derived from the atmosphere by red clover averaged across the season, estimated by the natural abundance technique using wheat as the reference crop, ranged from 0.42 in the LTER Low Input treatment to 0.78 in the LFL Conventional treatment (Figure 9). Significant differences between treatments were documented in both experiments. Within the LTER, fNdfa in the Zero Input treatment was 30% higher on average than in the Low Input treatment ($t_{15} = 2.14$, $P = 0.049$). The Conventional treatment fNdfa was 23% higher than the Low Input treatment across sampling times, but these differences were marginally significant ($t_{15} = 1.76$, $P = 0.098$). Fall fNdfa was 45% higher compared to the spring sample ($F_{1,13} = 34.48$, $P < 0.0001$).

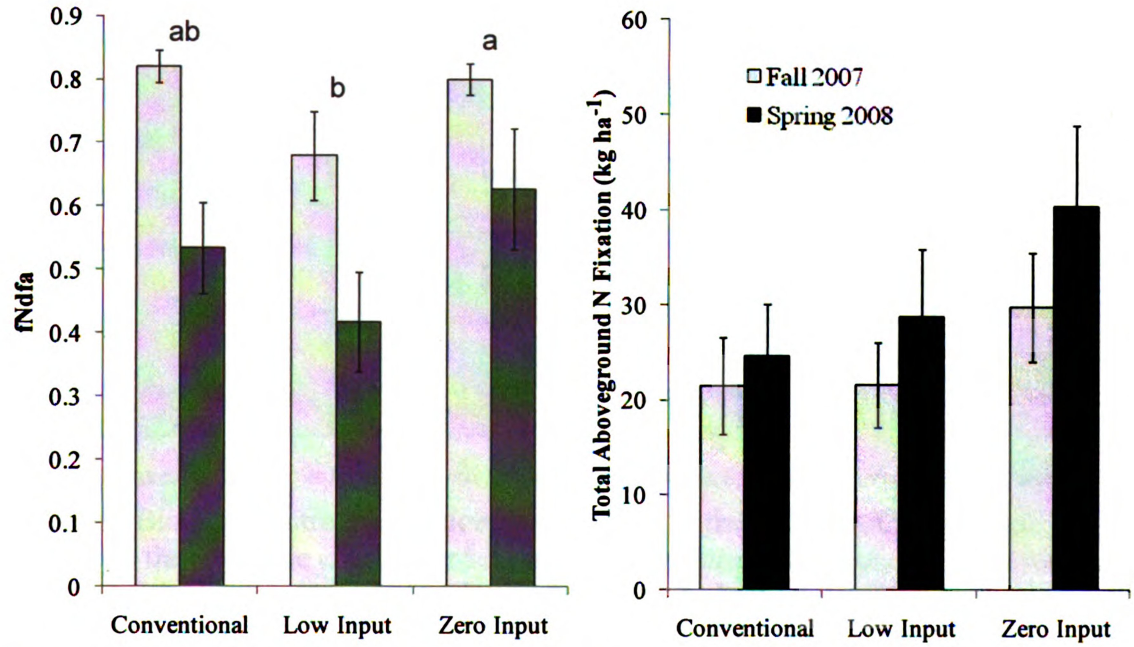

Figure 9. Biological nitrogen fixation by red clover in the LTER using wheat as the reference crop. Error bars indicate standard error. Statistical differences between treatments are indicated by letters on top of the bars ($P \leq 0.05$).

Differences in fNdfa did not translate to significant differences in total BNF between treatments in the LTER with wheat as the reference crop (Figure 9, Appendix:

Table 18). Total nitrogen fixation increased from 24 kg ha^{-1} to 31 kg ha^{-1} through time across treatments ($F_{1,13} = 5.24$, $P = 0.040$).

Differences between cropping system treatments observed with wheat as the reference crop were not confirmed by analogous comparisons when perennial ryegrass was the reference crop, which revealed no differences in fNdfa among cropping systems (Figure 10, Appendix: Table 19). Similar to previous comparisons with wheat as a reference crop, fNdfa estimates were lower during the spring sample compared to the fall sample ($F_{1,9} = 37.12$, $P = 0.0002$).

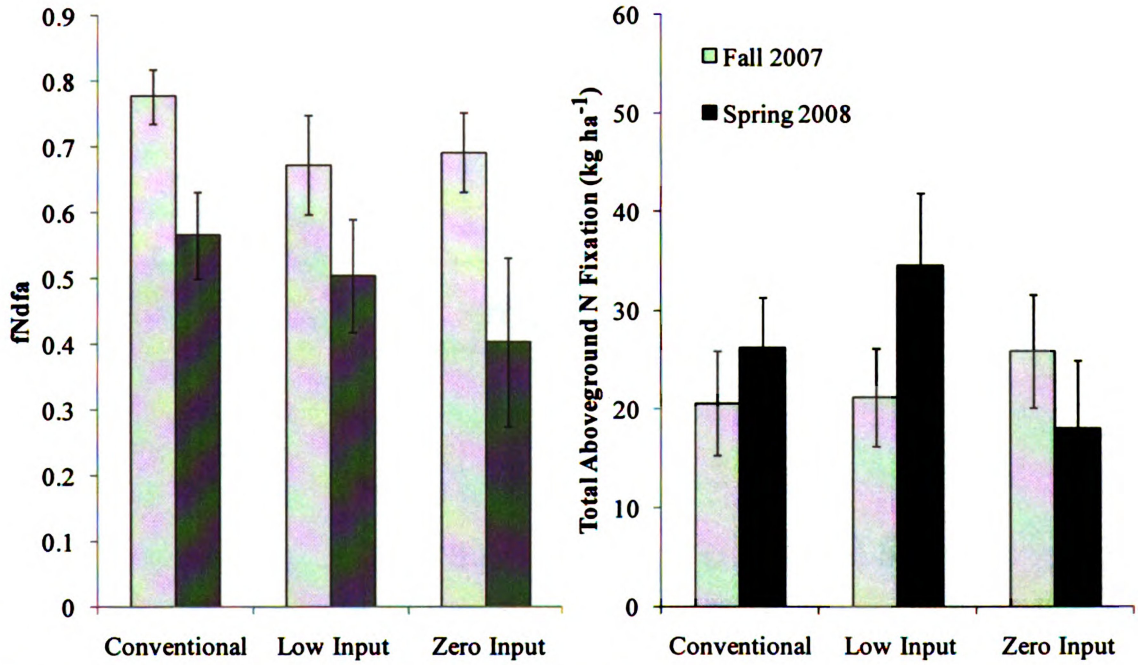

Figure 10. Biological nitrogen fixation by red clover in the LTER using perennial ryegrass as the reference crop. Error bars indicate standard error. Statistical differences between treatments are indicated by letters on top of the bars ($P \leq 0.05$).

Total BNF utilizing ryegrass as the reference crop (Figure 10) showed a marginally significant interaction between treatment and sampling time ($F_{2,9} = 2.95$, $P = 0.104$) stemming from greater growth in the Low Input system. Due to this interaction, we analyzed sampling times separately. At the time of spring sampling, the Low Input system exhibited 92% higher total fixed nitrogen relative to the Zero Input system, but was marginally statistically different ($t_9 = 2.08$, $P = 0.068$). No differences were detected between sampling dates.

In the LFL, fNdfa values averaged across both seasons were 28% higher in the Conventional treatment compared to the Compost treatment ($t_{18} = 2.45$, $P = 0.025$) while the Low Input treatment exhibited fNdfa values between the other two treatments, but not significantly different from either of the other two treatments (Figure 11, Appendix: Table 20). No differences were found between the two years for estimated fNdfa ($F_{1,18} = 0.35$, $P = 0.56$).

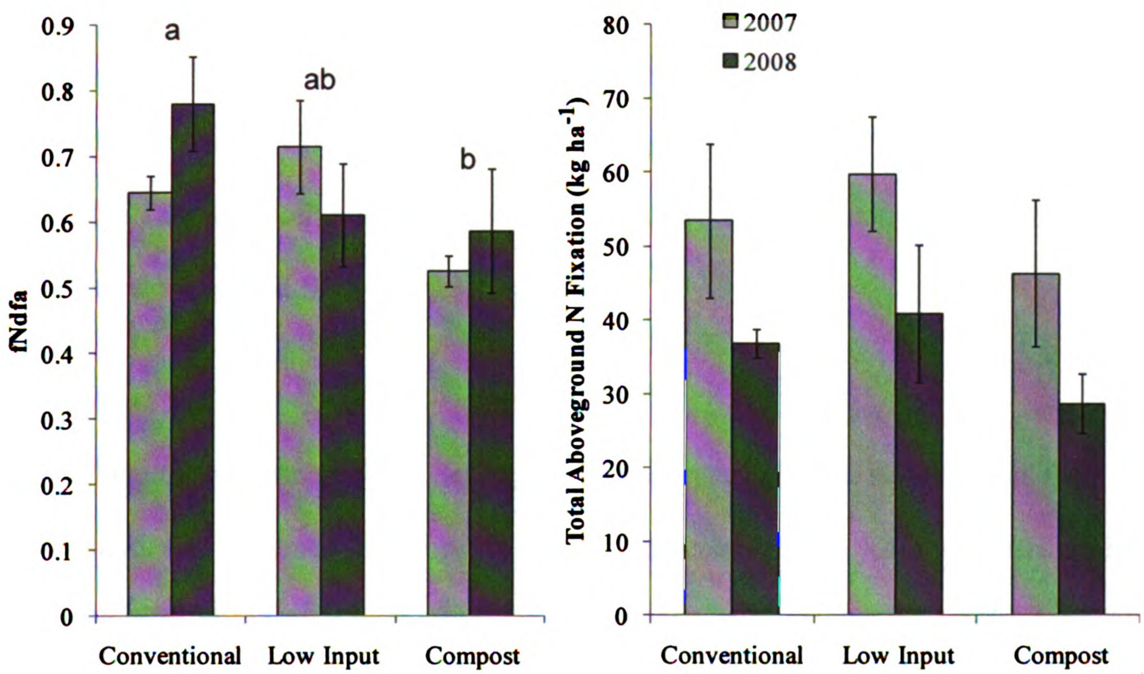

Figure 11. Biological nitrogen fixation in the LFL across the two experimental seasons. Error bars indicate standard error. Statistical differences between treatments are indicated by letters on top of the bars ($P \leq 0.05$).

Similar to the LTER, these differences in fNdfa did not translate to differences in total aboveground BNF, which ranged from 29 kg ha^{-1} in the Compost treatment in 2008 to 60 kg ha^{-1} in the Low Input treatment in 2007. Across treatments, total BNF values were 50% higher in 2007 compared to 2008 ($F_{1,18} = 7.83$, $P = 0.013$), consistent with increased total aboveground biomass in 2007 relative to 2008.

Is Nitrogen Fixation related to Soil Nitrogen Properties?

No significant differences between treatments were identified in either experiment for soil inorganic nitrogen or nitrogen mineralization potential (Appendix: Table 21, 22, 23, 24, 25). Inorganic nitrogen levels present in the soil on April 7, 2008 were 113% higher in the LFL relative to the LTER. Consequently, 30 day NMP levels were negative on average in the LFL, indicating net immobilization, whereas LTER values were positive.

No relationships were identified between soil nitrogen properties and fNdfa across both experiments; the overall models for all four regression analyses were not significant ($P > 0.05$). Likewise soil inorganic nitrogen levels and NMP were not significantly related to aboveground BNF in either experiment.

Discussion

Legume reliance on BNF, defined as the fraction of nitrogen derived from the atmosphere, was influenced by cropping system, particularly when wheat was used as the reference crop. Data trends were similar across both experiments, but were not entirely consistent. In the LTER, the Low Input cropping system exhibited lower percent of nitrogen from fixation (fNdfa) compared to the Zero Input system, suggesting more soil nitrogen was available to the clover plants in the Low Input system. Data from the LFL were consistent with the hypothesis that systems receiving organic nitrogen inputs over time exhibit lower percent of nitrogen from fixation values than systems without regular organic inputs, as the Compost system exhibited the lowest percent of nitrogen from fixation while the conventional system exhibited the highest percent.

Comparing results from these two experiments, systems managed with organic nitrogen sources and herbicides exhibited lower percent of nitrogen from fixation relative to systems that relied on inorganic fertilizers and herbicides. Yet, the system in the LTER that relied solely on organic nitrogen sources without herbicides displayed rates of nitrogen fixation similar to conventional systems. This finding could be explained by two prevailing hypotheses. First, utilizing legume cover crops in combination with compost may be more effective at increasing soil organic matter and subsequent nitrogen mineralization compared to utilizing cover crops alone as a fertility source, subsequently leading to reductions in nitrogen fixation by legumes in the system. Second, Fox *et al.* (2007) have shown that herbicides influence the community of nitrogen fixing bacteria in the soil, which could result in depressed nitrogen fixation in systems with regular herbicide additions relative to organically managed systems. In this experiment, the Zero Input system was the only treatment that did not receive regular herbicide treatments. But, based on wheat as a reference crop, the Zero Input system exhibited higher percent of nitrogen from fixation than the Low Input treatment in the LTER, which did receive regular herbicide treatments.

In the LTER, percent nitrogen in the plant samples increased while the percent of nitrogen from fixation decreased between the two sampling periods. These trends could be related as clover plants higher in nitrogen have lower needs for more atmospheric nitrogen. As for the decrease in the percent of nitrogen from fixation, the clover plants may have been able to access more soil nitrogen during the spring relative to the fall, potentially taking up nitrogen from parts of the clover plants that senesced during the winter season. This possibility could result in over-estimates of the percent of nitrogen from fixation and BNF using the natural abundance technique. In the LFL, the percent of nitrogen from fixation did not differ between years even though clover biomass and percent nitrogen were higher in 2007 relative to 2008, indicating relative constancy in the percent of nitrogen from fixation across variable growing conditions.

The utilization of organic plus inorganic nitrogen sources in the LTER Low Input systems resulted in decreased rates of nitrogen fixation relative to the Zero Input system. Differences in the percent of nitrogen from fixation between systems did not translate to statistical differences in total nitrogen fixation and aboveground biomass. This result supports the hypothesis that legumes with relatively high percent of nitrogen from fixation grow at slower rates than plants with relatively lower percent of nitrogen from fixation. There are at least two distinct possibilities to explain this phenomenon. First, legumes growing in poor soil conditions, likely low in available nitrogen, compensate by accessing more atmospheric nitrogen via associations with *Rhizobia* (van Kessel and Hartley, 2000). Second, nitrogen fixation is energetically costly for legumes (Vitousek *et al.*, 2002). Thus, legume plants that access a high proportion of nitrogen from the atmosphere may grow at slower rates than comparable plants that access a lower proportion of nitrogen from the atmosphere. In fact, it is likely that both mechanisms are operating simultaneously, limiting the total amount of nitrogen fixed from the atmosphere by any given plant.

Wheat grain was a superior reference crop compared to perennial ryegrass, as $\delta^{15}N$ values for perennial ryegrass varied widely (ryegrass CV = 5.0, wheat CV = 2.8) and were negative in three instances. Negative $\delta^{15}N$ for the reference plant are abnormal, but not impossible, and may indicate that the reference plant was accessing nitrogen derived from neighboring legumes rather than mineralized soil N (Ledgard and Steele, 1992). Less likely is that negative $\delta^{15}N$ values could also indicate the presence of associative N fixation by perennial grasses, which has been demonstrated in dune grasses (Dalton *et al.* 2004) and several tropical C4 grasses (Lima *et al.*, 1987; Urquiaga *et al.*, 1992; Dobereiner, 1993; Boddey and Dobereiner, 1995). Two of the three negative fNdfa estimates were calculated in the Zero Input treatment of the LTER. If associative nitrogen fixation does occur with perennial ryegrass, it is possible that herbicides influence this relationship (Fox *et al.*, 2007).

Soil nitrogen properties did not significantly differ between treatments in either experiment, although there was a trend towards increasing soil nitrogen from conventional to organically managed systems. Nitrogen fixation was not significantly influenced by soil nitrogen characteristics based on the results of the multiple regression analyses, which was contrary to the prediction in Figure 8. The lack of a relationship between nitrogen fixation and soil nitrogen at the plot scale might stem from inherent spatial variability in soil nitrogen availability, as soil samples and plant samples were not taken at exactly the same point in the plot. Qualitative comparisons show that soil organic matter might be a better predictor of nitrogen fixation based on data in Table 12 compared to patterns of the percent of nitrogen from fixation in this experiment.

Legume seed is expensive for farmers to purchase, and legumes are often difficult to establish or exhibit poor growth. As soil nitrogen supply increases through time in ecologically based cropping systems utilizing diverse fertility sources, legumes may become less important for supplying biologically fixed nitrogen. Continued biological nitrogen fixation by legume cover crops that are not harvested for forage could lead to an excess supply of soil nitrogen, leading to environmentally significant losses to surface water and ground water, but evidence from this experiment suggests that legumes like red clover may effectively regulate nitrogen inputs, as the rate of nitrogen fixation was limited in systems managed with organic inputs. The symbiotic relationship between *Rhizobia* and legumes affect natural and managed ecosystems. The complexity of the response of this symbiosis to external drivers has stymied attempts to predict ecosystem outcomes. The global and local importance of this source of biologically-available nitrogen highlights the need for further integrated research.

Conclusion

Across experiments, red clover fixed 64% of its nitrogen from the atmosphere, corresponding to 40 kg N ha^{-1} in aboveground biomass when sampled in May prior to termination. Proportions of nitrogen fixed from the atmosphere varied by treatment, with 23% higher percent of nitrogen from fixation in the Low Input treatment of the LTER relative to the Zero Input treatment, and 22% higher percent in the Compost treatment of the LFL compared to the Conventional treatment. Nitrogen fixation was independent of soil nitrogen, which differed little to none between treatments. Overall results suggest support for the hypothesis that cropping system management influences rates of nitrogen fixation, but more work is needed to uncover the specific mechanisms governing these differences.

Appendix: Additional statistical analyses and data tables.

Table 16. Repeated measures ANOVA for LTER aboveground biomass, percent nitrogen and nitrogen in aboveground biomass.

Source of Variation	DF Num	DF Den	Probability of significant F test		
			Aboveground Biomass (kg ha^{-1})	%N in Biomass	Aboveground N (kg ha^{-1})
Treatment	2	15	0.287	0.879	0.336
Time	1	13	<.0001	0.0002	<.0001
Treatment*Time	2	13	0.672	0.057	0.528

Table 17. Two-way ANOVA for LFL aboveground biomass, percent nitrogen and nitrogen in aboveground biomass.

Source of Variation	DF Num	DF Den	Probability of significant F test		
			Aboveground Biomass (kg ha^{-1})	%N in Biomass	Aboveground N (kg ha^{-1})
Treatment	2	18	0.717	0.024	0.380
Year	1	18	0.004	<.0001	<.0001
Treatment*Year	2	18	0.391	0.235	0.345

Table 18. Repeated measures ANOVA for LTER fNdfa and total biological nitrogen fixation with wheat as the reference crop.

Source of Variation	DF Num	DF Den	Probability of significant F test	
			fNdfa	Total BNF (kg ha^{-1})
Treatment	2	15	0.107	0.243
Time	1	13	<.0001	0.040
Treatment*Time	2	13	0.449	0.839

Table 19. Repeated measures ANOVA for LTER fNdfa and total biological nitrogen fixation with perennial ryegrass as the reference crop.

Source of Variation	DF Num	DF Den	Probability of significant F test	
			fNdfa	Total BNF (kg ha^{-1})
Treatment	2	18	0.396	0.580
Year	1	18	<.001	0.319
Treatment*Year	2	18	0.136	0.104

Table 20. Two-way ANOVA for LFL fNdfa and total biological nitrogen fixation.

Source of Variation	DF Num	DF Den	Probability of significant F test	
			fNdfa	Total BNF (kg ha^{-1})
Treatment	2	18	0.070	0.285
Year	1	18	0.561	0.013
Treatment*Year	2	18	0.182	0.989

Table 21. Soil inorganic nitrogen properties in the LTER. Means are shown with standard errors in parentheses (soil samples taken on 4/7/08)

Treatment	**NO_3^- (μg N g soil^{-1})**	**NH_4^+ (μg N g soil^{-1})**	**Total Inorganic N (μg N g soil^{-1})**
Conventional	3.53 (0.21)	2.58 (0.32)	6.10 (0.49)
Low Input	3.74 (0.07)	2.81 (0.28)	6.56 (0.32)
Zero Input	3.76 (0.12)	2.93 (0.19)	6.69 (0.28)

Table 22. Thirty-day nitrogen mineralization potential in the LTER. Means are shown with standard errors in parentheses (soil samples taken on 4/7/08).

Treatment	**NO_3^- NMP (μg N g soil^{-1} day^{-1})**	**NH_4^+ NMP (μg N g soil^{-1} day^{-1})**	**Total NMP (μg N g soil^{-1} day^{-1})**
Conventional	0.152 (0.037)	-0.022 (0.010)	0.129 (0.045)
Low Input	0.121 (0.026)	-0.003 (0.017)	0.118 (0.032)
Zero Input	0.125 (0.049)	-0.022 (0.008)	0.104 (0.050)

Table 23. Soil inorganic nitrogen properties in the LFL. Means are shown with standard errors in parentheses (soil samples taken on 4/7/08)

Treatment	**NO_3^- (μg N g soil^{-1})**	**NH_4^+ (μg N g soil^{-1})**	**Total Inorganic N (μg N g soil^{-1})**
Conventional	5.39 (0.40)	6.81 (1.37)	12.19 (1.69)
Low Input	4.70 (0.19)	8.36 (2.32)	13.06 (2.42)
Compost	5.67 (0.50)	10.30 (4.11)	15.96 (3.96)

Table 24. Thirty-day nitrogen mineralization potential in the LFL. Means are shown with standard errors in parentheses (soil samples taken on 4/7/08).

Treatment	**NO_3^- NMP (μg N g soil^{-1} day^{-1})**	**NH_4^+ NMP (μg N g soil^{-1} day^{-1})**	**Total NMP (μg N g soil^{-1} day^{-1})**
Conventional	0.155 (0.045)	-0.150 (0.046)	0.005 (0.082)
Low Input	0.147 (0.014)	-0.264 (0.137)	-0.019 (0.051)
Compost	0.162 (0.044)	-0.181 (0.072)	-0.117 (0.129)

Table 25. ANOVA results within both experiments for soil nitrogen properties.

Experiment	Source of Variation	DF Num	DF Den	Probability of significant *F* test	
				Inorganic N	NMP
LTER	Treatment	2	15	0.519	0.913
LFL	Treatment	2	9	0.634	0.637

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CHAPTER FOUR

Wilke, B.J., Kunkle, J., 2009. What does Agriculture have to do with Climate Change? Teaching Issues and Experiments in Ecology (TIEE) 6, Figure Sets. http://tiee.ecoed.net/vol/v6/figure_sets/climate_change/abstract.html.

CHAPTER FOUR

What does agriculture have to do with climate change?

Abstract*: Agriculture is a substantial contributor of greenhouse gases to the atmosphere. Yet, agricultural ecosystems can be managed for reduced greenhouse gas emissions, and can even become net sequesterers of greenhouse gases. In this chapter, we review literature pertaining to greenhouse gas emissions from agroecosystems, and present it in a format for educational purposes, particularly in college science classrooms. Three major greenhouse gases are emitted from agroecosystems; carbon dioxide, methane and nitrous oxide. We used published figures and data to guide students through the process of how each of these gases are produced in agroecosystems. In turn, we present the process by which soil can be a carbon reservoir and how agroecosystems can sequester carbon dioxide from the atmosphere. Finally, we examine a range of agroecosystems that vary from emitting to sequestering greenhouse gases, and explore the management scenarios that lead to these different outcomes.

***Key Words*:** Agriculture, Climate Change, Education, Greenhouse Gas, Global Warming Potential

Figure Set Homepage

Title: What does agriculture have to do with climate change?

The Issue: Agriculture is a major contributor of greenhouse gases, Certain management practices can substantially reduce greenhouse gas emissions, but these practices are not always economically viable for farmers.

Ecological Content: Oxidation of soil organic carbon due to agricultural management, sources of methane in agriculture, conversion of soil nitrogen to nitrous oxide, radiative forcing of greenhouse gases, carbon sequestration in agricultural soils, global warming potential from agricultural ecosystems. Other key words include carbon cycle, fertilizer, organic agriculture, no-till, carbon sources and carbon sinks.

Student-active Approaches: Turn to your Neighbor, Think Pair Share, Guided Class Discussion, Paired Think Aloud, Citizen's Argument

Student Assessments: Short Essay, Minute Paper, Land Management Activity

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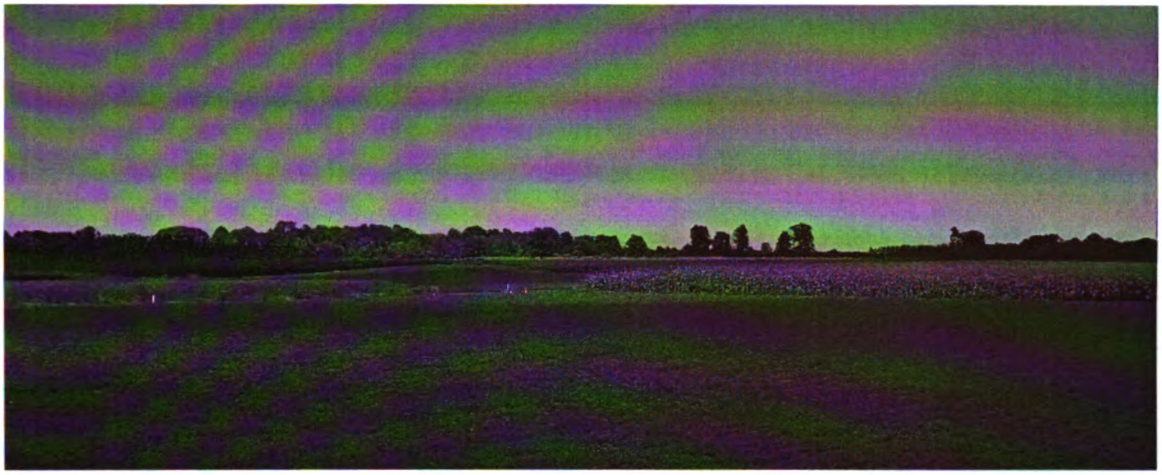

Figure 12. Cover Image Legend: Agricultural management practices in the Long Term Ecological Research (LTER) experiment at the W.K. Kellogg Biological Station (KBS) range from high intensity (Conventional Row Crop Management) to low intensity (Old Growth Forest). Many of these practices are visible in this mid-summer photo. Alfalfa, which will be harvested for animal feed, is growing in the foreground. Corn harvested for grain is growing on the right side of the photo while an old field successional plot is on the left side. Poplar trees, which are harvested for biomass, and hardwood forests are visible in the background.

Figure 12 Copyright: Photo taken from the W.K. Kellogg Biological Station Long Term Ecological Research website (www.lter.kbs.msu.edu).

Overview

What Is the Ecological Issue?

Agriculture provides important ecosystem services in the forms of food and fiber, but can also convey many disservices to agroecosystems themselves and to the ecosystems affected by agricultural practices. In particular, agricultural activities contribute substantial amounts of greenhouse gases, including more methane and nitrous oxide than any other human activity. For example, Duxbury (1994; PDF included) estimated that agriculture contributes 25%, 65% and 90% of all anthropogenic emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), respectively.

Several processes identified below are responsible for greenhouse gas emissions in production agriculture:

- Fossil fuels are oxidized to provide energy for machinery involved in tilling, planting and harvesting.
- Initial cultivation of previously untilled soil results in substantial losses of carbon previously stored in soil organic matter (Robertson and Grace 2004). This occurs because tillage increases oxygen supply to soil organisms and exposes previously protected soil organic matter to decomposers.
- Inputs such as nitrogen fertilizer, irrigation and manure can increase plant productivity and soil carbon sequestration, but don't necessarily result in a net decrease in carbon dioxide emissions due to the fossil fuel energy requirements to provide these inputs (Schlesinger 1999; PDF included).

- Nitrogen fertilization and tillage decrease the amount of CH_4 sequestered by soils because of a decrease in the abundance of methanotrophic bacteria in soil (Goulding *et al.* 1995).
- Nitrogen fertilization and tillage increase the amount of N_2O given off to the atmosphere through the processes of nitrification and denitrification (Mosier *et al.* 1991).
- Nitrogen fertilizer is produced using energy from fossil fuels, and applications of nitrogen fertilizer can result in high nitrous oxide emissions.

Certain management activities have been shown to reduce agricultural greenhouse gas emissions after accounting for all inputs and emissions (i.e., Net Global Warming Potential) (Robertson *et al.* 2000; pdf included). For example, no-till agriculture reduces soil disturbance, thus increasing soil aggregation and decreasing available oxygen for decomposition. Growing winter cover crops increases net primary productivity and inputs of organic carbon to the soil. Perennial plants have expansive root systems and have long growth periods, thus increasing soil carbon storage (Cox *et al.* 2006).

In this activity, students investigate three sources of greenhouse gas emissions from agriculture, and how different cropping methods, including no-till, organic and perennialization, affect global warming potential. In addition, students will discuss potential trade-offs that limit the broad application of these practices and identify tactics that may aid in the reduction of global warming potential from agriculture. The PDFs of several articles are included as resources with this Figure Set.

These Figure Sets have been developed over a period of time when they were used to teach high school ecology students, incoming first year college students and high school science teachers. We believe that these activities could be used in a range of classes, from high school biology up to graduate level biogeochemistry. Material is presented in a format that can be used directly in class, but instructors may need to modify the Figure Sets to better fit their objectives.

Table 26. Figure set summary table.

Figure Set and Ecological Question	**Student-active Approach**	**Cognitive Skill**	**Class Size/Time**
1 Cultivation and Soil Carbon losses (Robertson and Grace 2004)	Turn to Your Neighbor	Knowledge, interpretation, application	Any / Moderate
2 Methane Emissions from Agriculture (Moss *et al.* 2000; IPCC 2007)	Think Pair Share	Knowledge, interpretation	Any / Short
3 Nitrogen Fertilizers Increase Nitrous Oxide Emissions (McSwiney and Robertson 2005; IPCC 2007)	Guided Class Discussion	Knowledge, interpretation, synthesis	Any / Short
4 Carbon Sequestration in Degraded Agricultural Soils (Robertson *et al.* 2000)	Paired Think Aloud	Knowledge, interpretation, synthesis	Any / Short
5 Global Warming Potential – Temperate Agriculture (Robertson *et al.* 2000)	Citizens Argument	Knowledge, interpretation, analysis, synthesis	Small (can be adapted to large classes) - Long

Figure Sets*: figures or tables from published papers
Student-active approach*: a suggested approach appropriate for the cognitive skill, time, and class size
Cognitive skill*: One or more of Bloom's taxonomic skills (e.g. knowledge, comprehension, interpretation, analysis, application, synthesis; see http://tiee.ecoed.net/teach/teach_glossary.html#cognitive) that the exercise emphasizes. The editors are available to provide assistance.
Class size*: small class size > 26, medium 26-50, large is < 50; time is short, moderate, or long

FIGURE SET 1: CULTIVATION AND SOIL CARBON LOSSES

- **Purpose:** To teach students that cultivation of crops for food results in the oxidation of soil organic carbon, which in turn contributes a substantial amount of carbon dioxide to the atmosphere.
- **Teaching Approach:** Turn to your neighbor
- **Cognitive Skills:** Knowledge, Interpretation, Application
- **Student Assessment:** Post Lesson Assessment Essay

Figure Set 1 Background

Prior to European colonization of the U.S. Great Plains, prairies were the dominant plant communities. The soils of the prairie landscapes contained relatively high amounts of organic carbon, possibly more than 50,000 kg of carbon per hectare stored in the topsoil, which is equivalent to the amount of carbon found in 20,000 gallons of gasoline (calculations based on 2.5% soil carbon, 20 cm deep topsoil and soil bulk density of 1 g cm^{-3}).

In Figure 13, Robertson and Grace (2004) redrew this graph from Haas (1957) to show how cultivation of crops for food decreases soil carbon. Soil carbon at two sites in Kansas was measured prior to initiation of cultivation and was monitored for over 40 years to track soil carbon losses. Cultivation (tillage, fertilization, long fallow periods) resulted in the oxidation of soil organic carbon and although the two sites differed in total carbon loss, both sites exhibited a negative exponential trend. It is assumed that soil carbon eventually reaches a steady state if cultivation continues for many years.

Figure Set 1 Figures and Tables

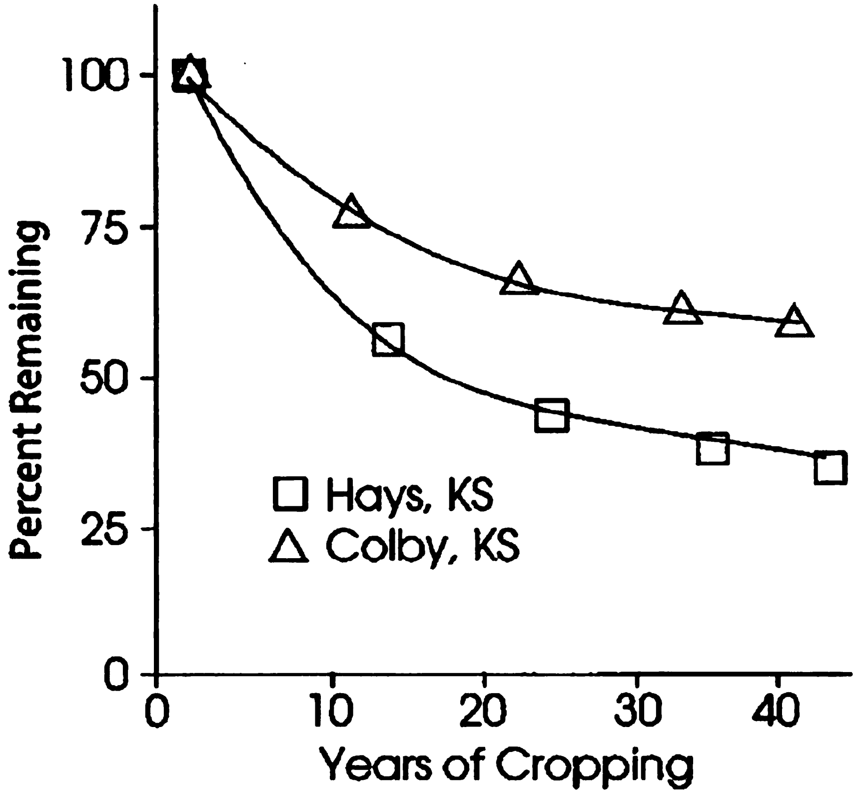

Figure 13. Average percent soil carbon remaining in the top 15 cm of soil in two Kansas locations following the initiation of cultivation on previously undisturbed prairie soils. Data presented in the figure represent the average across a variety of crop rotations from 1903-1946. Initial soil organic carbon levels were higher in Hays, KS (2.47%) than in Colby, KS (1.83%). Both locations were cultivated using traditional agricultural practices including tilling the soil and growing annual crops that included wheat, oats, barley, corn, kafir and milo. No manure was applied to the fields. Baseline measurements were calculated using adjacent undisturbed prairies at the culmination of the study.

Figure 13 Copyright: The image is published as Figure 1 in Robertson and Grace (2004) in the journal *Environment, Development and Sustainability.*

Figure Set 1 Student Instructions

Warm Up Exercise – Turn to your neighbor and take five minutes to answer the following question. You are allowed to use a calculator if you have one.

One morning, two old men are drinking coffee and are debating who contributed more carbon dioxide to the atmosphere during their lifetime. The first man (Fred) was a farmer and converted 50 hectares of prairie to corn fields 40 years ago (50,000 kg soil carbon per hectare), growing corn ever since while using draft horses for power. The amount of soil carbon in his fields declined by 50% during this period. The second man (Joe) was a carpenter, and drove an average of 130 kilometers round trip to work in his pickup truck 200 days per year for 40 years, getting an average of 6 kilometers per liter of gasoline. There are 0.72 kg of carbon per liter of gasoline.

- Who was responsible for more carbon dioxide emissions during their lifetime, Fred or Joe? If you feel stuck, start here: what is the source of carbon dioxide for Joe's and Fred's activities? Then, how would you start doing this calculation? What do you need to know?

Discuss the following questions with a neighbor. After each set of questions, your instructor will talk about the question set with the entire class before moving on to the next question set.

Question 1. Look at Figure 13 and make sure that you understand the X and Y axes. Next, describe the pattern displayed by the two lines.

- During what time period was the rate of change of C loss the highest?
- What happens to the rate of loss over time?
- How would you describe the pattern of change over the 40 years?
- Why do you think the decline in soil C levels off with time?

Question 2. If carbon is lost from the agricultural soil over time, this carbon has a source (comes from somewhere) and goes somewhere (sink). Prior to cultivation, one hectare (about two football fields) of agricultural land in the Midwest could have contained more than 50,000 kg of carbon, which is equivalent to the amount of carbon in about 20,000 gallons of gasoline.

- Where did that much carbon come from and how did it get into the top soil?
- During the cultivation of crop plants, do you think organic carbon was still entering the top soil?
- Since the grain (seeds of crop plants) is removed from the field for food, is there more or less carbon left on the soil surface compared to the original prairie?
- What plant parts other than the grain might remain in the soil as organic carbon?

Question 3. Soil contains many different types of microorganisms such as bacteria and fungi. There is a very common microbial process in which these microorganisms use organic carbon in the soil.

- What it is this process called?
- Why to microorganisms engage in this process?
- What ultimately happens to the organic carbon?

Question 4.

- How could tillage increase the rates of decomposition by soil microorganisms?
- What gas do these microorganisms require for cellular respiration?
- Would tilling the soil change the size of particles composed of organic carbon (e.g. dead plant material)?
- In agriculture, there are often periods when the soil is bare and no plants are growing. Does decomposition of soil organic matter by microorganisms stop when plants stop growing?

Question 5. Cellular respiration by microorganisms results in the conversion of soil organic carbon to carbon dioxide.

- Why is it important to monitor the amount of carbon dioxide in the atmosphere?

Figure Set 1 Notes to Faculty

The student active approach suggested here is "Turn to your Neighbor." This approach asks students to "turn to your neighbor" to discuss problems in small groups (2-4 students). Problems must be challenging to the degree that students will want to discuss it in groups, but not to the point that it becomes frustrating. We do not expect that students will have adequate background knowledge to answer all questions in this Figure Set. However, they should be able to come up with some well thought out responses. It is your responsibility as the teacher to stop the class after each question or set of questions (depending on whether students are more or less advanced) to discuss their answers and questions before moving on. Some questions are not directly related to the figure, but are important for the students to understand so that they connect soil carbon loss to climate change.

Depending on your area of expertise, this Figure Set may require you to do some background reading on the topics included. The resource section includes some references for this purpose, especially the suggested textbook (Schlesinger 1997). The questions will also require a substantial amount of interaction during class between the students and yourself. We've included a basic carbon cycle figure as Figure 14 for your general reference and as a potential handout for students.

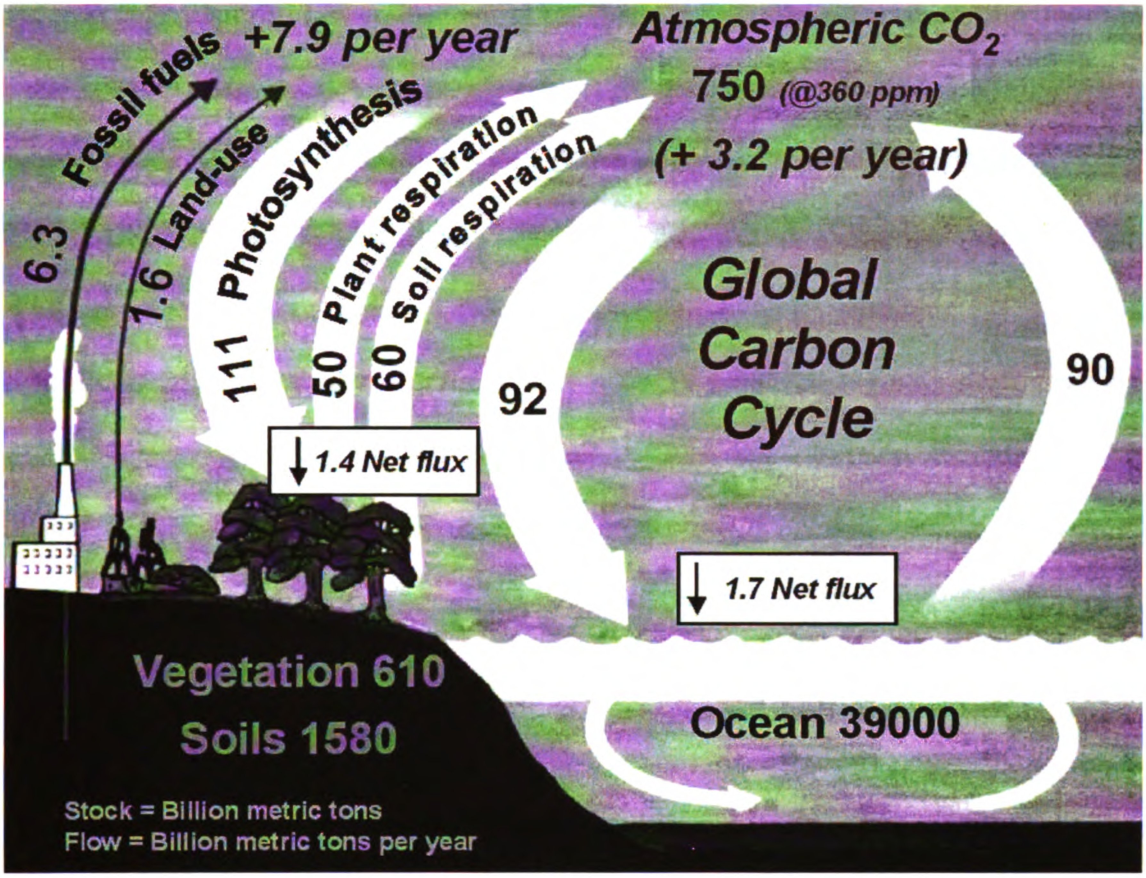

Figure 14. The global carbon cycle includes pathways, fluxes and pools of carbon. Soils contain nearly three times more carbon than living vegetation.

Figure 14 Copyright: Photo courtesy of Dr. George Kling at the University of Michigan. http://www.globalchange.umich.edu/globalchange1/current/lectures/kling/carbon_cycle/carbon_cycle_new.html

Prior to European colonization, prairies were the dominant plant communities in the Great Plains region of the United States. Settlers realized that these areas were very fertile and plowed up the prairies to plant crops such as corn, oats and wheat. After the first 40 years of cropping, approximately 40% of soil carbon was lost in the topsoil of an agricultural field in Hays, KS and 60% of soil carbon was lost in another field in Colby, KS. Figure 13 shows these trends in soil carbon from 1903-1946. Hays is in west, central Kansas while Colby is in the northwest corner of Kansas. Initial soil organic matter levels were lower in Colby than in Hays (see figure legend), which could help to explain the differences in total soil carbon lost during the study period. The data presented represent the average of a variety of crop rotations on soils not amended with fertilizers. Baseline data was collected from adjacent undisturbed prairie soils at the end of the study (1946).

These losses in soil carbon occurred because cultivation (tillage and periods of no plant growth) resulted in increased decomposition of soil organic carbon by soil organisms, such as bacteria and fungi. These soil organisms use the carbon for energy via cellular respiration, converting the organic carbon in soil to carbon dioxide in the atmosphere. Figure 15 provides a visual comparison of soils from an annually tilled field and a native tallgrass prairie. The color difference between soils is assumed to reflect the differences in organic carbon in these soils.

Figure 15. Soils from two adjacent Kansas fields, an annually tilled agricultural field (left) and a native tallgrass prairie (right), are shown in palms of a research scientist. The soil from the native prairie is dark in color and contains substantial amounts of roots and soil aggregates whereas the soil from the tilled field is lighter in color, lacks roots and clumps together.

Figure 15 Copyright: Photo courtesy of Steve Culman, graduate student at Cornell University. (http://www.people.cornell.edu/pages/swc25/landinstitute.html)

Many students have trouble understanding the complete carbon cycle. This activity will take them step by step through the basics of the soil carbon cycle, from fixation of carbon by plants to decomposition by soil microorganisms. Simultaneously, they will learn that cultivation results in the loss of organic carbon from soil. The figure used in this set (Haas 1957) is fairly easy to comprehend and should not cause too much confusion. It simply outlines how much carbon is lost from soil during cultivation. Students are asked to examine the rate at which the carbon is lost through time, and to identify that carbon is lost from soil at a faster rate immediately after initiation of cultivation compared to later years of cultivation.

Students are asked to consider a series of five question sets. After each question set, the instructor should discuss the question with the class to make sure they understand the answers before proceeding to the next question. Notes for specific questions are listed below.

Warm Up Exercise

Students are asked to do some simple calculations to find out whether a farmer using horses for power or a carpenter driving to work every day is responsible for more carbon dioxide emissions during their lifetime. This activity is designed to make students realize that a substantial amount of carbon is lost from soil during cultivation, and to pique their interest in the topic before the rest of the activity. The idea is that they might start asking how so much carbon can be lost from soil during cultivation.

The farmer (Fred) is responsible for 1,250,000 kg of carbon released to the atmosphere from soil (50,000 kg C / ha x 50 ha x 50% soil carbon loss). The carpenter (Joe) is responsible for 124,800 kg of carbon released to the atmosphere from gasoline oxidation ((130 km x 200 days per year x 40 years) / 6 km per liter of gasoline x 0.72 kg carbon per liter of gasoline).

Question 1.

Students are asked to describe Figure 13 and explain what happened. They must understand the figure before moving on to the next questions. It is essential that the students understand that the rate of carbon loss from soil is greatest during the first several years after the initiation of cultivation, but the rate of carbon loss slows through time and the amount of soil carbon approaches a steady state. This may also be a good time to make sure that students correctly understand the definition of cultivation. Cultivation is agricultural production of food by preparing the land to grow crops and includes tillage, fertilization, planting, harvesting, and other methods of creating an optimal habitat for crop species.

The amount of soil carbon approaches a steady state through time. This new equilibrium occurs when inputs of new plant biomass are balanced by outputs through microbial respiration, and is a function of climate, soil characteristics, agronomic management and residue management.

Question 2.

Students are asked to consider where the soil carbon came from originally, and where it came from during the years of cultivation. Important points are as follows: (1) soil carbon comes from plant biomass that is fixed during photosynthesis (roots, shoots, root carbon exudation); (2) removal of crop biomass for food reduces the amount of carbon entering the soil and (3) carbon is stored in soil as dead plant material or dissolved organic carbon in soil water. One potential problem here is that students may just say 'crops' and really not understand the processes of how plant roots, etc. end up as particulate organic carbon and dissolved organic carbon. You may choose to introduce the term 'senescence' while discussing this topic.

Figure 15 and Figure 16 are presented here as optional figures to use in this activity. These figures are useful during classroom discussion of Question 2, as they highlight differences in root biomass between tilled and untilled fields, helping students understand why untilled crop fields retain soil carbon and tilled fields lose soil carbon. As the teacher, you may choose whether or not to use them in the discussion with your students.

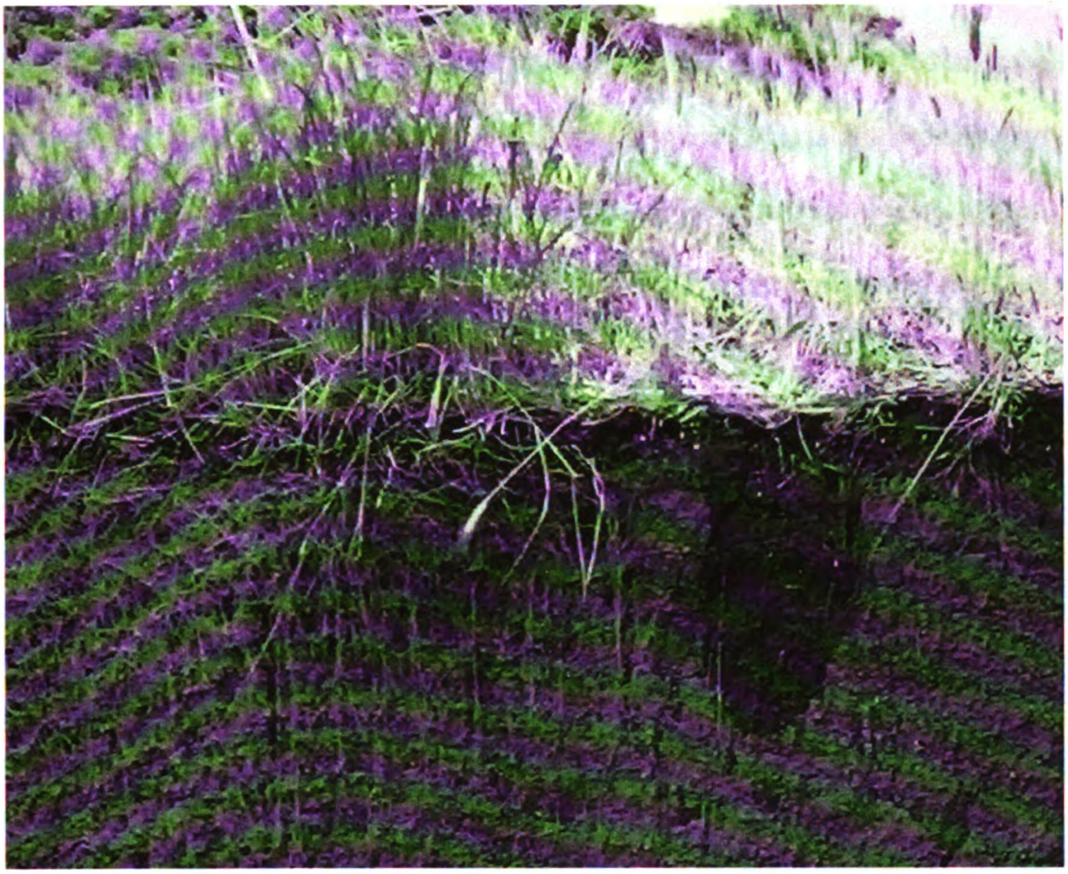

Figure 16. The soil profile can be seen for a perennial prairie plant on the left (intermediate wheatgrass – *Thinopyrum intermedium*) and annual wheat on the right (*Triticum aestivum*). Many roots can be seen underneath the intermediate wheatgrass plants. These roots, in addition to the shoots, will turn over at the end of the growing season resulting in the organic carbon inputs to the soil.

Question 3

Students consider what microorganisms live in soil and how they transform carbon. Important points for students to understand are that microorganisms utilize the potential energy in soil organic carbon via cellular respiration, and that the organic carbon is converted into carbon dioxide. A common misconception here is that students think the carbon is converted into energy, and thus disappears.

Question 4

Students consider why agricultural practices might result in increased microbial decomposition rates. These practices include tillage (Figure 17), which increases oxygen concentrations in the soil and breaks up soil aggregates, which protect organic carbon from decomposers. Decomposition also continues to occur during periods of no plant growth, which are common in agricultural fields.

Figure 17. Tillage in agriculture is a major disturbance to soil, breaking up soil aggregates and increasing the amount of oxygen available to aerobic decomposers. The field shown here has recently been tilled prior to spring planting.

Figure 17 Copyright: Photo taken by Brook Wilke

Question 5

To bring the lesson back to climate change, students are asked why carbon dioxide in the atmosphere is important to measure. They may need some guidance understanding the greenhouse effect. It may be beneficial to show them a graph of global atmospheric CO_2 concentrations, such as those measured at Mauna Loa, Hawaii.

The optional short essay assessment below asks students to identify reasons for soil carbon loss after initiation of agriculture. They should be able to answer this essay based on the class discussions, as it requires little critical thinking beyond explaining what they've learned in class.

Figure Set 1 Post Lesson Assessment: Short Essay (100-200 Words)

Converting prairie soils to agricultural fields results in decreases in the amount of carbon that is stored in soil. Identify two reasons why soil organic carbon decreases after the initiation of agriculture (Consider how carbon enters the soil and how carbon leaves the soil).

An alternative assessment for smaller classes is listed below. Students are asked to use boxes and arrows to draw out the processes of soil carbon loss as they understand them. Students are given the components of the system and are asked to draw boxes and arrows, and to label the processes (arrows). You will need to explain how to do this in class. A basic example diagram that exhibits a correct student drawing is shown below in Figure 18. You may want to use the carbon cycle in Figure 14 as an example for students, where arrows indicate processes and pools are indicated at the ends of arrows.

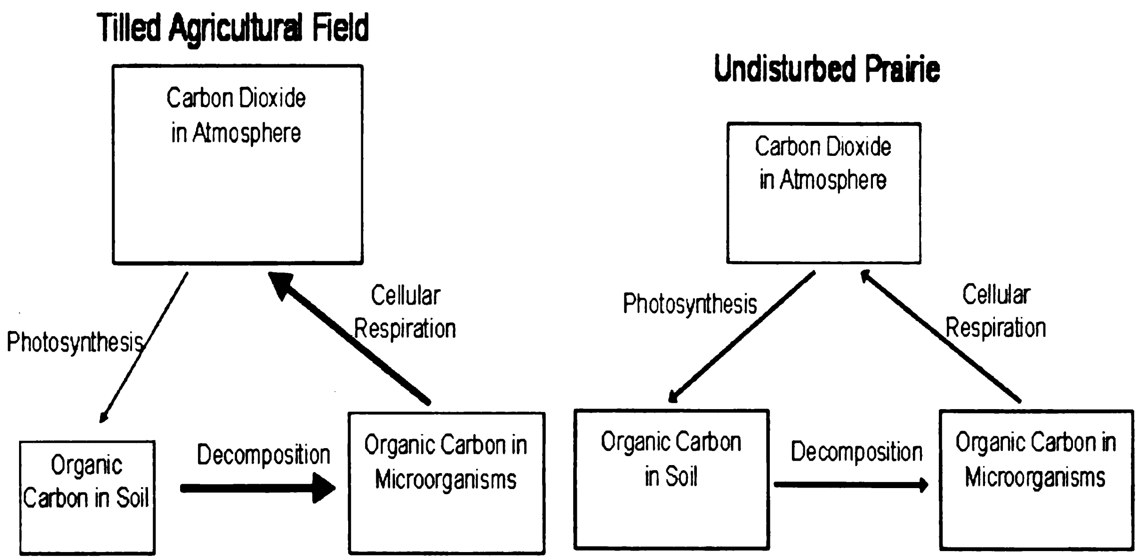

Figure 18. An example box and arrow diagram is shown to illustrate the carbon cycle in an undisturbed prairie and a cultivated agricultural field. Student answers to the Post Lesson Assessment below could look similar to this. The agricultural diagram contains a larger box for atmospheric carbon dioxide and a smaller soil organic carbon box. Arrow sizes also change between the two scenarios.

Figure 18 Copyright: Created by Brook Wilke

Figure Set 1 Post Lesson Assessment: Box and Arrow Diagram

As we have discussed in class, ecologists use box and arrow diagrams to show different parts of the carbon cycle, including sources and sinks for carbon. Use boxes and arrows to illustrate the carbon cycle in an undisturbed prairie and one that has been converted to a tilled agricultural field. Include the following as “boxes”: organic carbon in the soil, organic matter in microorganisms, and carbon dioxide in the atmosphere. Label the processes (the arrows). Briefly explain each drawing.

FIGURE SET 2: METHANE EMISSIONS FROM AGRICULTURE

- **Purpose:** To teach students that methane is a powerful greenhouse gas, and to teach the mechanisms by which agriculture contributes a substantial amount of methane to the atmosphere.
- **Teaching Approach:** Think – Pair - Share
- **Cognitive Skills:** Knowledge, Interpretation
- **Student Assessment:** Minute Paper

Figure Set 2 Background

Although methane concentrations are much lower than carbon dioxide, per kilogram, methane is 25 times more effective at trapping heat in the Earth's atmosphere compared to carbon dioxide. Methane concentrations have increased by more than 100% since pre-industrial times, indicating that the increased sources due to human activity are much larger than the sinks (reaction with OH^- in atmosphere and oxidation by soil bacteria). Every year, 84 Teragrams (Tg) are in excess in the Earth's atmosphere. Moss *et al.* (2000) combined literature values into one graph to show that agricultural activities contribute about half of all anthropogenic methane emissions, largely from animal digestion, waste, and rice paddies. More information about methane can be found on the U.S. EPA website: http://epa.gov/methane/.

The table in this set (Table 27) was reconstructed from the Intergovernmental Panel on Climate Change (IPCC) reports from 2007, while the figure in this set is taken from Moss *et al.* (2000). The IPCC 2007 report, which compiled information from various scientific sources, provides detailed information about methane's contribution to climate change.

Figure Set 2 Figures and Tables

Table 27. This table was constructed using data from the Intergovernmental Panel on Climate Change (IPCC) report in 2007. Information is shown regarding two important greenhouse gases, Carbon Dioxide (CO_2) and Methane (CH_4). Atmospheric concentration data are reported as parts per million (ppm) in the atmosphere, while the % increase indicates the change in ppm for each gas between pre-industrial and present time points.

	Carbon Dioxide (CO_2)	Methane (CH_4)
Atmospheric concentration	(ppm)*	(ppm)
Pre-industrial	280	0.8
Present (2005)	379	1.77
% Increase	36%	121%
Atmospheric lifetime (years)	50-200	12
Relative radiative effectiveness		
Per unit mass over 100 years	1	25

* ppm: parts per million

Table 27 Copyright: Figure created using data from the Intergovernmental Panel on Climate Change Fourth Assessment Report (2007).

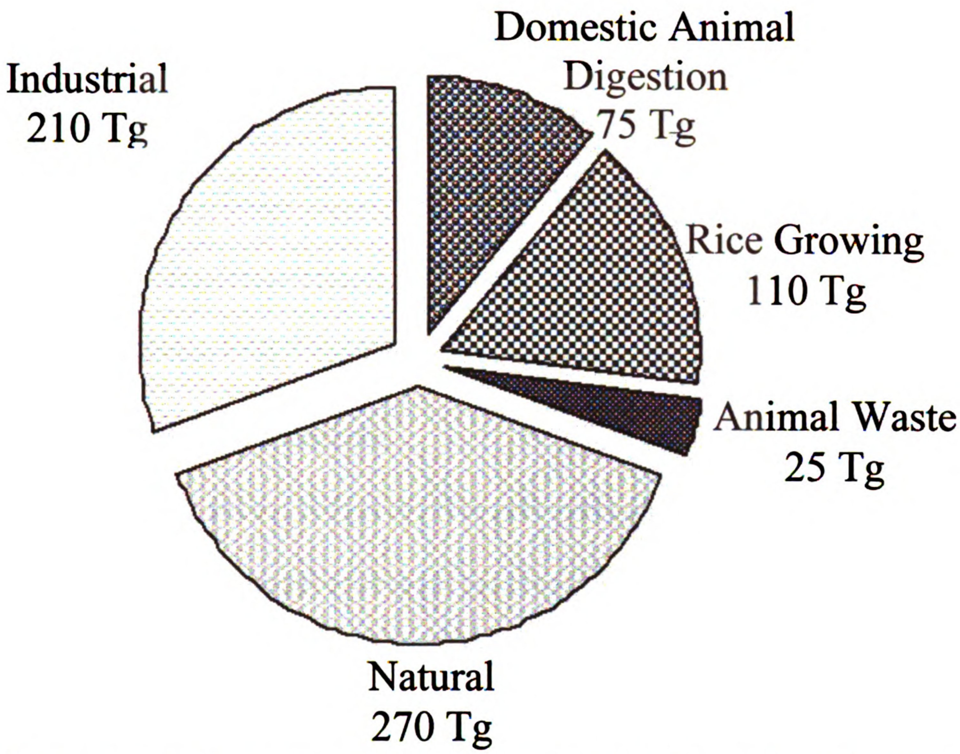

Figure 19. This figure shows total global production of methane per year divided into five categories. Units are in teragrams (Tg), which is equivalent to 10^{12} grams. A total of 690 Tg of methane are emitted to the atmosphere each year. Agricultural processes (i.e., domestic animal digestion, rice growing, animal waste) produce 210 Tg of methane, which is 30% of total methane emissions and 50% of anthropogenic methane emissions. Natural processes in soil and the atmosphere convert this methane to carbon dioxide by oxidizing the carbon, but 84 Tg of methane do not get oxidized every year and remain in the atmosphere. This imbalance between methane emissions and oxidation has resulted in a 121% increase in atmospheric methane concentrations since pre-industrial times.

Figure 19 Copyright: Figure 19 was drawn from data in Figure 1 in Moss *et al.* (2000), which is published in the journal '*Annales de Zootechnie.*'

Figure Set 2 Student Instructions

Part 1

There are several gases produced by human activities that contribute to climate change. Carbon dioxide (CO_2) receives the most attention in the media, but other gases are also very important contributors to climate change. In this activity, we'll specifically examine methane (CH_4), including its relative importance for climate change and why it is increasing in the atmosphere.

Table 27 shows carbon dioxide and methane abundance in the atmosphere, as well as the percent increase for each gas since pre-industrial times. Concentrations of the two gases are reported in parts per million (ppm), which indicates the number of parts of a particular gas relative to one million parts of all gases in the atmosphere. Radiative effectiveness is a term used to describe the ability of a gas to trap radiation energy near the surface of the Earth, and is reported per unit mass.

For the following two questions, come up with an answer on your own. Then, find a partner and discuss this together and write down your answer.

1. Which gas, CO_2 or CH_4, is more abundant in the atmosphere? Which one has had the highest proportional increase since pre-industrial times?
2. If one kilogram of methane trapped 125 units of radiation energy, how many units of radiation energy would a molecule of CO_2 trap?
3. Based on the data in Table 27 (atmospheric concentration and relative radiative effectiveness), which gas, carbon dioxide or methane, is a larger overall contributor to atmospheric warming? Why?

Part 2

Agriculture is one of the most important anthropogenic (human caused) sources of methane to the atmosphere. Rice is cultivated in wetlands, where there is little oxygen available in the soil. Decomposition of organic matter in these anaerobic environments produces methane instead of carbon dioxide. Methane is also produced in the digestive tracts of ruminant animals (e.g., cows, sheep, etc.) during the digestion process, which is then released to the atmosphere. In fact, one cow can release as much as 500 liters of methane per day (Johnson and Johnson 1995). Animal manure is stored in large holding ponds, where anaerobic bacteria decomposing the manure also release methane to the atmosphere. Most of the methane released to the atmosphere is consumed by a reaction with hydroxyl radicals (OH^-) or is oxidized by soil bacteria to carbon dioxide (these reactions are called "sinks"), but a portion (84 Tg per year) remains in excess in the atmosphere.

Together with a partner, discuss the following:

1. What agriculture activities produce significant amounts of methane?
2. Can you think of other agricultural activities that are not listed on Figure 19, and therefore do not contribute substantial amounts of methane to the atmosphere?
3. As consumers of food, are there any decisions we could make to reduce the amount of methane emitted to the atmosphere from agriculture?

Write down your answers to these questions and be prepared to share your answers with the rest of the class.

Figure Set 2 Notes to Faculty

The suggested student active approach suggested for Figure Set 2 is "Think-Pair-Share." This approach is similar to "Turn to your Neighbor" and requires students to think about the question, turn to their neighbor to discuss the question, and then share their answer with the class.

Students may need help understanding some of the terms in Table 1. For instance, relative radiative effectiveness may need to be described as the potential for each of the gases to trap heat in the atmosphere, and thus contribute to global warming. Therefore, one kilogram of methane is 25 times more effective at trapping heat compared to carbon dioxide. Two factors influence relative radiative effectiveness, which are physical chemistry (including radiation absorption properties) and lifetime of a molecule in the atmosphere. Physical chemistry of a molecule determines the infrared (IR) wavelength absorbed. Gases with absorption bands in the non-visible portion of the IR spectrum, particularly between 1,000-1,200 wavenumbers, have the highest radiative forcing effect. Carbon dioxide absorption peaks occur at 2350 and 650 wavenumbers while methane absorption peaks occur at 3,000 and 1,300 wavenumbers.

In order to calculate the answer for part 1, question 3, students must consider not only the ppm increase of CO_2 and CH_4 in the atmosphere, but also the molecular mass of these gases. This is necessary since relative radiative effectiveness is calculated per unit mass (e.g. per kilogram), and not per molecule.

As stated before, methane absorbs frequencies of IR radiation emitted from the Earth's surface that would otherwise continue out to space. Even though methane is more effective per molecule than carbon dioxide at radiating heat, carbon dioxide is still the most important greenhouse gas because the quantity of carbon dioxide created by human activities is much greater than the quantity of methane. The students are assigned to discuss this with a partner. Continue this discussion with the entire class, to make sure they know that methane is a substantial contributor to climate change, but is still not as important as carbon dioxide.

We've included Figure 20 here for faculty reference. Use Figure 20 in a more advanced class if you wish. Figure 20 describes methane sources and sinks. Methane source and sink values in Figure 20 were calculated in individual studies, and were then compiled and scaled to a global level by Moss *et al.* (2000). There are slight disagreements between the values in the figure, reflecting the error involved when scaling up from multiple scientific studies. Students may be confused regarding why there is methane left over and it does not all get consumed by the reaction with hydroxyl molecules in the atmosphere. They may also notice that there should be much more methane in excess after considering how much methane comes from human activities. However, the methane sinks (oxidation in the atmosphere by hydroxyl radicals or oxidation in soil by methanotrophic bacteria to CO_2 and H_2O) have the capacity to oxidize some of this excess methane produced by human activities, but these sinks cannot oxidize all of the excess methane. Methane is gradually broken down to CO_2 and H_2O through a series of chemical reactions, which explains its relatively short life in the atmosphere.

Methanogens are the only living organisms that produce methane as a way of life. The biochemistry of their metabolism is unique and definitively delineates the group. The terminal electron acceptor in methanogenesis is not oxygen, but carbon. The two best described pathways involve the use of carbon dioxide and acetic acid as terminal electron acceptors.

Methanogenesis – $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$ or $CH_3COOH \rightarrow CH_4 + CO_2$

Methanotrophs are bacteria that can use methane as their only source of carbon and energy. They are responsible for methane oxidation in soil.

Methane oxidation in soil – $CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$

Students are asked to consider what individuals might do to cut down on methane emissions. Some examples may include eat less meat or drink less milk. They may also say eat less rice or use less fuel. Answers may vary and, hopefully, will be creative. Bringing in the social dimension will make it more interesting and relevant for students.

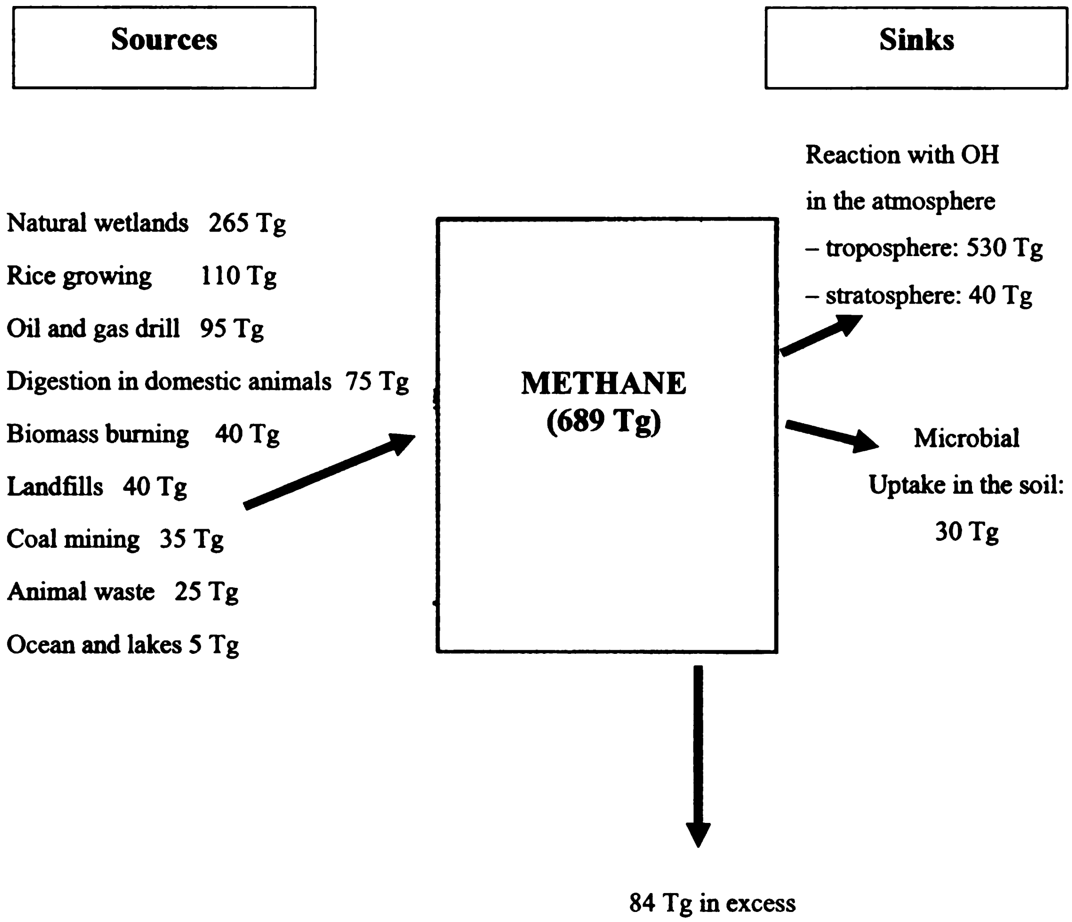

Figure 20. Methane sources consist of natural and anthropogenic locations. Two major methane sinks exist; oxidation by hydroxyl (OH^-) in the atmosphere and oxidation by methanotrophic bacteria in soil.

Figure 20 Copyright: This figure is taken directly from Figure 1 in Moss *et al.* (2000), which is published in the journal '*Annales de Zootechnie.*'

Students should complete the "Minute Paper" below for an assessment of the material presented. Be sure to discuss a couple of the main ideas found in the Minute Paper's during the next class period to show to the students that the Minute Paper is valuable.

Figure Set 2 Post Lesson Assessment: Minute Paper

Students take two minutes at the end of the class period to write an answer to the following questions on a piece of paper – to be turned in.

- How do agricultural practices produce methane?
- Why is it important to consider methane as a greenhouse gas when there is 350 times more carbon dioxide in the atmosphere than methane?

FIGURE SET 3: NITROGEN FERTILIZERS INCREASE NITROUS OXIDE EMISSIONS

- **Purpose:** To teach students that nitrous oxide is a very important greenhouse gas produced in soil, and that excess nitrogen fertilizer results in high levels of greenhouse gas emissions.
- **Teaching Approach:** Guided Class Discussion
- **Cognitive Skills:** knowledge, interpretation, synthesis
- **Student Assessment:** Short Essay

Figure Set 3 Background

Although the cumulative radiative forcing estimates for nitrous oxide (N_2O) are lower than either carbon dioxide or methane, N_2O contributes substantially to total radiative forcing by the Earth's atmosphere. Per unit mass, the radiative effectiveness of N_2O is 298 times more than carbon dioxide, making each kilogram of N_2O 298 times more relevant. The Intergovernmental Panel on Climate Change (IPCC) reported in 2007 that N_2O has increased by 10% since pre-industrial time periods, but lasts in the atmosphere for approximately 114 years. In soil, bacteria produce N_2O during the processes of nitrification and denitrification. During nitrification, ammonium is converted to nitrate, and N_2O is a byproduct. Denitrification, the reduction of nitrate to nitrogen gas (N_2), is an anaerobic process. Nitrous oxide is an intermediate product for many denitrifyers but can be the end product for some denitrifying bacteria (Robertson and Grace 2004). More information about nitrous oxide can be found on the U.S. EPA website: http://www.epa.gov/nitrousoxide/index.html.

Nitrous oxide is an important greenhouse gas because of its high relative radiative effectiveness. Two factors influence relative radiative effectiveness, which are physical chemistry (including radiation absorption properties) and lifetime of a molecule in the atmosphere. Physical chemistry of a molecule determines the infrared (IR) wavelength absorbed. Gases with absorption bands in the non-visible portion of the IR spectrum, particularly between 1,000-1,200 wavenumbers, have the highest radiative forcing effect. Carbon dioxide absorption peaks occur at 2350 and 650 wavenumbers while nitrous oxide absorption peaks occur at 2,200 and 1,250 wavenumbers.

Agricultural soils high in available nitrogen are a major contributor of N_2O to the atmosphere. Reactive (biologically available) nitrogen in the biosphere is twice as high as pre-industrial times, largely due to agricultural practices of fertilization and increased growth of nitrogen fixing crops (Vitousek *et al.* 1997). A good general source about human alteration of the global nitrogen cycle can be found on the Ecological Society of America website: http://www.esa.org/science_resources/issues.php.

Figure Set 3 Figures and Tables

Table 28. This table was constructed using data from the Intergovernmental Panel on Climate Change (IPCC) report in 2007. Information is shown regarding two important greenhouse gases, Carbon Dioxide (CO_2) and Nitrous Oxide (N_2O). Atmospheric concentration data are reported as parts per million (ppm) in the atmosphere, while the % increase indicates the change in ppm for each gas between pre-industrial and present time points.

	Carbon Dioxide (CO_2)	N_2O
Atmospheric concentration	(ppm)*	(ppm)
Pre-industrial	280	0.29
Present (2005)	379	0.32
% Increase	36%	10%
Atmospheric lifetime (years)	50-200	114
Relative radiative effectiveness		
Per unit mass over 100 years	1	298

* ppm: parts per million

Table 28 Copyright: Figure created using data from the Intergovernmental Panel on Climate Change Fourth Assessment Report (2007).

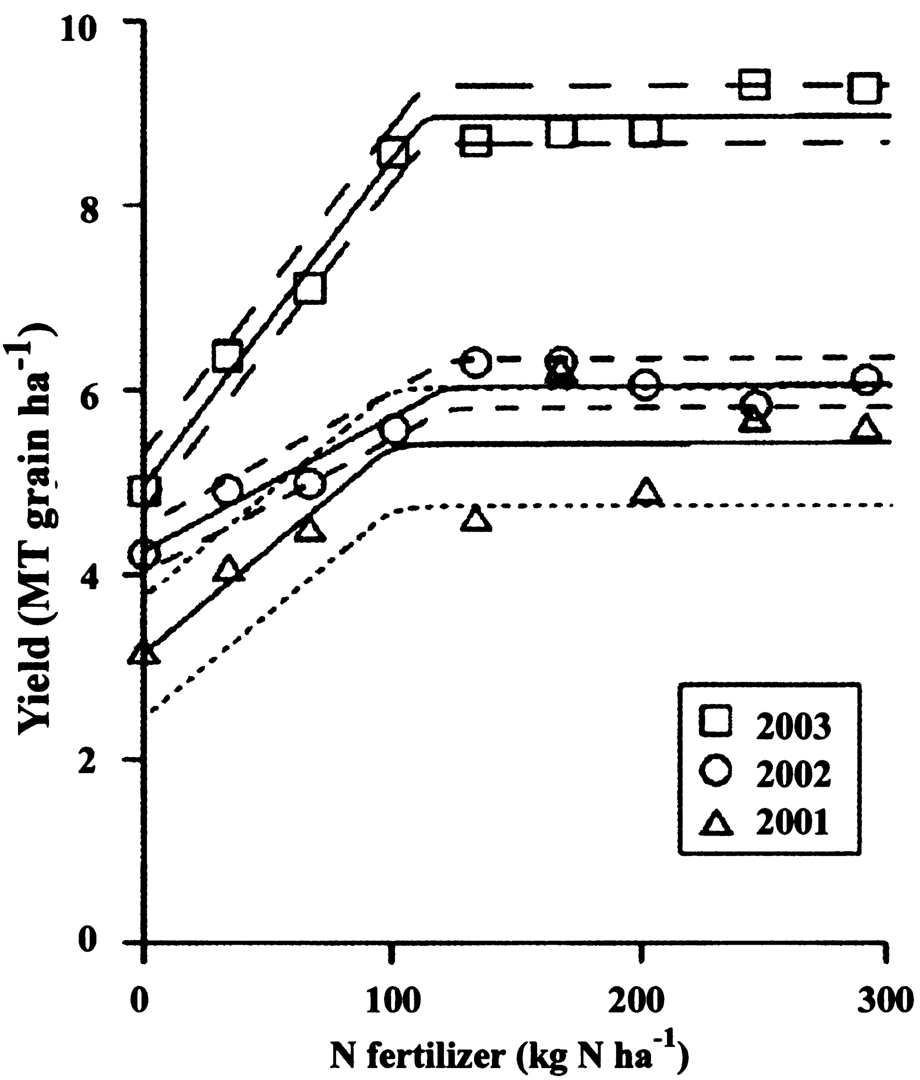

Figure 21. Corn yields were measured in six replicate fields across a gradient of nitrogen fertilizer rates at the W.K. Kellogg Biological Station in SW Michigan from 2001 – 2003. Solid lines indicate modeled grain yields while dashed lines indicate standard error. The term "kg N ha^{-1}" in the x-axis label indicates kilograms of nitrogen fertilizer applied per hectare (one hectare is approximately 2.2 acres). The term "MT grain ha^{-1}" in the y-axis label indicates metric tons of grain produced per hectare (one metric ton is 1,000 kilograms).

Figure 21 Copyright: This figure was taken directly from Figure 2 in McSwiney and Robertson (2005), which is published in the journal '*Global Change Biology*.'

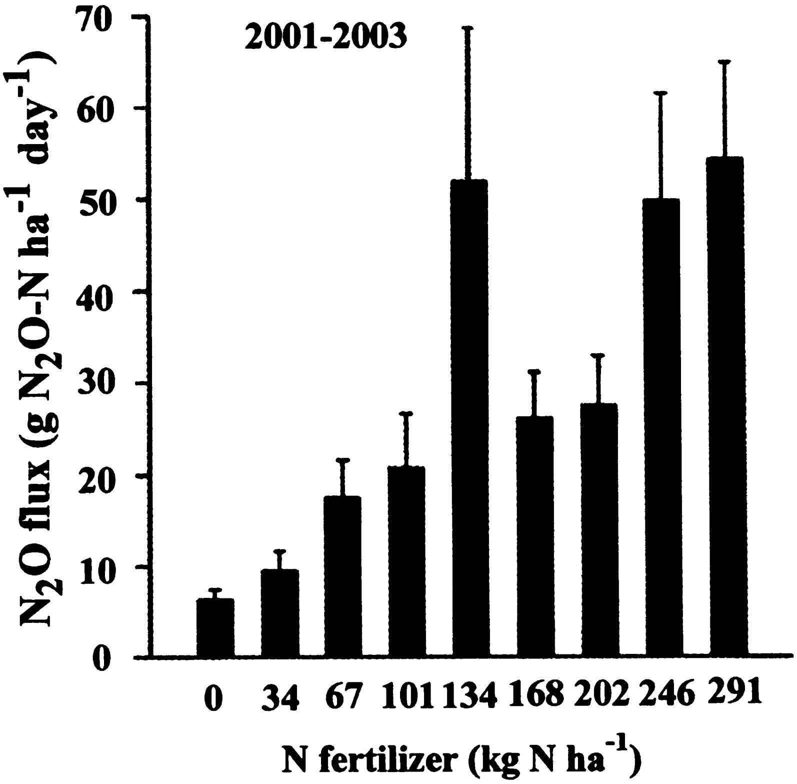

Figure 22. Nitrous oxide (N_2O) emissions from soil were measured in six replicate corn fields across a gradient of nitrogen fertilizer rates at the W.K. Kellogg Biological Station in SW Michigan from 2001 – 2003. Nitrous oxide was measured by placing closed chambers over the soil and monitoring the rate of change in N_2O in the chambers over time as it was released from the soil. Error bars represent standard error.

Figure Set 3 Student Instructions

Part 1

There are several gases produced by human activities that contribute to climate change. Carbon dioxide (CO_2) receives the most attention in the media, but other gases are also very important contributors to climate change by trapping heat in the Earth's atmosphere. Nitrous oxide (N_2O) is considered to be one of the other important greenhouse gases, as human activities have increased its concentration in the atmosphere since pre-industrial time periods.

Examine Table 28, which provides data from the Intergovernmental Panel on Climate Change (IPCC) 2007 report. Think about what the data mean and make sure you understand all of the terms. Ask your instructor if you are unfamiliar with any of the terms. After interpreting Table 28, consider the following questions.

Is there more carbon dioxide or nitrous oxide in the atmosphere?

- Which of the two gases (CO_2 or N_2O) has had larger increases in the atmosphere since pre-industrial times?
- In your own words, what do you think relative radiative effectiveness means?.
- Why do you think scientists pay attention to relative radiative effectiveness?
- The concentration of carbon dioxide in the atmosphere is more than 1,000 times higher than nitrous oxide. Why is nitrous oxide considered to be an important greenhouse gas?
- Why might nitrous oxide have a higher relative radiative effectiveness than carbon dioxide?

Part 2

It has been estimated that 50% of human induced nitrous oxide (N_2O) emissions are produced in agricultural soils (IPCC 2001). Nitrogen fertilizers applied to soil increase plant growth and food production, but plants are not the only organisms that use the nitrogen. Soil microorganisms also use nitrogen for growth and energy. Specifically, certain bacteria are involved in the process of nitrification and denitrification, and nitrous oxide is a minor product in both of these reactions.

Figure 21 and 23 show data of N_2O emissions and corn crop yields in a study conducted in corn fields of southwest Michigan. The researchers measured N_2O emissions from soil, but also measured corn crop yields.

Interpret Figure 21 and 23 for a moment on your own. Ask your instructor to describe anything that you do not understand. After examining the figure, consider the following questions.

- Using Figure 21, do corn yields increase linearly with increasing nitrogen fertilizer rates?
- Using Figure 22, what happens, in terms of N_2O emissions when a farmer applies more fertilizer than needed for maximum crop growth (more than 101 kg in this study)?
- Do you think that 101 kg of nitrogen fertilizer enough to maximize crop growth in all fields or for all crops?
- Why might a farmer apply more than enough nitrogen fertilizer?
- Corn crops are being grown to produce biofuel (ethanol) for fueling vehicles. This practice is intended to reduce greenhouse gas emissions because less fossil fuel is

being used to power vehicles. Based on the data in Figure 3, how might the strategy of growing corn for ethanol actually increase the heat trapping potential of the Earth's atmosphere?

Figure Set 3 Notes to Faculty

In this guided class discussion, the suggested strategy is to show Table 28 to your students, have them interpret the table on their own for a moment, and then discuss the Table as a class using the questions listed in the student instructions as prompts. Instead of asking for a show of hands to answer questions, call on randomly selected students to ensure participation by the entire class. Repeat the same strategy for Figure 3.

Part 1

Students are likely to struggle initially with the term "relative radiative effectiveness." This is a unit-less measure that compares the heat trapping potential of a molecule of different greenhouse gases to a molecule of carbon dioxide, where a molecule of carbon dioxide is set as the baseline. Greenhouse gases are also compared on a volume basis. There are several natural and anthropogenic sources of N_2O, which are listed in Table 29, which is included for faculty reference. Management practices on agricultural soils are the single largest category in terms of N_2O emissions, contributing 3.3 Tg N_2O-N per year.

Table 29. Global nitrous oxide budget based on calculations in 1997.

Nitrous Oxide Sources	Tg N_2O–N per Year
Ocean	3.0
Tropical	
Wet Forests	3.0
Dry Savannas	1.0
Temperate	
Forests	1.0
Grasslands	1.0
Agricultural Soils	3.3
Biomass Burning	0.5
Industrial	1.3
Feedlots	2.1
Total	16.2

Tg = teragrams (10^{12} grams)

Table 29 Copyright: Data are from the Intergovernmental Panel on Climate Change (IPCC) 1997 report.

Part 2

Students may have trouble answering the last question because it is designed to make them think through the problem. Guide them through the question to help them realize that nitrous oxide emissions may be very high during corn crop production, thus off-setting the climate benefits of growing corn as a biofuel. As stated in the background material, a good general source about human alteration of the global nitrogen cycle can be found on the Ecological Society of America website: http://www.esa.org/science_resources/issues.php.

Nitrous oxide is a kind of by-product of both nitrification and denitrification. Sometimes denitrification does not go all the way to nitrate, with nitrous oxide as the end product instead. The equations for nitrification and denitrification are listed below. The terminology here is very confusing, largely because the terms (e.g. nitrification) do not have an obvious meaning; therefore you need to decide what information you want students to remember, because the details can be overwhelming. It is not necessary that students learn the equations for nitrification and denitrification, but we've supplied the equations for your reference. During nitrification, ammonia (NH_3) is converted to nitrate (NO_3^-). However, nitrous oxide (N_2O) is also a minor byproduct of this reaction.

Nitrification – NH_3 (Ammonia) + $O_2 \rightarrow NO_2^-$ (Nitrite) + 3 H^+ + 2 e^- (First step of equation)

NO_2^- (Nitrite) + $H_2O \rightarrow NO_3^-$ (Nitrate) + 2 H^+ + 2 e^- (Second step of equation)

$$\begin{array}{l} N_2O \\ \uparrow \\ NH_3 \rightarrow NH_2OH \rightarrow NOH \\ NO \rightarrow NO_2^- \rightarrow NO_3^- \end{array}$$

(Steps of Nitrification leading to N_2O production)

Denitrification, the conversion of nitrate (NO_3^-) to nitrogen gas (N_2), occurs in low oxygen soil conditions. Nitrous oxide is an intermediate in the denitrification process and can be an end product for some bacterial taxa (Robertson and Grace 2004).

Denitrification – 2 NO_3^- (Nitrate) + 10 e^- + 12 H^+ $\rightarrow N_2$ (Nitrogen gas) + 6 H_2O (Redox reaction)

NO_3^- (Nitrate) $\rightarrow NO_2^-$ (Nitrate) $\rightarrow NO \rightarrow N_2O$ (Nitrous Oxide) $\rightarrow N_2$ (Nitrogen gas)

(Steps in denitrification leading to N_2O production)

In the nitrogen cycle, bacteria are engaged in very different processes. Nitrification is actually a type of chemoautotrophy in which bacteria use the energy released from oxidation of ammonium to nitrate to reduce carbon dioxide to carbohydrates. In contrast, denitrification is a type of respiration in which carbohydrates are oxidized for energy; in this case it is an anaerobic respiration and nitrate is used instead of oxygen. It may be wise not to tell students this level of detail, but it is good for you to recognize these differences.

McSwiney and Robertson (2005) found that N_2O emissions did not increase linearly with nitrogen fertilizer rates, but that "excess" nitrogen fertilizer resulted in substantially larger N_2O emissions. Nitrogen is a limiting nutrient for corn crop growth, but the application of nitrogen fertilizer can saturate the supply of nitrogen. Above 101 kg N per hectare, corn crops no longer responded with larger yields, and the supply of nitrogen in the soil was larger than the demand by the corn crops. Excess nitrogen in the soil resulted in much higher rates of N_2O production via the nitrification and denitrification processes. Previous studies (Bouwman 1996) had estimated a linear relationship between N_2O emissions and nitrogen fertilizer rates (see student assessment).

There appears to be a threshold where crop yields level off above 101 kg N per hectare while N_2O emissions increase dramatically. Nitrous oxide emissions were particularly high at 134 kg N per hectare, which cannot be directly explained. It could be due to experimental error, which would be unlikely due to randomization in the experimental design and multiple years of data collection. The authors of Figure 22 suggest a potential change in the microbial process by which N_2O is produced, or that another N sink (luxury plant uptake, microbial immobilization) could be competing for nitrogen at the higher N rates. This may be a good discussion point for the class – asking them why N_2O emissions were so much higher at 134 kg N than 168 and 202 kg N per hectare.

The assessment below is a short essay that requires students to describe the relationship between nitrogen fertilizer rates and nitrous oxide emissions. A linear equation does not appear to fit the data well, as nitrous oxide emissions are variable and quite high above fertilizer rates of 101 kg ha^{-1}. Students need to understand the equation for a linear model in order to answer the question completely. Introductory students may need some guidance to answer the question, but should be able to provide an answer using basic algebra and geometry.

Figure Set 3 Post Lesson Assessment: Short Essay (100 – 200 Words)

An article published in 1996 provides an equation for estimating nitrous oxide emissions from agricultural fields based on the amount of fertilizer applied (Bouwman 1996). The equation that they use to calculate nitrous oxide emissions is: $\mathbf{E = 1 + 1.25F}$ (E = kg nitrous oxide per hectare, F = kg nitrogen fertilizer per hectare).

Would this equation ($E = 1 + 1.25F$) accurately estimate the amount of nitrous oxide emitted from the fields that were studied to generate the data in Figure 3? Why or why not?

FIGURE SET 4: CARBON SEQUESTRATION IN AGRICULTURAL SOILS

- **Purpose:** To teach students that degraded agricultural soils can sequester carbon, and that there are certain management strategies that can maximize carbon storage in soil.
- **Teaching Approach:** Paired Think Aloud
- **Cognitive Skills:** Knowledge, interpretation, synthesis
- **Student Assessment:** Short Essay

Figure Set 4 Background

Many agricultural fields in temperate regions have been cultivated for hundreds of years. In these fields, much of the carbon stored in soil has been lost to the atmosphere due to enhanced decomposition during cultivation (See Figure Set 1). Under conventional crop management, soil carbon loss eventually levels out & remains at a steady state at approximately 50% of original carbon levels. However, certain management strategies can slowly increase soil carbon content back towards original levels, which is called soil carbon sequestration. This can occur when net primary productivity due to plant growth exceeds respiration of organic carbon by soil biota. See Schlesinger (1999 – pdf included) or Post and Kwon (2000) for more information about carbon sequestration on agricultural soils. A simple explanation of carbon sequestration can be found at the U.S. EPA website: http://www.epa.gov/sequestration/local_scale.html.

The data on soil carbon sequestration in Figure 23 were collected from a Long Term Ecological Research (LTER) Experiment at the W.K. Kellogg Biological Station in southwest Michigan. In this experiment, six ecosystems were established in 1989 and compared from 1989 to 1999 to characterize their ability to sequester carbon in the soil. These systems were compared to conventionally tilled (physically turned over) agricultural fields in which soil carbon concentrations were hypothesized to remain relatively unchanged over time. The first three ecosystems were cultivated with annual crops, in a corn-soybean-wheat rotation.

- The **"Conventional"** ecosystem received both soil tillage and pesticides to control weeds and was fertilized to maximize crop yields.
- **"Organic"** refers to an ecosystem that received no fertilizer or pesticides, but tillage was used to control weeds and legume (nitrogen fixing) cover crops were used as nitrogen fertilizer sources.
- **"No-Till"** refers to an ecosystem in which the soil was not disturbed after the start of the experiment in 1989. Instead, weeds were controlled using pesticides.

The last three agroecosystems contained perennial plants and no tillage.

- **"Alfalfa"** is a perennial nitrogen fixing plant that is grown for animal feed. Alfalfa was planted in 1989 and the aboveground growth was cut and removed from the fields 3-4 times per year. Although perennial, alfalfa was replanted every 5-7 years to maintain vigorous growth, as older plants died and weeds invaded the fields.
- Successional communities are those that are left fallow and receive no human induced disturbances. **"Early Successional"** ecosystems were last tilled in 1988, but were then left undisturbed, except for occasional burning to prevent trees from growing in the experimental plots.
- **"Poplar trees"** were first planted in 1989, and were harvested after 10 years of growth. Trees were cut and used as biofuel for electricity generation. After

harvest, the trees re-sprouted and will be harvested a second time for the same purposes.

Figure Set 4 Figures and Tables

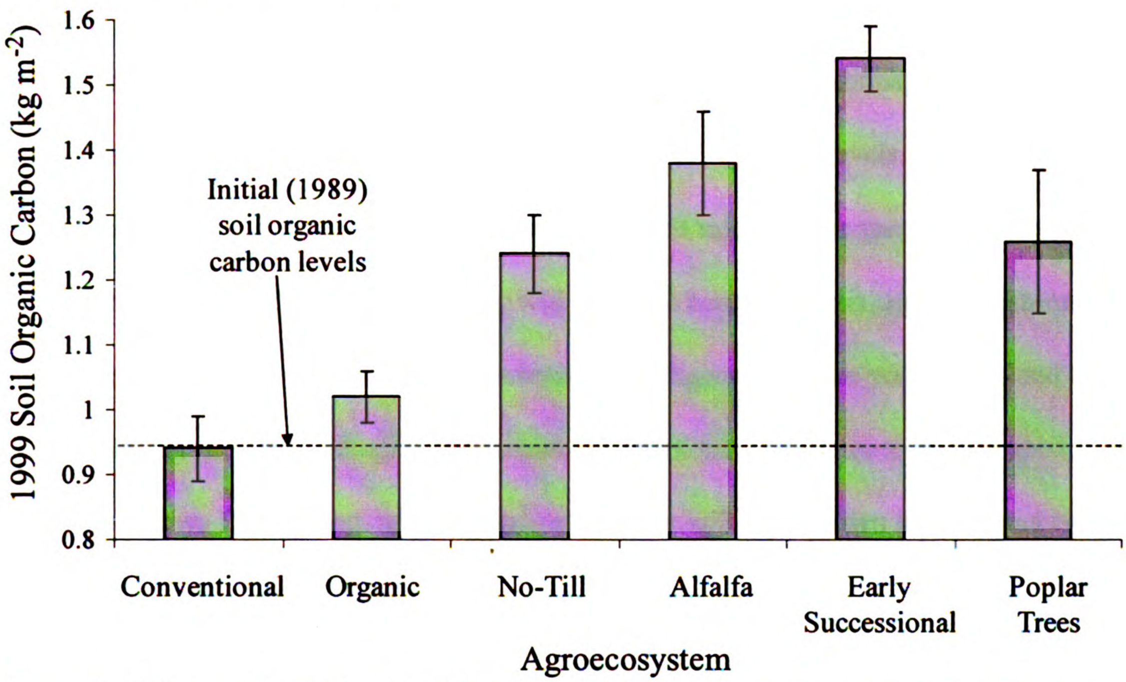

Figure 23. Soil organic carbon (SOC) levels in 1999 (kilograms per square meter) are shown for six experimental ecosystems in a long-term ecological research (LTER) experiment in southwest Michigan. Error bars represent standard error. Each Ecosystem Management treatment was initiated in 1989 and was replicated six times on randomly selected one hectare plots. The dashed line indicates average SOC for all treatments in 1989, when all treatments had similar SOC levels. Prior to 1989, the entire experimental area was farmed uniformly, where corn, soybeans and alfalfa were grown. Annual crops were harvested for agricultural production, alfalfa and poplar trees were harvested for biomass and the early successional community was left undisturbed, except for occasional spring burning to prevent colonization of tree species.

Figure 23 Copyright: Data to create Figure 23 was taken from Table 1 in Robertson *et al.* (2000), which is published in the journal '*Science*.'

Figure Set 4 Student Instructions

The cultivation of agricultural fields has resulted in the loss of soil carbon, which was part of the soil organic matter pool. Cultivation results in higher rates of decomposition relative to photosynthesis, causing this decline in soil carbon over time

(See Figure Set 1 for more details). Consequently, soils depleted in organic matter have the potential to store carbon – and therefore remove carbon dioxide from the atmosphere - if agricultural practices cause organic matter concentrations to increase. In this case net primary productivity by plants would be greater than decomposition of the dead plant material. Scientists are studying ways to remove carbon from the atmosphere and store it in soil, thus increasing soil carbon amounts back to their original levels.

Take a look at Figure 23, including the legend, and interpret the graph. Make sure to read the labels carefully. Your instructor will describe each of the six agroecosystems to you, which are also described in the Figure Set 4 Background. Once you understand the graph and what the different agroecosystems are, work with a partner to answer the questions below. The student with the earlier birthday will write down the answers while the person with the later birthday will answer the questions out loud, telling the recorder what to write down.

- Which experimental ecosystems appear to have had the lowest soil carbon content in 1999?
- Why might some agricultural practices (such as growing perennial plants) be more effective at sequestering carbon in the soil? To address this question, consider various sources of carbon to the soil
- Did any agroecosystems decline in soil carbon content from 1989 to 1999?
- What is the best management strategy to build soil organic matter among annual agroecosystems (conventional, no-till, organic)?

- In addition to removing carbon dioxide from the atmosphere, what benefits do you think a farmer might gain from having higher organic matter content in his/her soil?

Figure Set 4 Notes to Faculty

The suggested student active approach for Figure Set 4 is "Paired Think Aloud." This approach is designed to allow students that are shy to present their opinion to another student, without having to talk immediately in front of the whole class. Students work on the questions in pairs. We suggest that the older student answers the question while the younger student records the answers. The younger student may then present the answer to the larger group. You may choose another strategy for assigning the students to be talkers or recorders, such as using the last digit of a phone number.

Familiarize yourself with each of the six agroecosystems in Figure 23. Photos from the agroecosystems can be found at www.lter.kbs.msu.edu/photo_gallery/overview.php. Robertson *et al.* (2000) found that early successional plots dominated by herbaceous perennial plants (grasses, forbs, legumes) exhibited significant increases in soil organic carbon from 1989 to 1999, and sequestered the most carbon (>200 g CO_2 m^{-2} $year^{-1}$) when established on previously cultivated soils from (Tables 1 and 2 in Robertson *et al.* 2000). Alfalfa and poplar trees, both perennial crops, also sequestered substantial amounts of carbon in soil during this time period. Several factors contributed to carbon sequestration by perennial cropping systems, including year round plant cover and root growth / turnover, high root productivity, and no soil tillage, which enhances decomposition.

Among annually cropped fields, no-till strategies were the most effective at sequestering soil carbon. Much of the plant residue from these fields was left on the soil surface after harvest, and decomposed slower than the residue in the tilled plots (conventional and organic). Cover crops were grown during the winter in organic plots, thus continuing plant growth across the entire season and building soil carbon whereas conventional plots did not utilize cover crops. Nitrogen fertilizer was also applied in conventional plots, which has been shown to accelerate rates of decomposition within the light soil carbon fraction (decadal turnover time) (Neff *et al.* 2002).

In general, perennial crops sequestered more carbon in soil than annual crops. No-till strategies were the most effective at increasing soil carbon in annually planted plots, and no agroecosystems exhibited further decline in soil carbon. Students are asked what benefits a farmer might gain from having increased soil organic matter. These benefits can be increased nutrient supply from the soil, increased water holding capacity, reduced risk of erosion, pH buffering capacity and potentially reduced risk of soil pathogen outbreaks. Other answers not mentioned here may also be correct.

Agricultural researchers debate whether raising a productive agricultural crop, such as corn, for many years in a row can increase soil organic carbon levels. The answer to this question likely varies depending on climate and management conditions, but it may be an interesting question to bring up with your students. Perhaps consider the question of whether or not continuous corn cropping can build organic matter in conventional vs. no-till managed fields.

The short essay assessment below asks students to consider why ecosystems with perennial crops exhibit higher rates of carbon sequestration rates in soil than ecosystems where annual crops are grown. They could list several reasons for this, and it is your discretion to determine if an answer is satisfactory. Students could provide answers that include deep root systems of perennial plants, lack of soil tillage, plant cover during winter months, cooler soil temperatures in summer due to plants covering the soil, etc.. to explain the differences between annual and perennial dominated ecosystems.

Figure Set 4 Post Lesson Assessment: Short Essay (100 – 200 Words)

Why do agroecosystems with perennial crops (alfalfa, successional, poplar trees) build soil carbon faster than agroecosystems containing annual crops (conventional, no-till, organic)? Consider what you know about perennial and annual plants and the ways they are managed to develop your answer.

FIGURE SET 5: GLOBAL WARMING POTENTIAL – TEMPERATE AGRICULTURE

- **Purpose:** To teach students that land management can affect the amount of greenhouse gas emissions from temperate agricultural production and that cessation of agriculture results in net sequestration of greenhouse gases in the soil. Students will play roles of various citizen groups to identify ways in which agricultural land management can affect a variety of different people around the world.
- **Teaching Approach:** Citizens Argument
- **Cognitive Skills:** Knowledge, interpretation, analysis, synthesis
- **Student Assessment:** Land Management Activity

Figure Set 5 Background

Agriculture and climate change are inextricably linked, as was shown in Figure Sets 1-4. Not only will climate change affect agricultural crop production, but agriculture is a primary source of several greenhouse gases. As shown in Figure Set 1, cultivation of undisturbed soils results in the loss of soil carbon. The production of nitrogen fertilizer, burning of fossil fuels by machinery and lime applications also emit carbon dioxide to the atmosphere. Fertilized agricultural soils contribute a substantial amount of nitrous oxide to the atmosphere. Methane oxidation in soil is lower in agricultural soils compared to adjacent forested areas. All of these factors must be examined simultaneously to understand the cumulative global warming potential of agroecosystems.

Many agricultural soils in temperate regions have been cultivated for many years. In these fields, much of the carbon stored in soil has been lost to the atmosphere due to enhanced decomposition during cultivation (See Figure Set 1). Soil carbon loss eventually levels out and remains at a steady state under conventional crop management. However, soil carbon content can actually increase under certain crop management strategies, including conservation tillage, cover crop planting and perennial crop growth. Likewise, other management strategies such as reducing fertilizer applications can reduce the amount of greenhouse gases emitted during management activities. Taken together, the net global warming potential can be calculated for different agroecosystems. Negative global warming potential values indicate net decreases in atmospheric heat trapping potential and positive global warming potential values indicate net increases in atmospheric heat trapping potential.

The global warming potential (GWP) of five agroecosystems in the Long Term Ecological Research Experiment at the W.K. Kellogg Biological Station in SW Michigan were compared from 1989 – 1999 (Table 30). In this experiment, five ecosystems were compared from 1989 to 1999 for their total contribution to global warming. The first three ecosystems were cultivated with annual crops, in a corn-soybean-wheat rotation.

- The **"Conventional Agriculture"** ecosystem received both soil tillage and pesticides to control weeds and was fertilized to maximize crop yields.
- **"No Till Agriculture"** refers to an ecosystem in which the soil was not disturbed after the start of the experiment in 1989. Instead, weeds were controlled using pesticides.
- **"Organic Agriculture"** refers to an ecosystem that received no fertilizer or pesticides, but tillage was used to control weeds and legume (nitrogen fixing) cover crops were used as nitrogen fertilizer sources.

No-till and organic agricultural practices reduced greenhouse gas emissions compared to conventional management, but most farmers still use conventional practices. There are several reasons why farmers may not switch to using management activities that reduce greenhouse gas emissions. Farmers need to maintain a steady income to continue farming. For example, farmers may not switch to no-till agriculture because soil compaction may occur, and tillage helps to breakdown surface plant litter that may reduce germination in future planting exercises. Farmers may not use organic agriculture practices because of slightly reduced crop yields and more labor involved in organic agriculture. Fertilizers ensure that plants will have enough nutrients to grow during the growing season. Economics and sociological pressures also play a big role in farmer decisions.

Successional communities are those that are left fallow and receive minimal human induced disturbances.

- **"Early Successional"** ecosystems were last tilled in 1988, but were then left undisturbed, except for occasional burning to prevent trees from growing in the experimental plots.
- **"Late Successional Forest"** ecosystems had not been disturbed for over 100 years and were dominated by large, hardwood trees such as oaks and maples.

Three primary gases contribute to the global warming potential (GWP) of the different agroecosystems shown in Table 30. GWP refers to the relative radiative forcing (heat trapping) ability of each source. Nitrous Oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) molecules do not have the same ability to trap heat. A molecule of N_2O traps the most heat over its lifetime in the atmosphere while a molecule of CO_2 traps the least amount of heat over its lifetime. Therefore, a molecule of N_2O is given more weight in terms of GWP than the other two gases. The table already reflects this change. See IPCC (2007) for further information on greenhouse gas concentrations and relative radiative forcing.

Carbon dioxide is produced through several agricultural processes. Tillage often leads to the loss of soil carbon due to enhanced decomposition of organic matter. Nitrogen fertilizer production is an energy intensive process. Currently, fossil fuels are used to "fix" nitrogen from the atmosphere and transport it to fields. Lime is often applied to fields to increase the soil pH, which can lead to net emissions of carbon dioxide in certain circumstances. Fuel is needed to power tractors and other equipment used to complete various agricultural activities, such as planting, tilling, spraying pesticides and harvesting.

Nitrous oxide (N_2O) is a gas that is produced during nitrification and denitrification processes. Nitrification is the process by which certain types of bacteria convert ammonium (NH_4^+) to nitrate (NO_3^-). Denitrification occurs when no oxygen is available. Anaerobic bacteria utilize nitrate as an electron donor for the oxidation of organic matter, which leads to the production of N_2 and N_2O gases. High levels of soil nitrogen due to fertilization can lead to increased levels of N_2O production (McSwiney and Robertson 2005).

Methane (CH_4) is also produced under anaerobic conditions. Microbes that thrive in these oxygen poor environments produce methane as a byproduct of carbon mineralization (Segers 1998). However, some soil organisms are able to oxidize methane, effectively removing it from the atmosphere and producing carbon dioxide (Roslev *et al.* 1997).

Figure Set 5 Figures and Tables

Table 30. Global warming potential (GWP), by greenhouse gas and by specific source of CO_2, is shown for five different experimental ecosystems in a long-term ecological research (LTER) experiment in southwest Michigan. Each Ecosystem Management treatment was replicated six times on randomly selected one hectare plots. Positive numbers indicate net increase in global warming potential while negative numbers indicate a decrease in global warming potential. Annual crops were harvested for agricultural production while successional communities were left undisturbed, except for occasional burning of the Early Successional plots.

Ecosystem Management	**CO_2**				**N_2O**	**CH_4**	**Net GWP**
	Soil C	**N Fertilizer**	**Lime**	**Fuel**			
Conventional Agriculture	0	27	23	16	52	-4	**114**
No-Till Agriculture	-110	27	34	12	56	-5	**14**
Organic Agriculture	-29	0	0	19	56	-5	**41**
Early Successional	-220	0	0	0	15	-6	**-211**
Late Successional Forest	0	0	0	0	21	-25	**-4**

Table 30 Copyright: This table is redrawn from Table 2 in Robertson *et al.* (2000), which is published in the journal '*Science.*'

Figure Set 5 Student Instructions

Familiarize yourself with the Figure Set 5 Background and Table 30 before coming to class.

Figure Set 5 Class Activity - State of Michigan Hearing

The State of Michigan is considering passing a law that requires farmers to become GWP-neutral, which means that they no longer can be a net emitter of greenhouse gases. To do this, they would need to change their agricultural practices to no-till or organic methods and/or set aside some of their land in conservation areas (e.g. Early Successional), where greenhouse gases are sequestered in the soil.

You will be assigned to one of six stakeholder groups that have a chance to testify in front of the Michigan Legislature. In your group, you will construct a statement and will have three minutes to address the State of Michigan Legislature, presenting your argument. A seventh group of Michigan Legislators will also be formed from students. The six interest groups are:

1. Michigan corn farmers, who need to cultivate as much land as possible to maintain income levels, and make sure that they get enough use out of expensive equipment.
2. Landowners along the Florida coast that would lose their property with only a 1 meter rise in sea level if global warming continues. Global warming will cause thermal expansion of water and the melting of ice sheets on land in northern and southern areas, resulting in more water in the sea.
3. The Pheasants Forever organization. Pheasants thrive in conservation areas that are planted to prairie grasses and forbs.
4. The ethanol industry, which is increasing dramatically in size, and utilizes corn grain for ethanol production. Biomass from trees, grasses and crops are also being considered for use in the synthesis of ethanol. Ethanol can be partially substituted for gasoline.
5. The Sierra Club, which promotes conservation of prairie habitat and biological diversity.
6. The Food Industry, which uses corn to make many food items that are consumed in large quantities by the public.

After all arguments are presented, the State of Michigan Legislature group will decide whether or not to vote in favor of the law.

Figure Set 5 Notes to Faculty

Many details are described for students in the “Student Instructions” section. Refer to that section for any details about the figure. The suggested student active approach for Figure Set 5 is called “Citizen’s Argument.” This approach requires students to approach a situation from the perspective of various citizen groups, and to make arguments that are grounded scientifically, yet represent their point of view. This activity was tested in a small high school classroom, and there were some outstanding statements from students.

After giving the students some time to read the instructions and look over the table, divide them into seven different groups and assign them to either the Michigan State Legislature or one of the six interest groups. You may need to assign the students to groups a few days before class, so that they can familiarize themselves with the interest group that they will be representing. Give them 10-15 minutes to prepare their argument. There are many other interest groups that could be formed – so feel free to modify the activity.

Each group has 3 minutes to present their argument to the legislature, which is a total of 18 minutes. Give the legislature a few minutes to think about how they want to vote, and then have each member of the legislature vote for or against the GWP-neutral farm law. After the legislature vote, hold a final class discussion about the outcome.

While the students are planning in their groups, they can come and talk to you – or ask questions about the figure. This is a good time to make any clarifications. Remember to remind them that positive GWP numbers on the table mean that greenhouse gases are being emitted while negative numbers mean greenhouse gases are being sequestered. You may also point out that these numbers are for southwest Michigan, and may vary with the landscape.

The activity as written will work best in small classrooms, but could be adapted to larger classrooms. Instead of including the entire class in one single "citizen's argument," divide the class into groups of seven. Each group of seven students conducts their own citizen's argument, where one student represents the legislature while the other six argue for one of the six interest groups. After providing the students with ample time to discuss, the legislator for each group can report back to the entire class regarding their decision, and why they decided to support or disapprove of the law.

Give the students the Land Management Activity as a homework assignment. This assignment requires them to consider both environmental and economic reasons for implementing certain farm practices. Of course there are other reasons that are factors, such as quality of life and labor requirements, but economics and environmental impact are two of the largest factors. When asked to produce the most grain possible while maintaining GWP neutral status, students should plan their farm to include no-till acres and early successional acres. To maximize profit, students may want to use a mixture of organic, no-till and early successional acres, depending on how they want their farm to look. Students should also include pictures of streams, ditches, etc.. in their pictures to depict a real scenario.

Figure Set 5 Post Lesson Assessment: Land Management Activity

Data from the W.K. Kellogg Biological Station Long Term Ecological Research Experiment (KBS-LTER) has shown that the global warming potential (GWP) impact of different agricultural activities could be mitigated considerably by changing management strategies. Adopting specific strategies (e.g., early successional) that minimize GWP seems straightforward; however, in reality these decisions are complex due to the broader social and economic issues. In an agricultural setting, a farmer strives to maximize his profits, so allowing all of his/her land to revert to early successional fields is not an economically viable strategy. Likewise, this strategy would not be suitable in a social context either, because societies like to maximize food production.

In this activity, you will assume the role of a farmer that needs to develop a management plan for his/her **1000 acre farm**. However, assume that a recent mandate by the government states that all farms in the United States must be GWP-neutral (0 lbs CO_2 $acre^{-1}$ yr^{-1}). Therefore, the farmer needs to develop a management plan that uses different proportions of various cropping methods (conventional vs. no till vs. organic vs. early successional) to be certified as a GWP-neutral farm under two different scenarios:

(1) maximization of farmer profit

(2) maximization of food production

Use Table 31 below, generated using data from the Kellogg Biological Station Long Term Ecological Research (LTER) experiment, to develop **two** farm management plans.

1. Maximize grain production while maintaining GWP Neutral Status
2. Maximize profit while maintaining GWP Neutral Status.

Create your farm management plans on two separate sheets of paper. For each of the two plans, **include the following**:

1. A drawing of your fictional farm with plots of land labeled based on cropping method (Remember to consider that most farmland is not flat and some areas work better for agriculture than others)
2. The total amount of acres for each cropping method
3. The total annual grain yield from your farm
4. The total gross profit from your farm

Table 31. Data from the Kellogg Biological Station LTER experiment to be used in generating the farm management plan.

Cropping Method	**Global Warming Potential (lbs CO_2 equivalents / acre)[#]**	**Average Annual Grain Yield (bushels / acre)***	**Average Annual Gross Profit (dollars / acre)[+]**
Conventional	1014.6	55.2	$254.91
No-Till	124.6	56.9	$263.82
Organic	364.9	41.6	$336.06
Early Successional	-1877.9	0	0

[#] Positive values mean that greenhouse gases are being emitted into the atmosphere while negative values indicate that greenhouse gases are being sequestered by the cropping system.

[*] The average grain yield in bushels per acre includes all three crops (corn, soybeans, wheat) grown in the KBS LTER experiment. These three crops are grown in a three year rotation, so that each crop is grown every third year.

[+] The gross profit values are based on conventional and organic grain markets in Chicago for the week of April 17th, 2007.

Additional Resources

Chicago Climate Exchange:
http://www.chicagoclimatex.com/

Ecological Society of America - Issues in Ecology:
http://www.esa.org/science_resources/issues.php

Intergovernmental Panel on Climate Change Website
http://www.ipcc.ch/

Michigan Greenhouse Gas Emissions Calculator for agroecosystems:
http://lter.kbs.msu.edu/carboncalculator/

- The W.K. Kellogg Biological Station is in Kalamazoo County

Purdue University - Using Agricultural Land for Carbon Sequestration:
http://www.agry.purdue.edu/soils/Csequest.PDF

USDA NRCS Global Climate Change Website
http://soils.usda.gov/survey/global_climate_change.html

U.S. Environmental Protection Agency Climate Change website:
http://www.epa.gov/climatechange/index.html

U.S. Environmental Protection Agency Methane website:
http://epa.gov/methane/

U.S. Environmental Protection Agency Nitrous Oxide website:
http://www.epa.gov/nitrousoxide/index.html

W.K. Kellogg Biological Station Long Term Ecological Research website:
http://lter.kbs.msu.edu/

Included PDF's

Robertson *et al.* 2000
Duxbury 1994
Schlesinger 1999

Suggested Textbook

Schlesinger, W. H. 1997. Biogeochemistry: An analysis of global change. Academic Press, San Diego.

Acknowledgements

We thank numerous people at the W.K. Kellogg Biological Station (KBS), including Phil Robertson, Laurel Hartley and Sara Syswerda for inspiration, Drew Corbin for organizing years of data collection and everyone involved in the GK-12 Program, including graduate fellows and teachers. We thank the National Science Foundation and the Long Term Ecological Research network for providing funding for research and educational activities at KBS. We thank Charlene D'Avanzo for providing guidance in developing this activity and Katie Button, Jarad Mellard, Gary Mittelbach, Todd Robinson and Lindsey Walters for providing comments on earlier versions of the activity. Two anonymous reviewers were very supportive and provided excellent suggestions for revisions. This is KBS contribution #1444.

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