<u>Title: Diversity in biofuel feedstock production</u>

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Gap/Problem statement:

Strict monocultures may not be desirable for biofuel feedstock production due to the need to meet multiple sustainability and production goals while producing fuel. Efforts to increase the level of diversity in biofuel feedstock production can reduce ecological impacts, improve productivity, and increase system resilience to environmental perturbations (e.g., drought, contamination with unintended species).

Background:

The land that can be dedicated to biofuel feedstocks is of high value and needs to be used efficiently to justify diverting land to biofuels and to provide greater biofuel supply. Therefore, recently a great deal of interest has developed in using ecological knowledge to enhance the methods by which biofuel feedstocks are produced in order to increase overall yields and/or reduce environmental impacts. Some examples of these recent efforts include multiple cropping (growing multiple monoculture crops on the same land over the course of a year), mixed cropping (growing multiple species at the same time to meet ecological or yield targets or to meet multiple product needs), and the use of diverse species in systems such as algal aquaculture (to increase system resilience and decrease “crop” failures and interventions to prevent invasion by unwanted organisms). In each case, using a suite of complementary species with regard to cultivation requirements, resource needs, and/or ecosystem function would provide improved productivity for the same land area. Management and cultivation strategies such as double or mixed cropping of feedstocks with food crops may help protect food production (Tilman et al. 2009) and reduce nutrient leaching and soil erosion that normally occur during fallow periods (Anex et al. 2007). Further work in this area is desperately needed to reduce food/feed/fuel conflicts and improve the ecological value and resilience of biofuel production systems.

Multiple cropping entails the rotation of species with complementary seasonal growing requirements to allow sequential monocultures to be grown on the same land over the course of a year. Another example might be replacing a fallow year in which no crop is grown with a season of biofuel feedstock production, particularly if the feedstock is different ecologically from the food crop. A recent review of *Brassica* species suggested that rotation of these crops with wheat may be beneficial for wheat production as well as oilseed crop production (Angus et al. 2011). At least one *Brassica* species (*Brassica* *carinata*) is being tested for it's potential as a winter crop in southern wheat production (Crampton, Agrisoma, pers. comm. 10/15/2012). These types of double cropping offers the efficiency of monocultures (including mechanized seeding and harvesting, simplified agricultural additions, etc.), reduces soil erosion compared to fallow, offers the possibility of pest life cycle disruption (by growing a different kind of crop on the land), and may possibly improve soil fertility if the rotational crop is leguminous (nitrogen-fixing) or has other soil fertility benefits.

Mixed cropping involves the growing of more than one species at the same time (rather than sequentially as with multiple cropping). The potential advantages of mixed cropping are the possibility of meeting several needs at the same time (i.e., food and fuel) and/or achieving ecological/sustainability goals while providing substantial biofuel production. Feedstock cultivation can be used to enhance biodiversity and ecosystem functions. Tilman et al. have suggested that the use of low-input, mixed prairie grasses that grow well on moderately degraded former agricultural lands could provide greater biomass accumulation over time compared to monocultures, and would also provide substantial benefits for maintenance of plant biodiversity and ecosystem functions associated with native prairie (Tilman et al. 2006). The use of mixed cropping in this form would likely not include (human) food production, although grazing animals could conceivably be included in the rotational mix. Research has shown that over seeding a cool-season leguminous species in a stand of warm season grasses can eliminate the need for chemical nitrogen fertilizer application (Holmberg et al. 2012). However, it should be noted that separation of multiple crops poses significant logistical and cost challenges, and therefore it may be better to focus on mixed cropping of biomass crops which do not need to be separated after harvest. But regardless of whether co-production of food is undertaken, the use of mixed prairie grasses can meet significant conservation goals if managed properly. Feedstock production can be managed to maximize insect and bird habitat, including avoiding harvesting perennial grasses during bird nesting season, maintaining a mosaic of harvested and unharvested grasslands to maximize bird diversity based on varying preferences for grass height, and planting of mixed crops rather than monocultures (Fargione et al. 2009). Fargione et al. (2009) also recommend research to develop biodiversity management approaches in conjunction with development of major bioenergy projects to maximize diversity/wildlife benefits. Another major potential benefit of using native prairie grasses is that it avoids the introduction of non-native species that may pose risks of becoming invasive in natural areas. Some incentive programs restrict benefits to projects that use native spp and/or enhance local environmental conditions (e.g., Minnesota RIM-CE, BCAP). Challenges include maximizing productivity while meeting these multiple needs, providing high quality (controlled quality) output, proper valuation of ecosystem functions to provide incentive for slightly less "efficient" but more beneficial feedstock production.

A third recently proposed approach to using diversity for the benefit of biofuel production is the development of diverse algal cultures. Thus far, algal systems are generally grown as monocultures, or intended monocultures. However, growers who use open pond or raceway cultivation systems, which generally have lower costs and lower environmental impact than closed photobioreactors, are engaged in a constant battle to reduce losses from the invasion of unwanted organisms that either outcompete the target species, thus reducing their abundance and/or performance, or feed upon the species. Even closed systems periodically suffer the same issues, resulting in population collapse and requiring sterilization of components and restarting of the algal culture. Recently, it has been proposed that based on what is known about the ecology of microorganisms, systems that incorporate synthetic ecology of carefully chosen suites of organisms should be more flexible and resilient in response to the invasion by a toxic, predatory, or otherwise undesirable species and that they should therefore be better at maintaining productivity with fewer inputs and restarts (Kazamia et al. 2012). This approach has been suggested based on ecological knowledge and some research regarding control of algae through community ecology. In at least one case, such a system has provided benefits for maintenance of productive filamentous algal species such as *Spirulina* species over less productive *Chlorella* species (Mitchell and Richmond 1987). Similar general approaches have also been used in animal agriculture; farmers use probiotic bacteria inoculum in animals to prevent infection by pathogenic organisms (Callaway et al. 2008). While this practice has had mixed results, the successful cases suggest a potential model system for biofuel production from mixed cultures, according to Kazamia et al (2012). Similarly to mixed cropping, part of the approach is to cultivate several algal species with complementary resource needs. In addition, carefully selected predators in the system are intended to feed selectively on undesirable species (Mitchell and Richmond 1987). Thus, one of the outcomes of the synthetic ecology approach to algal production is anticipated to be increased biomass production, which can then be used either for lipid extraction or biomass-based process fuel, or both.

Current Status:

A number of organizations are currently exploring the potential to use oilseed crops (such as *Camelina sativa* or *Brassica carinata*) as rotation crops with wheat, although current research suggests that such crops are likely to need additional breeding and development to provide fallow period production without impinging on wheat yields(Yao 2010). Some production is currently being achieved in the upper Midwest (e.g., Montana) and in Pennsylvania using this approach. Rotation overseeding of canola into harvested native grass has been conducted at Mississippi State University (Baldwin unpublished data), but it is certainly still in the incipient stages of adoption. We are not currently aware of any mixed prairie grass or other mixed cropping production systems (other than the experimental ones described by Tilman et al.) in use to cultivate feedstocks for biofuels. The synthetic ecology approach to algal or microbial biofuel production systems has been proposed on the basis of theoretical effects on production but to our knowledge has not been implemented even on the test scale.

Solvability and Approaches

In order to facilitate multicropping, it is necessary to identify good alternate crops for key agricultural systems that take advantage of fallow/off-season without impinging on food crop production, and that preferably also enhance soil fertility, etc. Crops such as *Camelina* are expected to require significant breeding and optimization to make it more appropriate for rotation crop use (Yao 2010). Recent research has already led to release of varieties of *Camelina* improved with regard to yield (e.g., (Sustainable Oils 2010)) suggesting that additional improvement may eventually lead to suitable rotation varieties. Leguminous species other than soybeans have not been extensively screened for biofuel production potential, but there is both precedent for leguminous oilseed production on large scales (i.e., soybeans) and the additional benefit of nitrogen fixation by plants in this family, which adds to soil fertility over time, providing a nitrogen source for crops grown in double crop system or in rotation.

To facilitate mixed cropping, the agricultural community needs to perform research to develop suites of crops that grow well together in mixed cropping systems for food/biofuel production or for the enhancement of ecosystem functions and the maintenance of biodiversity. Multiple assemblage options would need to be developed for different regions of the country (i.e., beyond native prairie concept). Mixed cropping assemblages are likely to be different from multicropping options in which species are sequentially grown in monocultures. Therefore, we suggest two different research areas with similar overarching goals (maintenance of biofuel and food yields, improvement of soil quality and fertility, and benefits to biodiversity) in order to maximize benefits and minimize costs/trade-offs for each cultivation system.

Although in theory algal diversity may protect synthetic systems from population crashes and catastrophic invasions by unwanted organisms, little work in biofuel production systems has been performed to date; in fact, it appears that most previous work has focused on synthetic ecology to clear algae from water rather than enhance growth (Kazamia et al. 2012), although some work with Spirulina suggests that carefully assembled communities may enhance productivity (Mitchell and Richmond 1987). Experimentation is needed to identify the level of diversity and suites of organisms that provide protective diversity without reducing productivity with regard to lipid or biofuel production. Furthermore, Kazamia et al suggest that the organismal assembly will need to vary by location to address local wild species that may colonize open algal production facilities. Thus, it may be necessary to collect information on potential colonizing species in a variety of settings to develop appropriate "plug-and-play" assemblies for different locations. Furthermore, a number of biofuel producers intend to use genetically engineered (GE) organisms that process a substrate (CO2 or sugars) into hydrocarbons or other high value products. These products may be toxic to many otherwise suitable assemblage members. Furthermore, GE species may have specific nutrient requirements or other control mechanisms that may affect community members. Therefore, it is anticipated that a separate research effort would be required to identify suitable communities to coexist with and facilitate stability and productivity for GE organisms.

Benefits to industry as a whole

The use of synthetic ecology to develop multicropping, mixed cropping and/or algal community approaches to biofuel production could substantially reduce food/feed/fuel conflict by reducing the land use requirements for biofuels, through improved yields. Multi- and mixed-cropping also provide the possibility of producing food and fuel on the same land. Furthermore, mixed cropping and algal synthetic ecology strategies could result in improved system resilience and productivity by buffering against environmental perturbations such as drought, invasion by unintended organisms, etc. This would stabilize feedstock yields/supply.

Potential benefits of both multiple and mixed cropping include reduced erosion, benefits to soil fertility (e.g., use of nitrogen-fixing legumes as one of crop options, use of mixed types of crops (mono- vs dicot) to break pest cycles. These effects may also reduce agricultural inputs (fertilizer, pesticides).

The benefits of synthetic ecology for algal systems include greater system stability (fewer restarts) and improved productivity of biomass and possibly lipid production. Ina addition, reduced maintenance costs should reduce the final fuel costs from open algal systems.

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