

Productivity of Gramineae Species from Nitrogen Produced by Autumn Olive and Cowpea Grown in Soils of Differing Quality

Joel Betts

Mentored by Dr. David Dornbos Jr.

Biology Department, Calvin College

ABSTRACT

Applying ecological principles to the current unsustainable industrial agriculture system in the United States has the potential to lessen human impact on the environment and ensure long-term food sustainability. Interplanting crops with biologically nitrogen fixing (BNF) plants is an agroecological method that offers promise for enhancing agricultural systems in a sustainable way. In this study, six treatment pairs were used to analyze the weekly and seasonal effects of the BNF plants autumn olive and cowpea on the Gramineae species corn and switchgrass. We collected measurements of leaf chlorophyll content, leaf number, rate of photosynthesis, transpiration rate, and biomass accumulation for treatment pairs grown in two soils of different type and quality. Acetylene reduction was used to confirm nitrogen fixation in the BNF plants and to estimate nitrogen availability in the soil to both plants. Autumn olive was shown to have a high rate of nitrogen fixation at 3.8 ethylene-acetylene GC peak area ratio per gram fresh weight of nodules per minute of incubation. Leaf number and biomass were shown to be higher in switchgrass growing with autumn olive as compared to switchgrass monoculture. Our data strongly suggest a nitrogen benefit to switchgrass from interplanting with autumn olive. Corn leaf number and leaf chlorophyll content were also significantly higher when grown with autumn olive, although this effect is potentially exaggerated because the autumn olive plants were smaller than cowpea or corn plants, resulting in reduced water competition between the autumn olive and corn and therefore more corn growth in comparison to other treatments. Water competition and shading effects may have also nullified a potential nitrogen bonus from cowpea on adjacent plants. Corn, cowpea, and autumn olive exhibited greater productivity in Perrinton (higher quality) soils than in the coarser textured Oshtemo soil. Although autumn olive is an aggressive invasive, our results indicate that growing it in interplanted production systems could provide a sustainable nitrogen alternative to the current agricultural system's reliance on fossil-fuel derived nitrogen fertilizer.

Introduction

The industrialized food production system widely used throughout the United States is not sustainable (Raven 2002). The current system depends on fossil fuels to produce fertilizers and pesticides, and to process and transport food products. Not only do fertilizers and pesticides have a large carbon footprint, but they also tend to run off farmland and harm aquatic ecosystems. The large machinery used for tilling and harvesting can degrade soil and encourage soil erosion. At least twenty percent of topsoil has been eroded away globally over the past 60 years, and today, more nitrogen fertilizer is applied to farmland than is produced by natural processes (Raven 2002). Although the industrialized food production system results in high yields, production systems must change if we are to sustainably feed a growing world population in the long run.

The mechanized industrial systems in current use require that crops be grown in monocultures. The lack of plant diversity in these production systems makes for an unstable agro-ecosystem that is vulnerable to diseases and pests (Altieri 1998). An alternative method to this environmentally costly farming system is needed. Agroecology represents a particular approach of sustainable agriculture (SA), a more radical approach to changing the way food is produced than those SA techniques being widely adopted in agricultural systems today. Agroecology uses ecological principles as the basis for the development of agricultural systems. It focuses on using the natural processes of regeneration, plant-to-plant and pest-to-predator interactions, nutrient cycling, and hydrologic cycling to sustainably produce quality yields. It has much lower environmental costs than the industrial system as it does not depend nearly as much on fertilizers, pesticides, herbicides, or machinery. Agroecological systems are generally much more diverse and integrated, more like a natural ecosystem. This reduces vulnerability to a wide variety of disease and insect pests that require pesticide treatment in monoculture systems. Agroecological principles can be applied in many different ways and to varying extents. Research shows that agroecosystems can maintain high productivity without chemical inputs. Ecologist David Tilman of the University of Minnesota published a study in *Science* that showed that a mix of 16 legumes, grasses, and forbs resulted in the production of 238 percent more bioenergy after ten years, in comparison with a monoculture grown under similar conditions (Tilman 2006). In Havana, Cuba mixed systems of maize-sweet potato, cassava-maize-beans,

and tomato-cassava-maize have proved to be 1.45 to 2.82 times more productive than monocultures of the individual species (Altieri, Rossett, and Thrupp 1998).

Nitrogen is a macronutrient required in large quantity by plants and is absolutely essential to optimize crop yield. It is typically the primary limiting nutrient in both agroecosystems and natural ecosystems. Plants absorb nitrogen from soils as nitrate or ammonium ions. In industrial systems, nitrogen has been removed from soils faster than it is replenished by natural processes. Farmers compensate for the inability of soils to provide sufficient nitrogen by applying nitrogen fertilizer, most commonly in the form of ammonia (Howarth and Townsend 2010). Chemical nitrogen fertilizers must be produced using the energy-intensive Haber-Bosch process in a reaction that requires conditions of 500 °C and a pressure of 200 atmospheres in the presence of an appropriate catalyst to reduce atmospheric nitrogen to ammonia. In natural ecosystems, however, atmospheric nitrogen is fixed by species of bacteria. Several plant species form a symbiotic relationships with nitrogen-fixing soil bacteria. These plants attract the soil bacteria and house them in nodules on their roots. The nodules provide the anaerobic conditions necessary for biological nitrogen fixation (BNF) to occur. The bacterial species receive nutrients from the plant, while providing usable nitrogen to the plant. Enhanced use of BNF in agroecosystems would encourage sustainability. Currently, most Midwestern farmers rotate soybean (*Glycine max*) with corn (*Zea mays*) to take advantage of BNF by soybean. One study showed approximately 44.8 kg/ha of residual nitrogen available for corn from the previous year's soybean crop (Brunoehler 2003). Although a step towards sustainability, such rotations limit N supply from BNF plants to alternate years, requiring a greater proportion of the nitrogen required for crop growth to be applied as chemical fertilizer.

Plants from the *Leguminosae* family such as soybeans and cowpea (*Vigna unguiculata* (L.) Walp.) are capable of BNF through a symbiotic relationship with species of bacteria in the family Rhizobiaceae. Other species have exhibited nodulation with *Frankia* spp. bacteria. One of these species is *Elaeagnus umbellata* (Thunb.), commonly known as autumn olive (Mirza et al. 2009 and Oh et al. 2012). Rentschler and Bouma, (2010) determined that larger non-native and invasive autumn olive plants growing in poor soils produced a greater number of nodules and that high nodule number was correlated with high leaf chlorophyll content, faster photosynthetic rate, and competitive advantages over co-existing native shrubs. More interestingly, when black cherry (*Prunus serotina*), hawthorn (*Crataegus monogyna*), black

walnut (*Juglans nigra*), or gray dogwood (*Cornus rasemosa*) were grown in small pots with autumn olive the leaf chlorophyll content and rate of new leaf production in the native co-habiting trees were significantly higher than when grown with another plant of the same species (Rentschler and Bouma, 2010; VanKampen, 2011). Using acetylene reduction, VanKampen (2011) confirmed that autumn olive nodules are capable of very high rates of nitrogen fixation. Together, these results suggest that autumn olive is aiding the productivity of neighboring plants, likely by contributing residual nitrogen.

In view of these results, we hypothesized that autumn olive increases the growth of *Gramineae* species growing in close proximity by contributing biologically fixed nitrogen. Grass plants are incapable of nitrogen fixation and need substantial quantities of nitrogen to achieve optimum growth rates. By extension, we also hypothesized that *Leguminosae* species (specifically, cowpea) would provide similar benefit to neighboring *Gramineae* plants.

If supported by research, this principle could have positive implications for SA. As a method of natural nitrogen fertilization, carefully constructed polycultures with interplanted BNF and non-BNF plants could reduce or replace dependency on fossil fuel derived nitrogen fertilizer. Such a more integrated and diverse agroecosystem could also provide a series of other benefits, such as increased habitat for natural pest predators and other species, and less vulnerability to crop disease (Altieri 1998).

In Africa, growing legume cover crops on a plot of land before growing maize crops has been shown to increase maize crop yields (Ajavi 2007). Research on African agroforestry alley cropping systems showed that 45% of the nitrogen from prunings of leguminous shrubs were absorbed by and increased yields of various nearby crops such as maize (Mulongoy 1992). Clark, Hemery and Savill (2008) showed that, in England, when the common walnut was grown with autumn olive as its nurse species over a 7 year period, it exhibited higher nitrogen levels, greater overall height, trunk width, and plant biomass, in comparison to the control of common walnut grown by itself. These cases indicate that BNF plants, specifically BNF shrubs like autumn olive, can provide organic nitrogen fertilizer to neighboring shrubs.

The goal of this research is to test the potential of autumn olive and cowpea to provide beneficial nitrogen to a neighboring plant when growing in real-time next to it, without requiring pruning and mulching vegetation, or rotating crops for consecutive years. In order to model a key requirement of an agroecological polyculture system we tested the capacity of the BNF-

plants autumn olive and cowpea to directly enhance the growth of nitrogen-requiring *Gramineae* species. The objectives of this research were to:

1. Determine the leaf chlorophyll content, leaf number, and biomass accumulation of: corn adjacent to autumn olive and cowpea, and of switchgrass (*Panicum virgatum*) adjacent to autumn olive and cowpea in comparison with corn adjacent to corn and switchgrass adjacent to switchgrass.
2. Determine the effects of BNF on the other nitrogen requiring plants in two types of soil varying in quality.
3. Use acetylene reduction to confirm the nitrogen—fixing capacity of BNF plants.

Methods

Plot Establishment: The project was carried out at Piece Cedar Creek Institute (PCCI) near Hastings, Michigan. We identified two trial locations for research plots, one with Oshtemo sandy loam soils (poorer quality) west of Meadow Lodge, and one with Perrinton loam soils (higher quality) south of Hyla House. In determining these locations, we took care to ensure that they were similar in slope, sunlight exposure, and other environmental parameters besides those related to soil. Each research area was 7 by 10 m and was treated with glyphosate herbicide and later mowed to clear the early emergent vegetation. A 1.2 m tall fence was built to prevent herbivore damage to plants. Research plots were organized into a grid pattern of subplots with each subplot taking up a 1 m² area. In order to mitigate soil compaction, we tilled a 0.30 m deep by 0.30 m wide cylindrical hole in the center of each 1 m² research plot, leaving the layer of plant residue in the area around the tillage-holes to reduce weed emergence.

Planting Procedure and Experimental Design: We grew two plants types in each tillage hole; each pair representing one treatment using a split plot randomized complete block experimental

design. Treatments were represented by six plant-pair combinations randomized in each east-west row:

1. One corn (C) plant and one autumn olive (AO) plant C x AO etc.
2. One switchgrass (SG) plant and one autumn olive plant
3. One corn plant and one cowpea (CP) plant
4. One switchgrass and one cowpea plant
5. Two corn plants
6. Two switchgrass plants

There were ten replicates of the six plant combinations in north-south rows at each location (see Appendix A, Figure 1). Replicates were organized as blocks, approximately perpendicular to the topographic trend of slope (which was less than 7%). In each case, our goal was to have two plants in each plot. We placed plant pairs in each plot 0.10 m apart, sowing cowpea, corn, and switchgrass seeds at the Meadow lodge location on June 6 and the Hyla House location on June 8. For corn and cowpea, we initially planted three seeds and thinned down to one seedling soon after emergence. Five to eight switchgrass seeds were planted in each plot, and in many cases more than one switchgrass was left in each treatment because they grew up close together. In a few subplots, no switchgrass emerged, so switchgrass plants were transplanted from other subplots at the same location. In the case of AO, on June 11, 0.10-.25 m tall seedlings were collected from one location on the PCCI property and transplanted into the appropriate plots. We assumed that all transplanted AO plants possessed nodules capable of nitrogen fixation and confirmed this with a number of AO plants similar to those transplanted. In order to assure adequate bacteria availability for nodule formation in the cowpea plants, we inoculated the seeds during planting with an optimum *Rhizobium* strain (sent by Burpee with the seeds). We did not inoculate autumn olive with *Frankia* as it already possessed nodules. Additional AO and cowpea plants were placed in an adjacent 1 x 7 meter area on the east side of the plots for eventual acetylene reduction analysis of nitrogen fixation rate.

Watering and Weeds: Because it was an exceptionally hot and dry summer, we supplemented rainfall with 1 L additional water per plot, about twice a week. We pulled weeds manually every week to avoid unwanted resource competition. The plant residues were left around the tillage-holes and helped prevent weeds from emerging.

Plant Measurements: As the transplants and seedlings grew and developed through the summer, we made a number of weekly measurements on each plant, including leaf number and chlorophyll content. For each plant, leaf chlorophyll content was measured from a fully expanded leaf using a SPAD-502 chlorophyll meter. This meter does not have specific units, so units were not reported. It was used for proportional chlorophyll content comparison between plants and treatments. Switchgrass leaves were too small for chlorophyll measurement until just prior to harvest.

We used a LiCor 6400XTR system to measure photosynthesis and transpiration rates and leaf temperature during late-July and early-August on leaves of six replicates in both locations. Measurements were taken on the third-down fully expanded leaf, or in the case of AO which has smaller leaves, measurements were taken on a representative leaf that best fit the Li-Cor cuvette (often the biggest leaf). Switchgrass was too small for gas exchange measurements.

At the end of the growing season (mid-August), we removed three replicates of all plants from each location to count and weigh AO and CP root nodules and to estimate their rate of nitrogen fixation using acetylene reduction (AR) assays. In order to keep nodules alive, we transplanted these plants into pots and kept them in the greenhouse at Calvin College. We carried out AR in two batches, assaying nodules from the Oshtemo soils first (replicates 4-6), and from the Perrinton soils second (replicates 3-5). We carefully separated BNF plant roots from the soil and the roots of the partner Gramineae species. All above ground biomass was saved and labeled. We excised and counted all nodules with 1 cm of root and quickly placed them into a 6 mL gastight vial with 10 % acetylene gas. After a 3-h incubation period with periodic shaking, a 10 μ L sample of gas from the vial was injected into a gas chromatograph (GC) equipped with a 2 m column packed with 80/100 Poropak R and a flame ionization detector. Injection temperature was 21 $^{\circ}$ C, column temperature 420 $^{\circ}$ C, and flow rate 4.25 ml/min. Two gas samples were measured from each vial. Nodule fresh weight was taken after GC runs were finished. The ratio of ethylene to acetylene was measured using peak area ratio per gram fresh weight of nodules per minute of incubation, therefore showing relative rate of nitrogen fixation between plants and treatments. We harvested all plants by cutting them off at ground level, transported them to the lab, cut them into small pieces, dried them in convection ovens at 50 $^{\circ}$ C and determined dry weight.

Data Analysis: The data from each location was evaluated using Statistix 9 (Analytical Software, 2008) as randomized complete block design with six treatments and ten replicates for each location. Chlorophyll, photosynthesis, transpiration, and plant weight means among all treatments were compared using Tukey's mean separation test with an alpha level of 5 %. Mean groupings not significantly different from one another were given the same letter.

Results

Acetylene reduction for AO showed a higher nitrogen fixation rate, at 3.8 ethylene-acetylene GC peak area ratio per gram fresh weight of nodules per minute of incubation (see Appendix A, Table 1). Although cowpea plants were bigger and had many more nodules than AO, their nitrogen fixation rate was slower at 1.3 ($P=0.076$ where P is the probability of a greater F). These data confirm not only that both plant species were fixing N_2 , but that AO was likely fixing nitrogen faster than cowpea per unit of nodule mass. This could be a result of the differences in bacteria type or plant process efficiencies. There was no significant difference in N_2 fixation rates between the two locations, although fixation rates trended higher in the Perrinton soils at Hyla House ($P=0.21$).

Leaf chlorophyll content varied between treatments and more so between plant species (Appendix A, Table 2). Mean chlorophyll content of corn in the presence of AO was significantly higher (52.0) than that of corn with cowpea (49.5) or corn with corn (47.9) ($P=0.0004$). Corn plants growing in the presence of cowpea plants tended to have higher leaf chlorophyll content than corn with corn. Switchgrass did not show significant differences in chlorophyll content between treatments ($P=0.78$). Neither AO nor cowpea showed any significant difference in leaf chlorophyll content between treatments ($P=0.84$ and $P=0.20$ respectively). All corn plants increased chlorophyll content throughout the summer up to week six, and only corn in the presence of AO kept increasing in chlorophyll content through week 8 (Appendix A, Figure 2).

Plant leaf number also differed among treatments (Appendix A, Table 3). Mean leaf number of corn was significantly higher in the presence of AO than that of corn with cowpea or corn with corn. Mean leaf number of corn with corn was also significantly higher than corn with cowpea ($P<0.0001$). Corn with AO appeared to achieve maximum leaf number more quickly

than corn with cowpea or with another corn (Appendix A, Figure 3). Mean leaf number of switchgrass with AO was significantly higher than switchgrass with cowpea ($P=0.015$), while mean leaf number of switchgrass with switchgrass was not significantly different than leaf number of switchgrass with AO or cowpea. Leaf number of AO did not vary significantly between treatments, although there trended to be more leaves when it grew with corn ($P=.076$). Unlike AO, cowpea produced significantly more leaves when it grew with switchgrass compared to with corn ($P<0.0001$).

Switchgrass dry weight of above-ground biomass differed between treatments (Appendix A, Figures 4 and 5). Switchgrass had significantly more biomass when grown with AO than when grown with another switchgrass or with cowpea ($P=0.0041$). Switchgrass had a trend towards higher dry weight in the Oshtemo soils than in the Perrinton soils, but it was not statistically significant ($P=0.57$) (Appendix A, Figure 5). Corn and cowpea biomass are still being analyzed and will be reported at a later date.

Using the LiCor 6400XTS system, rates of photosynthesis for corn by AO were found to be the same as rates of corn by corn, and both of these were significantly higher than the rate of corn by cowpea ($P=0.034$) (Appendix A, Table 5). The pattern was similar with transpiration rates. Corn by AO was significantly higher than corn by cowpea ($P=0.0005$), but also appeared to have a higher rate than corn by corn. Corn leaf temperature did not vary significantly among treatments.

There were differences in plant growth between locations as well (Appendix A, Table 6). Across treatments, average leaf number for each plant was higher in the Perrinton soils (higher quality) than in Oshtemo soils (poorer quality) ($P<0.05$). Average corn leaf chlorophyll content was significantly higher for plants grown in Perrinton soils ($P<0.0001$), while switchgrass had significantly higher chlorophyll when grown in Oshtemo soils ($P=0.045$). Average AO leaf chlorophyll content did not vary significantly between soil types ($P=0.43$).

Discussion

The objectives of this research were to evaluate the agro-ecological principle of interplanting a BNF species with one requiring significant amounts of nitrogen, and compare how the response differs in soils of varying type and quality. Our evidence supports the hypothesis that a real-time growth benefit can be achieved through the use of agroecological farming systems.

Acetylene reduction data provided evidence that indicated that our two BNF plants were indeed fixing nitrogen. But was this nitrogen available in soil to aid the growth of nearby plants? In the case of AO with switchgrass, it appears so. Average dry biomass weight of switchgrass in the presence of AO was significantly higher than both other switchgrass plant combinations (Appendix A, Figure 4). Also following this trend, switchgrass average leaf number in the presence of AO appeared to be higher than switchgrass with switchgrass, and was significantly higher than switchgrass with cowpea (Appendix A, Table 2). Average leaf chlorophyll content did not statistically vary between treatments, but this may partially be attributed to the difficulty in taking this measurement; leaves were just barely large enough to measure with the SPAD-502 chlorophyll meter on our last day of data collection. The switchgrass plants were planted from seed, and grew slowly during the summer months and remained small. It would be interesting to repeat the experiment with larger and more established switchgrass plants. Despite leaf chlorophyll content, these results show that AO had a positive effect on the growth of switchgrass. Given this positive effect, the rapid nitrogen fixation rate of AO (as shown by acetylene reduction), and the lack of other obvious benefits to switchgrass from growing next to AO, this evidence suggests that switchgrass indeed reaped a real-time benefit from the nitrogen fixed by neighboring AO.

Why was this not the case with switchgrass by cowpea, a nitrogen fixer who had even more nodules, and likely fixed more overall nitrogen than the AO plants? We suggest that even if the cowpea was contributing available nitrogen to the switchgrass plants, that this benefit was outweighed by competition for resources with cowpea (light and moisture). This was evident in the very small biomass (Appendix A, Figure 4) and the lower leaf number (Appendix A, Table 3) of the switchgrass plants growing next to the very large cowpea plants as the switchgrass plants likely spent most of their energy and resources producing a few thin leaves and elongated stems

in an attempt to get above their overarching neighboring cowpea. They appeared to be unable to expand horizontally with many leaves as in the other two treatments. This same sort of competition did not exist for switchgrass by AO because the AO plants were much smaller and did not overarch the young switchgrass plants.

Initially it appeared that available nitrogen from AO also benefitted corn plant growth. Leaf number and chlorophyll content were significantly higher in corn grown by AO than corn grown by another corn or by cowpea. But in the case of corn, potential competition for water leading to water stress must be taken into account. AO plants were much smaller than the cowpea and corn plants, and therefore used much less water overall. Transpiration rates of corn by cowpea were significantly lower than those of corn by AO or corn by corn (Appendix A, Table 5). A reasonable explanation for this is that there was less soil water in the plots where there were two larger plants compared to the plots with one large and one small plant. This research was conducted in a summer that was unusually hot and dry; there was little rain until mid-July, and it's likely that our water supplementation of 1 liter twice a week was not enough to prevent water stress from being a limiting variable. This could also explain why cowpea leaf number and chlorophyll content were higher when growing next to small switchgrass plants as opposed to next to larger corn plants (Appendix A, Tables 3 and 4). Therefore, the significantly higher leaf number and chlorophyll content seen in corn growing by AO could be attributed to the AO plants' small size and less competition for water with its corn partner than with the control and comparison plants, corn and cowpea. Alternatively, during these last weeks of summer, there was more sufficient rain, which likely reduced water stress in general. It is very possible that water competition was not as significant a factor to plant growth parameters as was the nitrogen boost from AO. At least some of corn's increase in leaf number and chlorophyll content could be due increased soil nitrogen from the neighboring AO plant. During the end of the growing season while water was abundant, chlorophyll content of corn growing with AO was still increasing, while corn in the other treatments saw slight decreases in chlorophyll content. This observation seems to support the hypothesis that corn was benefitting from increased nitrogen due to its proximity to AO. This would be consistent with the switchgrass results mentioned above.

Our data suggests that water competition was not an issue between AO and switchgrass. Switchgrass and AO did not appear to have this competition disadvantage, because the

switchgrass was a smaller plant than AO. If competition was a factor for switchgrass by AO we would have expected it to show reduced growth in comparison with switchgrass by switchgrass (opposite to the corn treatments), because of AO's comparative size advantage. As all evidence pointed to the opposite having occurred, we can reject the idea that competition for water was a limiting factor in the switchgrass by AO treatment.

Finally, our data also suggests that Perrinton soils produced larger and more productive plants than those from the Oshtemo soils (Appendix A, Table 6). Leaf number averages were significantly higher for all plants in higher quality soil, as was chlorophyll content of corn. AO chlorophyll content was the same between both soil types. But chlorophyll content was significantly higher for switchgrass in the Oshtemo soil. Besides switchgrass, our results were as we hypothesized—the better quality soils typically support more productive plants. But the exception to the pattern with regard to switchgrass chlorophyll content should be noted. That switchgrass has higher chlorophyll content in the poorer quality soil shows that it at least was not lacking the resources (principally nitrogen) that it needed to grow well. This may be attributed to a C4 photosynthesis coping mechanism present in the two *Gramineae* species. The BNF plants could also be fixing more accessible nitrogen into the soil in the poorer quality soils than in the higher quality soils, as a coping mechanism for the BNF. The higher chlorophyll content could be a result from the benefits of increased soil nitrogen in the Oshtemo soils in the presence of BNF plant species.

Our experimental plots do not closely resemble the real ecological system Tillman (2006) used to demonstrate a 238 % increase in productivity resulting from a mix of 16 legumes, grasses, and forbs compared with monocultures, but our data does demonstrate a similar agro-ecological principle. Growing AO 10 cm apart from seedling switchgrass plants over just an 8-week period showed an increase in biomass and leaf number, a pattern followed by corn as well. Given enough water, it seems like this effect would only increase as the plants matured and if there were more than two plants of each type per 1 m² area as in Tillman's experiment. If managed in an efficient and ecological way, the nitrogen benefit given by BNF plants like autumn olive could supplement the environmentally costly need for fossil-fuel produced N fertilizer. Fossil fuel produced fertilizers adversely affect ecosystems by creating aquatic dead zones, contributing to climate change, and causing soil acidification. Using BNF plants in

agroecological systems could provide a long-term sustainable alternative to modern industrial agriculture.

Although societal-structural changes and additional research would have to occur before agro-ecological principles like this could be applied on a large scale, implementation of sustainable agriculture systems involving BNF plants is feasible. Similar small scale sustainable agriculture systems are already being used. As previously mentioned, mixed systems of maize-sweet potato, cassava-maize-beans, and tomato-cassava-maize in Cuba (Altieri 1998), alley cropping with legume shrubs in Africa (Mulongoy 1992), the increasing world-wide use of legume cover crops (Ajavi 2007), and even the crop rotation of soybeans and corn in the U.S. industrial system are agro-ecological systems already being used to increase production.

What about AO and switchgrass specifically? Besides demonstrating these agro-ecological potentials, we think this combination may have merit in a biomass production system on abandoned farm fields with low soil quality. Since both are perennials and re-grow from seasonal cuttings, this combination could be grown as a sustainable fuel source. This is just one example of how AO, a BNF shrub, could be used in a sustainable system. Clark, Hemery, and Savill's (2008) research with autumn olive and walnut trees in England provides another example.

Despite these opportunities with AO, it is important to consider its history as an aggressive invasive in North America. Originally from South Asia, autumn olive was introduced to the U.S as an ornamental plant in the 1830's and then used more extensively in the 1960s to restore soils along highways and to provide wildlife habitat for game animals. Since then, it has quickly expanded its range throughout the eastern, midwestern, and some of the northern Pacific United States, as well as Ontario, Canada (Appendix A, Figure 6) (USDA 2012). It can out-compete native shrubs and can overtake forest understory, squeezing out native flora. Because of its invasive nature, some might argue that it is not worth experimenting with such an ecologically dangerous plant. On the contrary, AO has already been naturalized to most abandoned fields throughout its range and as such is here to stay. Ecological benefits of using AO, such as less reliance on harmful fertilizers, could outweigh the costs of having AO plants in an area in which it is already well established as long as these plants are harvested annually for biomass thereby preventing additional seed production and dispersal. Autumn olive doesn't

produce fruits until its third or fourth year, so if harvested for biomass every year, it could potentially be properly controlled.

Although there is not a clear answer of how to use autumn olive as a specific plant in agro-ecological systems, our research suggests its potential to help neighboring plants grow more effectively through nitrogen contribution. AO and other related or non-related, less ecologically harmful BNF plants could be and have been used to improve production and sustainability in many different agricultural systems. In this way, agro-ecological systems including BNF plants like autumn olive have much yet untapped potential to change the current unsustainable industrial food system in North America, bringing it closer to sustainability.

Appendix A

B10	A10	E10	C10	D10	F10	Cowpea and Autumn Olive Plants
B9	A9	C9	E9	F9	D9	
D8	E8	B8	A8	F8	C8	
C7	F7	D7	A7	B7	E7	
A6	C6	F6	B6	D6	E6	
C5	D5	E5	F5	B5	A5	
B4	E4	C4	D4	F4	A4	
A3	E3	F3	B3	C3	D3	
E2	B2	F2	A2	D2	C2	
A1	B1	C1	D1	E1	F1	

Figure 1. Plot design at the Meadow lodge location on Perrinton soil. Each subplot is 1 m². In this figure, the letters A-F signify the different plant combinations (treatments), and the number signifies the replicate (A=AOxCorn, B=AOxSG, C=CPxCorn, D=CPxSG, E=CornxCorn, and F=SGxSG). Combinations were randomized in each row, and replicated north-south. The Hyla House plot was set up following the same protocol.

Table 1. A comparison of acetylene reduction results between autumn olive and cowpea, and between soil types, in terms of ethylene-acetylene GC peak area ratio per gram fresh weight of nodules per minute of incubation.

Comparison	Ethylene/g fresh wt/min	Probability
Autumn olive	3.8	0.076
Cowpea	1.3	
Perrinton Soils	3.8	0.21
Oshtemo Soils	1.4	

Table 2. A comparison of average leaf chlorophyll content measurements for each species by treatment. These values are an average of all plants in both locations over all four measurement dates (except for SG, which could only be measured on the last date). The letters A and B signify statistically significant groups within a species (Using Tukey Pair-wise comparisons). For example, in the corn treatments, the average AO x C chlorophyll content was significantly higher than the average CP x C chlorophyll content, and they therefore are in different statistical groups (A and B). But the average of CP x C is not significantly higher than that of C x C, and is therefore in the same statistical group (B). In the other three species, the chlorophyll content did not vary significantly between treatments, so there is only one statistical group (A) in each species.

Species	Leaf Chlorophyll Content						
	AO x C	AO x SG	CP x C	CP x SG	C x C	SG x SG	P
Corn	52 A	-	49.5 B	-	47.9 B	-	0.0004
SG	-	33.3 A	-	33.8 A	-	34.4 A	0.78
AO	41.9 A	42.3 A	-	-	-	-	0.84
Cowpea	-	-	75.6 A	77.1 A	-	-	0.20

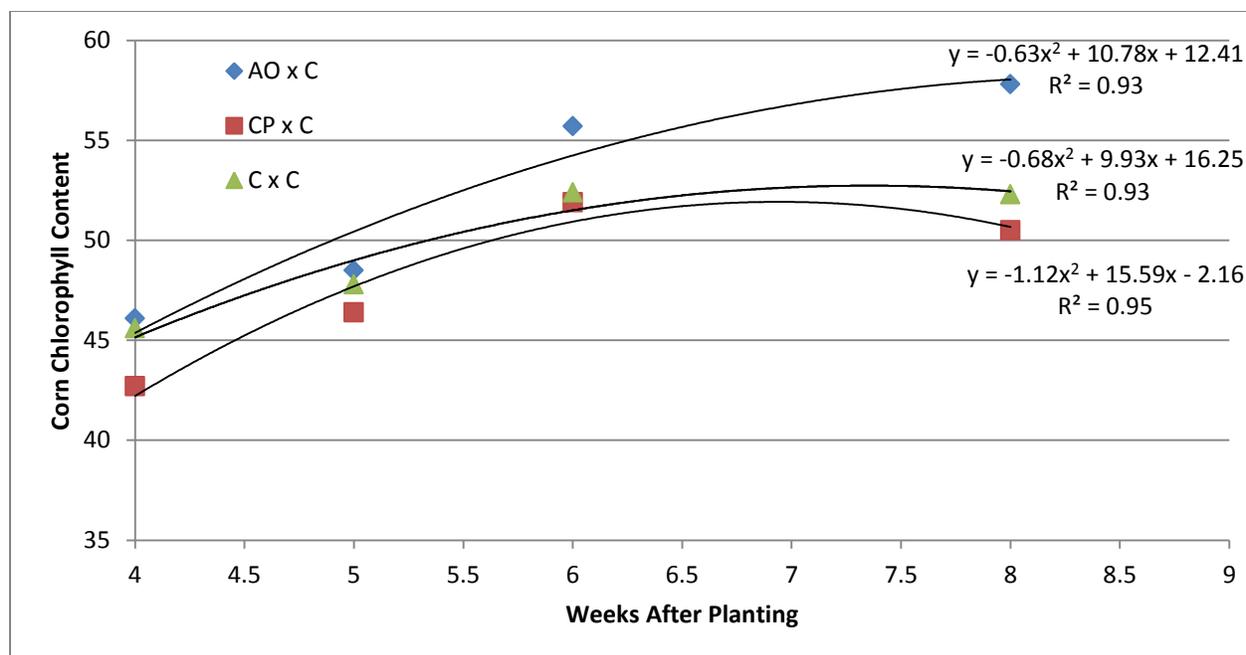


Figure 2. Corn leaf chlorophyll content over the 8 week growing season.

Table 3. A comparison of average leaf number for each species by treatment. These values are an average of all plants in both locations over all four measurement dates. The letters A, B, and C signify statistically significant groups within a species, as explained in Table 1. The numbers in this table represent the average number of leaves, combining fully and not fully expanded leaves; data on fully expanded leaves alone was also taken and displayed similar patterns.

Species	Leaf Number						
	AO x C	AO x SG	CP x C	CP x SG	C x C	SG x SG	P
Corn	15.1 A	-	14.2 C	-	14.6 B	-	<0.0001
SG	-	15.4 A	-	9.3 B	-	12.1 AB	0.015
AO	21.7 A	18.4 A	-	-	-	-	0.076
Cowpea	-	-	26.0 B	32.9 A	-	-	<0.0001

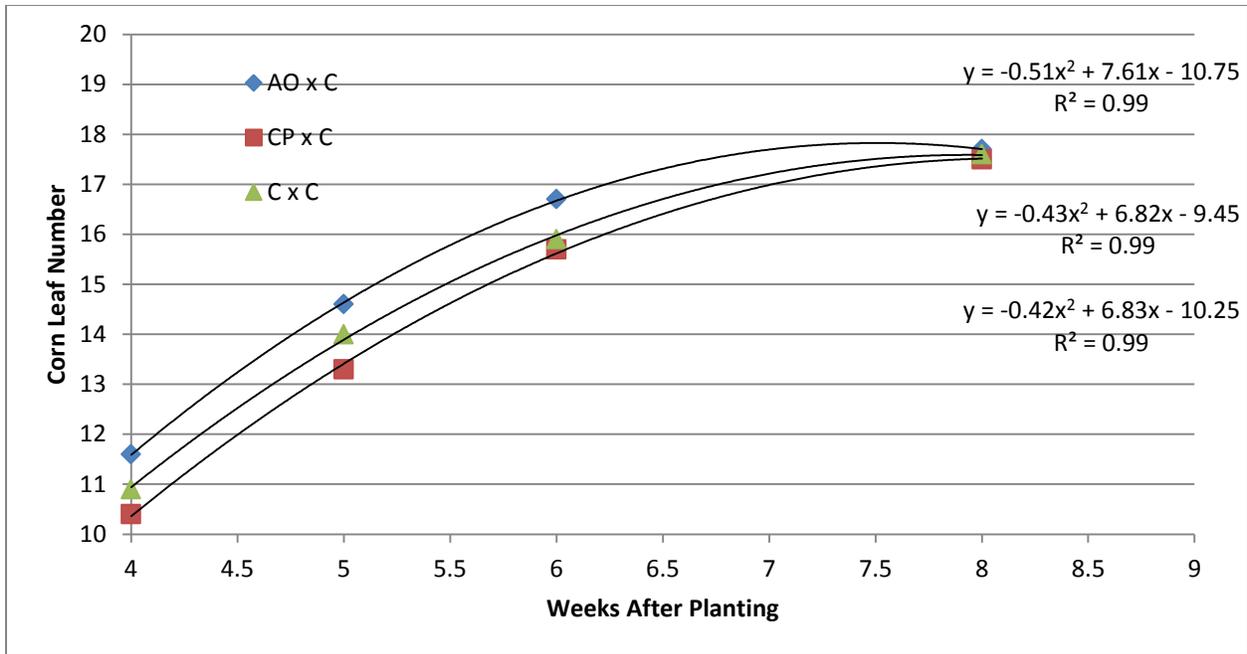


Figure 3. Number of corn leaves per plant over the 8 week growing season.

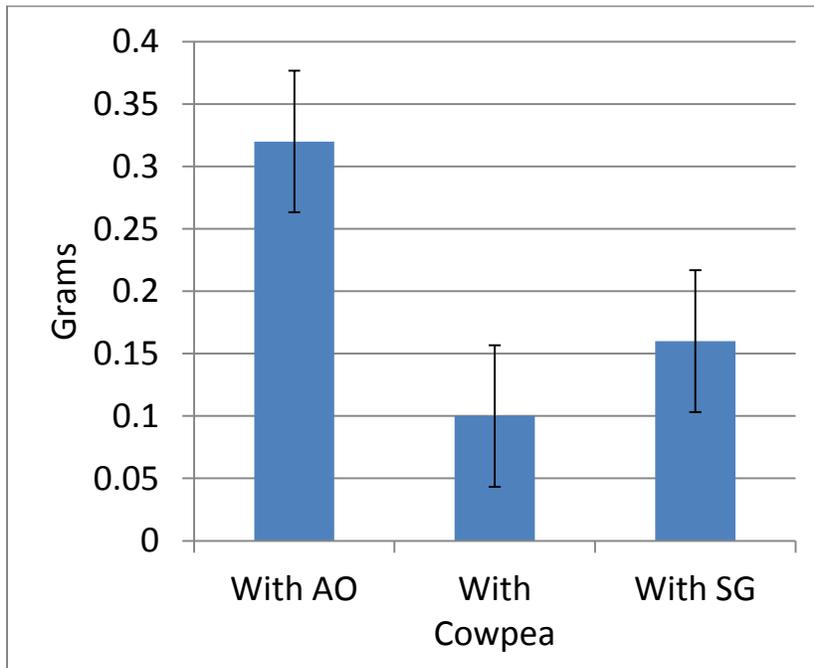


Figure 4. Average above-ground dry weight of switchgrass for three treatments. Error bars are for a 95% confidence interval.

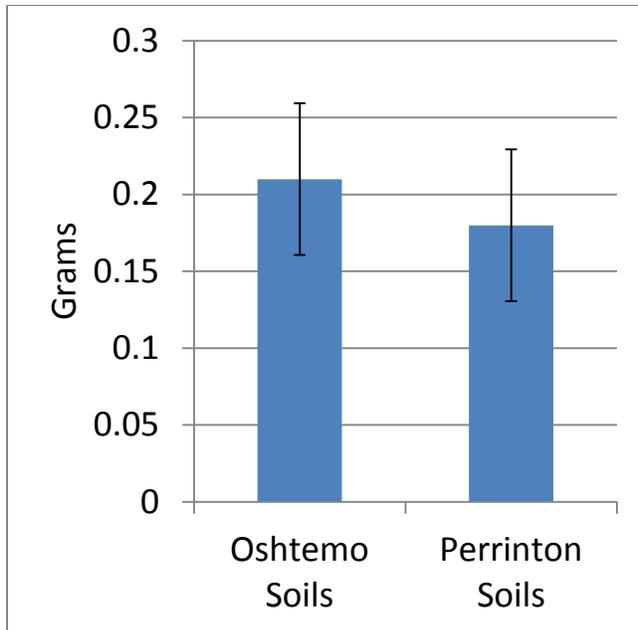


Figure 5. A comparison of the average above ground dry weight of switchgrass for two soil types. Error bars are with 95 percent confidence.

Table 5. A comparison of the photosynthetic rate, photosynthetic transpiration rate, and leaf temperature of corn plants for plant combination treatments. The letters A and B signify statistically significant groups within a species.

Parameter	AO x C	CP x C	C x C	P
Photosynthesis Rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	6.5 A	3.4 B	6.5 A	0.037
Transpiration Rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	3.6 A	2.3 B	2.9 AB	0.0005
Leaf Temperature ($^{\circ}\text{C}$)	33.1 A	32.3 A	32.7 A	0.45

Table 6. A comparison of leaf number and chlorophyll content for each plant species by soil type. The letters A and B signify statistically significant groups within a species.

Species	Location		
	Oshtemo Soils	Perrinton Soils	P
	Leaf Number		
Corn	14.5 B	14.8 A	0.039
Switchgrass	10.3 B	14.3 A	0.0083
Autumn olive	15.6 B	24.4 A	<0.0001
Cowpea	26.2 B	32.7 A	<0.0001
	Chlorophyll Content		
Corn	45.5 B	54.1 A	<0.0001
Switchgrass	35.1 A	32.5 B	0.045
Autumn olive	42.8 A	41.4 A	0.43

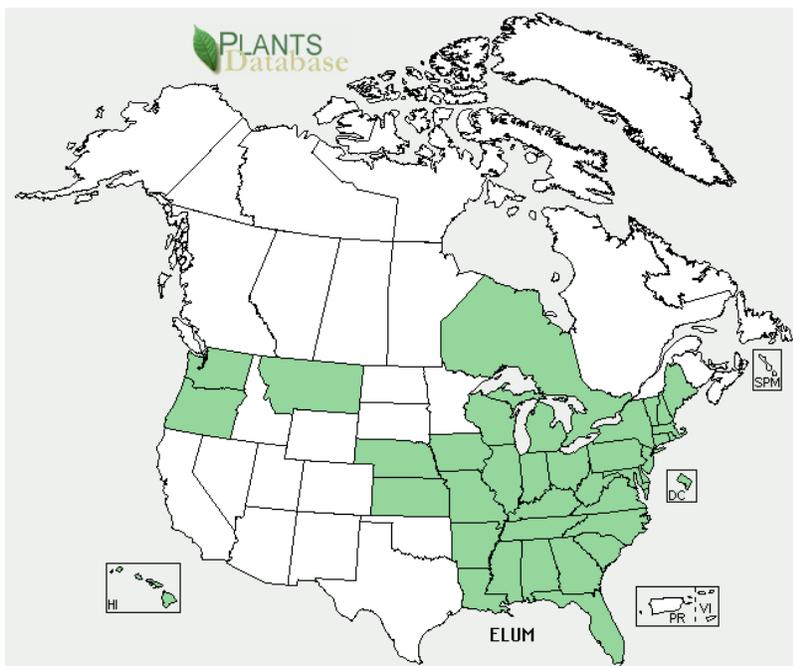


Figure 6. The distribution of autumn olive in North America (USDA 2012).

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