INFLUENCE OF VARIABLE PLANTING TIME ON A CORN-BEAN INTERCROPPING SYSTEM

by

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Sarah Eisenmenger

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LIST OF ABBREVIATIONS

Abbreviation	**Description**
LER	Land Equivalent Ratio
RY	Relative Yield
PO	Planting Offset
PO^2	Planting Offset Squared
Df	Degrees of freedom

1. INTRODUCTION

Intercropping, the growing of two or more crops together, is a common agricultural practice throughout the developing world and in developed countries as well. Farmers from rural subsistence farms to small organic farms practice intercropping as a low-input cropping system (a system that uses less synthetic fertilizer, pesticides, and fossil-fuel based machinery) that takes advantage of the natural tendency of certain crop mixtures to yield more than respective monocultures (Andrews and Kassam 1976).

Mechanized or industrial agriculture became the popular method for growing food when pesticides and tractors made monocropping more economical (Kass 1978; Horwith 1985; Andersen 2005). As machines became more specialized for certain crops, monocropping became the norm, while no technological improvements were targeted at making large scale intercropping more economical (Machado 2009). Though modern agriculture has been extremely successful producing affordable food for a growing human population, there are many problems that have developed because of it. Reliance on external inputs such as fertilizers, pesticides, combines, and tractors has created many unintended consequences in terms of climate change, air and water pollution, dead zones in coastal seas, biodiversity loss, and soil degradation (Montgomery 2007). For example, industrial agriculture has degraded ecosystems by reducing soil fertility and organic matter, increased soil erosion, negatively impacted water quality through fertilizer runoff, and reduced beneficial insects through insecticide application (Mortan and Suciu 2013). Conventional tillage methods, intensive livestock systems, and inorganic fertilizers all impact the air and climate by contributing to CO_2 emissions, CH_4 and N_2O emissions, as well as smog and acid rain (Mortan and Suciu 2013). Additionally, natural ecosystem services such as preserving soil fertility through nutrient recycling and preservation, water storage, natural pest and disease control, and pollination are impaired in industrial agriculture (Kremen, Iles, and Bacon 2012). Excessive use of pesticides and fertilizers can potentially lead to unstable yields since reliance on such additives must be continuous for their desired effect.

Nevertheless, low-input agriculture has survived in the developing world, often in the form of intercropping, the cultivation of mixed cropping systems where two or more species are growing side by side (Ngwira, Aune, and Mkwinda 2012). Using this practice, farmers capitalize on the greater resource use efficiency and sustainability of intercropping, since plants growing together will use a greater range of resources than one crop alone, as well as the greater degree of yield stability in the face of uncertainty (Horwith 1985). Yield stability results from the increased species and functional diversity of intercropping systems. For example, if one crop species fails due to susceptibility to uncontrolled harmful factors, such as drought or insect attack, a second more tolerant species can partially compensate for the yield loss through release from competition. Therefore, on farms where environmental or biotic stresses are common and not fully controlled, intercropping can act as insurance against complete crop failure (Horwith 1985). Additionally, the intercropping practice often protects and improves soil quality by reducing the uptake of certain nutrients by one crop and reducing erosion through continuous tillage, thus ensuring future farm income (Rusinamhodzi et al. 2012).

Alternatively, in the industrialized world, intercropping will most likely find its best use in organic farming and sustainable agriculture, where fossil-fuel based inputs and/or synthetic fertilizers are used less (Machado 2009). Even though it may seem counter-intuitive that low-input agriculture can compete against industrial agriculture in rich countries, there is scant evidence that industrial agriculture always outperforms other forms of agriculture (African Union 2008). The same crops produced commercially and in low–input intercropped settings can have the same productivity (Ponisio and Ehrlich 2016). One study found that intercropping increased crop yields by between 33.2 and 84.7% compared to the equivalent monoculture crop yields (Li et al. 2009).

Intercropping with legumes in particular can affect soil nitrogen levels in a favorable way. Legumes are known for hosting bacteria that can fix atmospheric N_2 that can be utilized by other plants upon the death of a legume plant. There have been studies examining the use of legumes as a legitimate method of adding nitrogen into the soil. Pimentel et al. (2005) compared organic and conventional farming systems that used either animal-based (organic) nitrogen input, legume-based (organic) nitrogen input, or industrial fertilizer in a conventional cropping system. At the beginning of the experiment, there were similar percentages of soil nitrogen on all farms. However, after about 20 years, the two organic farming systems had significantly more soil nitrogen than the conventional systems. Similarly, Harris et al. (1994) found that more than twice the amount of nitrogen was preserved in the soil a year after application of fertilizer in organic animal and organic legume systems than in conventional systems. Since intercropping is more likely to occur on small organic farms, utilizing a legume-based intercropping system resembling the one in this experiment may increase soil nitrogen.

The benefits of intercropping are typically quantified by their capacity to 'over-yield'. Over-yielding refers to the situation where an intercropped plot produces more yield than would be expected if the two or more crops had been grown separately on the same area of land. The reason for this advantage is often rooted in some form of ecological niche separation between the crop species, i.e., species having different requirements for nutrient levels, water ability or light intensity. These kind of functional differences can reduce competitive interactions, so that a crop plant surrounded by neighbors of a different species can produce more yield than if the neighbors were conspecifics (Vandermeer 1992). Often, intercropping systems emphasize the prosperity of one crop (the main crop and usually cash crop) over that of subdominant crops. Subdominant crops may serve primarily to maintain or improve soil fertility and stability, thereby ensuring the sustainability of cash crop yields. Secondarily, they may also add to overall yield production (Sadeghi and Kazemeini 2012).

Mechanistically, there are many reasons why two or more species grown together produce more yield than grown separately. Below I provide a brief overview of how a yield advantage is quantified and produced.

Background

Definition of yield advantage

The standard mathematical formula for quantifying intercrop performance is the land equivalent ratio (LER) (Vandermeer and Goldberg 2013). For the calculation of LER, the relative yield (RY_i) of component crop "i" is calculated as P_i/M_i, where P_i is the yield in polyculture on a unit area on land and Mi is its yield in a monoculture of the same unit area. Relative yields are usually less than one, because there are typically less plants in the polyculture than in the monoculture, depending on the design. However, the sum of all relative yields may be above one, thus indicating an overall yield advantage for the polyculture:

$$LER = \sum_{i=1}^{n} RY_i \qquad \text{Equation 1}$$

An LER > 1.0 indicates that the polyculture resulted in higher yield per area of land than the same area divided into monocultures of equal land area (Vandermeer 1989). However, this does not guarantee that the polyculture is better than a monoculture planted to the same area. To distinguish if an intercrop merely has a yield advantage or is the cropping system with the highest yield, the term "transgressive over-yielding" was coined for the situation where the polyculture produces more yield than the monoculture with the greatest yield, as opposed to non-transgressive over-yielding where this is not the case (Schmid et al. 2008).

Yield advantage by resource use complementarity

Studies of the intercropping advantage are commonly framed in the context of competition theory (Loreau 2010). In many ways, the conditions for the coexistence of competitors are the same as the conditions for over-yielding. Both require that intraspecific competition is stronger than interspecific competition for both species (Loreau 2010). Thus, when plants are surrounded by neighbors of a different species, intraspecific interaction is reduced in favor of interspecific interaction, thereby reducing competitive suppression. When species have different resource requirements, mixtures often make more complete use of all available resources and therefore, they can be more productive (Loreau 2010).

Complementarity of resource use can occur when crops use resources in different amounts or obtain them in different ways resulting in a more effective use of resources by the community of species as a whole (Franco et al. 2015). This ensures that all species within a community ultimately use most resources within a certain space due to individual species requirements for different amounts of certain resources.

In the competition theory developed by Tilman (1994), plant species are characterized as requiring the same resources, but in different quantities and ratios. Coexistence is possible when each species is the strongest competitor for a different resource under environmental conditions. When no species can reduce available resource levels below that required by the other species, coexistence, as well as more complete resource use, is assured (Kinzig et al. 2002; Tilman et al. 1997; Hooper, Chapin III, and Ewel 2005).

A positive correlation between diversity and productivity has been observed in natural populations, for example Mediterranean shrublands (Montès et al. 2008) and grasslands (Cardinale et al. 2007). However, W. J. Li et al. (2010) suggested that a large portion of the diversity effect on grasslands productivity may be due to the inclusion of legume species, which are nitrogen fixers. Nitrogen fixers typically require more phosphorus but less soil nitrogen than non-fixers, indicating resource use complementarity.

Not surprisingly, cereal-legume intercropping systems often over-yield and are among the most common intercropping systems (Ofori and Stern 1987), especially where legumes are paired with fast-growing C_4 cereals such as maize, sorghum or millet. Besides the difference in nitrogen acquisition, cereal crops are taller than legumes, thus avoiding shade. Their roots grow to greater depth than legume roots, thus reducing competition for water (Willey 1990; Sadeghi and Kazemeini 2012).

However, resource complementarity can be reduced with the application of nitrogen fertilizers (Jarchow and Liebman 2012). Species with the capacity to grow fast and respond vigorously to fertilization can subdue species that lack these qualities, thereby eliminating the intercropping advantage (Jarchow and Liebman 2012). Therefore, it appears that resource complementarity as a means to produce a yield advantage is most successful when plant growth is resource limited.

Yield advantage by different sensitivity to environmental factors

Another niche differentiation model (Winemiller et al. 2015) considers species differentiation in tolerance to non-resource factors such as soil pH or temperature. In a habitat varying in these factors temporally and/or spatially, different species may maximize growth in different places or at different times. When or where conditions are optimum for one species, that species can gain dominance over less well adapted species. Those inferior species may encounter more suitable conditions at another time or elsewhere and dominate in turn. Thus, each species dominates under conditions in which it performs better than all other species and an intercrop of such species produces more yield than any monoculture could.

This principle is utilized in relay cropping, in which several crops are grown in the same field with overlap in growing periods. Yu et al. (2015) examined how this practice affects LER. They found that relay intercropping does result in higher LER values, since the intercropping systems leaves the soil uncovered for less time during the growing season than any of the monocultures. Furthermore, they found that relay cropping with C_3 and C_4 plants increased LER more than relay cropping with only C_3

plants, presumably because C_3 and C_4 plants are more specialized for early and later planting. This could be due to the typically different temperature optima of C_3 and C_4 photosynthesis (Sage and Kubien 2007) .Accordingly, an early-growing C_3 crop would generate more yield than a C_4 crop grown at the same time and vice versa (Sage and Kubien 2007).

The study also demonstrated that the LER of cropping systems using species with a difference in tolerance did not diminish as much with fertilizer input. When resource availability was high, the reduced temporal overlap of the relay crops mitigated the potential for strong competition, which maintained a high LER. However, when resource availability was low, LER was less than at high resource availability (Yu et al. 2015). This suggests that resource conditions influence what kind of niche differentiation is most effective in producing an intercrop advantage. Overall, relay intercropping and intercropping in general can have their advantages when resources are limited or conditions are intermittently poor, in addition to when resources are not limited.

Yield advantage by facilitation

Lastly, the interactions between plants are not always adversarial. Facilitation between species may also play a role, when the productivity of one species benefits from the presence of another (Vandermeer 1989). Facilitation can occur if a particular species can lessen harsh environmental conditions, add a critical resource for other species or enhance biomass decomposition rates (Hooper, Chapin III, and Ewel 2005). For example, legumes are potential facilitators because they fix atmospheric N_2. When they die, their decomposing tissues add assimilated N to the soil. According to a recent meta-analysis

(Gómez-Aparicio et al. 2004), facilitative interactions are most common between species that are functionally and morphologically very different, which would limit their potential for competitive interaction and allow positive relationships to dominate. Callaway and Walker (1997) concluded that the balance between facilitation and competition depends on the life stages and physiologies of the interacting species, indirect interactions with other neighbors, and environmental stress intensity. A good example of facilitative interactions producing a yield advantage are found in agroforestry systems, in which the tree crop may benefit the growth of the understory crop by creating a more benign microclimate and improve soil stability, without being itself negatively affected by the companion crop (Monteith, Ong, and Corlett 1991). Facilitation is rarely reciprocal though. Therefore, yield advantages are expected only when the negative effect of the 'facilitated' on the 'facilitator' is weak or economically less important, as may be the case in the cereal-legume cropping system.

Modifying mechanisms

In addition to the morphological and physiological differences between species that differentiate their niches, the intercrop advantage is also strongly influenced by planting density, the spatial arrangement of component crops, crop proportions and planting order (Vandermeer 1989). Theoretically, there are numerous permutations of planting density, arrangements and proportions, one of which may be optimal in a given situation. However, no theory currently exists that could predict or explain which is the ultimate, 'perfect' planting design for a pair of crops.

Adding to the uncertainty, planting density and arrangement also affect the yield of monocultures, which is used to calculate the LER. It is usually assumed in agronomic applications that the 'optimal' monoculture arrangement is known and is the monoculture that produces the highest yield to provide reference in LER calculations (Thorsted, Weiner, and Olesen 2006; Zhang et al. 2014). By contrast, the optimal intercrop arrangement is identified empirically by trial. A critical question in intercrop design is the relationship between monoculture design and component crop densities. In research applications, to facilitate analysis and biological interpretation, an additive design is typically used, in which each component crop is planted to the same density as in monoculture (Vandermeer 1989). But this design may constrain intercrop performance because of overcrowding. For example, denser planting in the intercrop means that plants begin to interact sooner, which could increase the competitive suppression of the inferior species, and increase yield in the superior species at the cost of decreasing the yield advantage. An alternative design is the replacement series in which polycultures are formed by replacing a certain proportion of plants in the monoculture with the other crop species (Snaydon 1991). In this design, monocultures and polycultures would have the same total number of plants. The downside of this approach is that interactions between plants could be diminished due to lower densities of the component crops. A compromise between these two approaches is a design in which the combined crop density is higher than in monoculture, but less than in a strictly additive design.

A design aspect that has received much less attention than planting density and spatial arrangement is planting order or variation in the time of planting between crop components (Gillhaussen et al. 2014). Variation in the time of planting manipulates the size relationships between seedlings at the onset of competition, which should have consequences for their subsequent interactions and the resulting intercropping advantage (Thorsted, Weiner, and Olesen 2006). If competition is size-asymmetric, which is generally the case in agricultural settings, then small initial size advantages can be greatly amplified over time (Weiner 1990). This should give a strong competitive advantage to any crop planted earlier (Thorsted, Weiner, and Olesen 2006). For instance, a short-stature legume would normally be become competitively suppressed by tall-stature and fast-growing C_4-cereal neighbors just days after emergence, thereby reducing potential benefits of nitrogen-fixation. However, earlier planting of the legume may be able to delay competitive suppression in favor of higher bean yield and nitrogen fixation. It seems reasonable to expect that planting order is a way to manipulate competitive interactions and change component yields in an intercropping design. Earlier planting of a temperate-climate C_3 legume and later-planting of a tropical C_4 cereal may also align better with the seasonal temperature changes, adding to the yield advantage.

Still, there is likely an optimal planting offset between the species of an intercrop. Too much of a size advantage given to any species could suppress the companion species too much, making the intercrop more similar to a monoculture of the species that is planted first. These potential tradeoffs of planting order and intervals, and their effects on component yields and the overall yield advantage, have received very little attention in the intercropping literature.

Research Goals and Hypotheses

My overall goal was to test the effect of planting order and interval on the yield advantage of an organically grown corn-bean intercropping system in Southcentral

Texas.

Hypothesis about planting order:

A legume-cereal intercrop can potentially benefit from several mechanisms of niche separation, including reduced resource use complementarity, facilitative interaction to the benefit of corn, and differential sensitivity to environmental conditions. The effect of planting order likely depends on which mechanism dominates the yield advantage. Therefore, I propose three alternative hypotheses (Table 1):

If the yield advantage is produced chiefly by facilitation, I hypothesize that early planting of bean (20 days to 10 days earlier) relative to corn will increase corn yields. An early planted bean crop would be greater in size when starting to interact with corn and will have already established nitrogen fixation, for the potential benefit of corn. If the bean is planted simultaneously or later, soil enrichment through nitrogen fixation may come too late to have a significant effect on corn growth and bean growth will be more strongly suppressed by corn. The relative yield of bean with respect to a simultaneously planted monoculture may not change, but the relative yield corn may increase with the earlier planting of bean, thereby increasing LER.

Alternatively, if the yield advantage is produced primarily by complementary resource use, I hypothesize that early planting of bean will increase bean yields but decrease corn yields and vice versa. Thus, the relative yields would change in opposite directions favoring the crop that was planted earlier. Effects on LER may thus be nearly neutral.

Yet a third alternative is yield advantage due to a difference in environmental sensitivity. If bean is planted earlier than corn, I hypothesize that bean yields will increase due to having a longer growing season (better environmental conditions), without ill effect on the yield of corn. With a purely environmental effect on yield, the relative yields of neither crop may be affected and the effect on LER would therefore also be neutral.

Table 1. Effects of planting times of corn and bean plants on RYs and LER depending on whether facilitation, resource complementarity, or a difference in environmental sensitivity dominates the yield advantage. Note that RY are calculated based on simultaneously planted monocultures.

Planting sequence	**Facilitation**	**Complementarity**	**Environmental sensitivity**
Bean earlier than corn	No effect on bean RY Increased corn RY Increased LER	Increased bean RY Decreased corn RY No effect on LER	No effect on bean RY No effect on corn RY No effect on LER
Bean later than corn	Decreased bean RY No effect on corn RY Decreased LER	Decreased bean RY Increased corn RY No effect on LER	No effect on bean RY No effect on corn RY No effect on LER

II. MATERIALS AND METHODS

Time and place

The experiment took place at My Fathers Farm in Seguin, TX 78155. This certified organic farm encompasses 52 acres. The experiment was conducted on a field of approximately one acre of land that had not been used for agriculture in several years. Previously, the area had been the site of a chicken coop and a dumping ground for scrap wood and metal. Prior to the experiment any remaining scrap had been removed. The area was plowed on 2/12/17. A drip line irrigation system was set up on 3/17/17.

Fertilizer composed of hydrolyzed fish, molasses and humic acid were applied in solution to the soil surface on 4/19/2017.

Experimental design

The main experimental factor was the bean planting time, with five levels, in which bean seeds were planted 20 or 10 days earlier than corn seeds, simultaneously with corn seeds or 10 or 20 says later than corn seeds. All corn seeds were planted at the same time. From here on I refer to these treatments as bean planting times of '-20', '-10', '0', '+10' and '+20' days relative to the corn planting time. The 10-day planting intervals were chosen as a compromise between giving enough time for seedling emergence, but not too much of a growth advantage (Dapaah 2016). These treatments were applied to both intercrops and bean monocultures, so that for the calculation of relative yield totals, intercrop yields could be compared to the monoculture yields of concurrently planted crops.

The intercrop was composed of a 1:2 mixture of corn:bean, varieties glass gem (*Zea mays*) and Provider (*Phaseolus vulgaris*). Seeds were obtained from Johnny's Selected Seeds (Winslow Maine, United States).

In the monoculture plots, corn seeds were spaced 10 cm from each other and bean were spaced 5 cm from each other, following producer recommendations. This resulted in planting densities of 12 corn plants/m^2 (48,583 plants/acre) and 24 bean plants/m^2 (97,166 plants/acre). In the intercrop plots, corn seeds were planted every 15 cm along the row, and two bean seeds were planted in-between with equal spacing of 5 cm apart. This resulted in approximately 8 corn plants/m^2 (32,389 plants/acre) and 16 bean plants/m^2 (64,777 plants/acre). Thus, both component crops in the intercrop were grown at 2/3 the density of the monoculture. This struck a compromise between the possibility of overcrowding in an additive design and the possibility of too distant spacing in a replacement design.

Since I expected significant variation in soil fertility across the field plot due to previous land use, I laid out a block design, with six blocks within which all 11 treatments (5 mixtures and 6 monocultures) were replicated once in a randomized arrangement (Fig. 1). Each plot was 3 m x 5 m, where the long side spanned across six rows with an alternate row spacing of 45 and 155 cm. The total area of the experiment encompassed 33 x 30 m. Drip lines for irrigation were placed between the more closely spaced rows. The row spacing was dictated by the available farm equipment.

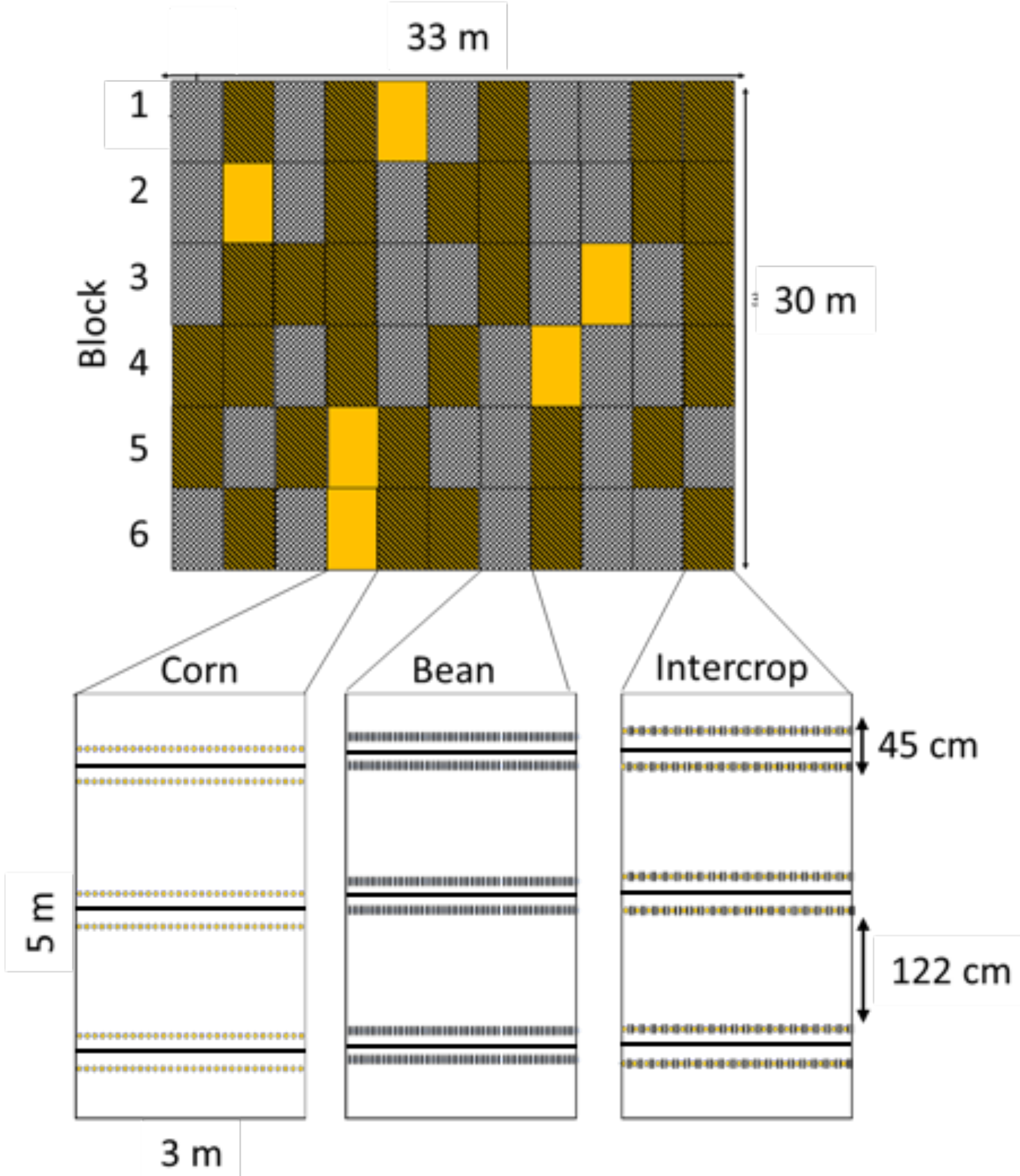

Figure 1. Experimental design. Individual plots are 5 m x 3 m and were composed either of corn monoculture (orange), bean monoculture (black stripes) or intercrop (orange with black stripes). Each block contained 11 plots in random order. Each plot had 6 rows with corn monoculture at a 10 cm spacing within row, bean monoculture and intercrop at a 5 cm spacing within row. In the intercrop, every third plant per row was corn. The drip irrigation lines were placed between the more closely spaced rows (black line).

Planting and upkeep

The planting dates were 3/6/17, 3/16/17 (beans only), 3/26/17 (bean and corn) and 4/5/17, 4/15/17 (beans only). Before planting the seeds, the soil was loosened with trowels or stirrup hoes. Corn and bean seeds were planted at 2.5 cm depth following producer recommendations. For the first two planting dates, I used toothpicks to mark where bean seeds had been planted to achieve a more accurate spacing of corn seeds planted later. For the later plantings, emerged corn plants guided the placement of the bean seeds.

The field was irrigated for two hours after each seed introduction to facilitate germination, except on 3/6/17, when the soil was still wet from a recent rainfall. Thereafter, the plots were watered every day for two hours.

Once a week, between rows were hand-weeded with stirrup hoes and within rows with a trowel. Four times during the experiment, a string trimmer was used.

Periodic measurements

To track the growth rate of plants non-destructively and to potentially determine the point in time when plants started to interact, I repeatedly measured plant heights of both species on three randomly selected plants in the center of all plots. At first, beans were measured from the soil up to the tallest node. In the first week of May, they were measured to the tallest leaf because the tallest node did not express the true height of the bean plants. Corn plants were measured from the soil up to the highest point where the whorl of leaves forms a closed tube until they started to tassel, at which point height was then measured from the soil up until the arch of the tallest leaf. For each Plot Type, growth was monitored starting two weeks after planting and then every week thereafter until the end of the experiment.

Harvest procedures

Bean pods were harvested approximately two months after planting, after ripening, on 5/5/17, 5/23/17, 6/6/17, 6/13/17, 6/22/17. Corn ears were harvested on 6/29/17. Both ears and bean pods were weighed immediately after harvesting using a counter-weight balance. Ears and pods were harvested from the entire plot area.

Additionally, the remaining above-ground biomass of three randomly chosen plants per species from each plot center was harvested on 7/7/17. By this time, both species had started or completed to senesce. These samples were placed into brown paper bags, first air-dried, then oven-dried at 70°C for 72 hours and then weighed.

Soil tests

After assessing the amount of spatial variation in the field used in this experiment, a decision was made to collect soil samples from each plot for soil nitrate analysis. Samples were taken on 7/7/17. Digging to approximately 12 cm with a trowel, samples were taken from three rows and mixed. These samples were air-dried for a week in the lab before subsamples were sifted through a 2mm mesh to exclude larger litter fractions and rocks. Samples were analyzed with a Palintest SKW500 complete soil kit (Palintest LTD, 1455 Jamike Avenue, Erlanger, Kentucky, United States), which included a nitrogen test kit to determine soluble nitrate/nitrite per dry soil volume. For the test, 2 ml of dry soil were mixed with 50 ml of deionized water. The test reagents reduce dissolved nitrate to nitrite, and a colorimetric reagent produces a color range from pale pink to deep violet depending on nitrite concentration. A spectrometer returns nitrate/nitrite concentrations in the range of 0 – 25 mg/L N   Around 27% of the soil samples had N values that fell above the calibrated range. A decision was made to classify soil N content into three categories, 1 for 0-10 mg/L N, 2 for 10-20 mg/L N, and 3 for > 20 mg/L N.

Data Analysis

Based on the results from the soil analysis, the original 6 blocks in the experimental design (Fig. 1) were collapsed into two 'N Blocks', referred to as 'high N' and 'low N', to examine potential effects of different soil nitrate/nitrite concentrations on yield relationships. Soil categories and log-transformed monoculture yields were analyzed by an analysis of variance (ANOVA).

RY's for corn and bean were calculated by dividing the intercrop values for each planting offset per original block by the corresponding monoculture yield. LER values were calculated as the sum of corn and bean RYs, per equation 1.

To test the main hypotheses, RY values and their sum LER were analyzed using GLM analysis with quadratic regression on planting offset and N Block as a categorical variable. If the quadratic term was found to be non-significant, the GLM analysis was repeated without the quadratic term. Since these indices are ratios, or the sum of ratios, they were log-transformed before analysis. Additionally, log transformed RY and LER values were compared against expected (Null Model) values through one-sample t-tests. RY values were compared to log(2/3), the expected value based on the relationship between the crop densities in intercrop:monoculture and an assumption of no-change in individual plant yields. LER values were compared to log(2 x 2/3), on the assumption that both crops maintained the same per plant yields, and to log(1) to test if there was a significant ‘intercrop advantage’ (i.e. LER >1).

Corn and bean crop yields per Plot Type and the vegetative above-ground biomass of individual plants were similarly log-transformed and analyzed by GLM analysis with quadratic regression on planting offset and with N Block, Plot Type (monoculture vs. intercrop) as well as all 2-way interactions (N Block-Plot Type, Plot Type-planting offset and N-Block-planting offset) as categorical variables. Interaction terms and the quadratic term were omitted from analysis if they proved to be non-significant.

To determine if and how plant-interactions affected plant growth over time, a repeated measures ANCOVA was performed on plant height data with quadratic regression on planting offset.

All statistical data analyses were performed using SPSS version 25.0 (IBM Corp, 2012), which was also used to draw the figures.

III. RESULTS

Soil nitrogen variability

The nitrogen categories were significantly lower across blocks 1-3 than across blocks 4-6 (Fig. 2) ($p = 0.034$). Corn monoculture yield was also significantly higher in the high N Block than in the low N Block; bean monoculture yield had only a marginally significant difference along this division (Fig 3).

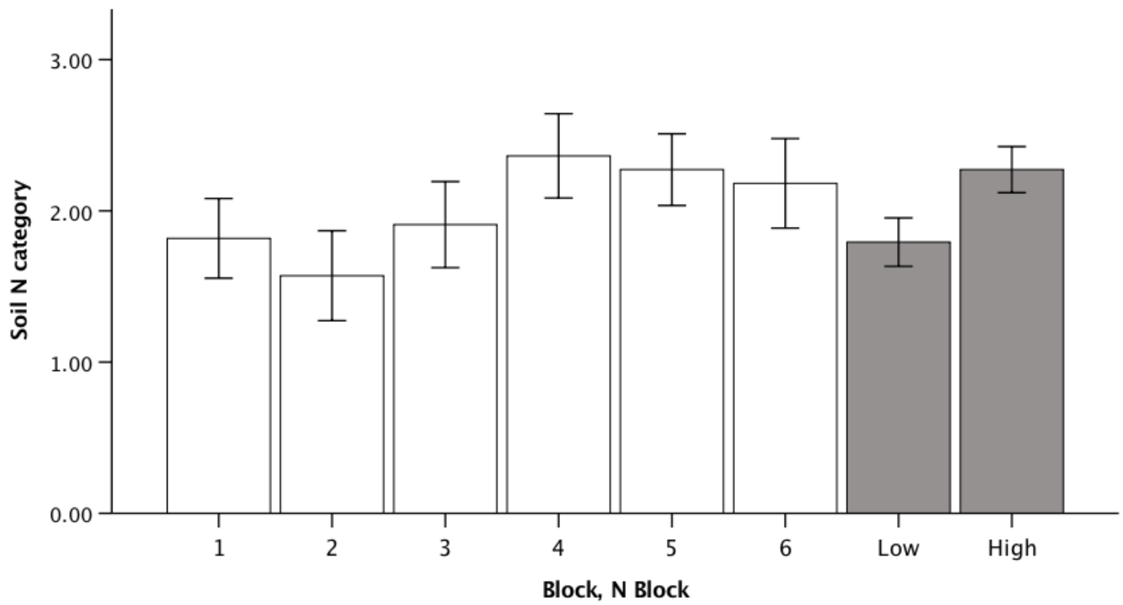

Figure 2. Average soil N category values, in which individual plots are categorized as level 1, 2 or 3. The new N Block division combines the original blocks 1-3 into one 'low N Block' and blocks 4-6 into one 'high N Block'. The average soil N values for the two N blocks are significantly different ($p = 0.034$). Error bars represent +/- 1 standard error of the mean.

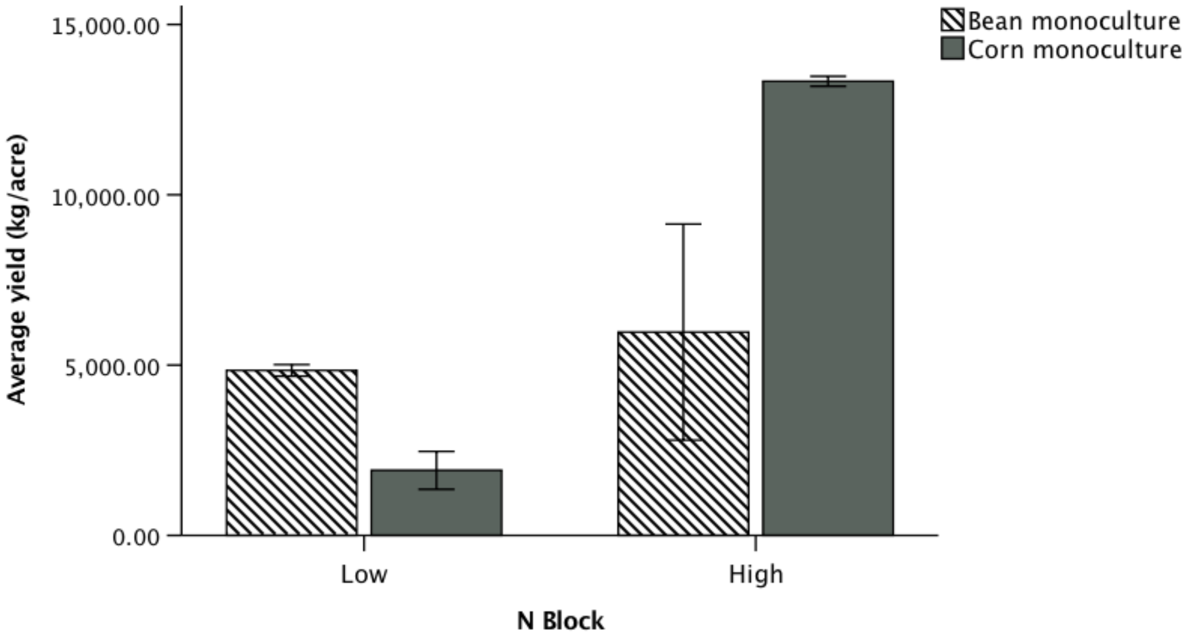

Figure 3. Average bean and corn monoculture yields by N Block. Error bars represent +/- 1 standard error of the mean. Bean yields are marginally significantly different between N Blocks (p = 0.061), corn yields are significantly different (p = < 0.001).

Analysis of intercrop advantage

Corn RY had a significantly quadratic relationship with planting offset (Table 2). The pattern indicated that while simultaneously and later-planted bean did not affect corn yields, earlier-planted beans significantly reduced corn RY. Corn RY was also significantly higher in the low N Block.

Table 2. GLM analysis for (log-transformed) Corn RY with quadratic regression on planting offset. The total sample size was 30. PO = Planting offset, PO^2 = the square of planting offset, b = regression parameters with standard error in parenthesis. N Block (high) is the reference state of the categorical factor and not shown.

Factor	B	df	Wald Chi- Square	Sign.
Intercept	-0.354 (0.121)	1	8.611	0.003
N Block (low)	0.549 (0.130)	1	17.822	**< 0.001**
PO	0.011 (0.005)	1	6.004	**0.014**
PO^2	-0.0010 (0.0004)	1	4.337	**0.037**

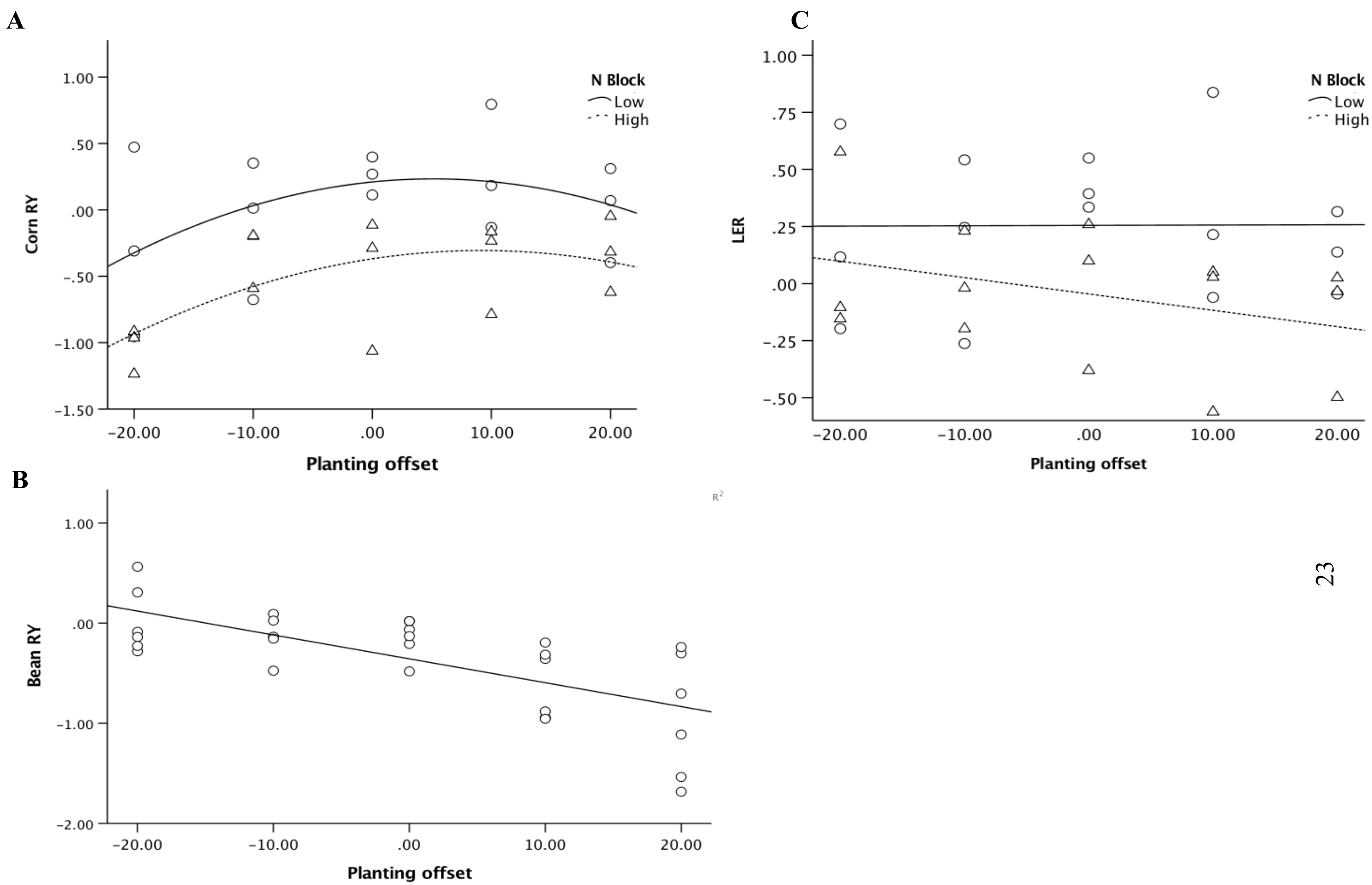

Figure 4. Quadratic regression of log-transformed corn RY, bean RY and LER separated by planting offset. The quadratic parameter was significantly different from zero for Corn RY (Table 2). Corn RY and LER were significantly affected by N Block.

The effect of planting offset on bean RY was not quadratic: the earlier bean was planted, the higher the bean RY (Table 3). This means bean plants that were planted earlier could achieve a greater portion of their potential yield in intercrops. In addition, the N Block effect on bean RY was not significant.

Table 3. GLM analysis for (log-transformed) Bean RY using planting offset as a covariate. The total sample size was 30. PO = Planting offset and b = regression parameters with standard error in parenthesis. N Block (high) is the reference state of the categorical factor and not shown.

Factor	B	df	Wald Chi-Square	Sign.
Intercept	-0.343 (0.097)	1	12.403	**< 0.001**
N Block (low)	-0.026 (0.135)	1	0.038	0.846
PO	-0.024 (0.005)	1	25.308	**< 0.001**

The LER value, the sum of the two relative yields, did not have a significant relationship with the planting offset (Table 4), as the positive effect of the planting offset on corn tended to cancel out the negative effect of the planting offset on bean. However, through the effect on corn RY, LER was significantly higher in the low N Block (Figure 4C).

Table 4. GLM analysis for (log-transformed) LER using planting offset as a covariate. The total sample size was 30. PO = Planting offset and b = regression parameters with standard error in parenthesis. N Block (high) is the reference state of the categorical factor and not shown.

Factor	B	df	Wald Chi-Square	Sign.
Intercept	-0.046 (0.076)	1	0.361	0.548
N Block (low)	0.30 (0.11)	1	7.868	**0.005**
PO	-0.004 (0.004)	1	0.857	0.355

Results from the one-sample t-tests indicated that in the low N Block, corn RY was significantly higher than 2/3 (p = 0.004) but bean RY was not significantly different from 2/3 (p = 0.202) (Fig. 5). LER was significantly higher than 4/3 (p = 0.037), indicating that the higher than expected corn yield generated a significant yield advantage overall. In the high N Block, neither corn nor bean RY were significantly different from 2/3 (corn: p = 0.109; bean: p = 0.148) and LER was not significantly different from 4/3 (p = 0.153) nor from 1 (p = 0.560)., indicating no intercrop advantage at high soil N levels.

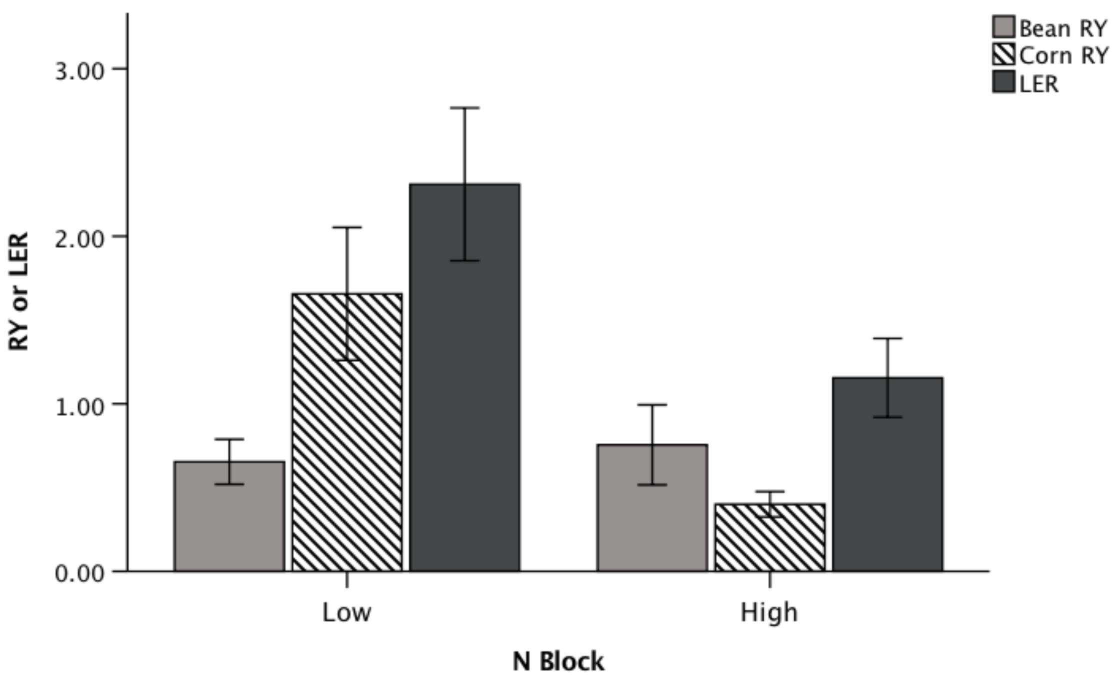

Figure 5. Corn and bean RY and their sums LER by N Block. RY and LER values were averaged across planting offsets. Block effects were significant for corn RY (Table 2) and LER (Table 4), and not for bean RY (Table 3). Error bars represent +/- one standard error of the mean.

Yield analysis

The planting offset had a highly significant negative effect on bean yields in the intercrop (p = <0.001; Table 5) and a weaker but still significant negative effect on bean yields in monoculture (p = 0.034). This explains why the bean RY decreased with the later planting of bean. Additionally, the bean planting offset had a significantly positive effect on corn yield in intercrops (p = 0.018) resulting in smaller yields at earlier planting offsets. Offset had a significant quadratic relationship with corn (p = 0.033) and not with bean.

N Block had a positive significant effect on corn monoculture yield and a marginally significant effect on bean monoculture yield (Fig. 3). For the intercrops, bean intercrop yield was not significantly affected by N Block (p = 0.092) and corn intercrop yield was significantly affected by N Block (p = < 0.001) with greater yields in the high N Blocks (Table 5).

Table 5. Average corn and bean yields by treatments and blocks. Standard errors are shown in brackets. Corn monocultures were planted at only on time (planting offset of 0).

N Block	Corn		Bean	
PO	Monoculture	Intercrop	Monoculture	Intercrop
	kg acre^{-1}			
High, -20	–	388 (95)	1610 (856)	1363 (297)
Low, -20	–	353 (167)	1307 (46)	1428 (534)
High, -10	–	1925 (353)	2648 (612)	1901 (321)
Low, -10	–	429 (149)	1165 (353)	1044 (494)
High, 0	3972 (396)	1851 (780)	1257 (282)	952 (349)
Low, 0	515 (150)	962 (340)	638 (127)	554 (177)
High, 10	–	1946 (397)	1604 (471)	446 (109)
Low, 10	–	963 (263)	789 (156)	182 (68)
High, 20	–	2032 (632)	755 (281)	97 (32)
Low, 20	–	490 (135)	840 (509)	77 (19)

Vegetative above-ground dry mass analysis

Above-ground dry mass of bean plants was significantly affected by planting offset, Plot Type, and the interaction between planting offset and N Block (Table 6). At high N Blocks, the above-ground dry mass of bean plants in both intercrop and monoculture plots declined steeply with planting offset but planting offset had no apparent effect on bean dry mass at low N Blocks (Fig. 7).

Table 6. GLM analysis for log-transformed bean vegetative above-ground dry mass at final harvest. A quadratic term for PO has been omitted from the analysis, since it was not significant. Each sample value is the average of three randomly sampled individuals. N Block (high) and Plot Type (mono) are the reference states of the categorical factors.

Factor	B	df	Wald Chi-Square	Sign.
Intercept	0.40 (0.08)	1	18.048	**< 0.001**
PO	-0.007 (0.004)	1	14.740	**< 0.001**
N Block (low)	0.231 (0.090)	1	5.462	0.128
Plot Type (inter)	0.285 (0.127)	1	5.070	**0.024**
N Block*PO	0.022 (0.006)	1	11.550	**0.001**

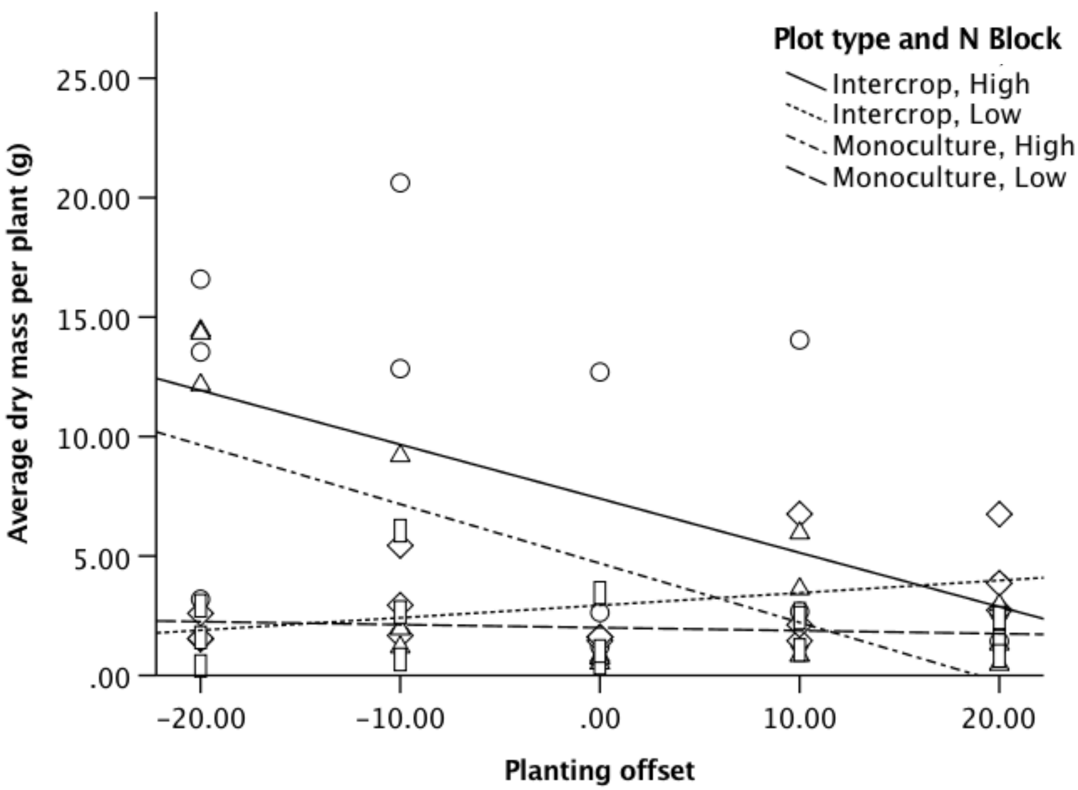

Figure 6. Average dry mass per bean plant as a function of Plot Type, N Block and planting offset. The effects of planting offset, Plot Type and the interaction between planting offset*N Block were significant (Table 6).

The above-ground dry mass of corn was not significantly affected by any treatment effect (Table 7).

Table 7. GLM analysis for log-transformed corn vegetative above-ground dry mass at final harvest. A quadratic term for PO has been omitted from the analysis, since it was not significant. Each sample value is the average of three randomly sampled individuals. N Block (high) and Plot Type (mono) are the reference states of the categorical factors, set to 0.

Factor	B	df	Wald Chi-Square	Sign.
Intercept	1.51 (0.07)	1	420.248	**< 0.001**
PO	0.005 (0.005)	1	1.003	0.317
N Block (low)	-0.1 (0.1)	1	1.938	0.164
Plot Type (inter)	-0.1 (0.2)	1	0.241	0.623

Analysis of plant height development

The bean planting offset had no significant effect on the development of corn height over time. However, corn plants in high N Blocks were significantly taller than in low N Blocks and there was a significant interaction between time after planting and N Block, indicating that height differences between plants in high and low N Blocks grew over time (Table 8, Fig. 8).

Table 8. Repeated measures ANCOVA for corn and bean heights, using planting offset as a covariate. Height values are compared across treatments on the basis of equal age (or time after planting). Interactions that are non-significant for both species have been omitted from the table.

	Corn height			Bean height		
Between-subjects	**df**	**F**	***p*-value**	**df**	**F**	***p*-value**
N Block	1	19.774	**0.001**	1	5.301	**0.026**
PO	1	2.189	0.165	1	8.957	**0.004**
PO^2	–	–	–	1	18.617	**< 0.001**
Plot Type	1	0.049	0.828	1	0.040	0.842
Within-subjects						
Time after planting	1	51.865	**< 0.001**	1	61.283	**< 0.001**
Time after planting*N Block	1	10.276	**0.008**	1	1.544	0.220
Time after planting*PO	1	1.898	0.193	1	8.549	**0.005**

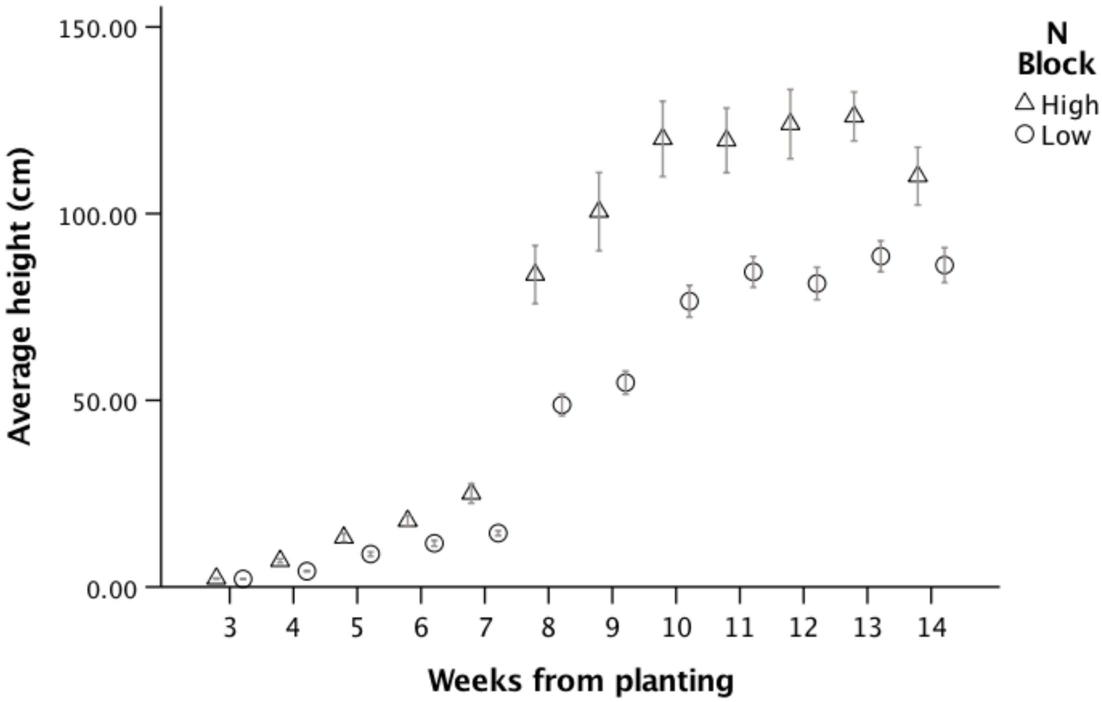

Figure 7. Corn height development. Shown are averages across Plot Type and planting offset, the two factors that did not have a significant effect on corn height development (Table 8). All corn plants were planted on March 26. Error bars represent +/- 1 standard error of the mean.

The height development of bean after planting had a significantly quadratic relationship with planting offset and there was a significant interaction between time after planting and planting offset (Table 8, Fig. 9). Beans planted earlier gained height more slowly with age. However, on the last measurement date, there was no significant difference in height based on planting offset ($p = 0.476$). In addition, bean plants were significantly taller in the high N Block and significantly shorter in the intercrop.

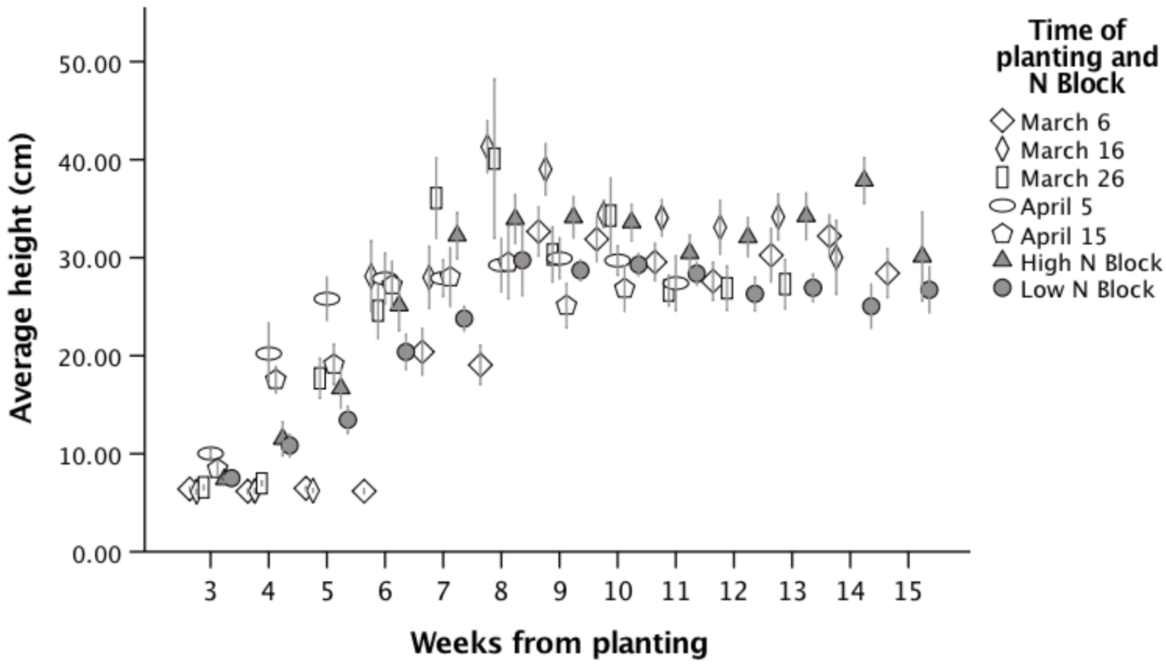

Figure 8. Bean plant height development as a function of time from planting. Shown are averages for different planting offsets averaged over N Blocks and Plot Type and averages for N Blocks averaged over planting offsets and Plot Type. Averages for Plot Types are not shown since this factor had no significant effect on bean plant height development (Table 8). The legends indicate the actual planting dates. Note that beans planted the earliest were observed for the longest time after planting. Error bars represent +/- 1 standard error of the mean.

IV. DISCUSSION

The results indicated that planting offsets favored the RY of the species planted earlier to the detriment of the RY of the species planted later. Since the effects on the two yield components were of similar magnitude, there were no significant effects of planting offset on their sum, the LER (Fig. 4C). This is the expected result for two species interacting competitively, in which one species' gain is another species' loss. The results therefore follow the prediction of an intercrop dynamic dominated by complementary resource use (Table 1).

The effect of planting offset on relative yields

Corn reached its maximal value for RY when bean plants were planted simultaneously with corn or later, while corn RY was significantly lower when beans were planted 10 or 20 days earlier (Fig. 4A). This indicated that the competitive dominance of corn over beans was already fully expressed in plots where corn and bean seeds were planted simultaneously. However, neither the height nor the above-ground biomass of corn plants were significantly affected by bean planting offset or Plot Type, indicating a relatively fixed developmental trajectory with respect to the competitive environment. Thus, the higher yield of individual corn plants in plots with reduced competition must have primarily been the result of plants allocating more biomass into fruit production (Paul-Victor and Turnbull 2009) rather than to accelerate growth.

By contrast, bean RY was consistently more negatively influenced the later beans were planted relative to corn (Fig. 4B). Comparison of the height of plants indicated that the time of planting would have had a strong effect on the height relationships of plants in the intercrop. For example, a 6-week-old corn plant was about 15 cm tall (Fig. 7). This would have been shorter than the ca. 29 cm of an 8-week-old bean plant, a little shorter than a 6-week-old bean plant and taller than the ca. 12 cm of a 4-week-old bean plant (Fig. 8). Thus, bean plants were increasingly more, or at an earlier age, overtopped and likely shaded by corn plants in the intercrop, the later they were planted. According to Sprent and Bradford (1977), shading slows the growth of bean plants and would explain the strong suppression of bean yield when planted later than corn plants.

The bean planting offset did however not significantly affect LER as the loss of corn yield with early planting of bean was offset by the greater bean yield. This indicates that it is possible to manipulate the yield ratios of corn and bean by changing planting dates, without negatively affecting the LER overall. This is not necessarily the case if other aspects of the intercrop design are changed such as the row ratios (Zhongmin and Guang 1990).

There are not many published studies with which to compare the results of this experiment. In one study by Mhlanga et al. (2016), cover crops were planted 8, 11, and 15 weeks after corn. Late planting of the cover crops resulted in lower biomass yields primarily due to the weakening of their development. However, the variable planting dates of the cover crops had no significant effects on corn grain and biomass yields, as in this experiment. However, the converse experiment, in which cover crops were planted earlier than corn, was not conducted and would have likely reduced the corn yield.

A study by Masvaya et al. (2017) showed that cowpea yield was compromised when relay intercropped (planted three weeks after corn) with corn and corn yield was either not affected or improved by the cowpea.

About the effect of nitrogen

Although the study was not designed to examine effects of soil fertility on intercrop performance, some conclusions in this regard were possible due to the high variability in soil nitrate/nitrate across the field. Higher nitrate/nitrite levels in one half of the field significantly increased monoculture yields (Fig. 3) and the height of corn plants (Fig. 8), but reduced LER (Fig. 5). Lower nitrate/nitrate levels made it possible for individual corn plants in intercrop to have greater yield than in monoculture, thus leading to a pronounced intercrop advantage. The decrease of corn RY with increasing nitrate means that the positive effects of nitrate in the corn intercrop must have been lower than in the corn monoculture. This indicates that corn yield was primarily limited by soil N levels in the low N Block and that fewer corn plants in the intercrop produced as much yield as corn plants in the monoculture, without apparent loss of yield due to competition with beans. Thus, beans are weak competitors for nitrogen, which is expected due to the nitrogen fixation capacity of legumes. Accordingly, bean plant growth and yield were only weakly affected by N Block, except in intercropping plots where the effect was probably indirect and related to the response of corn plants to N levels.

In the high N Block, where corn monoculture yields were much higher, individual corn plants did not yield any more in the intercrop than in the monoculture. In fact, corn RY were not significantly different from 2/3, the ratio of seeded plants in intercrop and monoculture. This signified that N supply per plant was close to saturation in both Plot Types and furthermore that the bean companion crop did not significantly diminish any other resource limiting corn growth.

The finding that a corn-bean intercrop has higher yield advantage at low soil fertility is not unusual. The customary explanation is that under high soil N conditions, corn is more competitive than legumes, which leads to a usually small legume contribution to LER and an LER close to unity. When soil N conditions are low, the competitiveness of the corn is diminished and legumes achieve higher growth, albeit no higher competitive ability. This reaction by bean is attributed to their N_2 fixing abilities, as well as release from competition for light is lessened since shading by the corn is reduced (Kermah et al. 2017; Chang and Shibles 1985; Midmore 1993). Kermah et al. (2017) found that low soil fertility led to more balanced competition between corn and bean, more similar contributions to the LER and greater LER values.

The average LER in the low N Block was 1.7, which was significantly greater than unity, indicating the overall advantage of a corn/bean intercrop. Additionally, the average LER in the high N Block was 0.9, which does not indicate a true intercrop advantage.

However, LER values do not tell the full story. As Li et al. (2011) pointed out, the total productivity of intercropping systems typically increases under high soil fertility or high rates of N application, and more so than the productivity of monocultures, on average. In this experiment, corn, across Plot Types, had significantly greater total yields at high N Blocks ($p = < 0.05$). Bean total yields were not significantly affected by the N Block.

Seasonality

If planting times are varied in an experimental design, it is inevitable that component crops also experience different environmental conditions, which could contribute to effects on yield advantages. For example, bean seeds require warm soil (around 30 ºC) for germination and seedling growth (Allman n.d.). Corn plants require temperatures between 18.33 °C to 29.44 °C for germination. In this experiment, temperature increased steeply during the first month. During the first 10 days, when the first bean plant cohort was growing, the average daytime temperature was 16.36 ºC, which was 4.9 ºC cooler than during the next 10 days and 4.8 ºC cooler than during next 10 days after that (National Weather Service, Primary Local Climatological Data Site in Austin Bergstrom). This probably explains why early-planted beans gained height more slowly. Nevertheless, early-planted beans produced more yield even in monoculture and in the intercrop were able to suppress corn yield. A later planting date for the first bean cohort could have had other negative consequences, such as sub-optimal high temperatures for growth.

Corn is typically planted later than beans due to their demand for, and tolerance to higher temperatures. Therefore, one may conclude that the apparent competitive inferiority of bean in corn-bean intercrops could at least in part be a consequence of sub-optimal planting time, rather than an intrinsically lower competitive ability of bean.

How yields in this experiment compare to the regional average

The National IPM Database (1730 Varsity Drive, STE 110, NCSU Centennial Campus, Raleigh, NC 27606) states that in general, green beans in Texas yield about 2040 kg/acre (Ipmdata.ipmcenters.org, 2003). This lies within the range of monocultures planted earlier (Table 5). One monoculture bean yield at high N Blocks was 2648 kg/acre in the high N Block.

According to USDA National Agricultural Statistics Service, corn grain yielded about 4490 kg/acre in 2017, which is just under the 3972 kg/acre achieved in the high N Block monoculture. Organic corn yields about 3050 kg/acre (McBride and Greene 2016), which is smaller than the monoculture yield from this experiment. Typically, the average corn plant population is around 30,000 plants/acre (Woli et al. 2014) which is lower than the 48,600 plant/acre (monoculture) used in this experiment. However, gem corn is a smaller corn plant. Thus, in general, monoculture yields from this experiment were comparable to yield from commercial farms.

As an example of bean yield in a corn-bean intercrop, Clark and Francis (1985), using an intercrop with slightly lower bean densities and significantly lower corn densities than used here, found that bush beans intercropped with corn yielded 583 kg/acre in an experiment in Palmira, Columbia and which is less than the approximately 1,000 kg/acre bean yield achieved here when bean and corn were planted simultaneously. In a study by Santalla, Casquero and De Ron (1999), they found that corn grown in monocultures yielded about 3355 kg/acre which is similar to the 3972 kg/acre in the high N Block from this experiment. Additionally, corn yield, when intercropped with bush bean, yielded around 1500 kg/acre which is generally less than intercropped corn yields in the high N Block.

Conclusions and implications for agriculture

The yields from this experiment was representative of commercial growing conditions and clearly demonstrated the effect of variation in planting time between component crops. Results followed closely the predictions associated with the complementarity hypothesis, which says that whichever crop component that is planted earlier achieves higher relative yield. Under facilitation, one expects a positive effect of early planted bean on corn yield, which was not evident. Similarly, under the environmental sensitivity hypothesis, one expects no effects on relative yields or LER, as performance is dominated by environmental responses influencing species in the intercrop and the monoculture in parallel ways.

Further research into planting offsets could prove helpful to farmers who are already using corn-bean intercrops and are searching for ways to fine-tune the yields of their component crops without jeopardizing the yield advantage that have been able to be produced.

One potential limitation of this study was the lack of multiple sites and years for data collection. Having duplicate plots would not only increase the sample size of the data collected, but it could also potentially increase accuracy in the findings. Using more than one site, however, is easier said than done, as experiments like this one require much time, effort, money, and resources to complete. The intense efforts needed to conduct just one of these experiments begs the question whether the advantage of more data would justify the difficulty in conducting the same experiment on multiple fields or increasing the number of replicates. A compromise in the form of undertaking the same experiment for multiple growing seasons might be the answer since it could lessen the financial burden of running a huge farming experiment at one time. However, there are many factors that can influence growth and yield of crops, many of which cannot be adequately impeded. These factors can potentially cause variance in the data, which larger experiments can help by giving more reliable mean responses.

In the context of planting offsets and facilitation, it seems unlikely that bean plants at early planting offsets would senesce in time for the corn to utilize the assimilated N from bean plant tissue. However, multiple growing seasons could impact the amount of N within the soil, thereby influencing the RY of component crops and LER. If this intercropping design would have been allowed to be repeated over several years, the results could have been different, as the longer growing season for early-planted bean could have added more nitrogen to the soil. In that case, early planting of bean could have ameliorated the negative competitive effect, at least under low soil N, thus increasing facilitative effects and resulting in a net-positive effect of early bean planting on LER

The conclusions from this experiment demonstrate that growing plants together in a plot with a certain goal in mind can be tricky; there are many influences that cannot be accounted for. Therefore, knowing more of the underlying relationships that plants have with one another and the environment can help in finding a way to maximize yields. More experiments like this one can increase knowledge in how the manipulation of competitive effects by planting intercrop components at different times utilizing planting offsets can benefit those who practice organic agriculture. For example, utilizing planting offsets with different types of component crops (but still functionally different crops) could result in results unlike those outlined here. Results found in this experiment, where resource use complementarity appeared to be the dominant form of species interaction, may not be the same in cropping systems dominated by facilitative interactions or those dominated by a difference in environmental tolerance. Additionally, different densities or spatial arrangements of corn and bean, could produce different results than seen here. Intercropping experiments are rarely identical in design, and research into multiple planting offsets is uncommon, so continued research in planting offsets could provide results that might be beneficial to farmers.

Ultimately, the usefulness of intercropping using planting offsets depends on the goals of a farmer. Given a choice, it may well be more desirable to have more yield at high soil fertility than greater yield advantage at low soil fertility, unless high resource inputs are considered undesirable in themselves.

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