Effect of Varying Phosphorus Availability on Non-nodulating Soybean Plants

Victor Wright

November 10, 2017

Abstract

Of all the elements on the periodic table, *P, N,* and *K*, are plant macronutrients. Nitrogen is the most important of the three because it has the highest domain over a plants growth and photosynthetic processes. Some species of plants can form symbiotic relationships with bacteria that convert N_2 into nitrogen that the species can devote to growth and photosynthesis. On the other hand, plant species that cannot fix nitrogen must uptake nitrogen from soil. However if the soil that a community of plants lives on is nitrogen poor, plant productivity such as growth, photosynthesis, and biomass accumulation can be severely limited by nitrogen deprivation whether the plants can fix N_2 or not. On the contrary, adding nitrogen as fertilizer to communities of plants that cannot fix nitrogen or live on nitrogen poor soils can increase plant productivity. On the other hand, it has also been discovered that when plants receive high volumes of P, biomass accumulation can be stunted as well their chlorophyll content and photosynthetic rate. In this experiment, we studied the effect of varying P as fertilizer on non-nodulating Glycine max chlorophyll a content, root, shoot, and leaf mass. We aimed to determine if differing qualities and volumes of phosphorus given to these plants as fertilizer would yield different biomass accumulation and chlorophlly a content in non-nodulating soybean plants. Our research shows that high volumes of strong phosphorus solutions can negatively impact non-nodulating soybean leaf mass and chlorophyll a content but not root or shoot mass.

1 Introduction

Flowers, seeds, or fruits are characteristic of most plants but, all plants have leaves, roots, and stems where each structure provides a different function engineered to aid the plants survival [How Plants Grow, 2017]. In a plants cells, photosynthesis takes place in a component of the plants cells called chloroplasts [Cunningham, 2011]. The substance chlorophyll, chlorophyll a being the most important in the photosynthetic process, is responsible for the plants celluar metabolism and is found in the chloroplasts [Kline, Cunningham, 2010, 2011]. Carbohydrates are the end product of photosynthesis. Carbohydrates allow plant reproduction and growth after the plant has converted the energy contained in the carbohydrates into a useable form [Robertson, 2007]. Phosphorus, calcium, nitrogen, sulfur, potassium and magnesium are nutrients that foster photosynthesis [Cunningham, 2011].

Plants use their roots to ingest phhhosphorus, calcium, nitrogen, sulfur, potassium and magnesium [How Plants Grow, 2017]. However, plant growth is mostly limited by availabilities of nitrogen, potassium and phosphorus. Thus, they are primary plant nutrients [Cunningham, 2011]. Some plants can produce their own nitrogen when an alliance forms between the plants roots and nitrogen-fixing microbes such as Frankia and Rhizobium [Alfrey, 2014]. Soybeans, alfalfa, lupine and other legumes are capable of forming this mutual relationship with Rhizobium [Alfrey, 2014]. In addition, a plant is nitrogen fixing if it is nodulating [Cavender-Bares, 2017]. If a plant is nodulating, its roots exhibit nodules where consumable nitrogen is produced by the N_2 converting microbes that live in the nodules [Cunningham, 2011]. On the contrary, some plants cannot fix nitrogen. This happens when the plant roots do not form nodules for microbes to live in [Cavender-Bares, 2017]. These plants are non-nodulating [Cavender-Bares, 2017]. Therefore, non-nodulating plants must meet their nitrogen requirement by aqcuiring it directly from the soil. Both non-nodulating and nodulating plants can comprise parts of plant communities and farmlands.

Many plant communities and agro-eco industry practices such as forest gardening, farming, and recreational gardening rely on macronutrient rich soils [Alfrey, 2014]. An agricultural study conducted in southern Minnesota by

Schmidt *et al.* showed that increasing applications of nitrogen to fields of non-nodulating soybean plants as fertilizer can produce higher crop yields [Schmidt, 2000]. Ritchie and Tilman studied plots of the leguminous plants Lathyrus venosus, Amorpha canescens, and Lespedeza capitata in eastern Minnesota [Ritchie and Tilman, 1995]. They added the macronutrients P, Na, K, S, Mg, and Mn to plots that exhibited exclosures limiting herbivorous pressure [Ritchie and Tilman, 1995]. They discovered that some of the leguminous plant species living on nitrogen poor soils were significantly limited by this particular factor [Ritchie and Tilman, 1995]. However, plant productivity can be stunted if a plant community absorbs too much P.

Plant bimoass accumulation and photosynthesis can be stunted when plant communities receive high volumes of P. In 2014, Corbella-Tena *et al.* studied the effect of varying P as fertilizer on Leucospermum Cordifolium [Corbella-Tena *et al.*, 2014]. They analyzed leaf, root, and stem mass of Leucospermum Cordifolium plants subject to phosphorus treatments of 5 mg, 10 mg, 15 mg, and 20 mg per L of nutrient solution and were grown in silica sand [Corbella-Tena *et al.*, 2014]. They observed that Leucospermum Cordifolium had the highest dry leaf weight on the condition that they received 5 mg of P per L as fertilizer compared to plants that received higher weights of phosphorus. [Corbella-Tena *et al.*, 2014]. Gianquinto *et al.* observed that the maximum photosynthetic rate of dwarf beans can be decreased when they endure a Zn deprivation that is often caused by high P absorption [Gianquinto *et al.*, 2000]. Furthermore, Gianquinto *et al.* alson discussed that when cauliflower exhibit Zn deficiency, the leaves often have low chlorophyll content and photosynthetic rates [Gianquinto et al., 2000]. Thus, we asked: does varying phosphorus availability have a significant effect on root mass, shoot mass, leaf mass, and chlorophyll a content of non-nodulating Glycine max, soybeans, living on nutrient poor soil? That is, we wished to discover if all soybean groups subject to unequal applications of phosphorus as fertilizer could be labeled the same with respect to soybean plant root mass, shoot mass, leaf mass, and chlorophyll a content. If not, we wished to uncover what applications had a significant effect on the previously mentioned variables. Therefore our hypotheses were

H_0: Varying phopshorus availability will have no significant effect on the chlorophyll a content and biomass accumulation between the groups of non-nodulating soybeans to be studied. That is, biomass accumulation and chlorophyll a content are the same for all groups of soybeans.

H_A: Varying phosphorus availability has a significant effect on chlorophyll a content and biomass accumulation between the groups of non-nodulating soybeans that will be studied. At least one treatment will have a significant effect on soybean biomass accumulation and chlorophyll a content.

2 Materials and Methods

Experimental Design

Non-nodulating soybean seeds were selected due to the fact that they cannot fix nitrogen and are easy to cultivate. Secondly, non-nodulating soybeans were selected due to the fact that our motivation was to observe the effect of varying phosphorus availability between groups. This was done to minimize the possible effect that nitrogen would have on biomass accumulation and chlorophyll a content. Thus, if differences between groups that received unequal application of phosphorus as fertilizer did exist, they could be easily detected. Similarly, the effect of herbivory was not simulated on any of the groups and light availability to all groups was made uniform to minimize further compilacations in our analysis. On the first day of the experiment, we selected each experimental unit to be a small flower pot where a coffee filter was placed at the bottom and then perlite was added to simulate nutrient poor soil. We then planted five surface-sterilized non-nodulating soybean seeds into each pot and innoculated them with Bradyrhizobium japonicum. We had twelve experimental units and sixty seeds planted in total. On this day, we did not add phosphorous to any of the experimental units. However, we did subdivide the experimental units into three groups where each group consisted of four pots. We then determined the quality and volume of phosphorus each treateatment group of pots was to recieve the following week and three weeks thereafter. Each pot was maintained to exhibit only three sprouts per pot for the duration of the experiment. The phosphorus solutions applied weekly to the plants as fertilizer were modified Hoagland's $P+$ (full strength phosphorus) and modified Hoagland's P- ($\frac{1}{10}$ strength phosphorus).

- Treatment group one pots recieved 25 mL of P- weekly
- Treatment group two pots recieved 12.5 mL of $P+$ and 12.5mL of P- weekly
- Treatment group three pots recieved 25 mL of $P+$ weekly

Data Collection

Weekly, we recorded the average of chlorophyll a in each soybean plant using a Minolta hand-held SPAD meter. Each average was taken over a total of five observations giving us a relative chlorophyll a content per leaf area per plant. However, on the second week of the experiment, we observed that one of the pots in treatment group three was dead. Weekly, each plants height was measured in centimeters and recorded. At the end of the experiment, we removed each plant from the perlite and divided them into their roots, shoots, and leaves with extreme care so that biomass lost in the process would be minimal. Each part of the plants were then placed in envelopes to dry for one week. The following week, we weighed each component of the dismantled plants in grams and recorded these data.

Statistical Methods and Models

To detect statistically significant differences between groups we computed one-way ANOVA tests with the hypotheses

H_0: $\beta_1 = \beta_2 = \beta_3$ *vs.* H_A: Not all β_js are equal

for $j = 1, 2, 3$ treatment groups. We declared that there was significant effect on non-nodulating soybean chlorophyll a content, root, shoot, or leaf mass if a one-way ANOVA test yielded a p-value < 0.05. In order to discover where the statistically significant differences were between groups, we compared group means of non-nodualting soybean chlorophyll a content, root, shoot, and leaf mass by constructing 95% Tukey Honest Significant Difference confidence intervals [Cannon *et al.*, 2013]. The form of these confidence intervals is

$$\bar{x_i} - \bar{x_j} \frac{+}{-} \frac{q}{\sqrt{2}} \sqrt{MSE \times (\frac{1}{n_i} + \frac{1}{n_j})}$$

for $i \neq j$ and the MSE is obtained from individual one-way ANOVA tests that examined non-nodulating soybean chlorophyll a content, root, shoot, and leaf mass. Tukey HSD confidence intervals that did not contain zero indicated that two phosphorus treatments of different volume and quality had a significantly different effect on chlorophyll a content, root, shoot, or leaf mass [Cannon *et al.*, 2013]. Tukey HSD confidence intervals that did contain zero indicated that the different volumes of phosphorus or quality had the "same" effect on non-nodulating chlorophyll a content, root, shoot, and leaf mass [Cannon *et al.*, 2013].

3 Results

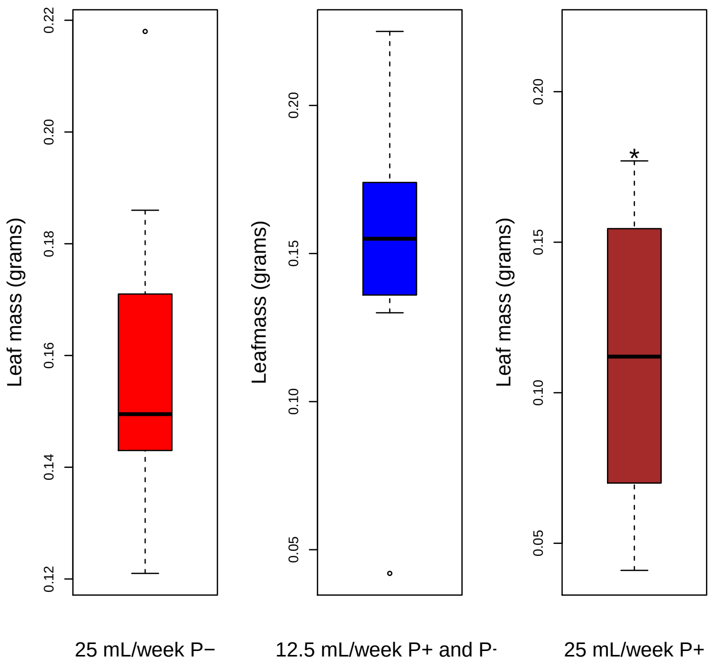

Figure 1: **Effect of varying phosphorus volume and quality on non-nodulating soybean leafmass.** The red, blue, and brown boxplots correspond to treatment groups one, two, and three respectively. Treatment group one pots recieved 25 mL of P- weekly, treatment group two pots recieved 12.5 mL of P+ and 12.5mL of P- weekly, and treatment group three pots recieved 25 mL of P+ weekly. The asterisk above the brown boxplot in (Fig. 1) indicates that adding 25 mL of P+ weekly to these groups of non-nodulating soybeans had significant effect on leaf mass.

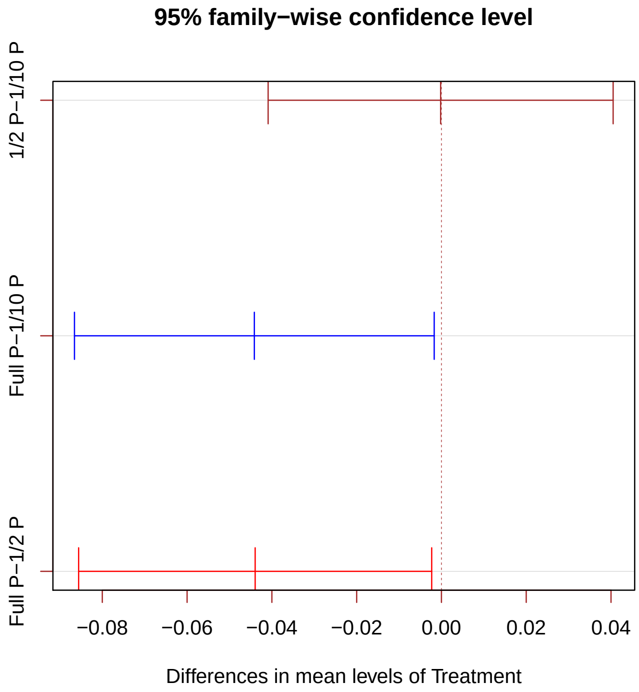

Figure 2: **Comparison of weekly phosphorus treatments on non-nodulating soybean plant mean leaf mass**. Fig. 2 contains 95% Tukey Honest Significant Difference confidence intervals that compare group means of non-nodulating soybean leaf mass. The brown 95% confidence interval in (Fig. 2) compares groups of non-nodulating soybeans that received 25 mL of P- weekly as fertilizer with groups of non-nodulating soybeans that 12.5 mL of $P+$ and 12.5mL of P- weekly as fertilizer. We concluded that those phosphorus applications to groups of non-nodulating soybeans had the same effect on leaf mass. On the contrary, these two treatments both had a significantly different effect on soybean leaf mass compared to soybean plants that received 25 mL of $P+$ weekly as fertilizer.

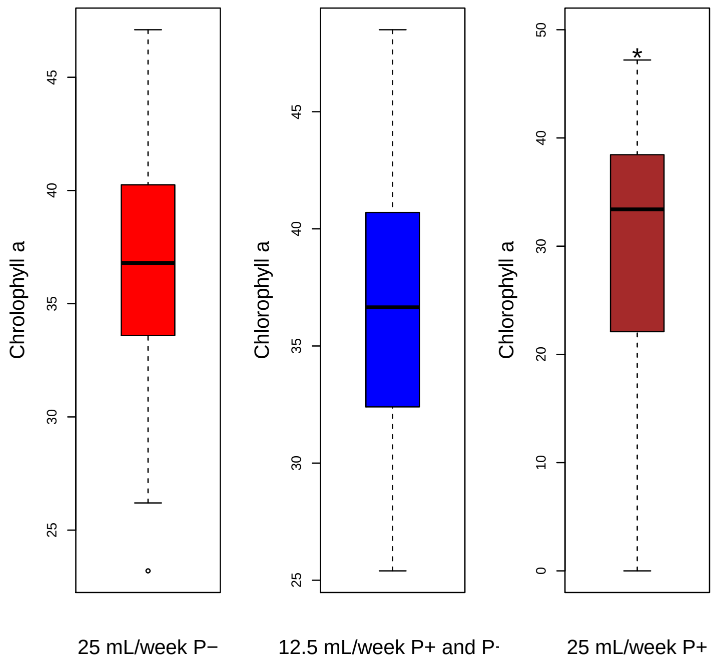

Figure 3: **Figure 3: Effect of varying phosphorus volume and quality on non-nodulating soybean chlorophyll a content.** The red, blue, and brown boxplots correspond to treatment groups one, two, and three respectively in (Fig. 3). Treatment group one pots recieved 25 *mL* of *P*- weekly, treatment group two pots recieved 12.5 *mL* of *P+* and 12.5*mL* of *P*- weekly, and treatment group three pots recieved 25 *mL* of *P+* weekly. The asterisk above the brown boxplot in (Fig. 3) indicates that adding 25 *mL* of P+ weekly to these groups of non-nodulating soybeans had significant effect on their cholorphyll a content.

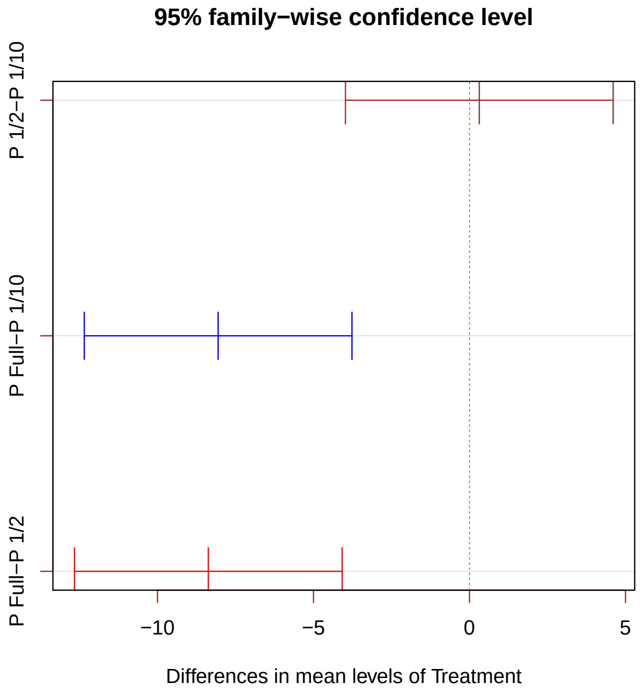

Figure 4: **Comparison of weekly phosphorus treatments on non-nodulating soybean chlorophyll a content** Fig. 4 contains 95% Tukey Honest Significant Difference confidence intervals that compare group means of soybean chlorophyll a content. The brown 95% confidence interval in (Fig. 4) compares groups of non-nodulating soybeans that received 25 mL of P- weekly as fertilizer with groups of non-nodulating soybeans that 12.5 mL of $P+$ and 12.5mL of P- weekly as fertilizer. We concluded that those phosphorus applications to groups of non-nodulating soybeans had the same effect on chlorophyll a content. On the contrary, these two treatments both had a significantly different effect on soybean chlorophyll a content compared to soybean plants that received 25 mL of $P+$ weekly as fertilizer.

Effect of Adding Differing Volumes and Qualities of Phosphorus to Non-nodulating Soybean Plants as Fertilizer

We explored the effect of phosphorus as fertilizer on non-nodulating soybean plant chlorophyll a content, root, shoot, and leaf mass by varying the quality and volume of the phosphorus that these plants received on a weekly basis. Three combinations of phosphorus volume and qualtiy were given to non-nodulating soybean plants. They were

- **Treatment one**: 25 mL of P- weekly
- **Treatment two**: 12.5 mL of $P+$ and 12.5mL of P- weekly
- **Treatment three**: 25 mL of $P+$ weekly

We discovered that treatment two and three both had similar effects on the leaf mass and chlorophyll a content of non-nodulating soybean plants. Therefore, we concluded that these combinations of phosphorus volume and quality given to non-nodulating soybeans as fertilizer were essentially the "same" in the sense that their effects on non-nodulating soybean plant leaf mass and chlorophyll a content were similar. However, when we compared treatment three to treatments one and two we discovered that treatment three was significantly different in the sense that it decreased non-nodulating soybean leaf mass and chlorophyll a content (See Figs. 1 and 3).

We also explored the effects of these three phosphorus combinations on soybean shoot and root mass. We discovered that all three treatments had similar effects on shoot and root mass. Hence, we concluded that these combinations of phosphorus volume and quality given to non-nodulating soybeans as fertilizer were essentially the “same” in the sense that their effects on non-nodulating soybean plant shoot and root mass were similar. This was due to the fact that all p-values obtained by one-way ANOVA tests were larger than 0.05. Thus, we chose to omit further statistical discussion and figures.

4 Discussion

In this study, we found that varying phosphorus availability had a significant effect on non-nodulating soybean leaf mass and chlorophyll a content. In particular, the volume and quality of phosphorus that had a negative impact on non-nodulating soybean plant leaf mass and cholorphyll a content was the weekly dose of 25 mL $P+$ compared to the other P treatments used. On the contrary, weekly doses of 25 mL of $P+$ and combined volumes of 12.5 mL $P+$ and 12.5 mL P- yielded higher chlorophyll a content and leaf mass in the non-nodulating soybean plants we studied. Therefore, we collected enough evidence to reject our null hypothesis and accept H_A as true. These findings are similar to what Corbella-Tena *et al.* documented in 2014 and Gianquito *et al.* discussed in 2000. If certain amounts and qualities phosphorus can have a negative impact on the biomass accumulation of crops such as soybeans, cauliflower, dwarf beans etc. how can we use this knowledge to minimize phosphorus induced eutrophication and increase crop yield?

Of the three plant macronutrients, the present-day agro-industry heavily relies on P due to the fact that it can accelerate plant maturity, deter root rot, increase stalk strength, etc. [USDA]. In addition, it has been documented that between 1950 and 2000 inorganic fertilizer usage, including N and P, has increased by more than fourfold [Daniel *et al.*, Cunningham, 1994, 2011]. The problem with increased inorganic fertilizer usage is that some P and N can run off of farm fields into rivers that lead to larger bodies of water such as seas creating a "dead zone" [USDA, Cunningham, 2011]. In addition, some species of algae can also fix N that arrives through runoff. Due to this fact, N induced eutrophication is said to be much more difficult to control compared to P since plants do not have mechanisms that allow them to fix P [Daniel *et al.*, 1994]. Therefore, the ultimate question is how do we minimize P field runoff? A theoretical approach is to use the tools of multivariable optimization and linear programming due to the fact that these mathematical programs are common in farm planning optimization problems [Williams and Meerschaert, 1999, 2013]. We choose to to conclude this paper by solving a multivariable optimization problem and illustrate how runoff can be minimized and while maximizing crop yield.

Suppose a family farms barley, peas, wheat, and canola on their 625 acre farm. Each week the members can devote 300 hours to agricultural practices. Finally, 1000 *acre-ft* of water is available for irrigation this growing season.

They, do not yet know how they should devote their acreage with respect to the four crops that they could plant. However, of the four crops, canola has the highest phosphate requirement which is 57 $\frac{lbs.}{acre}$ [reqs]. Peas, barley, and wheat require 46, 49, and 35 $\frac{lbs.}{acre}$ of phosphate respectively [reqs]. Assume that the farm is financially stable and could devote all 625 acres to canola. This implies that they can purchase 35625 pounds of phosphate if they wish. Suppose that they choose to purchase this amount of phosphate this year. It has been said that about five percent of P can wash off of fields when applied to crop [Daniel *et al.*, 1994]. If we assume five percent, then the maximum weight of P that could possibly runoff this farmland is 1781.25 *lbs.* if the 625 acre farm was devoted purely to canola crop. Finally, suppose that the family can earn \$50, \$100, \$150, and \$200 per acre of barley, peas, canola, and wheat. We want to maximize profit by finding how much of each crop should be planted but also possibly minimizing phorsphorus run off. The hours that must be devoted to each type of acreage weekly and irrigation requirement per acre crop will be found in the linear program that writes as

$$maximize\ Profit(x_1, x_2, x_3, x_4) = \$50x_1 + \$100x_2 + \$150x_3 + \$200x_4$$

subject to

$$x_1 + x_2 + x_3 + x_4 \leq 625$$

$$1.5x_1 + x_2 + x_3 + 1.5x_4 \leq 1000$$

$$0.2x_1 + 0.4x_2 + 0.2x_3 + 0.5x_4 \leq 300$$

$$49x_1 + 46x_2 + 57x_3 + 35x_4 \leq 35625$$

$$2.45x_1 + 2.3x_2 + 2.85x_3 + 1.75x_4 \leq 1781.25$$

and $x_1, ..., x_4 \geq 0$ where x_1, x_2, x_3, and x_4 represent possible acreages devoted to barley, peas, canola, and wheat respectively. In the matrix, rows two and three describe the irrigation requirements in *acre-ft.* of water and $\frac{person-hrs.}{week}$ that must be devoted to those plots of crops. Solving the mathematical program with juliabox yields that only canola and wheat should be farmed due to the fact that a simplex algorithm finds the optimal values to be $\tilde{x_3} = 42$ and $\tilde{x_4} = 583$ yielding a net profit of about \$122900 and using 916.5 *acre-ft.* of water. However, about 1140 *lbs.* of P is field runoff in this action plan. To possibly further mininmize field runoff, we consider minimizing a irrigation objective function in a linear program that writes as

$$\textit{minimize}\ Irrigation(x_1, x_2, x_3, x_4) = 1000 - 1.5x_1 - x_2 - x_3 - 1.5x_4$$

subject to

$$
\begin{aligned}
50x_1 + 100x_2 + 150x_3 + 200x_4 &\leq 122916.67 \\
x_1 + x_2 + x_3 + x_4 &\leq 625 \\
1.5x_1 + x_2 + x_3 + 1.5x_4 &\leq 1000 \\
0.2x_1 + 0.4x_2 + 0.2x_3 + 0.5x_4 &\leq 300 \\
49x_1 + 46x_2 + 57x_3 + 35x_4 &\leq 35625 \\
2.45x_1 + 2.3x_2 + 2.85x_3 + 1.75x_4 &\leq 1781.25
\end{aligned}
$$

where the first row is a "dictionary" from the optimal profit solution and the objective is written to consider total irrigation that is available to the farm for the year. The optimal solution is now $\tilde{x_1} = 42$ and $\tilde{x_4} = 583$ which yields a net profit of about $118700 for the farm but uses 937.5 *acre-ft.* of water. It seems that in this solution attempt that water usage was not minimized but, 1123.15 *lbs.* of P is runoff from the farmland in this action plan.

It definitely seems that there is a tradeoff between maximizing agricultural profit and minimizing P runoff from farms. As we saw in the theoretical example, it seems that minimizing field runoff in the agriculture industry should be economically viable since the reduction in net profit would be about $4200. The downside could is water conservation because this action plan requires 21 *acre-ft.* of water. It seems that there is a higher demand for irrigation due to the fact that the corn crop has a higher *acre-ft.* water requirement. The upside is that P runoff is in the case is ultimately less than that of P runoff caused by irrigating canola. Finally, crop yield would be maximized since all of the farmland was used in both cases.

Given our analyis the farm planning problem, it seems environmentally friendly to farm crops that have low P and irrigation requirements all while maintaining a strong argicultural economy. However this is just a theoretical hypothesis of what would happen if a farm followed such operating patterns. Of course the real world is much more complex but the solutions we obtained provide a nice simplification of reality. That is, it is definitely possible to farm such that crop yield is maxmimal, field runoff is minimized, and such outcomes can be economically feasible. On the contrary, it may take years of research and theoretical experimentation to conclude what agricultural functions and growing patterns such that global farming operations can be ecologically friendly while maintaining economically strong agricultural industries.