PHOSPHORUS LIMITATION OF SOYBEAN AND ALFALFA BIOLOGICAL NITROGEN FIXATION ON ORGANIC DAIRY FARMS

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ABSTRACT

Low plant available phosphorus limits legume growth and biological nitrogen fixation (BNF). This study examined, under controlled conditions, the relationship between soil phosphorus and alfalfa and soybean BNF on two contrasting low-P soils (Ontario and Nova Scotia) from organic dairy farms. Soluble P was applied up to 135 mg P kg^{-1}. An optimum range of 45 to 90 mg kg^{-1} applied P increased soybean plant growth, nodulation, N and P uptake and BNF. Significant effects of soil type reflected greater N supplying ability and lower P sorption for the Ontario soil. Alfalfa response to soluble P application was not as apparent. In addition three potentially organically acceptable amendments (MSW compost, Crystal Green® struvite and partially solubilized rock phosphate) were evaluated as alternate sources of plant available P. Compost and struvite, applied at moderate rates, sufficiently supplied P to increase plant growth and BNF comparably to that found for soluble P fertilizer.

LIST OF ABBREVIATIONS USED

AMF	arbuscular mycorrhiza fungi
BNF	biological nitrogen fixation
BNF-N	nitrogen derived from biological nitrogen fixation
C	carbon
CG	Crystal Green®
DAP	days after planting
DM	dry mass or dry matter
FC	field capacity
GM	green manure
ID	isotope dilution method
K	potassium
MF	moisture factor
MSW	Municipal Solid Waste compost
N	nitrogen
NA	natural abundance method
P	phosphorus
PR	phosphate rock
SAM	shoot apical meristem
STP	soil test phosphorus

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CHAPTER 1 : INTRODUCTION

Nitrogen (N) is one of the most important nutrients in cropping systems and is often the limiting factor in plant growth. Since the green revolution, the use of synthetic nitrogen fertilizer has negatively impacted the environment causing the pollution of air and water (Bohlool et al., 1992; Peoples et al., 1995; Vance, 2001). Additionally, the production of synthetic N fertilizer requires the use of fossil fuels which further damages the environment (Bohlool et al., 1992; Crews and Peoples, 2004) and contributes to the global warming effect. In an attempt to reduce the impact of agriculture on the environment, alternative production systems are being developed. These systems strive to maintain a high yield, crop quality and pest control in a manner which promotes sustainability. One such alternative is organic farming where the products used naturally occur. In organic and many traditional production systems, the key methods of increasing soil N are compost, manure and legume crops (Bohlool et al., 1992; Crews, 1993; Biro et al., 2000; Hardarson and Atkins, 2003; Bowatte et al., 2006; Canadian General Standards Board, 2009a; Canadian General Standards Board, 2009b; Warman et al., 2009). While manure and composts are added to the soil, legumes are incorporated into the soil using tillage to increase soil N content. In other cases, grain legumes are grown for their high protein content without the need for synthetic N fertilizer inputs.

The use of legumes in crop rotations has been in use for centuries and is a major source of N in areas where the cost of fertilizer is too high or import is difficult (Bohlool et al., 1992; Crews and Peoples, 2004). The ability of legume crops to obtain otherwise unavailable atmospheric N is due to the symbiotic relationship with several species of soil bacteria known collectivity as *Rhizobia*. Extensive research has been conducted to better understand the mechanisms and limitations of the legume-rhizobia relationship. Many studies have focused on quantifying the contribution of N from biological N fixation (BNF) in legumes. Crews (1993) found alfalfa in Mexico can fix between 232 and 550 kg N ha^{-1} yr^{-1}. Other studies have shown legumes can obtain up to 90% of their N from the atmosphere which allows the surrounding plants to access more of the soil N (Hardarson and Atkins, 2003). Legumes allow other plants to benefit from this N obtained from the atmosphere. During the normal turnover and decomposition of legume

roots, surrounding vegetation can access fixed N (Hardarson and Atkins, 2003) faster than through the breakdown of above-ground plant tissues. Although legumes have been shown to increase soil N, factors limiting the amount of N which can be fixed have been found (Lynch and Smith, 1993; Hardarson and Atkins, 2003; Bowatte et al., 2006; Zaman-Allah et al., 2007). It is important to understand these limiting factors in order to optimize the amount of N obtained though legume biological nitrogen fixation (BNF).

1.1 PHOSPHORUS LIMITATION OF LEGUME BIOLOGICAL N FIXATION

There are many known factors which limit the amount of N which can be obtained through legume BNF. Most of these factors can be related to abiotic conditions including: location (tropical vs. temperate), soil type, pH, soil temperature, water availability and nutrient levels (Bergersen et al., 1989a; Crews, 1993; Lynch and Smith, 1993; Ankomah et al., 1996; Bowatte et al., 2006). Often, the amount of N fixed by a species is largely dependent upon the variety and rhizobia strain (Ledgard and Steele, 1992; Houngnadan et al., 2008). This is likely due to genetic differences in the N fixation mechanism as well as the variety's ability to thrive within an environment. Some of the legume nutrient requirements are actually necessary for rhizobial growth and N production (Ledgard and Steele, 1992).

The effect of phosphorus on BNF has been extensively studied as the effect is localized to a specific soil, climate, legume variety and rhizobial strain (Plenchette and Morel, 1996; O'Hara, 2001; Hardarson and Atkins, 2003; Bowatte et al., 2006; Zaman-Allah et al., 2007). In Zimbabwe it was determined the number of nodules and plant growth of groundnut directly corresponded to plant available P (Lekberg and Koide, 2005). In a direct P rate response study using three varieties of cowpea (*Vigna unguiculata* cv. Amantin, It81D and Soronko), it was found that P application increased the size and number of nodules indicating the amount of BFN-N was increased. However, the extent of the difference was related to variety. In the same study, P did not increase the proportion of BNF-N in plant tissues rather it increased the total amount of N in the plant (Ankomah et al., 1996). In a contrasting study, Somado et al (2006) found P addition significantly increased the total N in *Crotalaria micans* plants while the ratio of soil N uptake to BNF-N shifted towards BNF-N but was not significant. These variable

responses to P may be attributed to cultivar differences, including P uptake and assimilation (Sanginga, 2003).

As well, P limitation of important North American crops has been studied, though not necessarily within North America. These crops include alfalfa and soybean. In Mexico it was found the leaf N content highly correlated with the amount of N fixed in alfalfa (Crews, 1993). In addition, Crews (1993) observed as plant available P increased the amount of N fixed by alfalfa also increased. When plants were not fertilized with P all above ground tissues were significantly smaller than the plants which received P fertilizer (Chaudhary et al., 2008). Additionally, the nodule dry mass (DM) per shoot DM was significantly reduced in the plants which had not received P fertilizer. BNF followed a similar trend and was significantly reduced in the plants which had not been P fertilized (Chaudhary et al., 2008). Chien (1993) evaluated the effectiveness of three PR for their ability to increase plant growth and BNF. It was found the PR increased plant growth and BNF compared to the control. Two of the PR's significantly increased N content in the shoot and leaves at 25 mg added P kg^{-1} while the third PR achieved this at 50 mg added P kg^{-1}. As well, the amount of BNF-N was significantly increased over the control for two of the PR's. The difference in response between the PR's was assumed to be caused by the PR ability to supply plant available P. In summary, both alfalfa and soybean crops have been shown to increase BNF-N with increase plant available P.

The mechanism involved in the P limitation on BNF has not been established. However, several theories have been formulated to explain the relationship. One theory states the plant's biomass limits the amount of N fixed by rhizobia. However, if P becomes a limiting factor of plant growth, P then becomes a secondary limiting factor of N fixation (Crews, 1993; Bowatte et al., 2006). Chaudhary and Fujita (1998) concluded BNF was a secondary response to added P since the leaf area increased as added P increased. Additional studies have arrived at the same conclusion (Somado et al., 2006; Rotaru and Sinclair, 2009). Although there appears to be a correlation between plant biomass and soil available P, Lekberg and Koide (2005) found this not to be the case. However, they did find nodule number to be directly and strongly correlated (r^2=0.98) with available soil P in groundnut (*Arachis hypogaea*) in Africa. Israel (1987) found P had a greater effect on plant N concentration than added nitrate. It was determined the

BNF process had a higher need for P than the plant therefore, the BNF process requires a certain level of P in order to optimized BNF-N. Additionally, it has been found P deficient plants contain a relatively high proportion of P in the nodules indicating the nodules require large amounts of P for BNF functioning (O'Hara et al., 1988). Additional studies have found the nodules have a higher response to added P than the plant itself (Almeida et al., 2000; Sulieman et al., 2008). In fact, Almeida et al (2002) found clover was unable to form nodules under severe P deficiency.

In a review paper O' Hara (2001) discusses several mechanisms which may cause the P limitation of rhizobial activity. First, rhizobia require P to synthesize the nitrogenase enzyme. The formation of enzymes relies on the amino acid arrangement as dictated by nucleic acids. Second, if P is limiting, the concentration of nitrogenase decreases which decreases the amount of N fixed. In addition, P is an important component of bacteria signaling systems. Third, if the rhizobium is P deficient, there will likely be an impairment of important cellular functions. This disruption of cellular processes affects the ability of the rhizobia to fix N. These theories explain the relationship between BNF and P on a basic cellular process level. In studying the regulation of BNF Suleiman et al (2008) found as available P decrease the levels of RNA decreased. Le Roux et al (2008) found nodules experiencing P deficiency switched metabolic processes which likely resulted in a feedback mechanism reducing BNF-N. It is very possible the P limitation of BNF may due to P effects at the intracellular level.

It is known that P limits the amount of N fixed by legumes through extensive studies. However, the mechanism or mechanisms of this limitation is still not fully understood. It may be the case, that the effect is not a result of a single factor but the result of interactions between the various plant organs and the rhizobia. Likely, part of this P limitation is due, in part, to the plant and rhizobia species.

1.2 MEASURING BIOLOGICAL NITROGEN FIXATION

There are many methods used to indicate the amount of N obtained through BNF; some of these methods only provide an estimation while other methods quantify the amount of N obtained. Methods used to provide an estimate of N obtained from BNF use specific plant organs including: the number and/or mass of the nodules on each plant

(Ankomah et al., 1996; Lekberg and Koide, 2005) or the difference in N uptake between legumes and a non-legume reference plant (Ledgard and Steele, 1992; Hardarson and Danso, 1993). There are two methods used to directly quantify the amount of N obtained from BNF. Both these methods use the naturally occurring stable ^{14}N and ^{15}N isotopes in the calculation. The atmosphere contains a low percentage of ^{15}N, 0.3663 percent of N atoms, while ^{14}N accounts for the remaining 99.6337 percent (Crews, 1993; Hogh-Jensen and Schjoerring, 1994; Hogberg, 1997). Natural biological processes in the soil concentrate ^{15}N due to the discrimination of biological N transformations towards the lighter ^{14}N isotope. The biological discrimination of N isotopes allows a distinction between the sources of N (Crews, 1993; Hogh-Jensen and Schjoerring, 1994; Hogberg, 1997). An N fixing plant obtaining most of its N through BNF will contain more ^{14}N than a plant which obtains its N exclusively from the soil. The Natural Abundance (NA) method relies on natural biological processes to concentrate ^{15}N in the soil (Crews, 1993; Hogh-Jensen and Schjoerring, 1994). In some instances the isotope difference between the soil and the atmosphere is too small to accurately quantify BNF (Hogh-Jensen and Schjoerring, 1994; Hogberg, 1997; Huss-Danell and Chaia, 2005). The other method, Isotopic Dilution (ID), ensures a large difference between isotope ratios by adding a small amount of ^{15}N enriched N fertilizer (Hogh-Jensen and Schjoerring, 1994; Huss-Danell and Chaia, 2005). Although the ID method ensures the N isotope ratio between the atmosphere and soil is large, the cost of ^{15}N enriched fertilizer is expensive. Therefore, the Natural Abundance method is often chosen due to a lower experimental cost.

When the Natural Abundance method is employed, an N isotope base line must be established. The N isotope base line can be determined using two different methods. In many cases, the isotope ratio of surrounding non-N fixing vegetation called reference plants is used (Allahdadi et al., 2004; Goh, 2007). It is best to have reference plants having a similar root structure and physiology as the plant of interest to provide the best result (Goh, 2007). In special cases, a non-BNF mutant (non-nod) may be used. The non-nods provide a better base line as these plants have the same root distribution and physiology as the N fixing plants (Allahdadi et al., 2004). This isotope base line is used to determine the percent of N obtained from BNF.

In addition to considering the cost of the chosen methodology, there are other factors which must be considered before choosing which method will be used to determine BNF-N. The time frame for studying BNF must be considered. The ID method provides a snap-shot view of BNF due to the limited penetration of the enriched ^{15}N fertilizer in the soil profile, while the NA method can provide a long term view of BNF (Huss-Danell and Chaia, 2005). In order to accurately determine BNF-N by the NA method, it is necessary for the $\delta^{15}N$ value to differ by five or six units between the legume and the reference material (Hogh-Jensen and Schjoerring, 1994; Huss-Danell and Chaia, 2005). If the ^{15}N difference is too low, it is recommended ^{15}N enriched fertilizer be added to the soil to further separate the isotope ratio, i.e. use the ID method. The isotope percentage in the plant material is determined using mass spectrometry.

The mathematical equation used to determine the amount BNF-N is dependent upon the method, NA or ID, used. This difference is due to the concentration of ^{15}N within the plant. The following equation is used to calculate the percent of N obtained through BNF when using the NA method:

$$\%N_{fixed} = 100 * \frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - \beta}$$

where $\delta^{15}N_{reference}$ is the concentration of ^{15}N in the non-BNF reference plant, $\delta^{15}N_{legume}$ is the ^{15}N concentration in the legume material and β is the ^{15}N concentration of the legume relying solely on BNF for N (Crews, 1993; Riffkin et al., 1999; Huss-Danell and Chaia, 2005). The value of β can be determined several ways. One method is to grow the legume in an N-free media, either hydroponically or in a soil-less medium (Crews, 1993; Riffkin et al., 1999). The other method is to estimate β by using the lowest $\delta^{15}N$ value in the legume material collected (Huss-Danell and Chaia, 2005). The preferred method is to grow the legume in an N-free media as this ensures all N within the plant has been obtained from BNF. The ID methodology uses the following equation to determine the percentage of BNF-N in legume material:

$$\%N_{fixed} = 100 * (1 - \frac{\%^{15}N_{legume}}{\%^{15}N_{reference}})$$

where $\%^{15}N_{legume}$ and $\%^{15}N_{reference}$ is the percentage of ^{15}N isotopes in the legume and

reference material, respectively, after subtracting the atmospheric percentage of ^{15}N, 0.6336 (Hardarson and Danso, 1993; Huss-Danell and Chaia, 2005; Goh, 2007).

1.3 LEGUME BIOLOGICAL FIXATION ON ORGANIC DAIRY FARMS

Soil nutrient inputs are often limited in organic dairy production systems due to the prohibited use of synthetic fertilizers (Canadian General Standards Board, 2009b). The most common materials used on organic dairy farms to increase soil nutrient levels are manures, composts and mined mineral nutrient deposits, *i.e.* rock phosphate (Nicholas et al., 2004; Roberts et al., 2008). Organic dairy farms in North America have a low-to-no P surplus whereas the opposite is true for their conventional counterparts (Anderson and Magdoff, 2000; Bengtsson et al., 2003; Martin et al., 2007; Roberts et al., 2008). This difference is attributed to management regimes, livestock density and land area. One of the largest contributors to the P surplus is imported feed (Anderson and Magdoff, 2000; Roberts et al., 2008). Due to the cost of organic feed, organic dairy producers often import as little feed as possible, with many producers opting to grow most of their own feed. Combined with the reduced livestock densities on these farms, this results in lower amounts of total P in manure being spread on the fields (Anderson and Magdoff, 2000; Martin et al., 2007). One of the largest losses of P on dairy farms, both organic and conventional, is in the exported milk (Anderson and Magdoff, 2000; Roberts et al., 2008). This reduced P cycle within the farm reduces P returning to the field each year.

A recent survey of long-term organic dairy farms in Ontario observed almost half of the farms had negative farm P balances. The average soil test P (STP), Olsen P, on the farms was 12 mg kg^{-1} (Roberts et al., 2008) where values below 10 mg kg^{-1} are considered low (Baute et al., 2002). In Norway, Loes and Ogaard (2001) observed a decline in STP values over several years on organically managed farms. However, the STP levels were still considered to be medium to high and it was concluded P deficits would not be observed in the near future. Loes and Ogaard (2001) suggest carefully monitoring STP levels in organically managed fields to ensure P levels do not drop below a critical P threshold. Watson et al (2002b) compiled research results from other organic studies around the globe in an attempt to further understand nutrient sustainability. The organic dairy average farm surplus of P was 8 kg ha^{-1} $year^{-1}$ but it is necessary to note

many of the studies examined did have negative P balances. It was concluded it is necessary to manage nutrient flows, including P, to ensure soil nutrient levels are able to support a high quality yield. Current studies of nutrient cycling, including P, indicate there will be a decline in STP which will affect farm productivity (Loes and Ogaard, 2001; Martin et al., 2007). However, with careful monitoring and an increased knowledge of nutrient cycling on organic farms it will be possible to effectively manage P deficits.

1.4 Soil Amendments to Increase Soil Phosphorus

The materials used in organic production systems are restricted to naturally sourced materials and are regulated by the Canadian Food Inspection Agency in Canada (Canadian General Standards Board, 2009a; Canadian General Standards Board, 2009b). Currently, the most commonly used non-synthetic sources of nutrients are: manure, green manure and compost (Berry et al., 2002; Warman et al., 2009). Another method is the inoculation or promotion of arbuscular mycrohhizal fungi (AMF) which assist in releasing P from the soil matrix. Phosphorus can be applied as rock phosphate (PR) which only supplies P to the soil when added in large quantities. However, these materials are complex and nutrient availability is difficult to predict (Arcand et al., 2010).

The nutrient availability of manure, compost and crop residues is difficult to predict due to biotic and abiotic factors (Warman, 1998; Berry et al., 2002; Sainju et al., 2002; Honeycutt et al., 2005; Griffin et al., 2008). One factor which impacts the availability of nutrients in these materials is the C:N ratio. If the ratio is too high soil microbes will immobilize N in the soil while a low ratio will allow soil microbes to mineralize N for plant uptake (Berry et al., 2002; Plaster, 2003). Another important ratio, focusing on the mineralization of P is the C:P ratio (Oehl et al., 2001; Oehl et al., 2004). In addition, some materials contain complex molecules which take longer to break down than materials with simple molecules. A mineralization study can be conducted to better estimate the nutrient availability in these products (Berry et al., 2002; Honeycutt et al., 2005; Burger and Venterea, 2008; Griffin et al., 2008). Unlike synthetic fertilizers where the correct ratio of nutrients needed for a crop can be supplied, organic amendment nutrient ratios are dictated by the material and are often uncontrollable. In many cases, it

becomes necessary to choose an application rate of an amendment based upon a single nutrient with other nutrients in the material being supplied either in excess or at insufficient levels (Warman, 1998; Mkhabela and Warman, 2005). However, many of these products supply additional secondary nutrients and micronutrients (Warman, 1998; Mkhabela and Warman, 2005; Warman et al., 2009). Even though the release of nutrients from organic soil amendments is difficult to predict, the addition of these materials has its advantages.

Manure is a suitable source of nutrients but its use is limited by availability and transportation costs. Additionally, there are organic farms that are crop based and do not contain livestock. These farms must find adequate sources of nutrients without manure inputs (Weinert et al., 2002; Martin et al., 2007).

Compost, another commonly used source, is subjected to similar restrictions as manure. The type of material composing the compost affects the type and availability of nutrients (Warman, 1998; Lynch et al., 2004; Parfitt et al., 2005; Burger and Venterea, 2008; Zai et al., 2008). As well, the method and time used for composting has a significant effect on the nutrient availability (Warman, 1998; Mkhabela and Warman, 2005). While composting reduces the volume of materials the composted material still maintains a large volume. A commonly available compost in Nova Scotia is an Municipal Solid Waste (MSW) compost collected from residents, containing source separated organic food and gardening wastes (Hargreaves et al., 2008). Previous studies have found MSW compost increases the water holding capacity, soil organic matter content, improves the soil microbial community, increases soil pH and supplies a host of plant nutrients, including P, as compared to soil without added amendments (Hargreaves et al., 2008).

Phosphate rock (PR) is a unique product used in organic agriculture. The main nutrient content is P though there can be other nutrients, especially calcium. The P availability in PR is dependent upon the source and size of the PR upon application (Loes and Ogaard, 2001; Arcand et al., 2006; Arcand and Schneider, 2006; Martin et al., 2007). Currently, the organic sector is studying methods to increase the predictability and release of P from PR. One method explored used a buckwheat (*Fagopyrum esculentum*) cover crop to increase the mineralization process from PR (Arcand et al., 2006). It was

found the source of the PR significantly affected the P uptake by buckwheat and the mineralization of P the following year. It was found buckwheat P mineralization was approximately 30 percent higher in the green manure incorporation plots over plots not receiving crop residues (Arcand et al., 2010). In addition, STP significantly increased in the plots where crop residues were incorporated. However, Arcand (2010) concluded the use of a cover crop in conjunction with a PR application did not significantly increase STP in agronomically sufficient levels to benefit subsequent crop yields. Another method being explored utilizes citric acid to partially solubilize P in PR. Schneider (2007) used a fungi, *Aspergillus niger*, to produce citric acid for the partial solublization of PR. It was found up to thirty percent P could be solubilized by the citric acid produced by *A. niger* depending on the method and PR composition. While using PR in conjunction with cover crops to increase P mineralization is organically acceptable, the acceptability of using citric acid for partially solubilizing PR prior to application remains to be determined. As such, solublization with organic acids does not involve synthetically produced materials and this method may become organically acceptable. Recently the partial stabilization of fish emulsions with acids is currently accepted under organic standards (Canadian General Standards Board, 2009b) which provides a possible precedent for processing other soil amendments including PR's. Research is needed to assess the agronomic benefits of partially solubilized PR's as a source of plant available P for legumes in organic production systems.

A new by-product derived from municipal waste water and sewage treatment systems, struvite, is being tested for nutrient supply in agriculture. The chemical name for struvite is magnesium ammonium phosphate. There is another type of struvite called K-struvite which is magnesium potassium phosphate (Qureshi et al., 2006) but is not as common as struvite. Struvite is a P rich product which contains other nutrients at lower concentrations including N, Mg, Ca and K (Ostra Nutrient Recovery Technologies Inc.; Pastor et al., 2008). Struvite is a naturally forming crystal in wastewater treatment plants and causes problematic blockages in the piping (Wu and Bishop, 2004; Pastor et al., 2008). Different methods have been developed to extract the P from sewage sludge. The general approach is to precipitate P from the wastewater using microbes, agitation and catalyst chemicals, often Mg and NaOH (Wu and Bishop, 2004; Qureshi et al., 2006;

Suzuki et al., 2007; Pastor et al., 2008). Suzuki et al (2007) significantly increased the precipitation of P from swine wastewater by adding a MgCl solution. The significance of this reaction was dependent upon the volume of the solution added. The nutrient content in struvite is largely dependent upon the initial nutrient content in the sewage sludge and the method used to form struvite (Qureshi et al., 2006; Suzuki et al., 2007; Pastor et al., 2008). In one study, the method used was able to precipitate 82% of the P in the wastewater (Qureshi et al., 2006) while another study reported efficiencies of 18 and 49% (Suzuki et al., 2007). The struvite crystallization process helps to reduce the potential phosphates pollution of the environment. In addition, there is a looming agricultural P shortage and this process, by helping to close the urban-rural nutrient loop, provides a much needed source of sustainable P. Estimates of mined P sources remaining vary between 3.6 to 22 million tons (Steen, 1998; Roberts and Stewart, 2002) and is estimated to last between 60 and 130 years (Steen, 1998; Vance et al., 2002). The majority of the reserves are in Africa and China. Table 1.1 provides a typical nutrient analysis of a struvite product, Crystal Green, being produced by Ostara Nutrient Recovery Technologies Inc. at a facility in Portland, USA (Ostra Nutrient Recovery Technologies Inc.).

Studies examining the effectiveness of struvite have shown the product is able to provide plants with sufficient P for growth and yield. Ponce and De Sa (2007) studied the ability of a struvite product to supply P to ryegrass (*Lolium perenne*) under controlled environment conditions. It was found the struvite product significantly increased the concentration of P in the plant tissue over a control and synthetic P fertilizers. While the response among the treatments varied between the five harvests of ryegrass, struvite always preformed as well as or better than the industry standard triple super phosphate (TSP). Massey et al (2009) compared PR and TSP with three different struvite products in an controlled environment study using wheat (*Triticum aestivum* L). All four P products were significantly different than the control. There was variability in response to the various struvite products, with magnesium ammonium phosphate performing at the same level as the TSP fertilizer.

Although struvite has the ability to be an excellent source of sustainable P fertilizer there are many questions with respect to its acceptability for organic agriculture.

Currently the product is not for sale in Canada. The Canadian organic standards currently do not permit the use of any type of sewage product (Canadian General Standards Board, 2009a) and the public perception of the current sewage sludge/biosolid products is negative (Delaney, 2010). However due to the looming shortage of P and the need to reduce environmental P pollution, struvite will have to be closely examined as a valuable source of P. A commitment to regional recycling of resources and closing nutrient loops is one of the seven key principles of the Canadian organic standards (Canadian General Standards Board, 2009a). Additionally, the organic sector, through a national Standards Implementation Committee, in concert with the Canadian General Standards Board continuously review the Canadian Organic standards using the most current research and will have to examine the use of sewage byproducts, such as in organic agriculture. As time progresses and nutrient sources become more expensive due to limited resources, sewage nutrient source byproducts such as struvite will likely become widely used and research is needed to examine the agronomic suitability of these products as P sources for organic production.

Table 1.1: The nutrient content of a struvite product marketed under the name Crystal Green® (Ostra Nutrient Recovery Technologies Inc.).

Nutrient	**Nutrient Content** (%)
Nitrogen (NH_4)	5
Phosphorus (P_2O_5)	28
Potassium (K_2O)	0
Magnesium (Mg)	10

The process of crystallizing struvite involves processing parts of the sludge through a struvite crystallizer. The crystallizer uses agitation, often air, to aid in the crystallization process. Chemicals such $MgOH_2$ or MgCl and NaOH are added to aid in the crystallization process (Ueno and Fujii, 2001; Wu and Bishop, 2004; Qureshi et al., 2006). Magnesium assists in crystal formation and NaOH is used to maintain an optimum pH of 8.2 to 8.8 (Ueno and Fujii, 2001). Other less common chemicals used to precipitate struvite are iron and aluminum salts (Gaterell et al., 2000). Additionally, microbes can be used to assist in the crystallization process (Gaterell et al., 2000). An

organically acceptable source of these catalysist's must be found to meet organic standards (Canadian General Standards Board, 2009b). The crystallization process has been found to produce a pure product, 98±1% (Bhuiyan et al., 2008), and has been found to have extremely low levels of heavy metals (Ueno and Fujii, 2001). The levels in a Japanese-produced struvite were lower than the levels set for commercial synthetic fertilizers (Ueno and Fujii, 2001). The amount of P which can be precipitated from the sludge dictates the feasibility of struvite fertilizer. In a mathematical model of the economical value of struvite, Gaterelle et al (2000) determined a minimum recovery rate of 80 percent must be achieved to be economically viable. An additional life cycle analysis suggested struvite has a lower environmental impact during production and transportation than commercial synthetic fertilizers if plants are established at local waste treatment plants (Gaterell et al., 2000). This reduction of transportation adds to the future popularity of struvite.

In some cases a crop is intentionally grown, such as a cover crop or green manure (GM), for its ability to add nutrients to the soil and the whole plant is incorporated using tillage. The selection of a cover crop is dependent upon many factors including: tolerance for weed species, nutrient content in the plant tissue and attraction of insects both beneficial and pest (Creamer and Baldwin, 2000; Hartwig and Ammon, 2002; Sainju et al., 2002; Frake et al., 2008). The time and type of tillage used to incorporate crop residues into the soil affects the timing of mineralization and amount of nutrients released (Sainju et al., 2002). Although GM and cover crops provide nutrients to the cropping system there are many drawbacks to its use, which may include the loss of a cropping year.

Arbuscular mycrohhizal fungi (AMF) have the ability to form a relationship with plants. Often this relationship is symbiotic; the plant receives P while the AMF receive a C source (Gosling et al., 2006). The AMF hyphae extend well beyond the root zone and are able to extract P well beyond the reach of roots (Grant et al., 2005; Gosling et al., 2006). Also, other compounds can be secreted by AMF to promote the release of adsorbed P including: phosphatases and H^+ ions to acidify the soil (Grant et al., 2005). However, management practices have an impact on AMF colonization including: tillage which destroys the hyphal network, fertilization or high P levels reducing the need for the

relationship and application of certain pesticides (Kahiluoto et al., 2000; Lekberg and Koide, 2005; Gosling et al., 2006). Many crops have shown an increase in growth, yield and yield quality when colonized by AMF (Kahiluoto et al., 2000; Lekberg and Koide, 2005). The inoculation of crops with AMF has shown an increase in plant growth and P uptake (Plenchette and Morel, 1996; Biro et al., 2000). However, the practice of inoculation is not commonly used but will likely gain popularity as alternate sources of nutrients must be found, including methods which allow plants to access otherwise unavailable soil P.

1.5 SUMMARY

To ensure the long term sustainability and productivity of organic dairy farms in North America, it is necessary to understand the relationship between legume BNF and plant available P in characteristic soils from these systems. Also, it is necessary to evaluate potential new sources of soil P. This study examined the relationship between legume BNF and available P using alfalfa and soybean cultivars with related non-nod plants. The study first evaluated (Chapter 2) the response of soybean and alfalfa BNF, grown in two different soil types, to increasing soil available P. Subsequently (Chapter 3) the effectiveness of three organic or potentially organically acceptable soil amendments to supply a readily available source of P to optimize BNF in soybean was evaluated.

CHAPTER 2 : SOYBEAN AND ALFALFA BIOLOGICAL NITROGEN FIXATION AND GROWTH RESPONSE TO AVAILABLE SOIL PHOSPHORUS

2.1 INTRODUCTION

A recent survey of long-term organic dairy farms in Ontario found roughly half of the farms had low soil test phosphorus (STP) levels, below 9 mg kg^{-1} as measured by Olsen P (Roberts et al., 2008). This study found the top three sources of N were BNF, atmospheric deposition and imported feed, while the top three sources of P were mineral livestock supplements, imported feed and bedding. The export of milk accounted for the largest losses of N and P from these farms. The average farm P balance across 15 farms was 1 kg ha^{-1} $year^{-1}$. While the whole farm balance was slightly positive, Roberts et al (2008) theorize some individual fields on the farms were experiencing P deficits. This Canadian observation is consistent with studies conducted elsewhere. In reviewing 67 organic dairy farm nutrient balances in temperate regions, Watson et al (2002b) found an average farm N surplus of 82.1 kg N ha^{-1} $year^{-1}$ and 3.1 kg P ha^{-1} $year^{-1}$. An organic vs. conventional dairy comparison study in Switzerland found the organic dairy farm had a P surplus of 1.1 kg ha^{-1} $year^{-1}$ which was ten times lower than the conventional farm (Cederberg and Mattsson, 2000). From these observations, it is very likely many organic dairy farms are heading towards P deficiency. The full effects of a possible P deficiency in these systems are not yet clear.

Legume nitrogen fixation is a key source of N in organic and sustainable production systems (Peoples et al., 1998; Crews and Peoples, 2004). Not only does BNF have the ability to reduce the energy required for N inputs but it has the ability to reduce the pollution caused by excess N (Peoples and Craswell, 1992; Lynch, 2009). It has been estimated that forty-five million tons of N are fixed annually in permanent pastures globally (Peoples et al., 1995). The amount of N fixed by legumes varies greatly, both by crop, variety and management regime. In Canada it was estimated alfalfa (*Medicago sativa* L) fixes roughly 200 kg N ha^{-1} $year^{-1}$ (Ta and Faris, 1987) while soybean fixes approximately 100 kg N ha^{-1} $year^{-1}$ (Rochette et al., 2004). This fixed N can be released into the soil by several mechanisms including: root secretions and decomposition, animal excreta and decomposition of senesced tissue (Ledgard and Steele, 1992; Peoples and

Craswell, 1992). In addition, legume crops can be inter-cropped with other plants or tilled into the soil as a green manure in an attempt to reduce the need for synthetic N fertilizer (Hartwig and Ammon, 2002; Lynch et al., 2008; Zai et al., 2008; Olesen et al., 2009). However, the amount of N fixed is dependent upon many biotic and abiotic factors including: rhizobia and plant species, soil nutrient status, pH, salinity, temperature and energy availability (Bohlool et al., 1992; Ledgard and Steele, 1992; Lynch and Smith, 1993; Bordeleau and Prévost, 1994). A commonly studied but poorly understood limiting factor of BNF is P deficiency.

The effects of soil available P on BNF in a variety of crops has been studied. Crews (1993) studied the effects of low plant available P on alfalfa BNF in Mexico. It was observed P affected the amount of N which could be fixed by the alfalfa as measured by the ^{15}N Natural Abundance technique. In a correlation analysis, Crews (1993) was able to explain 85 percent of the variability of BNF in alfalfa by the P content in the leaves. It is assumed as soil available P increases the P content in the leaves increases too. Additional studies have observed an increase of soil available P increases nodule number and mass in various legume crops (Isreal, 1987; Ankomah et al., 1996; Lekberg and Koide, 2005). As well, shoot growth was observed to increase and the P content has been observed to increase (Plenchette and Morel, 1996). However, it has been shown the effect of P on BNF is due in part to the cultivar (Ankomah et al., 1996). The mechanism of this interaction is not understood. However, it has been proposed the symbiotic rhizobia requires a large amount of P in order to fix N (Crews, 1993; Yemane and Skjelvag, 2003; Lekberg and Koide, 2005). It is known P has the ability to limit the amount of N fixed by legumes.

Two important legumes in the Canadian context are soybean and alfalfa. In the USA it was found P content of soybean shoot increased significantly as available P increased (Israel, 1987). In addition, the number and mass of the nodule increased significantly as added P increased. Isreal (1987) measured BNF and found BNF-N increased as plant available P increased. Additional studies have had similar results (Plenchette and Morel, 1996; Chaudhary et al., 2008; Rotaru and Sinclair, 2009). Similar results have been observed in alfalfa (Crews, 1993).

One factor which is important for P availability in soil is soil type and its

associated chemistry. Often, collected soil samples are analyzed for plant available nutrients using a proven soil extract. These soil extraction procedures are vigorously tested and correlated to plant nutrient uptake (Sharifi et al., 2007). Some nutrients can be extracted by several different extracts and use of these soil tests are often restricted to a large defined geographical area by similarities in soil characteristics. For example, P is extracted in Ontario using a sodium bicarbonate extract due to the region's high pH while P in Nova Scotia is extracted by Mehlic Acid due to the low pH of the soil (Carter and Gregorich, 2007). In order to identify potential soil nutrient deficiencies it is necessary to perform the analysis with the recommended extract for the region. Furthermore, the degree of nutrient sorption within a soil is partially affected by the organic matter and clay content (Plaster, 2003). In soils where the nutrient sorption capacity is high it is necessary to increase the nutrient application in order to obtain a maximum yield. It is necessary to understand the sorption capacity of a soil for P to optimize fertilization for maximum plant growth while minimizing nutrient losses (Morel et al., 2000). Sorption capacity for P varies between soil types and must be studied on individual soil types to understand nutrient availability.

Often the P cycle in organically managed systems behaves differently than in conventionally managed systems. Under organic management, the plant available P pools are much lower than under conventionally managed systems (Mader et al., 2002). However, the organic matter (Stockdale et al., 2002) and the soil microbial biomass often contain a high percentage of soil P (Mader et al., 2002; Stockdale et al., 2002). As well, soil biological activity is often increased under organic management (Mader et al., 2002; Stockdale et al., 2002). This increased biological activity is responsible for the mineralization of organic P and is an important source of plant P (Mader et al., 2002; Stockdale et al., 2002; Watson et al., 2002a). Additionally, arbuscular mycorrhizal fungi (AMF) have been shown to increase the absorption of P from unavailable plant P pools (Biro et al., 2000; Mader et al., 2002; Gosling et al., 2006). Typically organically managed systems have a higher presence of AMF (Mader et al., 2000; Mader et al., 2002; Gosling et al., 2006) which has been negatively correlated with extractable P (Mader et al., 2000). The P in organic farms focuses on the mineralization of P from organic sources. The goal of this study was to examine the effect of increasing soil available P on

soybean and alfalfa BNF. Two contrasting soils typical of organic dairy farms were used to compare the response in different regions. Low STP soil was collected from a long term organic dairy farm in Ontario and a transitional organic dairy farm in Nova Scotia for use in this study.

2.2 MATERIALS AND METHODS

2.2.1 *SOIL COLLECTION AND CHARACTERIZATION*

Soil was collected from two dairy farms with known low soil test P, one in Ontario and one in Nova Scotia. The Ontario farm was located near the village of Chepstow, Bruce County, Ontario. The soil is classified as a Harriston silt loam (Hoffman and Richards, 1954). The Nova Scotia field was located outside Lower Burlington, Hants County. The soil is classified as a Hansford soil sandy loam (Cann et al., 1978). The Ontario soil was collected in the July 2008, following two green manures, field pea and buckwheat planted that season. The Ontario farm had been certified organic for roughly 20 years. Currently, there are no certified organic dairy farms in Nova Scotia. However, there are a number of farms in the transitional phase in the organic certification process. The selected Nova Scotia field was part of a transitional dairy farm. The field had been in forage production for a long period of time and had received no amendments during this time. The producer had not applied amendments during his lengthy oversight of the field and it is likely the previous producer had not applied amendments either. The field was sown with a clover-timothy mix two years prior. However, due to the low soil fertility the field contained primarily weeds and local flora at the time of sampling. At each location, the soil was collected from the top 20 cm of the soil profile and sieved through a ½" (12.7 mm) sieve in the field. Subsequently, the soil was air dried and passed through a ¼" (6.4 mm) sieve. Four composite samples were taken from the air dried soil collected in each province (called bulk soil samples) and sent to provincial soil test laboratories. The soils were analyzed for plant available P, and K, pH and several micronutrients, appendix 1. The Ontario soil was analyzed by the Soil and Nutrient Laboratory, University of Guelph Laboratory Services, Guelph, Ontario. The Nova Scotia soil was analyzed at the Nova Scotia Agriculture Quality

Evaluation Division Laboratory Services, Truro, Nova Scotia. The STP was determined by the Olsen method (Schoenau and O'Halloran, 2008) on the Ontario soil while the Nova Scotia STP was extracted using Mehlic Acid (Ziadi and Sen Tran, 2008) (Table 2.1). Additional analysis on the Ontario soil samples were performed at the Nova Scotia Agriculture Quality Evaluation Division Laboratory Services, Truro, Nova Scotia (see appendix 1 for full nutrient analysis). Soil texture was determined and the Ontario was determined to be a sandy loam while the Nova Scotia soil was a loam (Plaster, 1997).

In addition, each bulk soil was analyzed for total N and C content. To ensure soil was acceptably dry prior to processing, a sub-sample from each raw soil sample was dried in an oven at 50^{o}C for 24 hours. Following, the soil samples were fine ground before analysis. The samples were placed in square jars with one large square rod, a medium round rod and three small rods. The jars were placed on a roller grinder for 24 hours at 70 bottle revolutions per minute (Arnold and Schepers, 2004). Total C and N of the ground samples were determined by combustion (Viro MAX CN Macro Elemental Analyzer, Elementar America Inc., Mt. Laurel, New Jersey, U.S.A.) (Table 2.1).

Table 2.1: Soil chemical properties of the bulk Nova Scotia and Ontario soils.

Nutrient	Ontario	Nova Scotia
Total Carbon	25.4 mg kg^{-1*}	24.1 mg kg^{-1*}
Total Nitrogen*	2.56 mg kg^{-1*}	3.08 mg kg^{-1*}
Extractable Phosphorus	8.4 mg kg^{-1**a}	8.1 mg kg^{-1} *** ^{b}
pH	7.3**	6.5

* this test was performed by combustion (Viro MAX CN Macro Elemental Analyzer)
** these tests were performed by the University of Guelph Laboratory Services
*** these tests were performed by the Nova Scotia Provincial Soil Test Laboratory
^{a} Olsen extractable P
^{b} Mehlic extractable P

2.2.2 *P SORPTION*

Four composite bulk samples from each soil were sent to Dr. Ivan O'Halloran, Ridgetown Campus, University of Guelph, for determination of P sorption capacity using a modified method (Graetz and Nair, 2009). The soils were passed through a 2 mm screen prior to analysis.

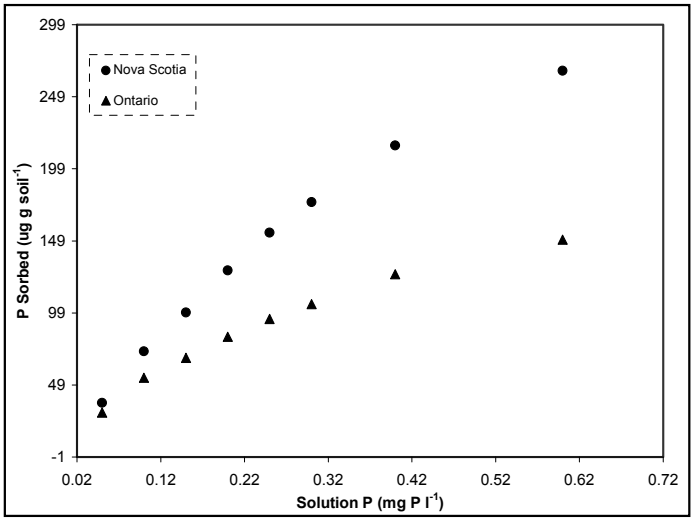


Figure 2.1: Mean phosphorus sorption capacity of the bulk Nova Scotia and Ontario soils.

The Ontario soil had less of an ability to fix P while the Nova Scotia was able to fix a substantially higher amount of the added P (Figure 2.1). The ability of the Nova Scotia soil to fix high amounts of P is partially due to a longer history of no P inputs on this field. As well, the Nova Scotia soil contained 196 percent more clay which has the ability to adsorb large amounts of P (Plaster, 2003). Sand content was only 46% for the Nova Scotia soil compared to 71% for the Ontario soil.

2.2.3 *Effect of Added P and Soil Type on Soybean Growth and Biological Nitrogen Fixation*

Two similar experiments were conducted on soybean (c.v. Evans) to determine the effects of soil type and increased soil available P on BNF. The goal was to determine if a threshold soil P level exists on organically managed soils for soybean BNF. The P threshold is defined as the point at which BNF stops increasing even as plant available P continues to increase. To gain greater control of environmental factors, these experiments were conducted in a growth chamber (Coviron, Controlled Environments, Canada). During the first experiment it was observed the plants were lacking red light and exhibited the classic symptoms of a shading response (Lambers et al., 1998). As well, many non-nod plants died shortly after transplanting and it was unsure if this was related

to the transplanting or plant genetics. A second experiment was conducted where seeds were planted directly into pots and the growth chamber photosynthetic active radiation was suitable. As well, in this experiment many of the non-nod seeds did not germinate.

2.2.3.1 *FIRST SOYBEAN EXPERIMENT*

Evans soybean (*Glycine max* cv Evans) was chosen for this study as the time to reach maturity was suitable for the Eastern Canadian growing season (Lambert and Kennedy, 1975). As well and more importantly, a closely related non-nodulating soybean cultivar was developed which has a very similar physiology and N isotope preference as the Evans cultivar. This relatedness results in a better estimate of BNF-N (Goh, 2007; Houngnadan et al., 2008). Soybean seeds were provided by the Minnesota Agricultural Experiment Station (Lambert and Kennedy, 1975) while a line of non-nod soybean seeds with Evans parentage was provided by Dr. Don Smith, McGill University, Montréal, Québec. Seeds were germinated in perlite for four days with a fourteen hour day at 22ºC ±2ºC and 163 µmol m^{-2} sec^{-1} at the growth chamber bottom while the night temperature was 20ºC ±2ºC.

Following the germination period, the seedlings were transplanted into 1.5 liter 6 inch pots containing the collected soil types with added amounts of P fertilizer. The P fertilizer used was $H_2NaPO_4 \bullet 2H_2O$ (Fisher Scientific) to ensure the P was 100% soluble upon application (Plenchette and Morel, 1996). Each soil type had five rates of P added to the soil (0, 5, 15, 45 and 135 mg P kg^{-1} soil). To ensure an even distribution of the fertilizer throughout the soil, the fertilizer was mixed into a quarter of the soil and the remaining three quarters of fertilizer additions were added one quarter at a time. The mass of the dried soil was 1340 and 1550 g per pot for the Nova Scotia and Ontario soil, respectively. The pots were watered with 40 ml of a full strength N and P-free Hoagland solution. Following, distilled water was added to bring the moisture content of the soil to 60% field capacity (FC). The seedlings were transplanted into the prepared pots and nodulating plants were immediately inoculated with 1 ml of a commercial rhizobial inoculant (Cell-Tech from EMD, Crop BioScience). All pots were returned to the growth chamber set at the above parameters. The pots were watered daily to approximate 60 % FC and were weighed once a week to ensure 60% FC was maintained. As well, the

soybeans were fertilized weekly with 20 ml of the N and P-free Hoagland solution.

At the pod development stage, 47 days after transplanting, the soybeans were harvested. Both the nodulating and non-nod plants reached this growth stage at the same time. First, the plants were removed from the pots and the soil was gently shaken from the roots. The shoot and roots were separated at the interface between the shoot and root; where the stem went from green to white. The height of the shoot was measured from this cut off point to the shoot apical meristem (SAM). All remaining root material was removed from the soil by hand and washed over a 0.5 mm screen. Immediately following harvest, shoot material was dried at 60ºC until a uniform dry state was reached before weighing. Roots were kept in a 4^{o}C cooler, for approximately one week, until an assessment of nodulation could be made. Visible nodules were removed from the roots, counted and dried in a 55ºC oven for 24 hours. Following, DM was determined. The roots were dried separately at 55ºC until uniformly dry and DM was taken.

Total leaf area per plant was determined using photographic analysis. Leaves were removed from the shoots at the base of the petiole and placed on a piece of wax paper for leaf area determination. The leaves were laid out on a white surface and a camera was positioned above. The photographs were taken with a Cannon Powershot SD1100IS Digital ELPH 8.0 megapixel camera. The photographs were analyzed using CIAS computer software (version 2.0, Jandel Scientific). Before analysis, the pictures were reduced by 50 percent using MS picture manager.

Following DM determination of the shoot including the leaves, the tissue was passed though a 2 mm screen on a Wiley mill (standard model number 3). To achieve a fine ground sample, approximately 2 g of the ground tissue was placed in a square jar with three small rods and ground on a roller grinder (Arnold and Schepers, 2004) at 70 ± 10 bottle revolutions per minute until a consistent fine powder was achieved, approximately 48 hours. Tissue samples less than 2 g were ground on a ball mill (Mixer Mill Type MM301, Retsch, Germany). Shoot N and C concentration was determined by combustion (Vario MAX CN Macro Elemental Analyzer) and was multiplied by the shoot DM to determine shoot N uptake. Shoot P concentration was determined using a modified ash procedure (Westerman, 1990; PEI Analytical Laboratories, 2008) and determined on an autoanalyzer (Technicon AutoAnalyizer III, Technicon Instruments

Corporation, NY, USA). The total shoot P uptake was determined by multiplying the tissue P concentration by the DM of the shoot. An estimation of BNF was calculated by the total N difference (n=4), section 2.2.3.2. To determine how well the roots were washed, four randomly selected nodulating plant roots were selected for determination of soil adhesion, section 2.2.3.3.

This experiment was designed as a split-plot in four blocks with five levels of added P. Since the non-nod plants were used to calculate BNF-N, non-nod plants were grown in separate pots using the same levels of added P and were randomly assigned to blocks. The data were analyzed using the proc mixed procedure in SAS (version 9.1, SAS Institute Inc., NC, USA). Assumptions for this model were tested using proc univariate. The alternate split-plot model was used where sub-plot error term becomes pooled with the block error and the interaction between the whole-plot and the sub-plot error (Montgomery, 2005).

2.2.3.2 *CALCULATION OF BNF- TOTAL N DIFFERENCE*

An estimation of BNF using the Total N difference was calculated using the following equation:

$$BNF = N_{nod+} - N_{nod-}$$

where N_{nod+} is the total shoot N in the nodulating legume plants and N_{nod-} is the total shoot N in the non-nod legume plants (Ledgard and Steele, 1992) . However, in cases where there were less than two non-nod plants at an added P level, then the non-nod plants at the P treatment immediately below and above were averaged as an estimate of shoot N uptake for the treatment level.

2.2.3.3 *DETERMINATION OF ACTUAL ROOT MASS*

Even though the roots were thoroughly washed, a small amount of soil remained on the root material. In order to correct for this additional weight the percent of soil adhesion was determined. The root DM was corrected for soil adhering onto the roots using the methodology as outlined by Janzen (2002). This method was performed on all soybean experiments. Four dry root samples were randomly selected from each study. A small section of the root was cleaned by gently rubbing a hard flat object over the root.

All root material was finely ground on a ball mill (Mixer Mill Type MM301, Retsch, Germany). The root samples were analyzed for total C using combustion (Vario MAX CN Macro Elemental Analyzer). The C content from the soil was assumed to be the same as the bulk soil. The following equation was used to determine the proportion of the analyzed sample which was true root C:

$$f_r = \frac{C_t - C_s}{C_r - C_s}$$

where f_r is the proportion of the sample which is truly root, C_t is the C content of the whole sample, C_s is the C content of the soil and C_r is the C content of the clean root. The following equation was used to determine the mass of the root DM which was truly a root sample:

$$M_r = f_r M_t$$

where M_r is the true root mass and M_t is the mass of the total root sample.

2.2.3.4 *SECOND SOYBEAN EXPERIMENT*

The procedure of this experiment was similar to the first soybean experiment, section 2.2.3.1, with the following changes: an additional P rate was included while the maximum P rate decreased (0, 5, 15, 30, 45 and 90 mg P kg^{-1}); the seeds were planted directly into the pots; and the Nova Scotia soil weight was increased to 1540 g per pot to account for soil settling observed in the first experiment. In addition, β reference plants were grown in a perlite, vermiculate and sand medium at a 1:1:1 ratio to provide an N-free environment. These β plants were watered with an N free Hoagland solution with the same amount as the other pots receiving an N and P-free Hoagland solution. The growth chamber (Controlled Environment, Canada) had a fourteen hour day at 25ºC ± 2ºC and a light intensity of 410 µmol m^{-2} sec^{-1} at the maximum height of the plants while the night temperature was 17ºC ± 2ºC. The plants were thinned 15 DAP to one plant per pot by cutting the seedlings off at the soil line. The plants were harvested at the late flowering stage. Total leaf area was calculated from photographs using ImageJ (National Institutes of Health, USA). Shoot N concentration was determined by dry combustion (LECO FP-228, LECO, MI, USA). BNF was calculated using the ^{15}N Natural Abundance (NA) technique, section 2.2.3.4.1. In addition, BNF-N by the total N

difference, section 2.2.3.2 was determined. Shoot P concentration and total shoot P were determined as in section 2.2.3.1. Shoot, root and nodule DM were recorded.

This experiment was designed as a split-plot six level with four blocks. The whole plot treatments were soil type and the subplot treatments were added P. Non-nod plants with the same levels of added P were potted in each block and used in BNF-N calculations. Due to the lack of germination, of almost all of the seeds planted in the Ontario soil the experiment was analyzed as a single factor randomized complete block experiment, using results obtained from nodulating plants grown in the Nova Scotia soil. The data were analyzed using the proc mixed procedure in SAS (version 9.1, SAS Institute Inc., NC, USA). Assumptions for this model were tested using proc univariate.

2.2.3.4.1 *CALCULATION OF BNF- NATURAL ABUNDANCE*

While the non-nod alfalfa MN-1008 plants are perfectly non-nod (Dudley and Long, 1989), Evans non-nod soybean are not (Smith, 2008, personal communication). Non-nod plants with nodules were assumed to have fixed N, due to their pinkish appearance, and were excluded from further analysis. Fine ground shoot samples, 2.2 ± 0.4 mg from both the nodulating and non-nod plants were encapsulated for ^{15}N analysis. An external reference material, green pea, was used as a check. Each sample of shoot tissue ^{15}N value varied by ± 0.15‰. The samples were sent to the College of Agriculture and Bioresources at the University of Saskatchewan and were analyzed on a mass spectrometer. The following equation was used to determine the percent of N fixed by the legumes:

$$\%NDFA = 100 * \frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - \beta}$$

where $\delta^{15}N_{reference}$ is the concentration of ^{15}N in the reference plant , $\delta^{15}N_{legume}$ is the ^{15}N concentration in the legume material, and β is the ^{15}N concentration of the nodulating plants growing in the soilless medium (Crews, 1993; Riffkin et al., 1999; Huss-Danell and Chaia, 2005). The $\delta^{15}N$ values for the β-reference plants were the same as the values from the plants grown in soil and it was determined these nodulating plants grown in the soilless medium did not accurately describe a plant solely relying on BNF for its N. Instead, the lowest ^{15}N value in the respective data set was used (Huss-Danell and Chaia,

2005). To determine the amount of BNF-N (mg N shoot^{-1}) in the nodulating plants, the following equation was used:

$$BNF - N = \%NDFA * TotalN$$

where %NDFA is the amount of N obtained through BNF, and Total N is the shoot N uptake.

2.2.4 *Effect of Added P and Soil Type on Alfalfa Growth and Biological Nitrogen Fixation*

A study was conducted on alfalfa to determine the effects of increased soil available P on perennial legume BNF over three forage harvests. This study was conducted under greenhouse conditions to gain better control over environmental conditions. The nodulating variety of alfalfa (*Medicago sativa*) Iroquois and non-nod MnN-1008 (Barnes et al., 1988) (supplied by Dr. JoAnn F.S. Lamb, USDA-ARS, St. Paul, MN) were used for this trial. This study was conducted under greenhouse conditions with moderate air temperature control. A datalogger with a temperature probe was installed in the greenhouse to record temperature, which averaged 26^{o}C to 19 ^{o}C during the day and night, respectively (Table 2.2). The greenhouse had supplemental lighting from high pressure sodium bulbs for a 16 hour photoperiod. Alfalfa seeds were sown April 27, 2009 and final harvest occurred August 1, 2009.

Table 2.2: A summary of the greenhouse temperatures during the alfalfa study.

Growth Period	Min Temperature	Max Temperature	Average Day Temperature	Average Night Temperature
Seeding to Cut 1	7.68	41.91	23.93	15.83
Cut 1 to Cut 2	10.02	42.88	26.27	18.55
Cut 2 to Harvest	14.63	44.52	26.73	19.97

The average day temperature is for the period 5:00 to 20:00 while the average night temperature is for the period 21:00 to 4:00. All temperatures are in ^{o}C.

Alfalfa seeds were planted in PVC pipes with an internal diameter of 15 cm and a

depth of 85 cm. Previous alfalfa studies have shown this size of pot suitable for alfalfa growth (Papadopoulos, personal communication, 2008). To prevent soil loss, the bottom of each tube was covered with standard nylon window screen and a piece of heavy silage plastic with a small hole in the center, approximately 1 cm^{-2}, to allow for drainage. The bottom 65 cm of each tube was filled with a mixture of perlite, vermiculate and fine gravel at a 1:1:1 ratio while the upper 25 cm was filled with 5400 and 5900 g of air dried Nova Scotia and Ontario soil, respectively. Before the soil was placed in the tubes, varying rates of P fertilizer (0, 5, 15, 45 and 90 mg P kg $soil^{-1}$) as $H_2NaPO_4 \bullet 2H_2O$ (Plenchette and Morel, 1996) were mixed into the soil. To ensure the fertilizer was evenly distributed throughout the soil, a quarter of the soil was mixed with a quarter of the fertilizer at a time. The soil was watered to approximately 60 percent FC and fertilized with 40 mL of an N and P-free Hoagland's Solution before planting. Each week following planting, the plants were fertilized with 20 mL of the N and P-free Hoagland solution. In addition, a β-plant in each replication was grown in a tube fully filled with the perlite, vermiculate and gravel to ensure an N free growing environment. These plants were watered with an N-free Hogland solution, 40 mL before sowing and 20 mL weekly.

Approximately 10 seeds were placed on top of the soil in each tube and inoculated with 1 mL commercial alfalfa inoculant (Nitragin) prepared as per supplier instructions. The seeds were watered with a spray bottle filled with distilled water until the seedlings had sufficient root growth to prevent migration when watering. When the seedlings reached sufficient height, approximately 4 cm, twenty-six DAP, the seedlings were thinned to two plants per pot by cutting the plants off at the soil line. Soil moisture was maintained at approximately 60% FC percent FC by estimation using distilled water. However, occasionally the soil moisture was intentionally raised to FC to ensure the soil throughout the tubes was moist. There was minimal leaching from the tubes following watering. However, if there was leaching, this excess was contained in individual saucers and returned to the tube at the next watering.

The alfalfa shoot tissue was harvested three times during the study. The first two harvests occurred when the majority of the plants had reached the late vegetative to early flowering stage. The first harvest occurred at 52 DAP and the second harvest at 80 DAP.

The third and final harvest occurred earlier than planned due to a greenhouse renovation and was only thirteen days after the previous harvest; otherwise this harvest would have occurred at the same plant growth stage as the previous two harvests. During the first two harvests only the shoot material was collected. At each harvest, the maximum height for each pot, to the nearest 0.5 cm, was determined by measuring from the soil line to the highest SAM. Following, the plants were cut 2.5 cm above the soil line keeping each material from individual plants separate and were dried at 55^{o}C. The DM of each plant was recorded. At the final harvest, 93 DAP, all plant material was carefully removed from the tubes. The shoot material was cut at the colour change interface between the shoot and root material. The branch heights of each plant were recorded and the shoot material was dried. The roots were cut at the division between the soil and the perlite mixture, 25 cm below the soil surface. All remaining visible root material in the soil was removed by hand and washed over a 1 mm screen. The visible root material in the perlite, vermiculite and gravel medium was removed and placed in a separate bag. All plant material was dried at 55^{o}C until a uniform dry state was reached.

The shoot material from the second harvest, 80 DAP, was processed for nutrient analysis. The plant material was analyzed as composite samples of both plants from each individual pot. If a pot only contained one plant, the pot was dropped from all analysis. This shoot material was ground to 2 mm on a Wiley Mill (standard model number 3) and finely ground on a roller grinder at 70 bottle revolutions per minute (Arnold and Schepers, 2004). Shoot tissue P concentration was determined using a modified ash procedure (Westerman, 1990; PEI Analytical Laboratories, 2008) and measured on an autoanalayzer (Technicon AutoAnalyizer III, Technicon Instruments Coroporation, NY, USA). Shoot P uptake was determined by multiplying the tissue P concentration by the shoot DM. BNF was determined using the ^{15}N Natural Abundance technique, section 2.2.3.4.1. Shoot N concentration and N uptake were determined using combustion (Vario MAX CN Macro Elemental Analyzer).

This experiment was designed as a split-plot in four blocks with five levels of added P. Since the non-nod plants were used only to calculate BNF-N, non-nod plants were grown in separate pots using the same levels of added P and were randomly assigned to blocks. The data were analyzed using the alternate split-plot model

(Montgomery, 2005) in proc mixed procedure in SAS (version 9.1, SAS Institute Inc., NC, USA). Assumptions for this model were tested using proc univariate.

2.2.5 *STATISTICAL ANALYSIS*

All experimental data were analyzed using SAS (version 9.1, SAS Institute Inc., NC, USA) using the proc mixed procedure. All assumptions were checked before performing analysis using proc univariate. Significant differences were determined using Tukey's LSD with a p-value < 0.05 considered significant. Split-plot designs were analyzed using the alternative model, section 2.2.3.1.

Regression analysis was conducted using Minitab (version 15.1.0.0, Minitab Inc. 2006). The menu selection *fitted line plot* was used to test all three regression lines: linear, quadratic and cubic. The equation with the best fit, highest r^2 value, was determined to be the equation which best described the relationship with the data.

2.3 RESULTS

2.3.1 *EFFECT OF ADDED P AND SOIL TYPE ON SOYBEAN GROWTH AND BIOLOGICAL NITROGEN FIXATION*

2.3.1.1 *FIRST SOYBEAN EXPERIMENT*

The high mortality rate of the non-nod plant after transplanting was likely caused by the age of the seeds, which were four years old. Ten DAP the primary leaves began to open. By 28 DAP clear signs of chlorosis and N deficiency was observed on the surviving non-nod plants. As well, slight chlorosis was visible on the nodulating plants during the experiment but soon disappeared after BNF began. Visible pods were observed 39 DAP. At harvest, it was observed the nodulating plants in the Ontario soil at 90 and 135 mg P kg^{-1} had excess vegetative material as auxiliary branches (Figure 2.2), growing from the bottom auxiliary meristems.

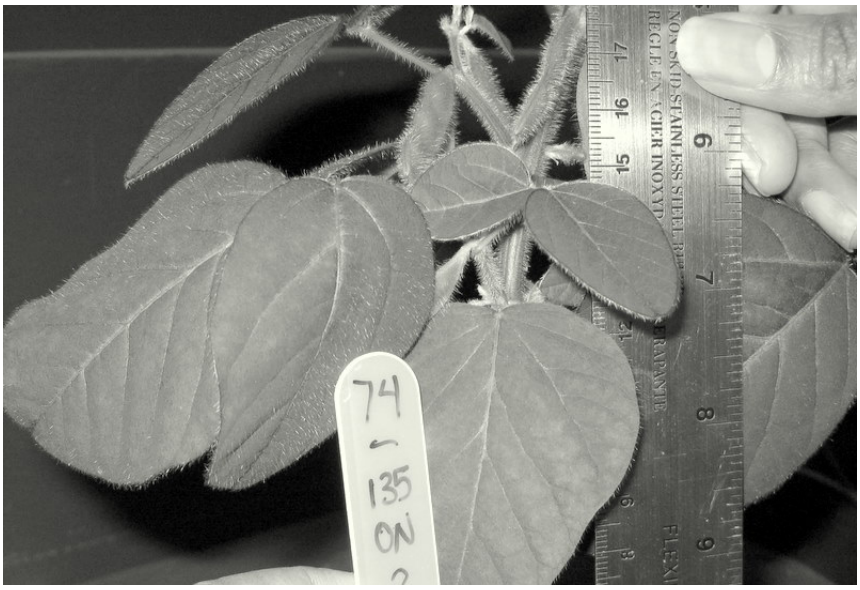


Figure 2.2: Excess vegetative material from the auxiliary meristems

Shoot height, DM and leaf area were significantly affected by added P with similar increasing trends as added P increased. Soil type significantly affected shoot DM alone, although a trend of larger shoot height and greater leaf area for plants grown in Ontario soil was consistent with the response for shoot DM (Table 2.3). The shoot height at 45 and 135 mg kg^{-1} added P was significantly greater than that for 0, 5 and 15 mg kg^{-1} added P. It was observed all the plants were very tall and spindly with larger than normal spaces between leaf sets. The shoot dry mass at 0 and 5 mg kg^{-1} added P was significantly lower than at 45 and 135 mg kg^{-1} added P, while at 15 mg kg^{-1} added P shoot DM was not different than other treatments. The regression analysis (Figure 2.3) indicates the strong response of shoot DM to added P (r^2=0.99). The figure clearly shows a leveling off and a decrease between 45 and 135 mg kg^{-1} added P. Total leaf area followed a similar trend and was significantly increased by 210 percent at 45 mg kg^{-1} added P than at 0 and 5 mg kg^{-1} added P (Table 2.3). The root correction factor was determined to be 0.4. Root DM, which averaged approximately 10% of the shoot DM at harvest, was more variable (greater SE) and neither soil type nor P fertilizer treatment significantly affected this parameter.

Table 2.3: Shoot height, dry matter, total leaf area and corrected root DM as affected by soil type and added P on soybeans.

		Shoot Height[1] (cm)	Shoot DM[1] (g)	Total Leaf Area[1] (cm^2)	Root DM[1] (g)
Added P (mg P kg^{-1})	0[2]	43.7 (6.2)[a]	1.96 (0.29)[a]	201.38 (37.40)[a]	0.24 (0.15)
	5	56.7 (5.8)[a]	2.35 (0.27)[a]	276.04 (34.68)[ab]	0.31 (0.12)
	15	63.0 (5.8)[a]	2.92 (0.27)[ab]	311.49 (34.68)[abc]	0.29 (0.12)
	45	78.4 (6.2)[b]	3.80 (0.29)[b]	427.97 (37.40)[c]	0.40 (0.13)
	135	82.2 (6.2)[b]	3.72 (0.27)[b]	407.48 (34.68)[bc]	0.36 (0.12)
Soil	Nova Scotia[3]	59.6 (4.1)	2.36 (0.20)[a]	280.01 (26.53)	0.30 (0.12)
	Ontario	70.0 (4.0)	3.54 (0.19)[b]	369.73 (25.35)	0.34 (0.11)
p-value	Soil	0.1713	0.0250	0.0921	0.5955
	Added P	0.0012	0.0002	0.0008	0.1588
	Soil * Added P	0.1391	0.4497	0.4841	0.8346

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=8; [3] n=20

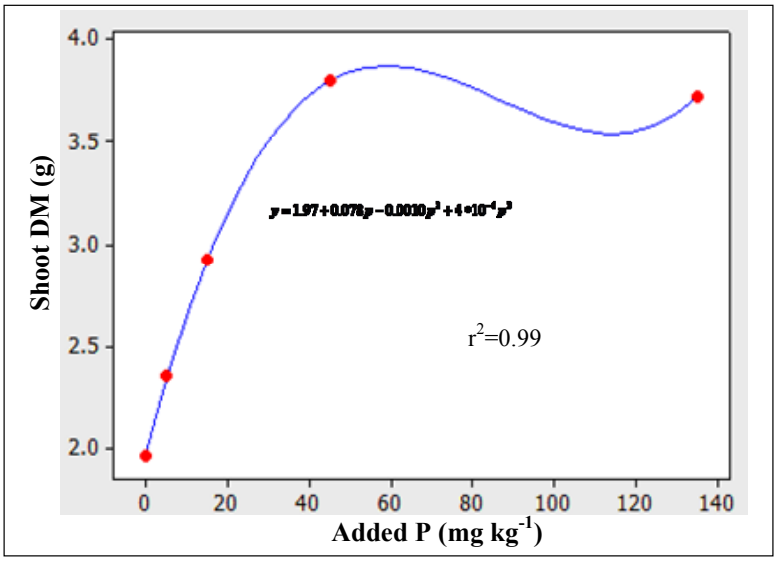


Figure 2.3: Shoot DM as it relates to added P in soybean.

Similar to shoot growth, nodulation response was primarily a function of added P and increased in response to added P (Table 2.4). Just as with the shoot responses, the various measures of nodulation response at 0 and 5 mg kg^{-1} added P were all significantly

lower than those at 45 and 135 mg kg^{-1} added P (Table 2.4). Furthermore, as with the shoot growth responses, there were no significant increases in nodulation and nodule DM (i.e. nodule number, nodule total and average DM and nodule DM:shoot DM ratio) response between 45 and 135 mg kg^{-1} added P. The effect of soil type was only significant for the average nodule mass. The plants grown in the Ontario soil had larger nodules than the Nova Scotia plants. Nodule DM (mg $plant^{-1}$) increased by 548 percent between 0 and 135 mg kg^{-1} added P (Table 2.4). In fact, nodule DM closely relates (r^2=0.99) to added P (Figure 2.4). The average nodule mass per plant increased by 244 percent between the plants grown in 0 and 135 mg kg^{-1} added P, (Table 2.4).

Table 2.4: Nodule number, dry mass, average nodule dry mass per plant and nodule DM per shoot DM as affected by soil type and added P on soybeans.

		Number of Nodules[1] (number plant^{-1})	**Nodule Dry Mass**[1] (mg plant^{-1})	**Average Nodule DM**[1] (mg nodule^{-1})	**Dry Nodule Mass per Shoot Dry Mass**[1] (mg g^{-1})
Added P (mg P kg^{-1})	0[2]	35 (9.2) [a]	42.3 (16.61) [a]	1.12 (0.22) [a]	19.69 (3.02) [a]
	5	46 (8.6) [a]	75.0 (15.44) [ab]	1.61 (0.20) [a]	30.00 (2.80) [a]
	15	52 (8.6) [a]	138.6 (16.61) [b]	2.62 (0.22) [b]	46.35 (3.02) [b]
	45	89 (9.2) [b]	207.2 (16.61) [c]	2.68 (0.22) [b]	52.73 (3.02) [bc]
	135	92 (9.2) [b]	231.90 (21.50) [c]	2.73 (0.25) [b]	59.10 (3.40) [c]
Soil	Nova Scotia[3]	70 (6.4)	120.1 (12.06)	1.68 (0.15) [a]	42.65 (2.53)
	Ontario	56 (5.9)	157.8 (11.26)	2.62 (0.13) [b]	40.50 (2.26)
p-value	Soil	0.1463	0.0877	0.0175	0.5712
	Added P	0.0002	< 0.0001	< 0.0001	< 0.001
	Soil * Added P	0.1337	0.3487	0.0761	0.5143

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=8; [3] n=20

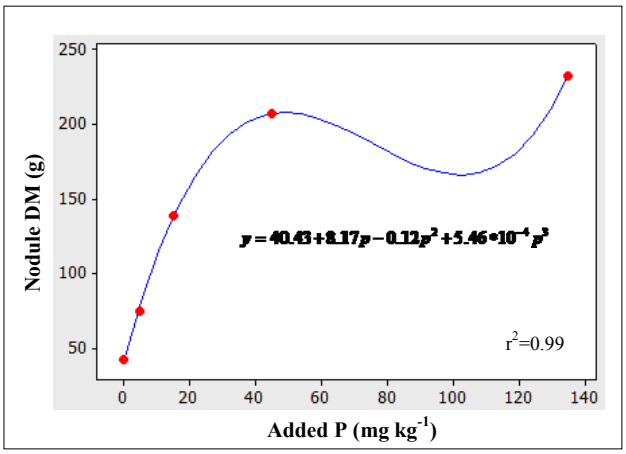


Figure 2.4: Nodule DM as it relates to added P in soybean.

The shoot nutrient content and estimation of BNF were significantly different among the added P rates. Shoot N and P concentrations were significantly different between the treatments with a significant soil by added P interaction (Table 2.5). Both shoot tissue N and P concentration responses exhibited consistent increasing trends from 0 to 45 mg kg^{-1} added P. The Nova Scotia shoot N concentration was significant higher at 135 than at 45 mg kg^{-1} added P and lower fertilization rates. However, the Ontario soil N concentration was higher at the three higher P rates than the lower P rates. The Nova Scotia plants had a maximum shoot P concentration at 45 mg kg^{-1} added P while this occurred at 135 mg kg^{-1} added P in the Ontario soil.

Both shoot N and P uptake varied significantly in response to the main effects of soil type and by added P (Table 2.5). The shoot N uptake increased by 254 percent and the shoot P by 360 percent between the lowest and highest level of added P. Additionally, average shoot N and P uptake were greater in the Ontario soil by 177 and 203 percent, respectively. For both soils there was not an additional response to shoot N and P uptake above 45 mg kg^{-1} added P. Both shoot N (r^2=1.00) and P uptake (r^2=0.99) was positively related to added P (Figure 2.5). Interestingly, the equation describing the relationship between added P and N uptake is cubic while P uptake is quadratic.

An estimation of BNF-N (mg N $shoot^{-1}$) by total shoot N difference exhibited an

increasing trend as added P increased. The two upper levels of added P resulted in significantly greater BNF-N estimations than the three lower levels of added P. BNF-N increased by over three times between the control and the two highest P rates (Table 2.7). In addition, BNF-N was highly correlated to added P (Figure 2.6). Interestingly, the relationship between added P and BNF-N is described as a quadratic equation while many of the other responses are described by a cubic equation. The percent of shoot N from BNF was lowest in the Nova Scotia soil at 0 mg kg^{-1} added P and increased with increasing added P from 21.5% to 88% of N derived from BNF (Table 2.6). The Ontario soil did not follow this trend and the amount of N derived from BNF was generally lower than found for the Nova Scotia soil. The lowest percent of N from BNF occurred at 15 mg kg^{-1} added P while the higher percents were at 0, 45 and 135 mg kg^{-1} added P.

Similar trends were observed in the non-nod soybeans (data not shown). The shoot height, shoot DM and leaf area increased with increased added P. In addition, shoot N and P concentration and shoot P and N uptake increased with increasing added P. However, the responses of non-nod plants were always lower than those of the nodulating plants at the same added P level.

Table 2.5: N and P concentration and uptake of the soybean shoot tissue as affected by soil type and added P.

		Shoot N Concentration[1] (mg N g^{-1})		**Shoot P Concentration**[1] (mg P g^{-1})		**Shoot N Uptake**[1, 2] (mg)	**Shoot P Uptake**[1, 2] (mg)
		Nova Scotia[3]	Ontario[3]	Nova Scotia[3]	Ontario[3]		
Added P (mg P kg^{-1})	0	28.17 (1.95) [a]	36.69 (1.68) [a]	0.97 (0.25) [a]	2.61 (0.18) [a]	63.77 (9.77) [a]	4.33 (0.74)[a]
	5	28.38 (1.53) [a]	36.62 (1.53) [a]	1.25 (0.18) [a]	2.90 (0.18) [b]	78.36 (9.09) [ab]	5.27 (0.61)[a]
	15	33.07 (1.53) [a]	37.93 (1.53) [b]	1.93 (0.18) [a]	3.62 (0.20) [b]	103.51 (9.09) [bc]	7.72 (0.66)[b]
	45	29.50 (1.68) [a]	40.08 (1.53) [b]	3.01 (0.20) [c]	3.72 (0.18) [bc]	134.82 (9.76) [cd]	12.90 (0.66)[c]
	135	37.33 (1.68) [b]	39.22 (1.68) [b]	3.01 (0.20) [c]	4.41 (0.18) [c]	162.05 (10.39) [d]	15.55 (0.74)[c]
Soil	Nova Scotia[4]	31.29 (1.21)		2.19 (0.09)		78.29 (7.67) [a]	6.04 (0.51) [a]
	Ontario	38.11 (1.18)		3.44 (0.08)		138.72 (7.38) [b]	12.28 (0.51) [b]
p-value	Soil	0.0201		0.0020		0.0101	0.0032
	Added P	0.0011		< 0.0001		< 0.0001	<0.0001
	Soil * Added P	0.0217		0.0140		0.3103	0.4426

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=8; [3] n=4; [4] n=20

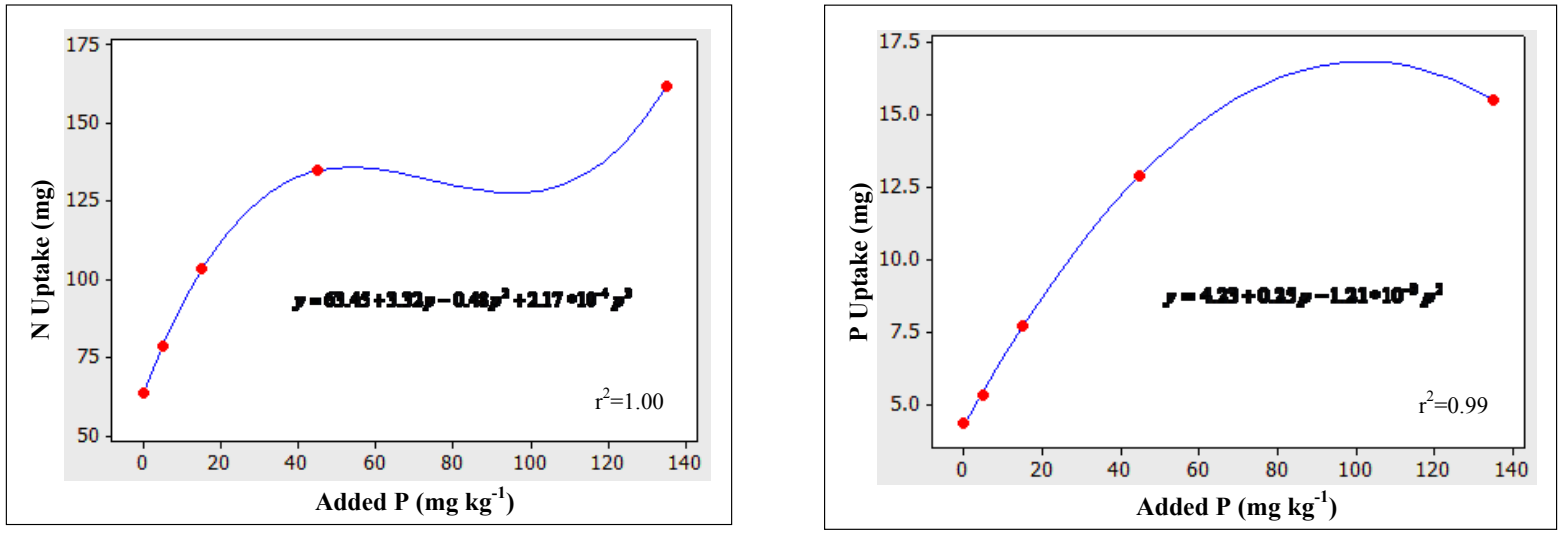


Figure 2.5: Shoot N (left) and P uptake (right) as it relates to added P in soybean.

Table 2.6: Estimation of shoot BNF-N and percent BNF-N as affected by soil type and added P.

		BNF-N[1, 2] (mg N shoot^{-1})	**BNF-N Percent**[1] (% of total shoot N uptake)	
			Nova Scotia[3]	Ontario[3]
Added P (mg P kg^{-1})	0	30.08 (11.10) [a]	21.48 (3.26) [a]	55.49 (3.26) [b]
	5	35.46 (9.24) [a]	50.75 (2.31) [b]	42.34 (2.31) [ab]
	15	47.75 (9.24) [a]	75.08 (2.31) [c]	30.22 (2.66) [a]
	45	93.68 (9.90) [b]	82.62 (2.66) [c]	60.03 (2.31) [b]
	135	99.06 (10.51) [b]	88.14 (2.66) [d]	52.22 (3.26) [b]
Soil	Nova Scotia[4]	57.84 (7.83)	63.62 (1.19)	
	Ontario	64.56 (7.25)	48.05 (1.25)	
p-value	Soil	0.5356	0.0029	
	Added P	<0.0001	<0.0001	
	Soil * Added P	0.0770	<0.001	

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=8; [3] n=4; [4] n=20

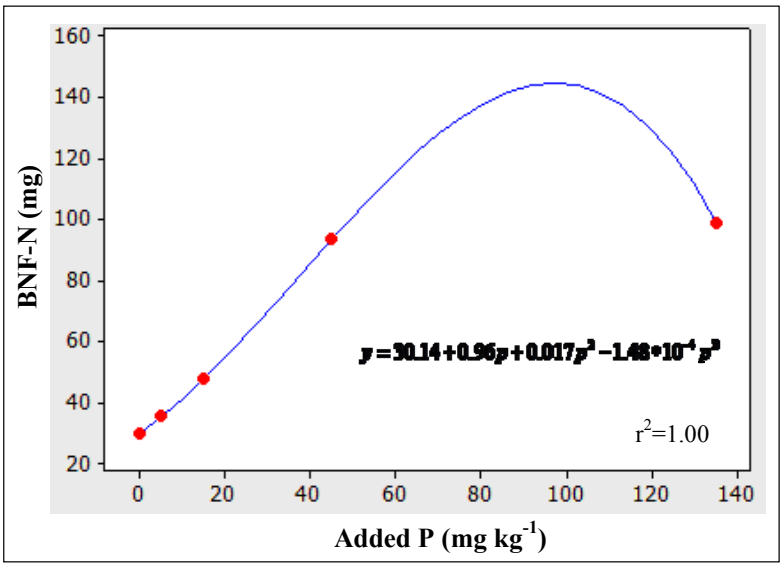


Figure 2.6: BNF-N as calculated by the Total N Difference as it relates to added P in soybean.

2.3.1.2 *Second Soybean Experiment*

In the second soybean experiment, the majority of the soybeans planted in the Ontario soil did not germinate. The nodulating plants in the Nova Scotia soil grew well but the non-nod plants did not fare as well. This experiment was analyzed as a single factor completely randomized block design, using only the nodulating plants in Nova Scotia soil. The range of added P in the previous experiment exhibited a maximum at approximately 45 mg kg^{-1} added P. To further explore the effects of available P on BNF a finer range of added P was utilized, with the addition of a 30 mg kg^{-1} added P treatment and lowering the highest application rate to 90 mg kg^{-1}. When the plants were thinned, 15 DAP, the majority of the plants had open primary leaves and the secondary leaf buds were present. By 23 DAP, all plants were exhibiting chlorosis, however, after the nodulating plants began BNF this symptom quickly disappeared.

As found for soybean experiment 1, shoot height, shoot DM and leaf area exhibited a consistent trend of increasing with added P (Table 2.7). While the shoot height almost doubled between the control and the higher levels of added P there was no significant difference between the treatments; this non-significance is marginal at p=0.0566. The shoot DM increased by 273 percent between the control and 90 mg added P and was significantly different. The treatments 15, 30 and 45 mg kg^{-1} added P shared

non-significance both with the lowest and highest treatments. As added P increased the shoot DM increased in a closely related relationship (Figure 2.7). The total leaf area increased by 238 percent with significant differences between the highest P rate and all other treatments (Table 2.7). The root correction factor was 0.37.

Table 2.7: Shoot height, shoot dry matter, total leaf area and corrected root DM as affected by added P on soybeans.

		Shoot Height (cm)[1]	Shoot DM (g)	Total Leaf Area (cm^2)	Root DM (g)
Added P ($mg\ kg^{-1}$)	0[2]	33.6 (7.6)	1.17 (0.32) [a]	193.92 (37.19) [a]	0.50 (0.28) [a]
	5	45.1 (7.6)	1.69 (0.32) [a]	251.85 (44.27) [a]	0.63 (0.28) [ab]
	15	68.1 (7.6)	2.35 (0.32) [ab]	342.84 (37.19) [a]	1.00 (0.28) [b]
	30	62.3 (7.6)	2.10 (0.32) [ab]	332.25 (37.19) [a]	0.69 (0.28) [ab]
	45	67.0 (11.4)	2.70 (0.47) [ab]	366.97 (81.06) [ab]	0.72 (0.42) [ab]
	90	60.3 (9.1)	3.19 (0.32) [b]	461.62 (37.19) [b]	0.98 (0.28) [ab]
p-value	Added P	0.0566	0.0099	0.0073	0.0290

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=4

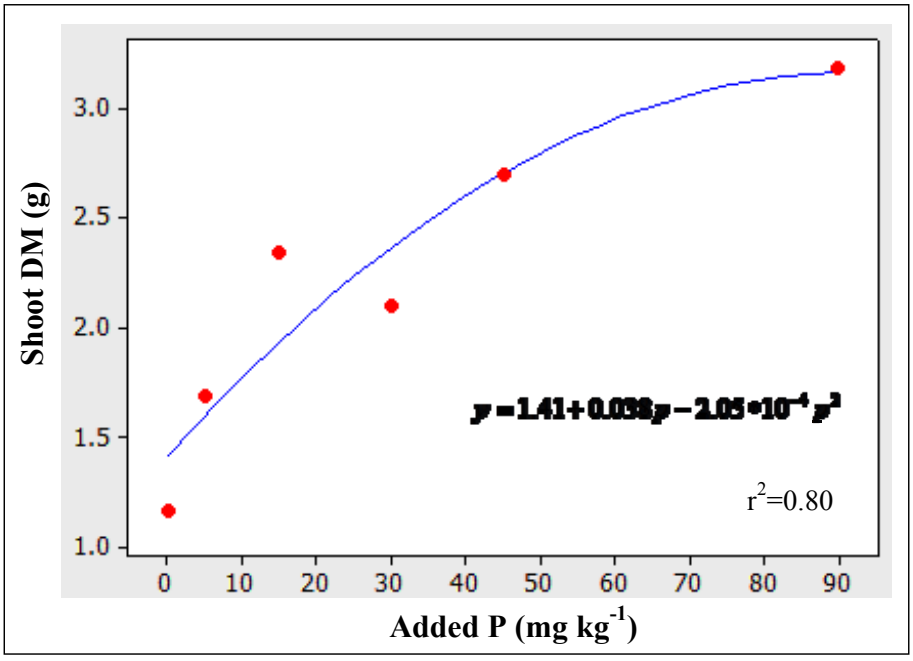


Figure 2.7: Effect of Added P on soybean shoot DM.

Similar to soybean experiment 1, the number of nodules per plant, nodule DM, average nodule DM and nodule DM per shoot DM increased significantly as added P increased (Table 2.8). The number of nodules per plant increased significantly, three fold, between the control and 90 mg P kg^{-1} added P. The dry nodule mass per plant was significantly different between the treatments with 15 to 90 mg kg^{-1} added P having the maximum nodule DM. The nodule DM increased by 180 percent between the control and 90 mg kg^{-1} added P. The average nodule mass increased significantly by 634 percent (Table 2.8). Nodule DM was closely (r^2=0.95) related to added P (Figure 2.8). The slope of the regression line begins to decline at 75 mg kg^{-1} added P. The nodule DM per shoot DM increased significantly by 725 percent between the control and highest added P rate (Table 2.8). Also, this was closely related (r^2=0.96) to added P (Figure 2.8). As well, it is apparent the slope of the line decreases as added P increases indicating a lessened response to added P.

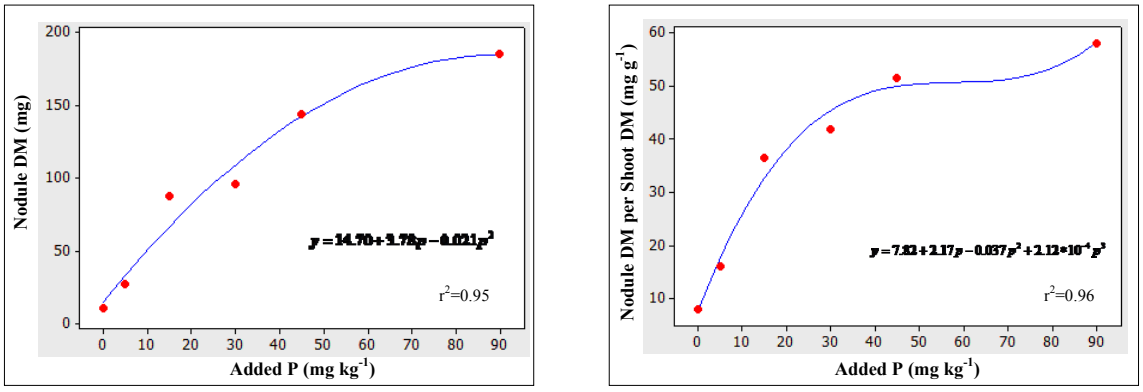


Figure 2.8: Effect of Added P on soybean nodule DM (left) and nodule DM per shoot DM (right).

The shoot tissue concentration of N and P was significantly different between the rates of added P (Table 2.9). However, the trend is opposite between the two parameters. The concentration of N in the tissue was highest in the control and lowest at 90 mg kg^{-1} added P decreasing by 179 percent. For the concentration of tissue P, the control was lowest was and the initial highest significantly different concentration occurred at 30 mg kg^{-1} added P, an increase of 265 percent. However, the P concentration did continue to increase slightly as added P increased to 90 mg kg^{-1}.

Shoot N uptake, estimation of BNF-N and percent of N from BNF were not

significantly different between the rates of added P (Table 2.9). However, shoot P uptake did vary significantly between the rates of added P. The shoot P uptake increased by 726 percent from the control to the highest rate. Shoot P uptake did increase with added P and was closely related (r^2=0.93) to added P. As with previous responses, the shoot P slowly levels off as added P increases. Between the control and the highest applied P rates, as found for shoot DM and in Experiment 1, the two highest levels of added P failed to differ in P uptake response.

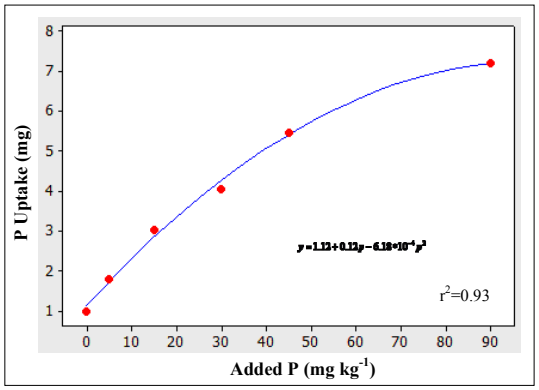


Figure 2.9: Effect of Added P on soybean P uptake.

Table 2.8: Nodule number, DM, average nodule mass and nodule DM per shoot DM as affected by added P on soybean.

		Number of Nodules (number plant^{-1})[1]	**Dry Nodule Mass** (mg plant^{-1})	**Average Nodule Mass** (mg)	**Dry Nodule Mass per Shoot Dry Mass** (mg g^{-1})
Added P (mg kg^{-1})	0[2]	33 (9.34) [a]	10.77 (20.09) [a]	0.29 (0.20) [a]	7.96 (4.62) [a]
	5	39 (9.34) [a]	27.50 (20.09) [ab]	0.75 (0.20) [ab]	16.12 (4.62) [ab]
	15	55 (9.34) [a]	87.50 (20.09) [abc]	1.59 (0.20) [bc]	36.42 (4.62) [bc]
	30	51 (9.34) [a]	92.50 (20.09) [abc]	1.77 (0.20) [c]	41.86 (4.62) [c]
	45	63 (13.85) [ab]	143.7 (29.80) [bc]	2.18 (0.30) [c]	51.38 (6.86) [c]
	90	100 (9.34) [b]	185.0 (20.09) [c]	1.84 (0.20) [c]	58.04 (4.62) [c]
p-value	Added P	0.0031	0.0004	0.0002	< 0.0001

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=4

Table 2.9: Soybean BNF-N estimated by total N difference, shoot N and P concentration and uptake as affected by added P on soybean.

		Shoot N Concentration (mg N g^{-1})[1]	**Shoot P Concentration** (mg P g^{-1})	**Shoot N Uptake** (mg $shoot^{-1}$)	**Shoot P Uptake** (mg $shoot^{-1}$)	**BNF-N Estimation** (mg $plant^{-1}$)	**% BNF-N**
Added P (mg kg^{-1})	0[2]	34.03 (1.80) [c]	0.85 (0.07) [a]	39.21 (6.28)	0.99 (0.63) [a]	8.27 (5.74)	20.44 (7.00)
	5	28.15 (1.80) [bc]	1.06 (0.07) [ab]	46.48 (6.28)	1.80 (0.63) [ab]	13.48 (5.74)	27.97 (7.00)
	15	21.40 (1.80) [ab]	1.32 (0.07) [b]	49.88 (6.28)	3.03 (0.63) [ab]	17.52 (5.74)	33.25 (7.00)
	30	23.45 (1.80) [abc]	1.96 (0.07) [c]	48.19 (6.28)	4.05 (0.63) [b]	15.83 (5.74)	30.33 (7.00)
	45	22.59 (2.66) [abc]	2.03 (0.10) [c]	59.77 (10.11)	5.46 (0.93) [bc]	29.27 (8.55)	46.67 (10.42)
	90	19.08 (1.80) [a]	2.23 (0.07) [c]	61.81 (6.28)	7.19 (0.63) [c]	37.98 (6.84)	49.28 (8.33)
p-value	Added P	< 0.0001	< 0.0001	0.3025	0.0001	0.0727	0.1810

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=4

While calculating the %NDFA it was found the values calculated using the $\delta^{15}N$ value from the β-plants did not result in realistic values and β values were more realistic if the lowest ^{15}N value from the nodulating plants was used (Huss-Danell and Chaia, 2005) or -2.77. The $\delta^{15}N$ value of reference material was -0.66. In addition, the majority of the $\delta^{15}N$ values were closer, 0.02 to 2.0, than a recommended minimum of five units between the reference material and the legumes (Huss-Danell and Chaia, 2005). Since the ^{15}N values of the reference material and the legumes were much closer than the recommended values the data set was not statistically analyzed as it was assumed the results of the calculations would be erroneous. However, the results of the ^{15}N NA analysis are presented in (Table 2.10).

Table 2.10: Percent of BNF-N, calculation of BNF-N by the NA method and δ15N values as affected by added P on soybean.

		%NDFA[1]	**BNF-N Calculation[1]** (mg plant^{-1})	**$\delta^{15}N$[1]**
Added P (mg kg^{-1})	0[2]	34.85 (39.25)	13.33 (14.98)	-1.12 (0.63)
	5	64.20 (14.63)	28.86 (7.43)	-1.69 (0.24)
	15	68.82 (30.12)	43.64 (9.85)	-1.89 (0.47)
	30	5.91 (9.21)	1.07 (7.38)	-0.66 (0.24)
	45	1.71 (2.42)	-15.33 (25.25)	-0.18 (0.75)
	90	14.38 (17.93)	8.10 (18.03)	-0.82 (0.37)

Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=4

2.3.2 *Effect of Added P and Soil Type on Alfalfa Growth and Biological Nitrogen Fixation*

The goal of this experiment was to determine how perennial legume growth and BNF are affected by increasing plant available P. In addition, the responses of legumes growth tp P fertility levels when grown in two contrasting soils found on organic dairy farms were observed. Only the nodulating alfalfa plants were analyzed to determine the effects of P and soil type on BNF. The plants were thinned to two plants per pot at 26 DAP. Plants in two of the pots with 45 mg kg^{-1} added P died during the experiment and were not included in the statistical analysis; only the plants in block 1 and 2 were

analyzed. One of the pots was flooded by a rain event while the greenhouse windows were open and the other pot died due to unknown reasons.

The first harvest of the alfalfa shoots occurred 52 DAP, the second harvest 80 DAP and the final harvest 93 DAP. It was constantly observed that the plants in block 4 were larger than the plants in all other blocks. In addition, this block effect was significant in all measured parameters and was attributed to light availability at the south end of the greenhouse. The plants at the south end did not have a period of the day where there was shade while the remaining blocks experienced a shading effect at different times of the day and at different levels.

The maximum shoot heights of the plants at first harvest (52 DAP) increased significantly as added P increased (Table 2.11) but only in the Nova Scotia soil. There was a significant interaction effect between added P and soil type.

The largest treatment effect on shoot DM was apparent at 52 DAP ((Table 2.11). There was a significant effect of added P and soil type. The Ontario soil had a produced a higher shoot DM, 53 percent, than the Nova Scotia soil. The shoot dry matter increased by 428 percent between the control and 90 mg kg^{-1} added P (Table 2.11). The shoot DM was closely related (r^2=0.98) to added P (Figure 2.10) however, in the cubic response there was a drop in shoot DM at 45 mg kg^{-1} added P.

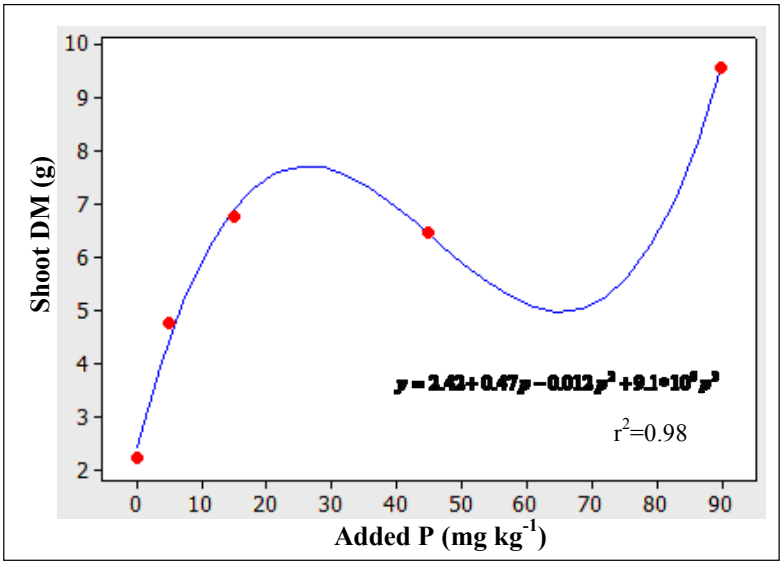


Figure 2.10: Effects of added P on alfalfa shoot DM at cut 1.

Table 2.11: Effects of added P and soil type on the height and shoot dry matter on the first cut* of alfalfa.

		Max Height [1] (cm)		Shoot Dry Matter[1,2] (g pot^{-})
		Nova Scotia[3]	Ontario[3]	
Added P (mg P kg^{-1})	0	26.7 (6.5) [a]	52.7 (5.4)	2.24 (1.21) [a]
	5	50.9 (4.7) [ab]	51.4 (4.7)	4.78 (0.99) [ab]
	15	59.3 (4.7) [b]	55.1 (5.4)	6.77 (1.10) [bc]
	45	52.8 (4.7) [ab]	59.6 (4.7)	6.46 (1.10) [abc]
	90	68.0 (4.7) [b]	63.0 (4.7)	9.58 (0.99) [c]
Soil	Nova Scotia[2]	51.52 (2.83)		4.15 (0.83) [a]
	Ontario	56.36 (2.78)		7.78 (0.81) [b]
p-value	Soil	0.1980		0.0205
	Added P	0.0009		0.0005
	Soil * Added P	0.0456		0.5085

* 50 DAP
Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=8; [3] n=4; [4] n=24

The second cut (80 DAP) did not produce significant differences between treatments for maximum plant height, shoot DM, total N uptake, shoot N concentration and shoot P uptake (Table 2.12 and 2.13). The cumulative DM, cut 1 and cut 2, was significantly greater for the Ontario soil but not for added P treatments.

Alfalfa shoot P concentration increased significantly by 50 percent between the control and the highest added P treatment (Table 2.13). However, there was no significant difference between the soil types. The shoot P uptake did not vary significantly between the added P treatments or soil types. Shoot N concentration (Table 2.13) did not differ while shoot N uptake was significantly different only between the two soil types, and was greater for the Ontario soil.

The non-nod alfalfa exhibited a response to added P (data not shown). The shoot height almost doubled between the control and 90 mg kg^{-1} added P. The shoot DM increased as added P increased for cut 1 and cut 3. The N uptake of the Nova Scotia plants varied between 1 and 150 mg N shoot^{-1} with a general increase as added P

increased. The Ontario plants varied between 25 and 200 mg N shoot^{-1} following a similar trend as the Nova Scotia plants. The P concentration and uptake were not measured on the non-nod soybeans.

Table 2.12: Effects of added P and soil type on the height, shoot dry matter and cumulative DM on the second cut* of nodulating alfalfa.

		Max Height (cm)[1]	**Shoot Dry Matter**[1] (g pot^{-1})	**Cumulative Dry Matter**[1] (g pot^{-})
Added P (mg P kg^{-1})	0[2]	59.7 (6.3)	6.68 (2.15)	9.23 (3.03)
	5	60.9 (4.9)	7.94 (1.77)	12.71 (2.42)
	15	62.2 (5.3)	8.87 (1.87)	15.33 (2.73)
	45	57.3 (5.9)	6.38 (1.77)	13.23 (2.73)
	90	59.5 (4.9)	8.47 (1.77)	18.05 (2.42)
Soil	Nova Scotia[2]	57.2 (4.0)	5.78 (1.45)	9.65 (1.95) [a]
	Ontario	62.6 (3.6)	9.55 (1.43)	17.78 (1.89) [b]
p-value	Soil	0.3262	0.0696	0.0319
	Added P	0.9732	0.7209	0.1673
	Soil * Added P	0.7120	0.2382	0.2394

* 80 DAP
Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=4; [3] n=24

Table 2.13: Effects of added P and soil type on shoot P and N concentration and shoot P and N uptake on alfalfa at the second* forage cut.

		Shoot P Concentration[1] (mg g^{-1})	**Shoot N Concentration**[1] (mg N g^{-1})	**Shoot P Uptake**[1] (mg P $shoot^{-1}$ pot^{-1})	**Shoot N Uptake**[1] (mg N $shoot^{-1}$ pot^{-1})
Added P (mg P kg^{-1})	0[2]	2.18 (0.23) [ab]	26.8 (3.1)	12.75 (4.71)	151.5 (40.9)
	5	1.98 (0.20) [a]	24.5 (2.7)	15.11 (3.77)	181.1 (33.2)
	15	2.35 (0.21) [ab]	25.4 (2.7)	20.70 (4.02)	207.5 (35.2)
	45	2.90 (0.20) [b]	29.8 (2.7)	17.54 (3.77)	168.9 (33.2)
	90	2.93 (0.20) [b]	29.8 .2.7)	23.28 (3.77)	217.4 (33.2)
Soil	Nova Scotia[3]	2.30 (0.16)	27.2 (2.2)	12.83 (2.95)	143.1 (26.7) [a]
	Ontario	2.64 (0.15)	27.1 (2.1)	22.92 (2.89)	227.5 (26.3) [b]
p-value	Soil	0.1259	0.9553	0.0501	0.0505
	Added P	0.0019	0.3180	0.2854	0.5515
	Soil * Added P	0.0625	0.5799	0.3558	0.2413

* 80 DAP

Letters denote significant differences at p=0.05

[1] Standard error in brackets

[2] n=4; [3] n=24

When calculating %NDFA, it was observed the β-value as calculated from the plants grown in an N-free environment did not result in realistic values. In fact, the majority of the calculated values were negative. It was determined the lowest $\delta^{15}N$ value from the nodulating alfalfa in each soil type would be used as the β-value for the %NDFA calculation (Huss-Danell and Chaia, 2005). The lowest $\delta^{15}N$ value in the Nova Scotia data set was -2.93 and -0.37 from Ontario. Since there was little difference in $\delta^{15}N$ values between the reference material and the legumes, it is assumed the %NDFA calculations are incorrect and the resulting data set was not statically analyzed. Previous researchers have found a difference of 5 $\delta^{15}N$ units between the legume and reference plants are necessary in order to accurately calculate BNF-N (Huss-Danell and Chaia, 2005). Nevertheless, the results of the NA analysis are presented in Table 2.14.

Table 2.14: Effects of added P and soil type on percent of BNF-N, calculation of shoot BNF-N by the NA method and δ15N on alfalfa at second cut.

	Added P (mg kg^{-1})	%NDFA[1]	BNF-N Calculation[1] (mg $shoot^{-1}$ pot^{-1})	$\delta^{15}N$[1] Reference	$\delta^{15}N$[1] Legume
Nova Scotia	0[2]	5.95 (14.92)	9.53 (16.87)	-0.30 (0.85)	-0.46 (0.39)
	5	28.06 (12.57)	42.29 (23.72)	-2.37 (1.73)	-1.04 (0.33)
	15	44.12 (41.11)	46.57 (28.27)	-1.13 (0.38)	-1.46 (1.08)
	45	39.04 (19.59)	45.68 (14.96)	-1.27 (0.57)	-1.33 (0.52)
	90	24.05 (25.46)	34.38 (28.65)	-1.06 (1.06)	-0.93 (0.67)
Ontario	0	31.61 (31.27)	59.29 (68.50)	2.72 (1.61)	1.89 (0.83)
	5	-7.89 (24.80)	-12.45 (50.24)	1.70 (0.92)	1.26 (0.37)
	15	37.16 (16.64)	134.74 (101.11)	2.93 (2.26)	1.70 (0.55)
	45	-27.89 (54.73)	-22.52 (58.05)	2.01 (0.55)	2.67 (1.30)
	90	36.46 (43.75)	43.51 (31.70)	1.78 (0.40)	1.00 (0.94)

[*] 80 DAP
[1] Standard error in brackets
[2] n=4

The estimation of alfalfa BNF-N using the total N difference method was not significantly different between the treatments (Table 2.15). The percent of N from BNF did vary significantly between treatments and was negatively related (r^2=0.86) to added P with a quadratic response (Figure 2.11). Soil type marginally (p=0.534) influenced BNF-

N and was greater for the Ontario than Nova Scotia soil.

Table 2.15: Effects of added P and soil type on alfalfa on BNF-N and percent BNF-N as estimated by the total N difference. The data is from the second harvest.

		BNF-N[1] (mg shoot^{-1} pot^{-1})		**% BNF-N**[1,2]
		Nova Scotia[3]	Ontario[3]	
Added P (mg kg^{-1})	0	13.3 (59.8)	196.1 (49.0)	49.32 (12.39) [ab]
	5	98.7 (42.6)	174.9 (42.6)	72.03 (9.59) [b]
	15	61.0 (42.6)	245.7 (49.0)	63.55 (10.36) [ab]
	45	26.9 (42.6)	84.3 (42.6)	24.54 (9.59) [a]
	90	138.7 (42.6)	72.8 (42.6)	39.95 (9.59) [ab]
Soil	Nova Scotia[3]	6.77 (2.30)		38.78 (6.65)
	Ontario	15.47 (2.25)		60.90 (6.46)
p-value	Soil	0.0534		0.0970
	Added P	0.2202		0.0189
	Soil * Added P	0.0557		0.0982

[*]80 DAP
Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=8; [3] n=4; [3] n=24

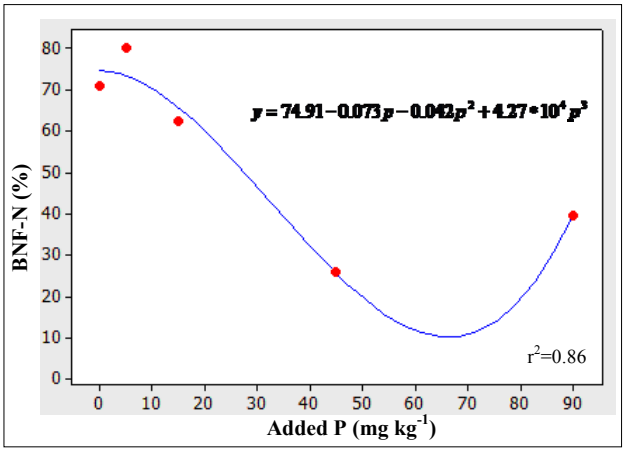


Figure 2.11: Effects of added P on alfalfa shoot percentage BNF-N.

The third cut and final harvest of alfalfa occurred 13 days after the second harvest due to greenhouse renovation. Therefore, the plant growth for this harvest is smaller due to the short time allowed for re-growth. The shoot DM was significantly higher in the Ontario soil by 168 percent (Table 2.16). The shoot DM was not significantly different between added P treatments. There was no significant difference in the plant height nor root DM between added P treatments or soil type (Table 2.16). Combined over all three harvests cumulative shoot DM was affected by soil type alone and was 100% greater for plants grown in Ontario compared to the Nova Scotia soil.

Table 2.16: Effects of added P and soil type on the height and shoot DM on the third* cut of alfalfa.

		Max Height[1] (cm)	Shoot Dry Matter[1] (g pot^{-})	Cumulative Dry Matter[1] (g pot^{-})	Root Dry Matter[1] (g pot^{-})
Added P (mg P kg^{-1})	0[2]	28.3 (2.8)	1.62 (0.36)	11.05 (3.27)	22.37 (6.91)
	5	29.5 (2.2)	2.18 (0.28)	14.90 (2.61)	16.68 (5.25)
	15	31.3 (2.6)	2.30 (0.30)	17.57 (2.94)	25.32 (5.78)
	45	31.3 (2.4)	1.95 (0.30)	14.98 (2.94)	18.76 (6.56)
	90	32.0 (2.4)	2.51 (0.30)	21.69 (2.79)	29.26 (5.79)
Soil	Nova Scotia[3]	30.3 (1.7)	1.58 (0.24) [a]	11.21 (2.11) [a]	19.95 (3.81)
	Ontario	30.6 (1.7)	2.65 (0.24) [b]	20.86 (2.08) [b]	25.01 (3.91)
p-value	Soil	0.8753	0.0475	0.0258	0.4224
	Added P	0.8226	0.3151	0.1164	0.5547
	Soil * Added P	0.9477	0.7437	0.4031	0.3141

* 93 DAP
Letters denote significant differences at p=0.05
[1] Standard error in brackets
[2] n=4; [3] n=24

2.4 DISCUSSION

2.4.1 *EFFECT OF ADDED P AND SOIL TYPE ON SOYBEAN GROWTH AND BIOLOGICAL NITROGEN FIXATION*

The most noticeable difference between the soybean plants in the first and second experiment was plant size. The plants in the first experiment were much taller and

spindly compared to the plants in the second experiment. It is assumed a lack of light in the red spectrum invoked a shading response in the plants (Lambers et al., 1998). It was subsequently realized previous researchers have noted the same response in other plant material in the same growth chamber. The plants in the second soybean experiment were grown in a different growth chamber with a known suitable red spectrum light. The plants in this experiment appeared to have a normal stature.

In both soybean experiments the shoot height, shoot DM and total leaf area increased as added P increased. This response has been observed in previous studies (Israel, 1987; Plenchette and Morel, 1996; Chaudhary and Fujita, 1998; Chaudhary et al., 2008) although none of these studies were conduced on soil from organically managed farms. In organically managed soils, greater microbially-mediated processes, including higher enzyme activity, root length colonized by micorrhizae, and P content and flux through the microbial biomass, contribute proportionately more to plant P supply (Mader et al., 2002). Plenchette and Morel (1996) reported the shoot DM of Maple Arrow soybean ranged from 1.5 g at 0 added P to 7.5 g at 310 mg P kg^{-1} applied as $H_2NaPO_4 \bullet 2H_2O$. Their findings also exhibited three significantly different ranges of plant DM between the following added P rates, 0 to 30, 40 to 70, and 110 to 310 mg P kg^{-1}. However, the increase in shoot DM between 70 and 310 mg P kg^{-1} was only 1.8 g. The added cost of fertilizer to achieve this small but significant increase would likely be not economically viable for producers. Similarly, the results of the plant DM in the first soybean experiment exhibited the same significant distinction at 45 mg kg^{-1} added P as the second experiment. As well, this response at 45 mg kg^{-1} added P was observed in the shoot height and total leaf area in the first experiment. However, the leaf area distinction at 45 mg kg^{-1} added P was not as clear as the other two parameters. Chaudhary et al (2008) found soybean exhibited a lessened response in leaf area than mashbean and mungbean. The first soybean experiment suggest there is a significant difference in measured plant growth parameters which occurs at 45 mg kg^{-1} added P in both the Ontario and Nova Scotia soil. In the second trial conducted on Nova Scotia soil alone, using a finer range of added P, the greatest response of shoot height, shoot DM and total leaf area was similarly found at 45 mg kg^{-1} to 90 mg kg^{-1} added P.

Nodule DM, the number of nodules, and average mass increased as added P

increased. Previous studies have found similar results in a variety of legumes including soybean (Isreal, 1987; Ankomah et al., 1996; Almeida et al., 2000; Lekberg and Koide, 2005). Chaudhary et al (2008) observed an increased P supply to plants increased nodule DM. In this study, the soybeans were supplied with a P nutrient solution for the first three weeks of growth, following which half the plants did not receive a P nutrient solution while the other half continued to receive P in the nutrient solution twice a week. The soybean nodule DM was 42 mg in the plants which did not receive further P while the plants receiving P in the nutrient solution weighed 230 mg. Rotaru and Sinclair (2009) found soybean nodule DM ranged from 50 to 800 mg given different amounts of added P and Fe. The nodule DM response of the two experiments reported here falls in these previously established ranges. Of all nodule measurements, it has been concluded that nodule DM best corresponds to BNF-N in legumes (Ankomah et al., 1996). The increases in nodule DM suggest BNF-N increased with increasing plant available P.

In the first experiment, both the number of nodules and nodule DM have significantly increased at 45 mg kg^{-1} added P, the average nodule DM at 15 mg kg^{-1} added P and the nodule DM per shoot DM at 15 mg kg^{-1} added P and again at 135 mg kg^{-1} added P. The second experiment produced a clear distinction in added P for nodule number and DM at 90 mg kg^{-1} added P. In addition, the average nodule DM and nodule DM per shoot DM significantly increased at 30 mg kg^{-1} added P. Clearly, plant available P effects nodule growth of soybean with the optimum rate for these soils being between 45 mg kg^{-1} and 90 mg kg^{-1} added P. The significant increase of average nodule DM and the nodule DM per shoot DM suggests plant available P not only increases plant growth but nodule growth too. If nodule growth was primarily a function of shoot growth, the nodule DM per shoot DM would remain constant. However, this was not the case. This suggests the increase in nodule DM is not primarily a function of plant growth but is also related to plant available P.

The shoot concentration of N and P increased as added P increased in the first experiment which has been observed in previous studies (Isreal, 1987; Plenchette and Morel, 1996; Chaudhary et al., 2008). The concentration of P in Maple Arrow soybean was found to be between 0.3 and 1.3 mg g^{-1} (Plenchette and Morel, 1996), 8.6 mg g^{-1} for Tamahomare, (Chaudhary et al., 2008) and 2.3 to 7 mg g^{-1} for DP 3478 (Freeborn et al.,

2001). The concentration of N in Tamahomare soybean was found to be 7 mg kg^{-1} (Chaudhary et al., 2008), and 54 mg g^{-1} for DP 3478 (Freeborn et al., 2001). In the first soybean experiment soil type had an effect on the maximum N and P concentrations. The Ontario soil reached its maximum shoot N concentration (based on statistical significance) at 135 mg kg^{-1} added P, while the Nova Scotia soil did acquire the maximum at 15 mg kg^{-1} added P. While both soils reached a maximum P shoot P concentration at 45 mg kg^{-1} added P. This is likely due to the background N in the soil.

The estimate of BNF-N, by total N difference, in both experiments increased as added P increased. In the first trial BNF-N amount (mg N $shoot^{-1}$) was greatest for 45 mg kg^{-1} added P, but failed to increase further at 135 mg kg^{-1} added P. In the second trial, this response was marginally significant ($p=0.0727$) and was greatest for 90 mg kg^{-1} added P. Previous studies have observed this response to added P in legume crops including soybean (Isreal, 1987; Crews, 1993; Ankomah et al., 1996; Lekberg and Koide, 2005). Chaudhary et al (2008) determined BNF could be doubled if soybeans were provided with sufficient P. As well, BNF-N percent of total shoot N uptake increased. Previous researchers have found soybean fixes between 17 and 100 mg N $plant^{-1}$ (Houngnadan et al., 2008) and 15 to 30 mg N per plant (Oberson et al., 2007). Calculated BNF-N ranged from 8 to 99 mg N $plant^{-1}$ for both experiment reported here. This has been found to correspond to 57 to 80 percent of the shoot N uptake (Houngnadan et al., 2008), 10 to 48 percent at the late flowering stage (Oberson et al., 2007), and 33 to 73 percent in the early reproductive stage (Kohl et al., 1980). These ranges are keeping with the BNF-N ranges found in this study (22% to 83% and 30% to 64% for the Nova Scotia and Ontario soils respectively in Experiment 1, and 20% to 49% in Experiment 2). Part of this variance in BNF-N percent can be attributed to the rhizobial strain (Pauferro et al., 2010- in press) as well as other growth limiting factors (O'Hara, 2001). The increase in BNF-N may be attributed to increased nodule activity as the nodule DM increased as added P increased. There was a highly significant interaction of BNF-N with soil type, i.e. % BNF-N clearly responded to added P in the Nova Scotia soil and fell into four ranges: control, 15, 15-45 and 135 mg kg^{-1} added P. The highest % BNF-N fell at 135 mg kg^{-1}. For the Ontario soil, % BNF-N fell within a narrower range 30% to 63 %. This increase is likely due to the increase of nodule DM per shoot DM. It appears

nodule activity is directly related to plant available P and may be the cause of an increased percent BNF-N as added P increased.

In the first soybean experiment, BNF-N is very closely related to added P (Figure 2.6). In fact, it is so closely related the calculated r^2 value was determined to be very close to 1- indicating the close relationship. In this experiment it appears BNF-N was maximized between 45 and 90 mg kg^{-1} added P. However, in both soils the percent BNF-N does not continue to increase after 45 mg kg^{-1} added P suggesting the BNF mechanism has reached its maximum. In the second experiment, Nova Scotia soil only, the BNF-N increases with added P and appears to still increase after 90 mg kg^{-1}. It appears BNF-N is optimized around or above 45 mg kg^{-1} added in both soils.

The two soil types differed in the magnitude of soybean shoot growth response and shoot nutrient uptake, N and P. This failed to differ in terms of nodulation and total nodule DM response. In addition the proportion of shoot N derived from BNF tended to be larger for the Nova Scotia soil, although shoot N uptake was substantially lower. These results likely reflect the combined effect of greater soil N availability in the Ontario soil and a higher P sorption of the applied P in the Nova Scotia soil. In a controlled environment study, Crews (1993) found alfalfa BNF-N differed five soils collected across Mexico. A part of this difference was attributed to soil characteristics and background nutrient levels.

The relationship between the measured growth parameters and added P in the first soybean experiment suggest there is a a plateau in soybean response reached between 45 and 135 mg kg^{-1} added P. As well, the measured parameters are closely related to added P. The measured parameters in the second soybean experiment suggest a plateau is being reached nearer to 90 mg kg^{-1} added P. In this experiment some of the parameters are not as closely related to added P as those in experiment one but the parameters are strongly related to added P. In summary, it appears the optimum added P rate for both soils occurs around or above 45 mg kg^{-1} added P.

2.4.2 *Effect of Added P and Soil Type on Alfalfa Growth and Biological Nitrogen Fixation*

This experiment had clearly different results from the soybean experiments. Part

of this difference is due in part to the life cycle of both the plants and the rhizobia. Soybean is an annual plant with determinate growth while alfalfa is a perennial plant with indeterminate growth. In addition, the effects of added P was observed over three forage harvests of alfalfa further differencing the alfalfa experiment from the soybean experiments.

The shoot DM and plant heights appeared to be slightly affected by added P. However, this significance only was observed for the first cut material. As well, the root DM was not affected by added P. Following three months of growth and three forage harvests, Biro et al (2000) observed alfalfa shoot DM varied between 1.6 and 2.5 g and the roots weighed between 1.8 and 3.2 g. Although the shoot DM range is consistent with previously defined values, there is little explanation for the non-significance between added P rates. This lack of response was likely due to the availability of P in the soils studied. It is very likely the applied P in each treatment was taken up and removed during the first forage harvest, 52 DAP, leaving the plants in the second and third harvest accessing similar amounts of P across all treatments.

Unexpectedly the nutrient content of the shoots at the second harvest did not exhibit significant increases. However, the range of shoot N concentration is consistent with previous studies. In a survey of pastures in Australia it was found alfalfa shoots contained between 26 and 31 mg N g^{-1} (Bowman et al., 2004), to be 48 to 51 mg N g^{-1} in Québec (Bélanger and Richards, 2000) and 40 to 50 mg N g^{-1} in Argentina (Guinazu et al., 2010). Similarly, the P range is consistent with previously observed values of 0.3 to 0.6 mg g^{-1} leaf DM (Crews, 1993). The shoot N and P uptake were not statistically significant but did increase slightly as added P increased. This is contradictory to other studies which found N and P uptake increased with added P (Crews, 1993; Allahdadi et al., 2004).

Both the N concentration and estimates of BNF greatly vary among the blocks. Allabdadi et al (2004) noted this as well. The observed variability in BNF-N and nutrient uptake in the alfalfa may be due in part to the genetic variability. Alfalfa plants are self-incompatible and require pollen from another plant to fertilize the seed (Viands et al., 1988). This cross-pollination creates genetic variability within the species allowing each plant to react to the added P to a different degree. This variation made it difficult to

accurately calculate BNF-N using non-nod alfalfa plants. The amount of BNF-N in each pot varied between the two soil types likely due to the availability of N and P in the soils. The response was lessened in the Nova Scotia soil due to the high sorption capacity of the soil. Previous studies have determined alfalfa fix between 78 and 96 percent of the shoot N (Allahdadi et al., 2004) and 45 to 64 percent (Crews, 1993). Several of the BNF-N percentages in this study were lower than previously observed values. These lower values may be due to the low availability of P.

2.4.3 *CALCULATION OF BNF USING THE NATURAL ABUNDANCE METHOD*

Previous studies have found the range of $\delta^{15}N$ values for nodulating soybean shoot tissue to be between -1.3 to -1.1 (Bergersen et al., 1989b), -1.6 to -0.4 (Pauferro et al., 2010- in press). The $\delta^{15}N$ value of non-nod soybean has been 8.4 (Pauferro et al., 2010- in press) in previous studies. Oberson et al (2007) grew β-value soybeans and determined its shoot $\delta^{15}N$ value to be -1.172. Alfalfa $\delta^{15}N$ values have been found to be an average of -3.0 (Hossain et al., 1995). Previous alfalfa β-values have been -0.4 (Brockwell et al., 1995). The soybean $\delta^{15}N$ values in this study are in the range of the literature values but the reference value is considerably lower. In this study the nodulating alfalfa $\delta^{15}N$ values are close to the literature values.

The NA method used to calculate BNF-N was inconclusive in both the alfalfa and soybean studies. Two of the % NDFA values in the soybean were extremely low and in one instance resulted in a negative 15 mg of BNF-N at 45 mg kg^{-1} added P. While in the alfalfa experiment the % NDFA and BNF-N values were negative at 5 and 45 mg kg^{-1} added P in the Ontario soil. It was determined the %NDFA values were closer to reality if the β value used was from the lowest $\delta^{15}N$ value from the legumes. Huss-Danell and Chaia (2005) showed it is possible to achieve acceptable values of %NDFA using the lowest $\delta^{15}N$ from the legumes. The results from the NA method were not statistically analyzed due to the assumed problems with the data sets. Previous studies have determined BNF-N is closely correlated with nodule DM, shoot DM and total shoot N (Ankomah et al., 1996; Rotaru and Sinclair, 2009), since the NA data set did not reflect this relationship it was decided to exclude the data from analysis. The NA method may have failed for several reasons: the ^{15}N signature of the soil was diluted by the number of

legumes grown in the collected soil; the $\delta^{15}N$ values between the legumes and the reference material was less than 5 $\delta^{15}N$ units; and the choice of reference material affected the results (Crews, 1993; Huss-Danell and Chaia, 2005; Goh, 2007). It may be possible that due to the long term presence of legume material grown previously in the two soils the ^{15}N signature was greatly decreased. This would have occurred due to the decomposition of ^{14}N enriched legume material resulting in a larger proportion of ^{14}N in the soil. Previous studies have observed non-nodulation legumes had a lower concentration of ^{15}N than expected due to the ability of the plants to discriminate against the isotope (Kohl and Schearer, 1980) and that the ^{15}N signature of the non-nod legumes was largely variable (Allahdadi et al., 2004). Additional studies have determined the selection of the reference plant largely determines the %NDFA value (Houngnadan et al., 2008). Thus the selection of the reference material must be carefully made.

2.5 CONCLUSION

The soybean study showed there was a significant increase in plant growth and BNF-N with increased added P. The results suggest plant growth, nodulation, and N and P uptake were optimized between 45 to 90 mg kg^{-1} added P. However, the expectation of nodule number and mass, soil type significantly impacted on the magnitude of plant growth response to added P. This resulted in greater overall values for the Ontario soil compared to the Nova Scotia soil. Also, BNF-N increased with added P up to 45 to 90 mg kg^{-1} added P but the percent of shoot N derived from BNF varied strongly with soil type. This influence of organic dairy farm soil type on soybean response to added P is likely due to the greater P sorption capacity and lower soil available N for the Nova Scotia soil. Further studies will be necessary to determine if these effects extend to field conditions.

The alfalfa study did not show a significant increase in plant growth or BNF-N with increased added P. To further understand the relationship between available P and alfalfa BNF, it is suggested the alfalfa trial be re-run with P fertilizer added to both the collected soil and the soilless medium, below 25 cm. Additional field studies would have to be performed to determine if the relationship extends to field conditions.

CHAPTER 3 : EVALUATION OF ALTERNATIVE PHOSPHOROUS SOURCES

3.1 INTRODUCTION

As the number of farms practicing organic and sustainable farming increases the need for reliable sources of nutrients must be found. A survey of long-term organic dairy farms in Ontario have shown almost half of the farms had low plant available P (Roberts et al., 2008). The same study observed the annual P budget was 1 kg ha^{-1}. Additional studies conducted on organic farms have shown P balances of 3.6 kg P ha^{-1} $year^{-1}$ in Vermont, (Anderson and Magdoff, 2000), 1 kg P ha^{-1} $year^{-1}$ in Sweden (Bengtsson et al., 2003) and an average of 8 kg P ha^{-1} $year^{-1}$ across the global organic dairy farms (Watson et al., 2002b). While the average P balance in these studies was positive some of the farms and/or fields did have negative P balances between -27 and -2.5 kg ha^{-1} $year^{-1}$. Both Anderson and Magdoff (2000) and Bengtsson et al (2003) surveyed conventional dairy farms along side the organic farms. The conventional farms had over three times the average P surplus as the organic farms. The differences between organic and conventional yearly P balances have both positive and negative effects. Since the organic farms have P balances closer to 0, it is expected less P is leaching into water systems reducing environmental pollution caused by agriculture. However, these balances indicate organic farms could become P deficient within a short time. It is very likely these farms will experience a decline in production and plant quality.

Currently there is a range of organically acceptable sources of nutrients. These materials include: livestock manure, ash, materials from naturally occurring mineral deposits, by-products from processing plants and compost (Canadian General Standards Board, 2009b). However, the source and processing involved in the production of the materials impacts their eligibility for organic certification (Canadian General Standards Board, 2009a; Canadian General Standards Board, 2009b). In fact, some of the aforementioned products have restrictions on their use due to production methods, parent materials, heavy metal content and environmental toxicity. The nutrient content of these products is variable and depends upon the parent material (Berry et al., 2002; Lynch et al., 2004; Warman et al., 2009). Often, the ratio of nutrients is not ideal for a specific

crop and must be applied on a single nutrient basis (Lynch et al., 2004; Mkhabela and Warman, 2005; Warman et al., 2009) resulting in other nutrients being applied in excess or deficiency. While these products may not apply nutrients in the optimum ratios, there are added benefits of using organic soil amendments. Many of these amendments have the ability to improve soil structure and quality (Mkhabela and Warman, 2005; Hargreaves et al., 2008).

There are products which have the potential to become organically acceptable. The addition of products to the organically acceptable Permitted Substance List requires that the product meet strict standards and is approved for use by the review board. The standards which must be met include: origin of the material, production methods of the material and impact the material has during and after processing on the environment (Canadian General Standards Board, 2009a). Sewage sludge is currently prohibited from use in organic agriculture (Canadian General Standards Board, 2009b). There are many concerns when it comes to the application of sewage products on agricultural land: public perception of the material, toxic heavy metal levels (Table 3.1) pathogens and environmental toxins including benzene, pesticides, synthetic hormones, phosphate esters and other organic chemicals (Harrison et al., 2006; Renoux et al., 2007; Delaney, 2010). However, research continues to identify methods which may reduce the potential environmental and health challenges associated with the use of municipal sewage sludges (Ueno and Fujii, 2001; Renoux et al., 2007; Suzuki et al., 2007).

Table 3.1: Heavy metal limits for compost material (Canadian Council of Ministers of the Environment, 2005)**.** These restrictions will likely be similar to sewage derived products.

Metal	Maximum Product Concentration (mg kg^{-1} DM)	Metal	Maximum Product Concentration (mg kg^{-1} DM)
Arsenic	13	Mercury	0.8
Cadmium	3	Molybdenum	5
Cobalt	34	Nickel	62
Chromium	210	Selenium	2
Copper	400	Zinc	700
Lead	150		

A new P rich product is currently being tested for its ability to improve soil P and

reduce environmental pollution. The product is called struvite and its nutritional content is listed in Table 1.1. Struvite is a natural forming crystal from sewage sludge and often causes restrictions in flow in processing plants (Gaterell et al., 2000; Wu and Bishop, 2004). Recent research has investigated the possibility of extracting the minerals causing the struvite formation and using it as a fertilizer (Gaterell et al., 2000; Ueno and Fujii, 2001). The intentional precipitation of struvite provides a sustainable source of P and reduces the P pollution risk to the environment form the use of raw sludges (Ueno and Fujii, 2001). In Japan the crystallization process removes over 90 percent of P in the sludge (Ueno and Fujii, 2001). Struvite has the ability to improve sewage processing while providing a sustainable source of P fertilizer.

Although struvite has great promise to provide a much needed sustainable source of P from urban sources, the organic acceptability of the product must be questioned. Most importantly, the current organic standards prohibit the use of sewage products (Canadian General Standards Board, 2009a; Canadian General Standards Board, 2009b) primarily on the basis of concerns over heavy metals and other contaminants present in bulk municipal sludges. However, the standards are constantly being reviewed as updated as new information is provided by research and as new materials develop. As such, it is possible this product could be added to the permitted substance list in the future. Struvite is a naturally occurring product but the chemicals currently utilized in the precipitation procedure, NaOH and MgCl, would either have to be replaced or come from naturally occurring sources (Canadian General Standards Board, 2009a; Canadian General Standards Board, 2009b). The product has the ability to close regional and urban-rural P nutrient cycles, as a key principle of organic standards while reducing environmental pollution.

MSW compost is a commonly used organic soil amendment. In Nova Scotia, all kitchen and yard scraps from private residences are source separated and collected curbside for composting locally (Hargreaves et al., 2008). Previous studies have shown the nutrient composition of the compost is variable depending on the parent material, composting method and composting facility (Hargreaves et al., 2008; Warman et al., 2009). Warman et al (2009) found MSW compost was able to support winter squash growth but high application rates were necessary to achieve the same nutrient levels as

synthetic fertilizers. Additionally, there were significant increases in many extractable nutrients in the soil. As well, MSW compost has the ability to improve soil characteristics including: pH, organic matter, water holding capacity, microbial activity, and nutrient availability (Zheljazkov et al., 2006; Hargreaves et al., 2008; Melero et al., 2008). MSW is a good source of nutrients for organic agriculture although the application rate must be matched with the nutrient content of the applied compost.

Rock phosphate is commonly used to increase soil P. However, the release of P from PR is slow and unpredictable (Martin et al., 2007; Ponce and De Sa, 2007; Arcand et al., 2010). In order to increase the release of P various methods have been examined. One such method involves partially solubilizing PR using acid. Acid can be naturally produced by various biological processes including by-products from microbes. Schneider (2007) investigated the potential of a fungi, *Aspergillus niger*, to partially solubilize P in PR. *A. niger* produces several acids as by-products with citric acid being the acid in largest quantities. It was found the partial solubilization by the naturally produced acids could increase the solubilization of P from PR. The partial solubilization of PR by *A. niger* resulted in 220 to 2 000 mg P l^{-1} becoming immediately plant available depending upon the type and source of PR. Schneider (2007) compared the P solubilized to several different acids and strengths. It was determined 100 mM citric acid solution solubilized approximately the same amount of P as *A. niger*. This form of partially solubilized PR is not known to have been tested for its effect on plant growth. However, it has been theorized P uptake by plants would be improved by first partially solubilizing PR with organically produced acids, including those produced by *A. niger*.

The previous soybean studies, chapter 2, have shown a relationship between plant available P and BNF on soils collected from organic dairy farms. These studies used a synthetic P fertilizer to better understand the relationship between BNF and P. However, synthetic P fertilizer is prohibited in organic agriculture. The goal of this study is to evaluate three organic or potentially organic soil amendments in the ability to provide plant available P thus increasing BNF in soybean. The amendments evaluated were MSW compost, struvite and PR partially solubilized by citric acid (mimicking the action of *A. niger* by-products).

3.2 MATERIALS AND METHODS

3.2.1 *NITROGEN AND PHOSPHORUS MINERALIZATION FROM VARYING SOIL AMENDMENTS*

A N and P mineralization study was first carried out to determine the amount and timing of mineralization from selected amendments. The N and P mineralization procedure was preformed as suggested by Honeycutt et al (2005) with several changes as indicated by additional references.

Air dried, low STP soil collected from organic dairy farms in Nova Scotia and Ontario was used. The collection and processing procedure is noted in section 2.2.1 while the initial soil nutrient content can be found in appendix 1. The soil texture of the soils can be found in section 2.2.1. The amendments tested were: Crystal Green® (CG) (a struvite product provided by Ostara Nutrient Recovery Technologies Inc, Vancouver, BC), MSW compost from Colchester county Nova Scotia and Calphos PR partially solubilized in 100 mM citric acid, section 3.2.1.1 for the solubilization procedure. Additionally, two control treatments were included: a P fertilizer, $H_2NaPO_4 \bullet 2H_2O$ (Fisher Scientific) (Plenchette and Morel, 1996) and an unammended soil control.

The soil was passed though a 2 mm sieve prior to use. The soil was packed into 250 mL plastic containers at the same bulk density used in the previous soybean experiment, chapter 2. The bulk density of the Nova Scotia soil was 1.02 g cm^{-3} while the Ontario soil was 1.21 g cm^{-3}. The mass of the air dried soil was 265 g and 307 g for the Nova Scotia and Ontario soil, respectively. Five 1.6 mm holes were punched in the lid of each container to allow air flow (Neilson, 2009, personal communication). The mineralization experiment occurred in a dark incubator at 25^{o}C (LI20 , Shell Lab, Oregon, U.S.A.). The soil was wetted to 60 percent FC using distilled water and a one week pre-incubation period occurred prior to the addition of the amendments. The containers were weighed piror to adding the amendments and were watered twice weekly to maintain approximately 60 percent FC with spray bottles filled with distilled water.

Following the pre-incubation period, the amendments were mixed into the soil and packed into the containers at the original bulk density. The amendments were added to approximate 15 and 30 mg total P kg^{-1} soil DM. The nutrient content of the amendments are given in Table 3.2. These rates were selected as a result of the P

fertilizer trials in chapter 2. However, Crystal Green was applied at a rate double the target P rates due to its known slow release (Ostra Nutrient Recovery Technologies Inc.), for two rates 30 and 60 mg P kg^{-1}. After the addition of amendments, a soil sample, between 15 and 20 g, was taken for nutrient determination. To ensure a consistent sample was taken a soil core using a test tube cover (size small, Morton Culture Tube Closure). Two cores per container at each sampling date were taken.

Table 3.2: Nutrient content of Crystal Green, PR partially solubilized by citric acid, MSW compost and P fertilizer on a dry mass basis.

mg kg^{-1}	Crystal Green®[a]	Solubilized PR	MSW Compost[b*]	$H_2NaPO_4 \bullet 2H_2O$
Phosphorus	120	700[c]	5.3	130
Nitrogen	50	0	14	0
Potassium	0	0	2.1	0
Magnesium	100	0	2.7	0

[a] nutrient contents listed on the product label of Crystal Green and confirmed by analysis by Nova Scotia Department of Agriculture Soil Test Lab
[b] as preformed by the Nova Scotia Department of Agriculture Soil Test Lab
[c] mg l^{-1} solution

At each sampling, the soil was divided into three parts for nutrient determination. A five gram sample was oven dried at 105°C to determine the water content of the sample and to re-adjust watering as necessary. Another fresh five gram sample was weighed into jars for NO_3 and NH_4 determination using 2 M KCl (Mayndar et al., 2007). The remaining sample was air dried and later processed for available P determination. The Ontario soil under went an Olsen P extraction and the Nova Scotia soil a Mehlich extraction (Carter and Gregorich, 2007). The extracts were analyzed on an autoanalyzer (Technicon AutoAnalyzer III, Technicon Instruments Corporation, NY, USA).

Following the pre-incubation soil sampling, soil samples were taken every two weeks for the first month and once a month for the next three months (Curtin and Campbell, 2007). The mineralization study ran for a total period of 104 days or approximately four months. The sampling dates are listed in Table 3.3.

Table 3.3: Sampling period dates for the collection of soil from the mineralization trial.

Sampling Period	1	September 4
	2	September 20
	3	October 9
	4	November 9
	5	December 9

The experiment was designed as a single factorial experiment in four blocks. The experiment was analyzed as a repeated measured design (Elmi et al., 2005) using SAS (version 9.1, SAS Institute Inc., NC, USA). Significance differences determined by the ANOVA test were tested using orthogonal contrasts. All assumptions were checked before performing analysis using proc univariate. Normality was not found in either of the N data sets. The NO_3 data required a log transformation to satisfy the normality assumption while the NH_4 data required a square root transformation. All significance testing was completed using the transformed data while the means presented are untransformed.

3.2.1.1 *PARTIAL SOLUBILIZATION OF ROCK PHOSPHATE BY CITRIC ACID*

Schneider (2007) investigated the ability of *A. niger* acid by-products to partially solubilize P from PR. The main acid produced by *A. niger* is citric acid. Schneider determined the ability of the citric acid produced by *A. niger* matched a 100 mM citric acid solution to solublized PR.

The culturing of *A. niger* is a difficult process and it was decided to use a 100 mM citric acid solution to match the solubilizing power of the naturally produced acids (Schneider, 2007). Calphos PR was dried in a 60^{o}C oven for 48 hours to remove absorbed moisture. Following, the PR was ground using a mortar and pestle to a consistency of a fine powder (Table 3.4).

Table 3.4: The granular properties of ground Calphos PR.

Screen Size	Percent of PR Passed Through the Screen
125 µm	39
205 µm	19
1 mm	40
> 1 mm	2

To partially solubilize the PR, 50 mL of 100 mM citric acid was added to 125 mL Erlenmeyer flasks. Two grams of the ground Calphos was added to each flask. Following, the flasks were rotated on a rotary shaker for 24 hours at 120 ± 20 rpm (Schneider, 2007; Schneider, 2009). The pH of the finished product was 7.5. This material was directly mixed into the soil.

3.2.2 *Evaluation of Amendment Ability to Supply P to Soybeans*

This trial was established to evaluate the effectiveness of several soil amendments to supply plant available P to soybean in contrasting soil from organic dairy farms. Soybean seeds (*Glycine max* cv Evans) were provided by the Agriculture and Agri-Food Eastern Cereal and Oilseeds Research Center in Ottawa. A non-nod seed with Evans parentage was provided by Dr. Don Smith, McGill University, Montréal, Québec. Plastic 6 inch, 1.5 l pots were filled with known low STP P soil collected from Nova Scotia and Ontario, section 2.2.1. The weight of the Nova Scotia soil was 1540 g while the Ontario soil was 1550 g per pot. The amendments: partially solubilized PR, Crystal Green® (CG), MSW compost and P fertilizer; were mixed into the soil at 15 and 30 mg P kg^{-1}. The product CG was added to the soil with two additional added P levels, 45 and 60 mg P kg^{-1} due to the known slow release of the product and lack of previous experimental data with this product (Ostra Nutrient Recovery Technologies Inc.). All the amendments were added to Ontario soil while the Nova Scotia soil only received fertilizer and CG treatments. The amendments were mixed into the soil by dividing the soil into quarters and adding a quarter of the soil at a time to the amendment to ensure and even distribution throughout the soil. The pots were fertilized with 40 mL of an N and P-free Hoagland solution and distilled water to achieve 60 percent FC. Soybean seeds, two to

three per pot, were placed on top of the soil surface and each seed was inoculated (Cel-Tech from EMD, Crop Bioscience) with 1 mL commercial soybean inoculant. The seeds were placed on the top of the soil to increase the number of germinated seeds over the previous study in Chapter 2. Following, the pots were arranged in a growth chamber with a 14 hour day at 25^{o}C ± 2^{o}C and night temperature of 19 ^{o}C ± 2^{o}C. The pots were watered daily with distilled water to approximate 60 percent FC and were weighed once a week to ensure the desired FC was maintained. Additionally, the plants were fertilized with 20 mL of an N and P-free Hoagland solution weekly. The plants were thinned to one plant per pot 10 DAP by cutting the plants off at the soil line. At this stage of the experiment all the plants had the primary leaves open and the second leaf set was at various stages of opening. The plants began opening flowers 34 DAP. The plants were harvested at 42 DAP, between pod development and pod filling.

At harvest, the plants were gently removed from the soil. Following, the plant was divided into a shoot and a root portion by cutting the stem at the colour change interface near the soil surface. Remaining visible root material in the soil was removed by hand. The leaves were removed from the shoots at the petioles and photographed using a Fujifilm FinePix A340 digital camera. The total leaf area was determined using ImageJ (National Institutes of Health, USA). The shoot height was determined by measuring from the cut-off-point to the SAM. The root material was gently washed over a 1 mm screen to remove excess soil. Following, the shoot and root material was dried separately at 55^{o}C until uniformly dried. Root material containing nodules were kept at 4^{o}C for approximately two weeks until the nodules were be removed and processed. Visible nodules were removed from the roots, counted and dried at 55^{o}C for 24 hours. The mass of the dry nodules was determined.

The DM of the shoot material was determined. Following, the shoot material was ground in a Wiley Mill (standard model number 3) though a 2 mm screen. A fine ground sample was achieved by placing 2 to3 g of the shoot tissue into square glass jars with a small, medium and large rod and rolled on a roller grinder (Arnold and Schepers, 2004) at 70 ± 10 bottle revolutions per minute for 72 hours. Tissue samples less than 2 g were fine ground on a ball mill (Mixer Mill Type MM301, Retsch, Germany). Shoot P concentration was determined using a modified ash procedure (Westerman, 1990; PEI

Analytical Laboratories, 2008). The samples were analyzed on an autoanalyizer (Technicon AutoAnalyizer III, Technicon Instruments Coroporation, NY, USA) to determine P content. Shoot C and N were determined by combustion (Vario MAX CN Macro Elemental Analyzer). As well, root samples were analyzed for C using combustion (Vario MAX CN Macro Elemental Analyzer) to determine the additional weight of adhering soil, section 2.2.3.3. An estimation of BNF-N was determined using the total N difference method, section 2.2.3.2.

This experiment was a single factor blocked experiment in four blocks. It was analyzed using orthogonal contrasts in SAS using proc mixed (version 9.1, SAS Institute Inc., NC, USA). Only the nodulating plants were statistically analyzed. Due to the loss of nodule data in the Nova Scotia for CG 30 and 60, these treatments were omitted from analysis for the nodule data only.

3.3 RESULTS

3.3.1 *NITROGEN AND PHOSPHORUS MINERALIZATION FROM VARYING SOIL AMENDMENTS*

The mineralization of NO_3-N had a significant interaction effect between treatment and day. However, in a repeated measures analysis the independence of the data assumption is violated due to the relationship between the subjects and the passage of time thus the interaction effect between the treatments and time cannot be tested (Montgomery, 2005). The mineralization of NH_4-N was significant for day and PO_4-P was significant for treatment (Table 3.5). The NO_3-N plot (Figure 3.1 and 3.2) shows the mineralization of NO_3-N from the amendments occurs at different sampling periods. The following treatments had a decreased extractable NO_3-N over time: both Nova Scotia fertilizer rates, Nova Scotia CG at 30 mg kg^{-1} added P and the Ontario PR. The Nova Scotia CG 60 30 mg kg^{-1} added P had a consistent extractable NO_3-N over the five sampling periods. The Nova Scotia control had a decreasing extractable NO_3-N for the first four sampling periods while the fifth sampling increased dramatically. The following treatments had an increased extractable NO_3-N over time: Ontario MSW, Ontario fertilizer, Ontario control and Ontario CG. These varying trends of NO_3-N mineralization explain the significant treatment by sampling date interaction

The mineralization of NO_3-N in the Nova Scotia fertilizer treatment did not vary significantly over the sampling periods and followed a similar trend as the control treatment (Figure 3.1 (a)) where as the mineralization of NH_4-N was significantly changed between the sampling periods. The mineralization of P was not significantly different between sampling dates. The mineralization of NO_3-N was not significantly different for the CG treatments (Table 3.5) while the NH_4-N mineralization was significantly different over the sampling period. The CG60 mineralization of NH_4-N maintained a higher extractable NH_4-N over the control (Figure 3.1 (b)) while CG30 was equal or lower than the control for the last three samplings. The extractable P from the CG treatments was significantly different from the control and from the added P fertilizer. The CG30 P mineralization was higher than the fertilizer treatment (Figure 3.1). In addition, the extractable P was much higher than the control.

The mineralization of NO_3-N and NH_4-N was significantly different between the two contrasting soils while P mineralization was not. In both soils the mineralization of NO_3-N, NH_4-N and P from the fertilizer had an additive effect over the control treatment (Figures 3.1 (a) and 3.2 (c)). In the Nova Scotia soil, the CG treatments did not follow a similar pattern of release as the fertilizer and was significantly different. The mineralization of NO_3-N, NH_4-N and follows a similar trend of release between the control and the CG treatments in the Ontario soil (Figure 3.2 (d)). The mineralization of NO_3-N and NH_4-N follows a similar trend for the MSW treatments, (Figure 3.2 (e)). However, the mineralization of P appears to have increased with MSW and followed an additive trend when compared to the control. The mineralization of NO_3 appears to have decreased in the partially solubilized PR treatments as compared to the control (Figure 3.2 (f)). The extractable P at day 0 was higher in the PR treatments but is similar to the control for the following samplings.

Table 3.5: ANOVA p-value and orthogonal contrasts for the mineralization of nitrate, ammonia and phosphorus over three months.

		Nitrate	Ammonia	Phosphorus
Orthogonal Contrast*	NS[1] vs ON[2]	**<0.0001**	**<0.001**	0.6611
	NS Ctl vs All NS[3]	0.6402	0.8816	**<0.001**
	NS Fert 15 vs 30[3]	0.4197	0.6887	0.3370
	NS Fert vs CG[4]	**0.0341**	0.1193	**<0.001**
	NS CG 30 vs 60[3]	**0.0006**	0.6475	**0.0055**
	ON Ctl vs All ON[5]	**0.0038**	0.0709	**0.0006**
	ON Fert 15 vs 30[3]	0.4352	0.0505	0.1029
	ON Fert vs CG[4]	**<0.0001**	**0.0035**	**0.0279**
	ON CG 30 vs 60[3]	0.9767	**0.0399**	**0.0108**
	ON Fert vs MSW[4]	0.8024	0.5984	**0.0015**
	ON MSW 15 vs 30[3]	0.3734	0.8364	**0.0084**
	ON Fert vs PR[4]	**<0.0001**	**0.0276**	0.1318
	ON PR 15 vs 30[3]	0.9561	0.1668	0.8047
	Day 1 vs 2 3 4 5	-	0.5511	-
	Day 2 vs 3 4 5	-	**0.0288**	-
	Day 3 vs 4 5	-	**0.0005**	-
	Day 4 vs 5	-	**0.0014**	-
p-value	Treatment	**<0.0001**	**<0.0001**	**<0.0001**
	Sampling Day	0.6448	**<0.0001**	0.5412

* list of abbreviations in Appendix 6
[1] n=5; [2] n=9; [3] n=4; [4] n=8; [5] n=8

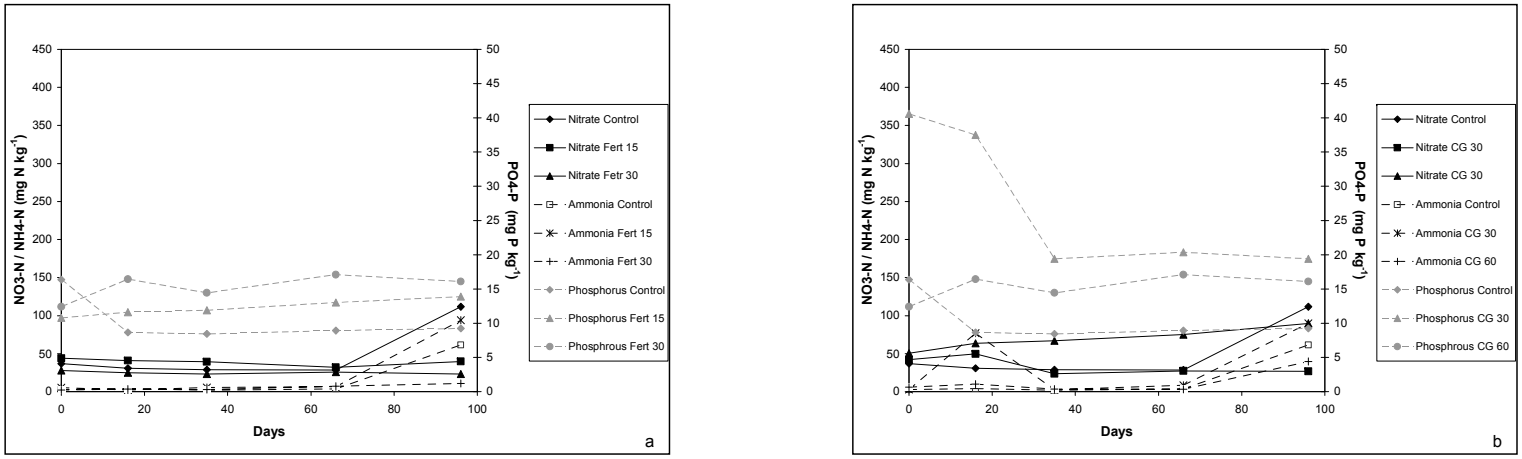
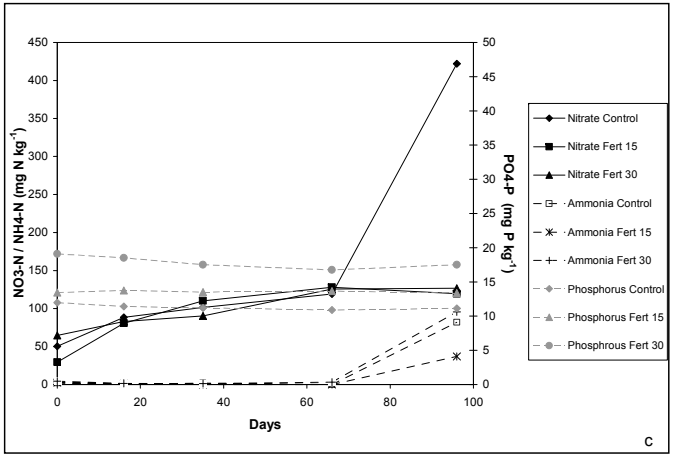
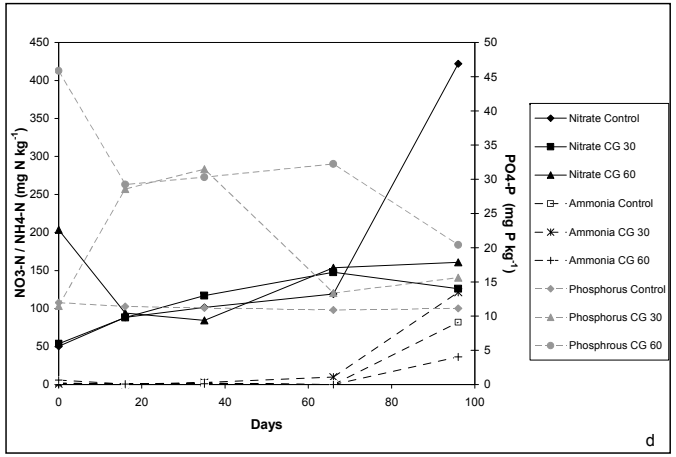
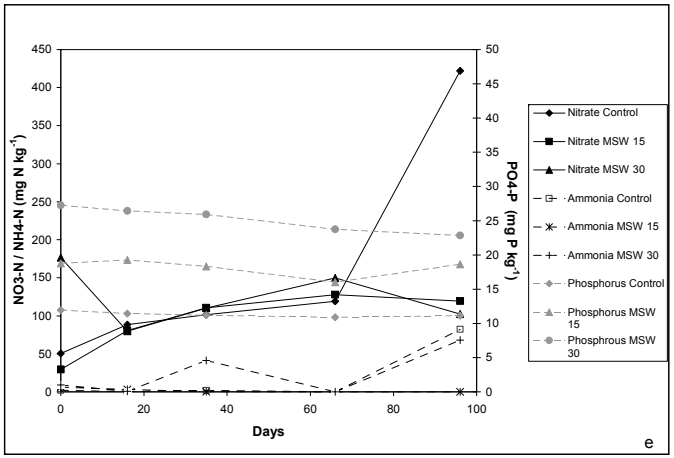


Figure 3.1: Mineralization of (a) fertilizer and (b) CG in the Nova Scotia soil.







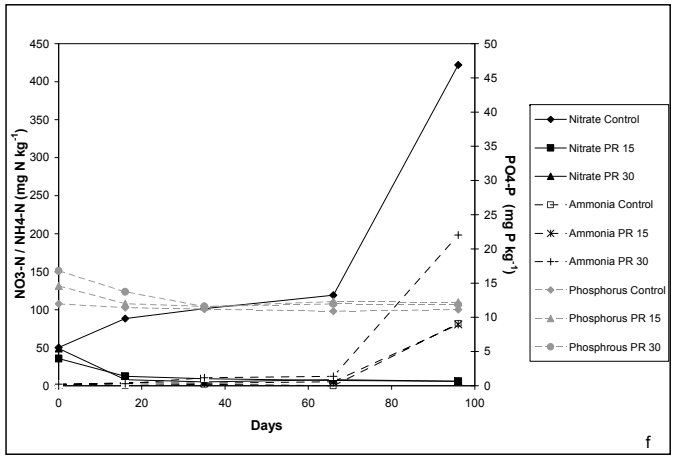


Figure 3.2: Mineralization of (c) fertilizer, (d) CG, (e) MSW compost and (f) partially solubilized PR amendments in Ontario soil.

Table 3.6: Standard error for nitrate, ammonia and phosphorus over three months.

Tretment[1]	Nitrate	Ammonia	Phosphorus
NS Ctl	37.22	14.10	4.06
NS Fert 15	39.40	35.67	1.95
NS Fert 30	15.87	4.81	2.63
NS CG 30	32.16	57.63	21.57
NS CG 60	51.55	12.16	13.56
ON Ctl	50.93	20.06	1.27
ON Fert 15	18.41	15.37	1.78
On Fert 30	16.07	36.42	2.00
ON CG 30	2.31	31.34	10.09
ON CG 60	29.75	8.63	21.00
ON MSW 15	18.49	2.75	2.28
ON MSW 30	71.30	45.59	3.83
ON PR 15	5.65	35.43	1.45
ON PR 30	4.4	6.48	2.32

[1] n=20

3.3.2 *Evaluation of Amendment Ability to Supply P to Soybeans*

The three amendments showed varying ability to provide P to soybean plants and enhance BNF. The non-nod plants began exhibiting chlorosis at 24 DAP. The chlorosis was slight at first and began to become more noticeable as the plants aged. By 34 DAP, all plants had fully developed buds and were beginning to open.

For the Nova Scotia, there were significant differences between all treatments and the controls for all plant growth measurements while the Ontario soil was only significant for shoot DM, total plant DM and leaf area (Table 3.7 and 3.9). The orthogonal contrast between the two soil types showed significant differences in all growth responses with total plant DM being marginally significant ($p=0.0716$). For soils, shoot height, shoot and root DM and leaf area were significantly higher in the Ontario soil than in the Nova Scotia soil (Table 3.7 and 3.8). As well for both soils, shoot DM increased with increasing added fertilizer P, from 15 to 30 mg P kg^{-1} to over 8 g shoot DM produced for the latter treatment, compared to 1.6 g and 3.7 g per shoot for the Nova Scotia and Ontario control treatments respectively. For both soil types, CG and fertilizer treatments did not differ with respect to their effects on plant growth. The mean of the values for

CG treatments was numerically higher than that obtained for fertilizer treatments (Table 3.7). For the CG treatments, shoot DM ranged between 4.4 g to 6.8 g in the Nova Scotia soil and increased to 5.9 and 7.7 g in the Ontario soil. The partially solubilized PR (Ontario soil only) differed from fertilizer treatments for most growth measurements with the majority of the parameters being lower. In contrast, the MSW treatment matched the plant growth response obtained with fertilizer,. As well,, the root DM was significantly different among many of the amendments. The root correction factor was 0.94. Interestingly, the total DM was not significantly different in the Nova Scotia soil between the fertilizer and CG, and in the Ontario soil between fertilizer and CG and fertilizer vs. MSW. Increasing the rate of CG, MSW or PR had very little effect on plant growth response.

The two contrasting soils were unable to be statistically analyzed for nodule responses due to the incomplete data at NS CG 30 and 60 (Table 3.10). The roots were accidentally placed in the dryer before the nodule data were collected and accurate data could not be collected after drying. Therefore, the nodule information on the Nova Scotia soil is incomplete (Table 3.9 and 3.10). Both soils had a significant increase in all measured nodule parameters for all treatments compared to the controls (Table 3.10). The p-value was unable to be calculated for the Ontario vs. Nova Scotia soil due to the missing values in Nova Scotia soil. Thus the contrasts were rerun with NS CG 30 and 60 omitted. The number of nodules significantly increased while the nodule DM was marginally significant in the Nova Scotia soil between the two levels of fertilizer. For the Ontario soil, the number of nodules was not significantly different between the levels of added amendments (CG, MSW and PR) and fertilizer treatments (Table 3.10). Also, this trend was apparent in the nodule DM (Table 3.10). As found in the general plant growth above, the increasing rates of alternative amendments (CG, MSW and PR) failed to influence nodulation response. Both the average nodule DM and nodule DM per shoot DM were only significantly different for the Nova Scotia control vs. amendments (Table 3.10).

Table 3.7: Effects of amendments on soybean shoot height, shoot DM, corrected root DM, total plant DM and total plant leaf area as affected by soil type and soil amendments.

		Shoot Height* (cm)	Shoot Dry Matter* (g)	Root Dry Matter* (g)	Total Plant Dry Matter* (g)	Total Plant Leaf Area* (cm^{-2})
Nova Scotia[1]	Control	81.2 (14.9)	1.64 (0.28)	0.19 (0.18)	1.83 (0.45)	301.19 (49.83)
	Fertilizer 15	112.8 (25.9)	4.44 (1.61)	0.38 (0.38)	4.84 (1.99)	716.24 (243.83)
	Fertilizer 30	138.0 (33.1)	8.09 (1.77)	1.22 (0.62)	9.39 (2.37)	1116.32 (54.57)
	CG 15	112.5 (35.5)	4.59 (0.53)	0.60 (0.06)	5.23 (0.58)	863.61 (149.16)
	CG 30	149.2 (4.2)	5.58 (0.26)	1.04 (0.51)	6.69 (0.50)	962.68 (146.60)
	CG 45	150.5 (28.8)	6.84 (2.97)	0.85 (0.91)	7.74 (3.82)	1183.14 (428.89)
	CG 60	105.0 (64.9)	4.37 (3.80)	1.49 (0.75)	8.11 (1.68)	727.08 (649.85)
Ontario[1]	Control	134.2 (17.3)	3.66 (0.15)	0.24 (0.03)	3.92 (0.18)	704.79 (129.93)
	Fertilizer 15	151.1 (28.5)	6.58 (2.40)	0.66 (0.28)	8.32 (1.68)	1519.30 (455.39)
	Fertilizer 30	158.3 (29.1)	8.53 (0.66)	0.85 (0.26)	9.43 (0.89)	1402.91 (120.52)
	CG 15	140.8 (37.8)	6.30 (0.02)	0.55 (0.03)	6.88 (0.05)	1131.02 (395.09)
	CG 30	169.0 (1.4)	7.69 (0.62)	0.54 (0.06)	8.26 (0.67)	1555.22 (325.15)
	CG 45	133.3 (30.3)	6.70 (0.75)	0.51 (0.32)	7.31 (1.19)	1251.48 (248.44)
	CG 60	262.5 (22.6)	5.88 (3.30)	0.51 (0.34)	7.22 (3.86)	1500.31 (1028.44)
	MSW 15	147.4 (33.7)	7.11 (0.82)	0.59 (0.24)	7.74 (1.04)	1296.37 (198.48)
	MSW 30	168.8 (26.4)	7.27 (2.76)	0.44 (0.37)	7.74 (3.08)	1337.30 (281.26)
	PR 15	144.5 (19.9)	5.72 (1.80)	0.31 (0.26)	6.05 (2.04)	1049.20 (373.15)
	PR 30	133.0 (39.9)	4.34 (0.68)	0.09 (0.14)	4.46 (1.19)	1017.00 (152.53)

* Standard error in brackets
[1] n=4

Table 3.8: Orthogonal contrast p-values for soybean shoot height, shoot DM, root DM, total plant DM and total leaf area plant^{-1} as affected by soil type and added amendments.

Orthogonal Contrasts[*]	Shoot Height (cm)	Shoot Dry Matter (g)	Root Dry Matter (g)	Total Plant Dry Matter (g)	Total Plant Leaf Area (cm^{-2})
NS[1] vs ON[2]	**0.0022**	**0.0009**	**0.0004**	0.0716	**<0.0001**
NC Ctl[3] vs All Other NS[4]	**0.0084**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0004**
NS Fert 15 vs 30[3]	0.2528	**0.0023**	**<0.0001**	**0.0014**	0.0725
NS Fert[5] vs NS CG[6]	0.7954	0.1722	0.5021	0.4823	0.9609
NS CG 15 vs 30[3]	0.1345	0.5915	0.0730	0.3946	0.8096
NS CG 30 vs 45[3]	0.8948	0.1971	**0.0189**	0.9487	0.2524
NS CG 45 vs 60[3]	0.0711	0.0562	**0.0006**	0.3650	0.0749
ON Ctl[3] vs All Other ON[7]	0.3148	**0.0023**	0.2429	**0.0103**	**0.0045**
ON Fert 15 vs 30[3]	0.5657	0.2396	0.3499	0.3600	0.2703
ON Fert[5] vs ON CG[6]	0.8915	0.0762	0.1597	0.1278	**0.0403**
ON CG 15 vs 30[3]	0.4033	0.3540	0.6502	0.3554	0.1163
ON CG 30 vs 45[3]	0.1865	0.4668	0.7008	0.9150	0.4594
ON CG 45 vs 60[3]	0.1906	0.4423	0.6137	0.4355	0.2134
ON Fert vs ON MSW[5]	0.8949	0.9024	0.2522	0.3251	0.1412
ON MSW 15 vs 30[3]	0.4512	0.1306	0.6477	0.9886	0.9133
ON Fert vs ON RP[5]	0.3392	**0.0011**	**0.0071**	**0.0043**	**0.0022**
ON PR 15 vs 30[3]	0.5434	0.2257	0.5672	0.4016	0.8787
ANOVA p-value	0.0441	<0.0001	<0.0001	0.0004	<0.0001

[*] list of abbreviations in Appendix 6
[1] n=7; [2] n=11; [3] n=4; [4] n=6; [5] n=8; [6] n=16; [7] n=10

Table 3.9: Soybean nodule growth measurements: number of nodules per plant, nodule DM, average nodule DM and nodule DM per shoot DM as affected by soil type and soil amendments.

		Number of Nodules* (plant^{-1})	**Nodule Dry Mass*** (mg plant^{-1})	**Average Nodule Dry Mass*** (mg nodule^{-1})	**Nodule Dry Mass per Shoot Dry Mass*** (mg g^{-1})
Nova Scotia[1]	Control	34 (13)	12.15 (7.49)	0.35 (0.18)	7.14 (3.97)
	Fertilizer 15	70 (31)	114.60 (87.45)	1.47 (0.69)	22.76 (11.45)
	Fertilizer 30	175 (49)	242.50 (55.60)	1.42 (0.24)	30.01 (1.77)
	CG 15	56 (34)	122.50 (25.00)	2.59 (1.05)	26.50 (2.65)
	CG 30	-	-	-	-
	CG 45	209 (117)	290.00 (81.85)	1.58 (0.58)	35.66 (2.50)
	CG 60	-	-	-	-
Ontario[1]	Control	83 (10)	85.00 (7.07)	1.03 (0.04)	23.31 (2.88)
	Fertilizer 15	137 (76)	247.50 (138.17)	1.78 (0.67)	34.82 (12.33)
	Fertilizer 30	140 (40)	370.00 (65.57)	2.71 (0.36)	43.41 (7.54)
	CG 15	115 (8)	260.00 (70.71)	2.25 (0.46)	41.32 (11.37)
	CG 30	113 (25)	270.00 (45.83)	2.43 (0.35)	35.25 (6.49)
	CG 45	103 (42)	202.50 (56.20)	2.19 (1.08)	29.90 (6.05)
	CG 60	84 (54)	173.38 (171.09)	1.64 (1.02)	22.74 (16.15)
	MSW 15	196 (51)	287.50 (78.48)	1.72 (0.22)	39.88 (7.76)
	MSW 30	142 (71)	293.33 (165.63)	2.08 (0.95)	38.32 (10.75)
	PR 15	135 (52)	285.00 (123.69)	2.08 (0.13)	48.48 (7.27)
	PR 30	135 (63)	230.00 (167.93)	1.56 (0.50)	50.66 (33.07)

* Standard error in brackets
[1] n=4

Table 3.10: p-values for orthogonal contrasts for soybean number of nodules per plant, nodule dry mass per plant, average nodule dry mass and nodule dry mass per shoot dry mass as affected by soil type and added amendments.

Orthogonal Contrasts[*]	**Number of Nodules** (plant^{-1})	**Nodule Dry Mass** (mg plant^{-1})	**Average Nodule Dry Mass** (mg nodule^{-1})	**Nodule Dry Mass per Shoot Dry Mass** (mg g^{-1})
NS[1] vs ON[2]	0.3114	**0.0044**	**0.0156**	**0.0030**
NC Ctl[3] vs All Other NS[4]	**0.0038**	**0.0029**	**0.0005**	**0.0058**
NS Fert 15 vs 30[3]	**0.0081**	0.0790	0.9299	0.4369
NS Fert[5] vs NS CG[6]	0.7252	0.6061	0.0696	0.5015
NS CG 15 vs 45[3**]	**0.0007**	**0.0372**	**0.0494**	0.3754
ON Ctl[3] vs All Other ON[7]	**0.0013**	**0.0002**	**0.0001**	**0.0005**
ON Fert 15 vs 30[3]	0.8177	0.0959	0.2158	0.1877
ON Fert[5] vs ON CG[6]	0.0853	0.0682	0.4420	0.3998
ON CG 15 vs 30[3]	0.9505	0.8473	0.6974	0.7611
ON CG 30 vs 45[3]	0.8169	0.3940	0.6755	0.6247
ON CG 45 vs 60[3]	0.6173	0.6821	0.2391	0.4425
ON Fert vs ON MSW[5]	0.8742	0.6579	0.2139	0.8236
ON MSW 15 vs 30[3]	0.5098	0.9512	0.4796	0.8645
ON Fert vs ON RP[5]	0.5937	0.2851	0.1369	0.0827
ON PR 15 vs 30[3]	1.000	0.4425	0.2628	0.8142
ANOVA p-value	0.0026	0.0022	0.0034	<0.0001

[*] list of abbreviations in Appendix 6
[**] nodule data lost for NS CG 30 and 60 and was omitted from analysis
[1] n=7; [2] n=11; [3] n=4; [4] n=6; [5] n=8; [6] n=16; [7] n=10

Table 3.11: Soybean shoot N and P concentration, total N and P, estimation of BNF-N (total N difference) and percent of N from BNF as affected by soil type and added amendments.

		Shoot N Concentration* (mg g^{-1})	**Shoot P Concentration*** (mg g^{-1})	**Total N Uptake*** (mg $shoot^{-1}$)	**Total P Uptake*** (mg $shoot^{-1}$)	**BNF-N Estimate*** (mg $plant^{-1}$)	**% BNF-N***	**Total N Uptake Non-nod*** (mg $shoot^{-1}$)
Nova Scotia[1]	Control	41.55 (5.72)	1.59 (0.15)	66.85 (3.71)	2.60 (0.38)	2.10 (3.08)	2.98 (4.33)	65.3 (22.6)
	Fertilizer 15	37.25 (1.55)	2.83 (1.03)	149.18 (57.05)	11.44 (2.03)	74.08 (57.05)	45.16 (17.75)	75. 1 (52.0)
	Fertilizer 30	29.86 (4.99)	2.24 (0.56)	115.20 (43.25)	17.52 (2.63)	157.97 (21.73)	66.64 (2.90)	77.3 (4.3)
	CG 15	28.44 (6.58)	2.22 (0.39)	132.6 (40.8)	10.06 (0.91)	41.66 (29.90)	27.10 (18.70)	96.7 (5.2)
	CG 30	20.47 (6.84)	2.59 (0.27)	115.2 (43.2)	14.41 (1.18)	17.08 (29.85)	10.37 (17.96)	113.5 (111.1)
	CG 45	33.59 (8.24)	2.94 (1.02)	213.0 (59.6)	17.68 (3.97)	0.00 (0.00)	0.00 (0.00)	283.9 (194.3)
	CG 60	17.73 (0.53)	3.28 (0.06)	115.6 (13.1)	21.48 (3.46)	0.00 (0.00)	0.00 (0.00)	165.3 (44.1)
Ontario[1]	Control	17.61 (3.42)	1.48 (0.27)	64.6 (15.1)	5.40 (0.78)	0.00 (0.00)	0.00 (0.00)	92.1 (17.1)
	Fertilizer 15	32.92 (3.80)	2.45 (0.32)	222.4 (95.3)	16.02 (5.680	130.27 (95.31)	48.78 (31.65)	92.1 (17.1)
	Fertilizer 30	36.13 (2.04)	2.60 (0.27)	307.8 (21.0)	22.04 (0.71)	158.30 (106.91)	68.48 (2.23)	96.7 (18.0)
	CG 15	35.19 (3.51)	-	221.5 (22.8)	-	128.53 (22.83)	57.80 (4.35)	93.0 (2.0)
	CG 30	34.82 (2.57)	2.67 (0.46)	267.3 (20.8)	20.40 (1.95)	147.49 (20.77)	55.01 (3.36)	119.8 (57.4)
	CG 45	36.21 (3.04)	3.46 (0.47)	241.3 (19.3)	21.90 (1.52)	146.54 (19.27)	60.53 (3.19)	94.8 (73.9)
	CG 60		2.83 (0.18)	229.6 (126.7)	15.79 (12.26)	122.94 (107.15)	40.61 (35.21)	118.0 914.2)
	MSW 15	35.19 (2.75)	2.63 (0.55)	248.9 (22.0)	18.36 (1.88)	201.93 (22.00)	81.01 (1.68)	47.0 (30.3)
	MSW 30	33.40 (4.63)	2.36 (0.38)	235.4 (104.2)	17.08 (6.97)	106.32 (110.71)	52.41 (26.84)	93.6 (20.3)
	PR 15	34.61 (1.70)	2.55 (0.22)	197.6 (59.9)	13.73 (4.80)	141.79 (59.95)	68.96 (12.47)	55.8 (37.3)
	PR 30	36.98 (0.75)	3.15 (0.28)	160.6 (26.6)	13.68 (2.57)	67.80 (26.57)	41.05 (9.48)	92.8 (80.0)

* Standard error in brackets
[1] n=4

Table 3.12: p-values for orthogonal contrasts for soybean shoot N and P concentration, total shoot N and P, estimation of BNF-N by the total N difference and percentage of N derived from BNF as affected by soil type and amendments.

Orthogonal Contrasts[*]	**Shoot N Concentration** (mg g^{-1})	**Shoot P** (mg g^{-1})	**N Uptake** (mg $shoot^{-1}$)	**P Uptake** (mg $shoot^{-1}$)	**BNF-N Estimate** (mg $plant^{-1}$)	**% BNF-N**
NS[1] vs ON[2]	**0.0066**	0.5197	**<0.0001**	**0.0095**	**<0.0001**	**<0.0001**
NC Ctl[3] vs All Other NS[4]	**<0.0001**	**0.0010**	**0.0043**	**<0.0001**	0.2524	0.5359
NS Fert 15 vs 30[3]	**0.0422**	0.1377	**0.0349**	**0.0176**	**0.0016**	0.7444
NS Fert[5] vs NS CG[6]	**0.0004**	0.3935	**0.0143**	0.3940	**<0.0001**	**<0.0001**
NS CG 15 vs 30[3]	**0.0234**	0.4006	0.4151	0.1410	0.1076	**<0.0001**
NS CG 30 vs 45[3]	**0.0004**	0.4008	**0.0040**	0.1603	0.2692	**0.0183**
NS CG 45 vs 60[3]	**0.0002**	0.5021	**0.0106**	0.2488	0.9127	0.7805
ON Ctl[3] vs All Other ON[7]	**<0.0001**	**0.0066**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
ON Fert 15 vs 30[3]	0.3828	0.8866	**0.0134**	0.1918	**0.0313**	0.9956
ON Fert[5] vs ON CG[6]	0.7991	0.2640	0.1917	0.2497	0.6072	0.9668
ON CG 15 vs 30[3]	0.9.78	0.6151	0.2289	0.1244	0.2525	0.9503
ON CG 30 vs 45[3]	0.5296	0.1929	0.7632	0.0683	0.2659	0.9958
ON CG 45 vs 60[3]	0.7358	0.0856	0.4588	0.5964	0.5162	0.9226
ON Fert vs ON MSW[5]	0.9498	0.9906	0.7515	0.1948	0.7282	0.8724
ON MSW 15 vs 30[3]	0.5748	0.6281	0.1706	0.6125	**0.3179**	0.7831
ON Fert vs ON RP[5]	0.5639	0.2657	**0.0007**	**0.0018**	**0.0026**	0.9405
ON PR 15 vs 30[3]	0.4572	0.2086	0.2697	0.9926	0.2596	0.8146
ANOVA p-value	<0.0001	0.0100	<0.0001	<0.0001	<0.0001	<0.0001

[*] list of abbreviations in Appendix 6
[1] n=7; [2] n=11; [3] n=4; [4] n=6; [5] n=8; [6] n=16; [7] n=10

N uptake and P uptake were significantly different between the two contrasting soils (Table 3.12). In the Nova Scotia soil the shoot P concentration and P uptake was significantly increased over the control treatment. The higher level fertilizer increased shoot P uptake and BNF-N over the lower level. Fertilizer and CG treatments differed with respect to N uptake and BNF estimates in the Nova Scotia soil but not in the Ontario soil. However, BNF-N and percent BNF-N significantly decreased as the level of CG increased in the Nova Scotia soil. In the Ontario soil fertilizer did not differ from CG and MSW effect with respect to total N and P uptake and BNF-N estimates. However, this was not true for the PR treatment which resulted in lower N and P uptake and BNF-N than fertilizer.

3.4 DISCUSSION

3.4.1 *NITROGEN AND PHOSPHORUS MINERALIZATION FROM VARYING SOIL AMENDMENTS*

The mineralization of N and P varied significantly among the different amendments. In both soils the mineralization trend followed a similar pattern: P remained relatively constant over the course of the study and both NH_4-N and NO_3-N remained constant over the first four sampling periods when the rate dramatically increased at the last sampling period. Other studies have found the mineralization of NH_4-N to be constant while NO_3-N increases steadily over time (Burger and Venterea, 2008). The extractable P in each of the individual treatments, except for the CG in the Nova Scotia, soil had the same amount of extractable P at each sampling.

Both the MSW compost and the fertilizer increased STP over the control. The mineralization showed the products had an additive effect suggesting a similar cycling of P from the products through the soil microbial biomass. The PR increased STP for the first two sampling periods then followed the same trend as the control. This indicates the partially solubilized PR does not have the ability to provide a sustainable P source over the growing period. The mineralization of P from MSW and PR follows a similar mineralization trend as the unamended soil.

Since struvite products are new, the agronomic characteristics are currently being explored and there are few peer-review articles on the subject. Those articles which have

been published focus on plant growth response rather than the mineralization of N and P. However, the results of such studies suggest the mineralization of P is similar or better than the standard P fertilizers (Johnston and Richards, 2003). The mineralization of CG in this study did not follow a constant pattern. However, STP was higher than the control throughout the study. The mineralization pattern suggests CG has the ability to supply P and sustain plant growth over the entire growing season.

The mineralization of NH_4-N was not significantly different among the amendments and it is assumed has a similar effect on plant growth across the amendments. The mineralization of NO_3-N was significantly different among the amendments. This indicates plant growth in these amendments may differ due to an effect of P and of N. When the plant growth studies are conducted, focusing on a single nutrient, it is important to remember the observed effects may be due, in part, to an interaction effect between several nutrients supplied by the amendments.

3.4.2 *Evaluation of Amendment Ability to Supply P to Soybeans*

The previous study in chapter 2 showed soybean BNF is limited by the availability of P. However, due to forecasted P shortage (Martin et al., 2007; Filippelli, 2008) it will not be possible to increase STP using commonly used PR fertilizer. However, the amendments contained other nutrients which confounded the effects. Most importantly, the effect of N availability is confounded with BNF. Generally, all measured growth parameters were equal or greater than the P fertilizer control.

The Ontario soil increased the majority of measured parameters significantly over the Nova Scotia soil. This could be caused by a higher functioning microbial community in the Ontario soil. It is more likely the better performance is due to the background nutrient levels and availability of nutrients in the soils as shown in the mineralization study (section 3.3.1). The zero BNF-N obtained for the control treatment for the Ontario soil compared to the 19.74% N derived from BNF in the Nova Scotia soil further suggests this. In chapter 2 it was observed the Ontario soil performed better than the Nova Scotia due to the lower sorption ability of the Ontario soil; a factor undoubtedly influencing growth in the response to the diverse amendments in this trial. The fertilizer treatments performed well across all measured parameters. The fertilizer 30 treatment

did not perform better than the fertilizer 15 treatment which was shown in Chapter 2. This is to be expected as the results of the previous study in chapter 2 suggests a strong response, in these soils, of soybean growth, nodulation and BNF to readily soluble P applied at rates up to 45 and 90 mg kg^{-1}. Using the fertilizer treatments as a guide the effectiveness of the other amendments can be critically evaluated.

The the MSW treatments preformed similarly to the fertilizer with respect to plant growth, nodulation, N and P uptake and BNF-N. This shows the MSW has the ability to supply additional nutrients causing different effects on the soybean growth. This effect has been observed in other studies (Hargreaves et al., 2008; Warman et al., 2009). In contrast, the shoot and total plant dry matter, leaf area, shoot N and P concentration, shoot N and P uptake and BNF-N decreased with PR compared to the fertilizer treatments. The MSW treatments do have higher N uptake and BNF-N than the fertilizer treatments suggesting the MSW treatments increased N availability.

The CG treatments also performed as well as or better than the fertilizer which has been observed in previous studies (Gaterell et al., 2000; Ueno and Fujii, 2001; Johnston and Richards, 2003; Ponce and De Sa, 2007). However, the CG 60 treatment constantly had numerically lower growth measurements than then CG 45 treatment in both soils. This suggests there is an optimal fertilization application of CG between 45 and 60 mg P kg^{-1} for soybean growth. As well, the nodule parameters and BNF were lower than in the CG treatments (Table 3.8). This decrease in nodule growth is likely due to the increased N availability in the amendment. However, this is not the case in the Nova Scotia soil. The nodule DM continued increase in nodule growth in the Nova Scotia soil is likely due to the P sorption capacity of the soil. Clearly, soil chemistry has an impact on the effects of CG.

All the tested amendments affected the soybean growth at the same level or higher than the P fertilizer, except for the partially solubilized PR. Even the shoot P concentration and P uptake were equal to or higher than the fertilized treatments. However, MSW and CG contained higher levels of plant available N which decreased BNF with increasing application rate. This decrease of nodule number and DM is a trend in response to N supplied with the amendments (Lynch et al., 2005), as noted, for the CG in particular. In addition, the plant response to the added N supplied with CG and MSW.

The latter is further supported by a general increase in non-nod N uptake with the increased application rate of these products.

An important factor to consider when considering which soil amendment to use is the amount and cost of the material. In terms of the three amendments used in this study, 0.5, 0.08 and 11 T ha^{-1}, Crystal Green, partially solubilized PR and MSW compost respectively, would need to be applied to achieve a target of 30 mg P kg $soil^{-1}$ (dry mass basis). The cost of the amendments would need to be considered and product recommended on cost vs. effectiveness may change.

CHAPTER 4 : CONCLUSION

The soybean experiments conducted using P fertilizer showed P limits BNF. In addition, soil type and chemistry affected the reaction of BNF to added P. It was determined BNF in soybeans was optimized at 45 to 90 mg kg^{-1} added P. This suggests the BNF on many organic dairy farms will be affected by low STP. It will become necessary to maintain STP levels in order to maintain a source of N input through legume BNF.

The alfalfa experiment conducted using P fertilizer showed a plant's life cycle affects the reaction to added P. The alfalfa growth was only affected by added P during the first growth period, following the second and third growth periods were unaffected by added P. However, the soil type and chemistry did have an effect on alfalfa growth, with generally greater growth in the Ontario soil. This increase in growth was observed in all experiments and both plants.

The final study evaluating the ability of three organically acceptable or potentially acceptable amendments showed there are products which have the ability to provide adequate P for plant growth. Two of the three products, MSW compost and Crystal Green®, positively affect soybean growth at the same level or higher than the synthetic P fertilizer, when applied at equivalent total P application rates while PR partially solubilized by citric acid (mimicking the action of *Aspergillus niger* by-products) had a decreased response compared to the P fertilizer. However, BNF was lessened by higher application rates of MSW and Crystal Green® due to the N availability in these amendments. In addition, the shoot P concentration and uptake was equal to or higher than in the fertilized soybeans. MSW and CG products were determined to be suitable P sources.

4.1 FUTURE OUTLOOK

This study is consistent with previous literature stating P limits the amount of N which can be obtained from legume BNF, but improves our understanding as to whether this is also true for soils typical of long term organically managed dairy farms. The results suggest organic dairy farms will likely experience a drop in BNF-N in the future

due to a decline in plant available P. The effects of the P limitation were influenced by the soil texture and chemistry. These sets of studies were conducted under a controlled environment which reduces the effects of other influencing factors including AMF colonization. To provide recommendations for STP levels to optimize BNF, it is necessary to test a wide range of P levels across varying soil types.

There are amendments which have the ability to supply adequate levels of P to sustain plant growth and BNF. Due to the forecasted PR depletion, further research will be necessary to determine soil amendments to supply P for plant growth. MSW compost is a suitable source, but is limited by its large volume and varying nutrient contents. Crystal Green®, a struvite product, has exhibited an ability to supply plant available P equal or greater than P fertilizer. However, the product did decrease BNF-N in the Nova Scotia soil, particularly at higher application rates, which was reflected in an equal N uptake in the non-nod reference plants. This means the Crystal Green® product had the ability to supply significant amounts of N. As the need for closing the rural-urban nutrient loop increases, it is expected the popularity of products like Crystal Green® will increase.

The future of BNF and alternative P sources is uncertain. However, as research continues to move forward the understanding of various options to address these challenges and their environmental sustainability will increase.

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APPENDIX 1: RAW SOIL NUTRIENT ANALYSIS

Table 1: Nutrient analysis of the bulk soil. All tests were performed by the Nova Scotia laboratory unless otherwise noted.

Nutrient	Ontario	Nova Scotia
Total Carbon**	25.4 mg kg^{-1}*	24.1 mg kg^{-1}*
Total Nitrogen**	2.56 mg kg^{-1}*	3.08 mg kg^{-1}*
Phosphorus	8.4 mg kg^{-1}**	8.1 mg kg^{-1} (37 P_2O_5 kg ha^{-1})
Potassium	245 mg kg^{-1}**	42 mg kg^{-1} (101 K_2O kg ha^{-1})
pH	7.3**	6.5
Magnesium	300 mg kg^{-1}**	335 mg kg^{-1} (671 kg/ha)
Calcium	2385 mg kg^{-1} (4771 kg ha^{-1})	1369 mg kg^{-1} (2737 kg ha^{-1})
Sodium	9.6 mg kg^{-1} (19 kg ha^{-1})	20 mg kg^{-1} (40 kg ha^{-1})
Sulphur	15 mg kg^{-1} (30 kg ha^{-1})	8 mg kg^{-1} (16 kg ha^{-1})
Aluminum	823 mg kg^{-1}	872 mg kg^{-1}
Iron	151 mg kg^{-1}	200 mg kg^{-1}
Manganese	126 mg kg^{-1}	116 mg kg^{-1}
Copper	1.50 mg kg^{-1}	1.19 mg kg^{-1}
Zinc	4.9 mg kg^{-1}	1.9 mg kg^{-1}
Boron	$\leq$ 0.50 mg kg^{-1}	1.64 mg kg^{-1}
Cation Exchange Capacity	13.1 meq $100gm^{-1}$	15.4 meq $100gm^{-1}$

* Viro MAX CN Macro Elemental Analyzer
** these tests were performed by the University of Guelph Laboratory Services

APPENDIX 2: ABBREVIATIONS USED IN CHAPTER 3 TABLES

NS	All treatments in Nova Scotia soil
NS Ctl	Nova Scotia Control or 0 mg kg^{-1} added P
All Other NS	All Nova Scotia treatments excluding the previously stated treatment
NS Fert	All Nova Scotia fertilizer treatments (15 and 30 mg kg^{-1} added P)
NS Fer 15	Nova Scotia fertilizer treatment at 15 mg kg^{-1} added P
NS Fert 30	Nova Scotia fertilizer treatment at 30 mg kg^{-1} added P
NS CG	All Nova Scotia Crystal Green® treatments (includes 15, 30, 45 and 60 mg kg^{-1} added P- depends upon the experiment)
NS CG 15	Nova Scotia Crystal Green® treatment at 15 mg kg^{-1} added P
NS CG 30	Nova Scotia Crystal Green® treatment at 30 mg kg^{-1} added P
NS CG 45	Nova Scotia Crystal Green® treatment at 45 mg kg^{-1} added P
NS CG 60	Nova Scotia Crystal Green® treatment at 60 mg kg^{-1} added P
ON	All treatments in Ontario soil
ON Ctl	Ontario Control or 0 mg kg^{-1} added P
All Other ON	All Ontario treatments excluding the previously stated treatment
ON Fert	All Ontario fertilizer treatments (15 and 30 mg kg^{-1} added P)
ON Fert 15	Ontario fertilizer treatment at 15 mg kg^{-1} added P
ON Fert 30	Ontario fertilizer treatment at 30 mg kg^{-1} added P
ON CG	All Ontario Crystal Green® treatments (includes 15, 30, 45 and 60 mg kg^{-1} added P- depends upon the experiment)
ON CG 15	Ontario Crystal Green® treatment at 15 mg kg^{-1} added P
ON CG 30	Ontario Crystal Green® treatment at 30 mg kg^{-1} added P
ON CG 45	Ontario Crystal Green® treatment at 45 mg kg^{-1} added P
ON CG 60	Ontario Crystal Green® treatment at 60mg kg^{-1} added P
ON MSW	All Ontario MSW Compost treatments (includes 15 and 30 mg kg^{-1} added P)
ON MSW 15	Ontario MSW Compost treatment at 15 mg kg^{-1} added P
ON MSW 30	Ontario MSW Compost treatment at 30 mg kg^{-1} added P
ON PR	All Ontario partially solubilized PR treatments (includes 15 and 30 mg kg^{-1} added P)
ON PR 15	Ontario partially solubilized PR treatment at 15 mg kg^{-1} added P
ON PR 30	Ontario partially solubilized PR treatment at 30 mg kg^{-1} added P