ABSTRACT

Title of Thesis: UTILIZING HYBRID POPLAR TREES TO PHYTOREMEDIATE SOILS WITH EXCESS PHOSPHORUS

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Phytoremediation, using plants to remove soil pollutants, has been suggested as a method to remove P from over-enriched soils. This research investigated the potential of utilizing hybrid poplar trees to remove excess P from soils associated with long-term poultry manure application. Hybrid poplar clones were planted in Snow Hill, MD, on three fields differing in previous poultry manure applications with Mehlich-3 soil-test P levels of 261, 478, and 982 mg P kg^{-1}. During this two year study, soil P decreased on fields planted with hybrid poplar; the magnitude of the reduction was positively associated with initial soil-test P. Plant tissue P concentrations increased with soil P concentration. However, factors other than plant uptake were hypothesized to contribute to the soil-test P reductions. Results suggest that hybrid poplars have the potential to phytoremediate soils with excess P but that soil chemistry also impacts the fate of available P in the soil.

UTILIZING HYBRID POPLAR TREES TO PHYTOREMEDIATE SOILS WITH EXCESS PHOSPHORUS

by

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CHAPTER 1: LITERATURE REVIEW

Phosphorus Impacts on Water Quality

Phosphorus (P) is an essential element for plant and animal growth. However, excessive P inputs to fresh water ecosystems contribute to water quality degradation by accelerating the eutrophication of the system.

The environmental impacts of eutrophication restrict water use for drinking, recreation and the fishing industry. This occurs due to an increase of algal blooms and other aquatic plant growth (mainly undesirable weeds) which can block sunlight from penetrating the water, thereby decreasing the photosynthesis rates of underwater grasses. Loss of these desirable freshwater grasses can destroy the habitats of many aquatic species and disrupts the food chain of the ecosystem.

The increase of algae biomass causes additional problems when the algae begins to die. Microorganisms that decompose the organic material can substantially deplete the dissolved oxygen levels in the water resulting in fish kills and shellfish mortality.

While algae can be harmful due to an unnaturally high abundance, some algae can also produce a toxin. While less than 2% of more than 700 known species of algae in the Chesapeake Bay produce toxic substances, economic losses have been associated with their effects. Maryland's recreation and fishing industry lost revenue with precautions taken to protect the public from blooms of harmful algae like the dinoflagellate *Pfiesteria piscicida* (Magnien, 2001). Increases in *Pfiesteria* growth have been associated with the presence of P in the water (Macilwain, 1997).

Excess nutrients can also produce dense blooms of cyanobacteria (previously known as blue-green algae) which can kill fish, cause bad odors and problems in drinking water. Cyanobacteria entering a water treatment system using chlorine can react with the chlorine to form carcinogens known as trihalomethanes (Carpenter et al., 1998). These toxins can kill livestock and pose health issues for humans.

Total P concentrations exceeding 0.05 mg/L in streams entering lakes/reservoirs and 0.025 mg/L within lakes/reservoirs accelerates eutrophication (Daniel et al., 1998). However, current water quality improvement criteria treat the cause of eutrophication rather than its effects. Therefore reduction of P inputs is targeted to lower the eutrophication of water bodies and improve water quality.

Sources of Phosphorus

Phosphorus pollution results from point and nonpoint sources. Point sources such as effluent piped from municipal sewage treatment facilities and other industries discharge P directly into a water system. While point sources are of concern, they are more manageable and easier to regulate with environmental policies than nonpoint sources. Point source P loads to the Chesapeake Bay declined 56% (2.3 million kg/yr) between 1985 and 2001 due to increased regulation (Chesapeake Bay Program, 2003).

Nonpoint sources of pollution caused by soil erosion and runoff have become the major source of water pollution in the US and they are less easily targeted for control (Carpenter et al., 1998). Nonpoint sources of P, mainly runoff from agricultural soils, are a major cause of eutrophication of water bodies in the US and Europe (Heathwaite et al., 2000).

In order to achieve their Chesapeake 2000 agreement commitments, the Chesapeake Bay program partners set an annual average target load for P entering the bay from all sources at 12.8 million pounds (5.8 thousand kg) (Chesapeake Bay Foundation, 2004). However, data for 2004 suggest that P levels entering the Bay were almost double the target levels (Chesapeake Bay Foundation, 2004). Of this total load, nonpoint sources of agriculture runoff were estimated to be responsible for 41% of the P entering the Chesapeake Bay (MDA, 2003).

Forms of Soil Phosphorus

Phosphorus exists in various chemical forms in the environment and can be classified into four general categories: dissolved P, labile organic P, stable organic P and inorganic solid-phase P (Coale, 2000). The pools of P are in equilibrium and therefore changes to one will affect the other forms of P in the soil.

Dissolved P or soil-solution P is dominated by orthophosphates, inorganic polyphosphates and organic P compounds that will pass through a 0.45 μm filter. Plants absorb P from the dissolved P pool in the form of orthophosphate ions. The specific chemical form the plants take up is influenced by the soil solution pH. In acidic soils, the monovalent anion $H_2PO_4^-$ dominates. Conversely, the divalent anion HPO_4^{-2} dominates in alkaline soils. In neutral soils, both forms have similar availability for plant uptake.

The labile organic P pool contains plant and animal residues and organic matter P as well as microbial populations within the soil. Additions of P to the soil from agricultural inputs affect all four pools.

The inorganic solid-phase P pool includes sediment bound P occluded in clay particles, amorphous P complexes and mineral P. The stable organic P pool is less well defined and acts more as a sink for reactive P.

Although P uptake by agronomic crops can stabilize soil P pools, excess P can accumulate due to unbalanced and excessive agricultural inputs. This reduces the soil's capacity to retain additional P, which accelerates the movement of P in runoff into watercourses.

Environmental thresholds for soil P based on specific soil tests have been established in many states. In Maryland and Delaware, 150 mg kg^{-1} Mehlich-3 P is the critical value above which P-based management should be implemented (Sims, 2002). Soil test P values above critical thresholds and limits indicate an increased potential for P accumulated in the soil to leave the site. The increase in P concentrations in agricultural drainage water over time reflects the accumulation of P in soils (Sharpley et al., 2000).

Phosphorus Additions to Soils

Agricultural soils receive P from the additions of animal manures, synthetic fertilizers, direct livestock excreta, municipal sludge, and wastewater. Precipitation also deposits P onto soils but in insignificant amounts.

Phosphorus Additions from Poultry Manure

Soil nutrient pollution has been associated with intense livestock production. For the past decade in the US, cattle, pig and poultry numbers have increased while the number of farms on which they are produced has decreased (Sharpley, 2004). This intensification of animal operations generates amounts of manure that often exceed the capacity of nearby farmland to use and retain the nutrients; thus limited areas receive excessive amounts of manure (Pant, 2004).

The type of animal operation also plays a role in the distribution of high P soils associated with farmland (Sims, 2000). The magnitude of P surpluses on farms usually follows the order: poultry > swine > dairy > beef > mixed animal production farms (Sims, 2000). Poultry manure contains high concentrations of P because broilers fail to digest more than 50% of the P in their feed (Ventsias, 2002). The P content in poultry manure is also higher than swine or dairy manure relative to N content. Manure application is typically based on the N application rate, and for the same N application rate, poultry manure supplies 87 kg P ha^{-1} compared to 52 kg P ha^{-1} for dairy manure and 77 kg P ha^{-1} for swine manure (Coale, 2000).

In Maryland, broiler production leads the state's agricultural cash receipts, accounting for 31% of their total cash revenue for the year 2002 (MDA, 2002). While poultry is still the leading agricultural product for the state, the production numbers have dropped in the past three years. In 2003, Maryland produced an estimated 2.9 million chickens compared to 3.5 million in 2002 (USDA, 2004).

The large number of high density poultry operations in Delaware, Maryland, and Virginia and the associated manure applications to farm land has been a regional focus for controlling the increased inputs of P to the water of the Chesapeake Bay. The farmers in the Delmarva Peninsula region produced an estimated 589 million broilers in 2000 which generated an estimated 635 thousand tons of poultry manure, most of which was applied to the land (Lichtenberg et al., 2002).

The most common disposal method for poultry litter is by land application as fertilizer. Approximately 50% of the cropland in the Delmarva Peninsula receives animal manure or sewage sludge (Boesch, 2001). Soil P build-up occurs and is especially apparent where land application of fertilizer and/or manure from animal operations has continuously occurred. The additions of poultry litter to the land in the Chesapeake Bay watershed have contributed to the increasing soil test P levels in agricultural soils over the past forty years (Coale, 2000).

Phosphorus and Crop Requirements

Farmers control the amount of P added to their soils through land management decisions. Imbalances occur because P is applied at levels that are in excess of the amounts required for optimum crop yields. Only an average of 30% of the fertilizer and feed P input to farming systems is output in crop and animal produce (Sharpley et al., 1999).

Until recently, recommended manure land application rates have been based on manure N content and the N requirement of crops. Since the ratio of N/P taken up by agronomic crops ranges from 7:1 to 11:1 and manures have N/P ratios from 2:1 to 6:1, more P was applied to the land than the crops used (Heathwaite et al., 2000). This has led to increased concentration of P in soils that received manure fertilization, especially in soils receiving poultry litter fertilization because the ratio of N/P required by crops is more than double the plant-available ratio in poultry manure (Boesch, 2001). Repeated applications of poultry manure based on crop N requirements result in P application rates several times greater than crop P removal rates. In states with intensive poultry operations like Delaware, Maryland and Virginia, this has led to a buildup of soil P.

Consequently, these states have established P-based nutrient management plans and have placed restrictions on manure applications in areas where soil test P values are high.

However, even with restrictions on fertilization, once P levels have become excessive from years of application, traditional agronomic crops alone will not adequately reduce the P levels of these soils. It has been estimated that without further P addition, 16 to 18 years of cropping corn (*Zea Mays* L.) or soybeans (*Glycine max* L. Merr.) would be needed to deplete soil test P (Mehlich-3) in a Portsmouth NC sandy Ultisol from 100 mg P kg^{-1} to the threshold agronomic level of 20 mg P kg^{-1} (McCollum, 1991). If manure continues to be applied to soils high in P, the P soil surplus will increase. Relying on traditional agronomic crops to correct this imbalance will increase the likelihood that the P will leave the site and enter a nearby water body.

Phosphorus Transport

Phosphorus from agricultural land is transported in drainage water through surface (overland flow) and subsurface pathways. Surface flow associated with erosion events and surface runoff carries particulate or dissolved forms of P offsite. It is the main mechanism of P transport and is the most manageable pathway.

Sediment or particulate P includes inorganic P attached to soil particles and plant residues bound to clay and in soil minerals and organic P in the form of labile and stable organic matter. Soil and plant material are eroded during rainfall or irrigation and flow events transport the particulate P through the landscape and ultimately into a body of water. As the number and severity of erosion events increase, the amount of particulate P in the runoff increases. Particulate P constitutes 60% to 90% of P transported in surface runoff from most cultivated land (Sharpley et al., 1992).

Dissolved P is released when rainfall or irrigation water interacts with a thin layer of surface soil and/or plant material before leaving the field as surface runoff (Sharpley et al., 1999). Dissolved P comprises a larger portion of the total P in runoff from uncultivated lands (pasture and forested land). This is due to the low sediment load associated with uncultivated lands where little sorption of the dissolved P occurs. Therefore, the loss of dissolved P in the runoff from uncultivated fields is higher than that from highly eroded cultivated fields.

The site specific characteristics that regulate P transport with surface runoff include erosion, type of P load, loss of P from crop residues, soil texture and structure, cultural practices, and the hydrology of the individual field (Coale, 2000). Distance of agricultural fields to sensitive water bodies like the Chesapeake Bay is also a factor when considering the impact of P transport on water quality (Daniel et al., 1998).

To a lesser extent, P is transported via subsurface pathways in which P flows laterally through the soil. P moves vertically from the soil surface through macropores, artificial drainage channels and preferential flow paths like fissures, cracks, root channels, earthworm burrows. Once the P enters into the saturated zone of the soil, it travels laterally and can enter into bodies of water.

The loss of P from subsurface flow is usually less than the loss from surface flow because as the water moves below the soil surface the P is sorbed to solid particles (Coale 2000). Subsurface pathways are more variable and dependent on the physical characteristics of the soil such as the water infiltration capacity, water percolation rate, and depth to the water table.

The loss of P from agricultural land is dependent on the relative importance of surface and subsurface runoff in a watershed area but also land management practices, and the amount, form, and availability of P in soil (Daniel et al., 1998). However, because of the interactions of the varying forms of P and the complexity of their transport, P transformation and fate are difficult to predict. In general terms, dissolved P is immediately available for plant uptake while particulate P is primarily a long term source of P for aquatic biota.

Phosphorus Management Practices

Efforts to reduce agricultural nonpoint source P pollution have focused on farmer education, technical assistance, and cost-share programs to promote the use of practices that reduce nutrient and sediment losses from agricultural land. These practices, referred to as Best Management Practices (BMPs), include Soil and Water Conservation Plans (SWCPs), Nutrient Management Plans (NMPs), and state agricultural cost-share programs.

Soil and Water Conservation Plans (SWCPs)

SWCPs involve recommended practices that focus on improving surface drainage and reducing sediment transport. In Maryland, riparian buffers or vegetated filter strips, including constructed wetlands and natural wetlands are recommended. Riparian buffers are forested lands near or along stream banks that are adjacent to agricultural land. The vegetation in the buffer strips serves to reduce soil erosion and sedimentation in the drainage waters. The strips help slow the velocity of agricultural surface runoff and retain nutrient-bearing sediments. The vegetation also helps to stabilize stream banks and shores protecting them from eroding due to storms and flood events. Riparian buffers have been more effective at sediment removal than in reducing total P (Abu-Zreig et al., 2003).

Other SWCPs involve management practices that control P loss from surface runoff include conservation tillage and crop residue management, terracing, contour tillage, cover crops, and settlement basins (Sharpley et al., 1999). These reduce the impact of rainfall and irrigation by decreasing runoff and erosion volume and velocity and increase the nutrient storage capacity of the land.

Nutrient Management Plans (NMPs)

In 1998, the Maryland General Assembly passed the Maryland Quality Improvement Act (WQIA) which requires farmers grossing $2,500 a year or more and those with livestock operations with 8,000 pounds or more of live animal weight to operate utilizing a NMP. The WQIA advocates NMPs that balance crop nutrient needs with fertilizer or manure applications in order to achieve realistic crop yields, minimize nutrient losses to surface water and maintain soil productivity. The plan does this by providing farmers with crop and field specific recommendations for manure, fertilizer, and/or sludge application based on past and present field practices, soil analysis and proximity to water bodies. Initially, the WQIA mandated that all applicable farmers implement NMPs based solely on N but by July 1, 2005, NMPs must be based on both N and P.

As of December 31, 2003, approximately 5,200 Maryland farmers submitted NMPs under the WQIA which encompassed over one million acres of land, accounting for approximately 85% of the land regulated by WQIA (MDA, 2003).

Virginia and Delaware, following Maryland's lead, passed nutrient management laws in 1999 that mandated P-based NMPs where animal manure is applied to fields.

Cost-Share Programs

Cost-share programs provide monetary incentives to landowners for implementing certain NMPs and the Maryland Agricultural Water Quality Cost-Share (MACS) Program provides financial assistance to farmers who hire professional consultants to prepare their NMPs. Maryland also has a manure transport program for animal producers with high soil P levels or limited land who can receive payments to transport excess manure to an alternative location.

The Conservation Reserve Program (CRP), USDA's largest conservation and environmental program, was authorized through the Food Security Act of 1985. CRP is a voluntary program that offers farmers payments to take portions of their land out of agricultural production and establish long-term conservation practices.

Effectiveness of Management Practices

Despite aggressive implementation of BMPs, P losses from croplands into the Chesapeake Bay watershed have not been significantly reduced (Boesch, 2001). For example, in the Choptank River drainage area of the watershed from 1990 to 1995 N concentrations increased and P concentrations remained at elevated levels (Boesch, 2001).

Although the intent of the NMPs is that crops will utilize the P in the soil if the levels applied are regulated, Coale (2000) suggests that with traditional agronomic crops this reduces the P level of very high P soils, but the rate of soil P reduction is slow and soil type specific.

Many management practices limit water P contamination by controlling P transport but *in-situ* reduction of high soil P concentrations is also important. Recent research suggests P management should focus both on minimizing P loss through surface runoff and reducing the P concentration of surface soils (Butler, 2005). One potential method to rapidly remove excess soil P in the field is to cultivate plant species that will take up significant amounts of soil P. Using plants to phytoremediate P has been suggested as a vegetative management technique by Delorme et al. (2000).

Phytoremediation

Phytoremediation utilizes plants to absorb and translocate targeted soil contaminants into their shoots and leaves. After the soil contaminant is accumulated in the plant tissue, it is harvested to remove the pollutant from the site. Plant species that are considered to have the greatest potential for phytoremediation are typically hyperaccumulators, plants that accumulate 100-1000 fold the levels of the targeted elements (typically metals) normally present in most plants. However, since the goal of phytoremediation is soil contaminant removal, the amount of harvestable material is also an important consideration in selecting species because the total uptake is dependent on the mass of the plant parts removed from the site and the concentrations of the element in the harvested plant parts.

Delorme et al. (2000) investigated traditional agronomic crops as potential phytoremediators of excess soil P. Although the plant species differed in their ability to extract soil P, the common row crops examined removed nominal quantities of soil P from excessive soil-test P fields. The crop species exhibiting the highest concentration of P were the shoots of collard (*Brassica oleracea*) and corn (*Zea mays*) (6.3 g P $kg^{-1}$and 4.9 g P kg^{-1} respectively), the lowest was the shoots of canola (*Brassica napus*) (2.0 g kg^{-1}). Corn (*Zea mays*) and Indian mustard (*Brassica juncea*) showed the highest potential P removal, with the harvest of the stover and seed P uptake was 114 kg P ha^{-1} and 108 kg P ha^{-1}, respectively.

Another study conducted in the Mid-Atlantic region of the USA measured nutrient uptake in harvested corn grain from 23 site-years (Heckman et al., 2003). They found grain P concentration ranging from 2.2 to 5.4 g P kg^{-1} coupled with a yield ranging from 4.9 to 16.7 Mg ha^{-1}. Although their results were similar to other published reference values for nutrient removal, the usefulness of the results was questioned due to high variation among averages. Based on the rates of uptake from traditional agricultural crops, it would take years to reduce P in highly contaminated soils to acceptable levels.

Total P contents of plant materials typically range from 2 to 8 g P kg^{-1} (Leinweber, 2002). For a plant to be a good candidate for P phytoremediation, it should have tissue P content at levels surpassing “normal” plant concentrations, high biomass and a post-harvest economic value (Delorme et al., 2000). Hybrid poplar trees, recognized for their rapid accumulation of biomass and adaptability to a wide range of environmental conditions, are an agro-forestry crop that could meet these criteria to maximize P phytoremediation.

Hybrid Poplar Trees

Hybrid poplars are progeny from crosses between plants of two or more species within the genus *Populus*. The genus *Populus* contains over 25 species of deciduous trees including black cottonwood, eastern cottonwood, lombardy poplar, and aspen. Hybrid poplar clones combine desired characteristics not found within one *Populus* species. Hybrid crosses also create heterosis (hybrid vigor) in which the progeny have increased performance of traits beyond what is capable by either parent. Hybridization can also increase developmental homeostasis, resulting in greater phenotypic stability in varied environments (Stettler et al., 1996).

The majority of hybrid poplar breeding utilizes three native species: *Populus deltoides* (eastern cottonwood), *Populus balsamifera* (balsam poplar) and *Populus trichocarpa* (western black cottonwood) and two non-native species: *Populus maximowiczii* (Asian black poplar) and *Populus nigra* (European black poplar) (Demchik, 2002). Select parents are usually intermated and superior progeny are selected for the next generation of mating. Selected trees can be vegetatively propagated (cloned) and commercially released during any generation of the breeding process.

<u>Phosphorus Content</u>

Few studies of total P uptake have been reported in the scientific literature. A study conducted in France on a low P soil developed on alluvial deposits (ranging from sandy silt to silty clay) estimated biomass and nutrient uptake of hybrid poplars (*Populus trichocarpa* x *deltoides*). Total above-ground biomass after eight years for the clone '*Beaupré*' reached 89 t ha^{-1} dry matter and had average annual nutrient uptake rates over eight years of 92, 15 and 87 kg ha^{-1} for N, P and K, respectively (Berthelot, 2000). The P content of hybrid poplars from this study was about double that of average broadleaf (deciduous) stands. Compiled world data from various soils show an average annual uptake for broadleaf stands of 95 kg of N, 6 kg of P and 48 kg of K (Berthelot, 2000). While these studies were conducted on relatively low P soils, growing hybrid poplars in soils with excess P has not been the focus of any published scientific study. However, increased biomass has been associated with high soil nutrients and increased P uptake may follow suit.

Biomass

Hybrid poplar trees are known to be among the fastest growing trees in North America (Eckenwalder, 1996), able to out-produce most natural stands by a factor of 5-6 times (Demchik, 2002). In most parts of the USA, hybrid poplars can grow up to 3 m per year (Landmeyer, 2001). Hybrid poplar are responsive to fertilizer and grow taller, produce more leaves and stems and biomass when grown under increased treatments of ammonium-N, nitrate-N and phosphate-P (Marler, 2001).

Hybrid poplars have also been shown to respond to applications of composted municipal sewage sludge. The addition of compost to the soil increased the nutrient concentration. Three years after transplanting, hybrid poplar tree heights averaged 2.3, 4.4 and 4.8 m on fields amended with 0, 150 and 300 Mg ha^{-1} of municipal sewage sludge, respectively (McIntosh et al., 1984).

In a greenhouse fertilization experiment, hybrid poplar biomass increased in response to P when other nutrients were not limiting. Increasing the P concentration in the growth medium from 37 to 356 mg P kg^{-1} increased the mean tree dry weight by 53%.

Increasing the P concentration in the growth medium from 37 to 500 mg kg^{-1} increased the mean stem volume 47% (Van Den Driessche, 2000).

Hybrid poplars possess several traits that allow for increased uptake of nutrients and therefore more biomass production including: high transpiration rates of approximately 100 liters/day optimally for a 5 year old tree (Chappell, 1997), a perennial root system which allows for a longer phytoremediation period, adaptability to environmental change, and the ability to produce vigorous regrowth after being harvested. Hybrid poplars also have deep root systems with a fine root mat at the soil surface that aids in nutrient absorption and limits nutrient movement into the ground water. A stand of hybrid poplars can have as much as 300,000 km per hectare of total root length (Quinn et al., 2001). The fibrous nature of the roots allow them to deeply penetrate the soil giving them the potential to remediate soil, groundwater and saturated soil media.

Economic Markets

Hybrid poplar trees are harvested and sold for fiber (pulp, lumber and plywood), bioenergy (heat, power and fuel) and biobased products (organic chemical and adhesives). Hybrid poplar production can offer farmers the opportunity to diversify their income. Hybrid poplar production in northern Minnesota was found to be competitive with corn and soybeans production for financial return on many farms (Demchik, 2002).

Additional Uses

In addition to income, hybrid poplar trees provide ecological services. These environmental benefits include serving as an odor block which is particularly important for use near livestock operations, providing wildlife habitats and protection, and sequestering significant amounts of carbon.

Hybrid poplar trees have also been effectively used for land reclamation projects. In Glen Burnie, MD, ERCO Inc. applied biosolids to abandoned gravel spoils and planted hybrid poplar trees. During the 6-9 year tree rotation, the N concentration of the biosolids dropped from between 3.4% to 1.2% N, indicating that the large root mass of hybrid poplar trees may have been acting like a nutrient sponge (Kays, 1999). With the help of hybrid poplars, this land application technique provides a method for disposing of biosolids while reclaiming the land and creating wildlife habitat.

Another land reclamation project utilized hybrid poplars for maximizing biomass production on gold mine tailing in the Black Hills (Bjugstad, 1986). In areas like mine tailings where adverse environmental conditions exist, hybrid poplars are able to become readily established and provide the necessary cover to stabilize the sites.

Overall Objectives

Hybrid poplar trees possess traits that make them a desirable candidate for P phytoremediation. This thesis seeks to examine the P uptake of two hybrid poplar clones at three fields that vary in soil P concentration and management practices. High tissue concentration and biomass could identify hybrid poplar clones for potential use for phytoremediaton of P. By measuring and comparing the P concentrations of the leaf and stem tissue of two hybrid poplar clones, this thesis will evaluate the capacity of these hybrid poplar clones for P phytoremediation. Based on soil-test P concentrations, this thesis also intends to determine if the hybrid poplars can significantly lower excess soil P levels during the first two years following transplanting. By examining hybrid poplars ability to phytoremediate excess soil P this thesis will provide preliminary data on the potential of this agroforestry practice to improve the water quality in the Chesapeake Bay Watershed.

CHAPTER 2: MATERIAL AND METHODS

Site Description

The experiment was conducted on privately owned farmland in Snow Hill, MD, Worchester County. Three agricultural fields were selected based on previous land use and prior application of poultry manure to represent low, medium, and high levels of soil P relative to levels found in soils associated with poultry production on the coastal plain of MD. Because these are relative P levels, even the Low P field contained excessive soil P in which the nutrient concentration was more than adequate for optimum plant growth.

Fields were designated as Low, Medium, and High based on Mehlich-3 P test values at the start of the experiment. Soils were classified as Mattapex silt loam for the Low field and Matapeake silt loam for the Medium and High fields (Hall, 1973).

The Low and Medium fields were previously used for soybean and corn production. The most recent applications of soil amendments to the Low and Medium fields were in 2001 when poultry litter (3.58% N, 2.84% P_2O_5) was spread at a rate of 6.7 Mg ha^{-1} prior to planting the fields with corn. No soil amendments were added to these fields for the years 1995-2000 and 2002 when they were planted to soybean. In 1993 and 1994, corn was planted in the Low and Medium fields and poultry litter was applied at a rate of 11.2 Mg ha^{-1}. The Medium field was formerly used for temporary periods of poultry litter retention.

The High field had not been tilled for approximately twenty years and was adjacent to a chicken house which had not been used since 1991 and was removed from the site in 2001.

Weather

The temperature and precipitation means for Snow Hill, MD are presented in Table 1 for years 2003 and 2004 and the 30 year average. For both growing seasons, the temperatures were very similar to the 30 year averages. The precipitation, however, at Snow Hill was higher than average in the spring of 2003 and the summer of 2004. These growing seasons had more than adequate rain fall for plant growth except for June 2004. The precipitation was below average in June 2004, which could have affected plant growth that spring. But as the temperatures in the summer months increased, precipitation did as well, especially in August 2004 when rainfall was more than double the 30 year average. Several high volume summer storms could have created such a large spike in precipitation measurements. Such storms while not presenting a problem for plant growth do disrupt the soil and can cause heavy erosion events.

Table 1: Temperature and precipitation means by month in 2003, 2004 and 30 year averages for Snow Hill, MD.

	Temperature (°C)			Precipitation (mm)		
Month	**2003**	**2004**	**1971-2000**	**2003**	**2004**	**1971-2000**
Jan.	0	1	5	48	86	102
Feb.	4	6	7	136	48	87
Mar.	17	16	15	124	51	119
Apr.	22	25	24	113	140	82
May	30	40	33	134	94	93
June	41	41	41	108	53	87
July	46	45	45	170	100	114
Aug.	46	42	43	153	314	137
Sept.	39	38	38	183	82	95
Oct.	26	26	26	93	29	87
Nov.	21	19	17	92	140	84
Dec.	9	10	9	207	66	87

Experimental Design

Layout

Three experimental fields designated as Low, Medium, and High were established in the spring of 2003. Each field measured 9 m by 17 m which included border trees surrounding each experimental field. Prior to planting hybrid poplar cuttings, an initial application of RoundUp® (Glyphosate) was sprayed on the fields to kill weeds. A repeat application of RoundUp® was applied to the fields in May 2004. Weeds growing in the fields were also manually cut several times throughout the experiment.

Hybrid Poplar Clones

Two clones of hybrid poplar trees, DN 34 and OP 367, were selected for the experiment based on their reported high biomass accumulation rates in Maryland (Flamino, 2003). Clone DN 34 was obtained from Lee Nursery, Fertile, Minnesota and clone OP 367 from Broadacres Nursery, Hubbard, Oregon. Both clones are crosses between *Populus deltoides* (Eastern Cottonwood) and *Populus nigra* (Black European Poplar). The clones were shipped as cuttings each 22–26 cm in length and 1-2 cm in diameter. They remained in climate controlled storage until the time of planting.

June 2003, fifty cuttings of each hybrid poplar clone, DN 34 and OP 367, were planted in a completely randomized design on each of the three fields. The cuttings were planted on 1.5 m centers.

Prior to planting the cuttings were soaked in water for one hour. After the soil had been loosened with a dibble stick, cuttings were positioned into the ground with 3-5 cm above the surface. Tree tubes, 2 m tall with a 15 cm diameter, were placed around each cutting to protect the trees from animal browsing and weed competition.

Sampling and Analysis

Soil Sampling

Initial soil samples were taken in June 2003 immediately following planting. Final soil samples were taken in October 2004 following the harvest of all trees. The samples were collected on an individual tree basis at two depths (0 to 15 cm and 15 to 30 cm). Four subsamples were collected in a 15 cm diameter circle around each tree and composited for analysis. Initial soil samples were collected at each tree in each of the three fields and air dried. Based on statistical analyses (see statistical analysis section) of initial soil samples, it was determined that forty soil samples per field provided sufficient statistical power to find biologically meaningful differences in soil P concentration. Thus, final soil samples were collected at forty randomly selected trees per field and dried at 50°C for seven days. All soil samples were ground to pass through a 2mm mesh sieve and stored at room temperature until analysis.

Soil compaction measurements were taken utilizing a penetrometer (Dickey-john Soil Compaction Tester®) in October 2004. Five readings were taken in random locations in each of the three plots by placing the 2 cm cone probe of the penetrometer into the soil until maximum resistance.

Soil Analysis

Soil pH was determined with a pH meter (Mettler Toledo MP220®) in a 2:1 slurry of deionized water and soil that was mixed intermittently for one hour. Percent organic matter was determined by loss on ignition where 1 ml soil samples were predried at 120° C for 1 hour, weighed, then heated at 360° C for 2 hours and reweighed (Storer, 1984).

The soil P, K, Ca, Mg, Mn, and Zn were obtained using the Mehlich-3 extractant procedure (Mehlich, 1984) measured using inductively coupled plasma atomic emission spectrometry (ICP-AES). Sample preparation involved placing approximately 2.5 g of soil into a 100 ml plastic extraction beaker and adding 25 ml of Mehlich-3 extracting solution (0.2 *N* CH_3COOH + 0.25 *N* NH_4NO_3 + 0.015 *N* NH_4F + 0.013 *N* HNO_3 + 0.001 *M* EDTA) to each soil sample. The samples were shaken at 200 oscillations per minute for 15 minutes on a reciprocating shaker, filtered through Whitman #42 filter paper into vials, and analyzed using the ICP-AES.

Total C, H, and N were measured using the combustion method (Campbell, 1992). Each soil sample was finely ground using a mortal and pestle and approximately 0.2 g was placed into an aluminum capsule. The capsules were then placed into the Leco CHN 2000 Carbon-Hydrogen-Nitrogen Analyzer®. Upon combustion, the machine determined the percent C, H and N of each sample.

<u>Tree Sampling</u>

Throughout the growing seasons, the trees were monitored for survival, vigor and growth. Tree mortality and vigor were noted. Tree height was measured from ground to tip of main branch in September 2003 and 2004.

September 17, 2003, ten random trees of each clone were coppiced (cut and left to regrow) from each field. September 20, 2004, all trees were harvested from each field. The trees were cut at the base of the trunk approximately 5 cm above the soil line. The leaves and petioles were separated from the woody stems for sample preparation and analyses. Samples were dried at 50°C for seven days and weighed to determine biomass and ground to pass through a 2 mm mesh sieve for nutrient analysis.

Tree Analysis

The leaf and stem samples were analyzed for P, K, Ca, Mg, Mn and Zn using ICP-AES. Plant samples were prepared using a dry ashing procedure adapted from AOAC method 3.014(a) (AOAC, 1984) for analysis with the ICP-AES. Approximately 2 g of each dry plant sample was placed into an acid-washed 100 ml beaker. The samples were then ashed in a muffle oven at 480° C for 16 hours. After cooling, deionized water was added to wet the samples followed by 2 ml of concentrated HNO_3. The samples were heated to evaporate to near dryness and ten ml of 3N HCl was added to each sample, covered with a watch glass and heated to reflux for two hours with intermittent agitation.

The watch glasses were rinsed into the beakers with 0.1N HCl. The solution was poured from the beaker through 0.1N HCl wetted Whatman #40 filter paper into acid-washed 25 ml volumetric flasks. The beakers were rinsed twice and the solution was poured into the filter followed by two rinses of the filter paper with 0.1N HCl. The filters were drained and the solution was brought to volume with 0.1N HCl. The flasks were shaken and the solution was transferred into vials. The plant samples were then analyzed for nutrient concentrations using the ICP-AES.

For quality control and assurance, blank, duplicate and standard samples were analyzed along with the plant samples.   One blank sample was run every 10 plant samples to correct for any microelements in the acids or any small level of inadvertent contamination. Duplicate plant samples were run every 10 samples to monitor sampling variation. One standard reference sample from the National Institute of Standards and Technology (NIST) was run for each batch of samples (approximately 47 samples). Sample duplicates were within 10% agreement and all concentrations measured for the NIST standard were within the range specified by NIST.

Leaf and stem tissue were analyzed for N using a Carlo Erba Nitrogen gas chromatograph based on a procedure adapted by J. R. Peters Inc. The method is based on flash combustion where inorganic and organic samples are converted into gas and thereupon N oxides are reduced to elemental N.

Statistical Analysis

The resulting data were analyzed using Statistical Applications Software Version 9.1 (SAS Institute, 2002). The General Linear Model (GLM) procedure was used to test for differences in soil and tissue elemental concentrations and plant growth across the three fields. Data were log transformed prior to analyses if needed to correct for correlations between means and variances. Statistical significance of factors and their interactions were determined based on F-tests using Type III sums of squares. F-values are presented as a measure of relative importance of significant sources of variation. Relationships between the soil elemental concentrations, hybrid poplar P concentration and uptake were examined using the correlations procedure (CORR). LSD values were calculated and used to determine significant differences between means at the 0.05 alpha level.

CHAPTER 3: RESULTS AND DISCUSSION

Soil

Overview of Fields

Soils from the three experimental fields were analyzed to establish baseline levels for macro and micronutrients of each field before planting hybrid poplars. However, the mean soil P was the element of primary importance for these experiments and the initial soil P levels were used to classify the fields. The initial soil-test P concentrations were also used to estimate the change in soil P levels following two growing seasons. The initial means and standard errors of soil nutrients at the 0 to 15 cm and 15 to 30 cm depths are given in Table 2 and Table 3, respectively. The initial P concentrations for the soil surface (0 to 15 cm depth) averaged 261, 478, and 982 mg P kg^{-1}. Thus, the surface P concentrations of the Medium and High fields were two and four times the concentration of the Low field, respectively. The sub-surface soil (15 to 30 cm depth) mean P concentrations were 180, 600, and 584 mg P kg^{-1}. The subsurface P concentrations of the Medium and High fields were both three times the concentration of the Low field. Similar to the surface, the subsurface mean P concentration was lowest for the Low field. However, the Medium and High field P means were not significantly different.

The soils in this study were chosen to be representative of soils in the Delmarva Peninsula associated with long term poultry production. As such, the Mehlich-3 P soil-test values, even the Low field, were excessive according to the Maryland State Nutrient Management Regulations (MDA, 1999). The Mehlich-3 soil test P is used to estimate the

P available for plant uptake and the optimum Mehlich-3 soil P for most crops is 50 mg P kg^{-1} (Maguire and Sims, 2002). Thus, the initial soil test P concentrations far exceeded the expected agronomic optimum indicating the buildup of soil P above levels required by crops.

The means and standard errors for soil elemental composition for the three fields at the surface and subsurface depths two years after the hybrid poplars were planted are given in Table 4 and Table 5, respectively. These final P concentrations of the fields were 229, 379, and 703 mg P kg^{-1} at the surface and 120, 412, and 259 mg P kg^{-1} at the subsurface for the Low, Medium, and High fields, respectively. Although the final soil P concentrations still exceeded the agronomic optimum, each field had a significant decrease in soil P from year 2003 to year 2004 (Figure 1). The soil P of the Low, Medium, and High fields decreased 12%, 21%, and 28% for the surface soils and 33%, 31%, and 55% for the subsurface soils, respectively (Tables 6-7).

Analyses of variance were conducted on the soil collected at the end of the experiment to determine significant differences among the fields and also whether there were significant differences due the effect of clones, the coppice treatment, or their interactions. Although differences remained highly significant between fields, differences between clones, coppice treatment, and interactions were not significant for any of the soil elements at either depth with two exceptions; the interaction between field and clone was significant for the surface soil pH (α=0.05) and the subsurface soil P (α=0.05). However, the relatively small F-values associated with these interactions suggest that the interactions were weak and not important compared to the field differences.

On all three fields, Mehlich-3 P decreased substantially in the sampled soil profile possibly due to transport, uptake, and/or change in P chemistry. P in the surface may have been decreased due to soil erosion and/or sediment flow. Soil P at either depth could have also moved in the soil water through vertical or lateral flow in soil channels. The soil P concentrations also decreased due to uptake by the hybrid poplars (discussed in a later section). But since a comparison to an unplanted control field can not be made in this study, only speculations can be made as to the cause of the soil P decrease. However, it is likely that the growth of the hybrid poplar trees disrupted the equilibrium of the soil P pools producing an exudate that altered the form of soil P detected by the Mehlich-3 extractant (Chaney, 2005). This change in the form of soil P may have caused a significant reduction in the extractable Mehlich-3 P while only insignificantly decreasing total soil P. It is also probable that a proportion of Mehlich-3 extractable P changed over time due to interactions with other soil elements as well as changes in soil pH.

Table 2: Elemental soil composition means and standard errors with F-Values for differences among fields at depth 0 to 15 cm for 2003.

		%OM	pH	P	K	Ca	Mg	C	N
Field				mg kg^{-1}					
Low		**2.57 ± 0.02**	**6.3 ± 0.02**	**261 ± 4**	**268 ± 5**	**891 ± 12**	**115 ± 2**	**13.4 ± 0.1**	**1.15 ± 0.01**
Medium		**1.88 ± 0.03**	**5.7 ± 0.02**	**478 ± 10**	**219 ± 5**	**597 ± 12**	**56 ± 2**	**9.9 ± 0.1**	**0.92 ± 0.01**
High		**2.70 ± 0.05**	**6.0 ± 0.03**	**982 ± 24**	**234 ± 6**	**1567 ± 49**	**130 ± 5**	**15.6 ± 0.4**	**1.58 ± 0.04**
Source of Variation	df	F- Value							
Field	**2**	**143*** **	**174*** **	**989*** **	**21*** **	**393*** **	**261*** **	**179*** **	**216*** **

***** denotes significance at $\alpha = 0.001$**

Values were $\log_{10}$ transformed to meet the ANOVA homogeneity of variance assumption.

Table 3: Elemental soil composition means and standard errors with F-Values for differences among fields at depth 15 to 30 cm for 2003.

		%OM	pH	P	K	Ca	Mg	C	N
Field				mg kg^{-1}					
Low		**1.88 ± 0.03**	**6.2 ± 0.03**	**180 ± 11**	**160 ± 5**	**642 ± 18**	**85 ± 2**	**8.4 ± 0.3**	**0.78 ± 0.02**
Medium		**1.22 ± 0.02**	**5.5 ± 0.03**	**600 ± 9**	**172 ± 4**	**467 ± 17**	**35 ± 1**	**5.8 ± 0.1**	**0.56 ± 0.01**
High		**1.12 ± 0.04**	**6.2 ± 0.06**	**584 ± 23**	**203 ± 9**	**708 ± 40**	**85 ± 5**	**5.3 ± 0.2**	**6.2 ± 0.02**
Source of Variation	df	F- Value							
Field	**2**	**150*** **	**103*** **	**227*** **	**10*** **	**24*** **	**172*** **	**55*** **	**31*** **

***** denotes significance at $\alpha = 0.0001$**

Values were $\log_{10}$ transformed to meet the ANOVA homogeneity of variance assumption.

Table 4: Elemental soil composition means and standard errors with Analyses of variance combined over fields for log-transformed soil elemental concentrations at depth 0 to 15 cm, fall 2004.

		pH	P	K	Ca	Mg
		Elemental Soil Concentration (mg kg^{-1})				
Field						
Low		**5.7 ± 0.02**	**229 ± 4**	**259 ± 7**	**668 ± 8**	**83 ± 1**
Medium		**5.3 ± 0.02**	**379 ± 7**	**178 ± 4**	**439 ± 13**	**40 ± 1**
High		**5.7 ± 0.05**	**703 ± 26**	**196 ± 8**	**1079 ± 54**	**84 ± 4**
Source of Variation	df	F-Value				
Field	**2**	**46*** **	**431*** **	**33*** **	**137*** **	**141*** **
Clone	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Coppice	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Field x Clone	**2**	**3***	**NS**	**NS**	**NS**	**NS**
Field x Coppice	**2**	**NS**	**NS**	**NS**	**NS**	**NS**
Clone x Coppice	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Field x Clone x Coppice	**2**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively; NS = Not Significant at α = 0.05.**

Values were log_{10} transformed to meet the ANOVA homogeneity of variance assumption.

Table 5: Elemental soil composition means and standard errors with analyses of variance combined over fields for log-transformed soil elemental concentrations at 15 to 30 cm depth, fall 2004.

		pH	P	K	Ca	Mg
		Elemental Soil Concentration (mg kg^{-1})				
Field						
Low		**5.3 ± 0.03**	**120 ± 8**	**167 ± 4**	**452 ± 13**	**65 ± 2**
Medium		**4.9 ± 0.06**	**412 ± 8**	**136 ± 4**	**309 ± 15**	**23 ± 1**
High		**5.7 ± 0.09**	**259 ± 15**	**193 ± 12**	**297 ± 20**	**58 ± 4**
Source of Variation	df	F-Value				
Field	**2**	**36*** **	**106*** **	**12*** **	**29*** **	**105*** **
Clone	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Coppice	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**NS**
Field x Coppice	**2**	**NS**	**4***	**NS**	**NS**	**NS**
Clone x Coppice	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Field x Clone x Coppice	**2**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively; NS = Not Significant at α = 0.05.**

Values were $\log_{10}$ transformed to meet the ANOVA homogeneity of variance assumption.

Figure 1: Mean soil P concentration and standard errors for 2003 and 2004.

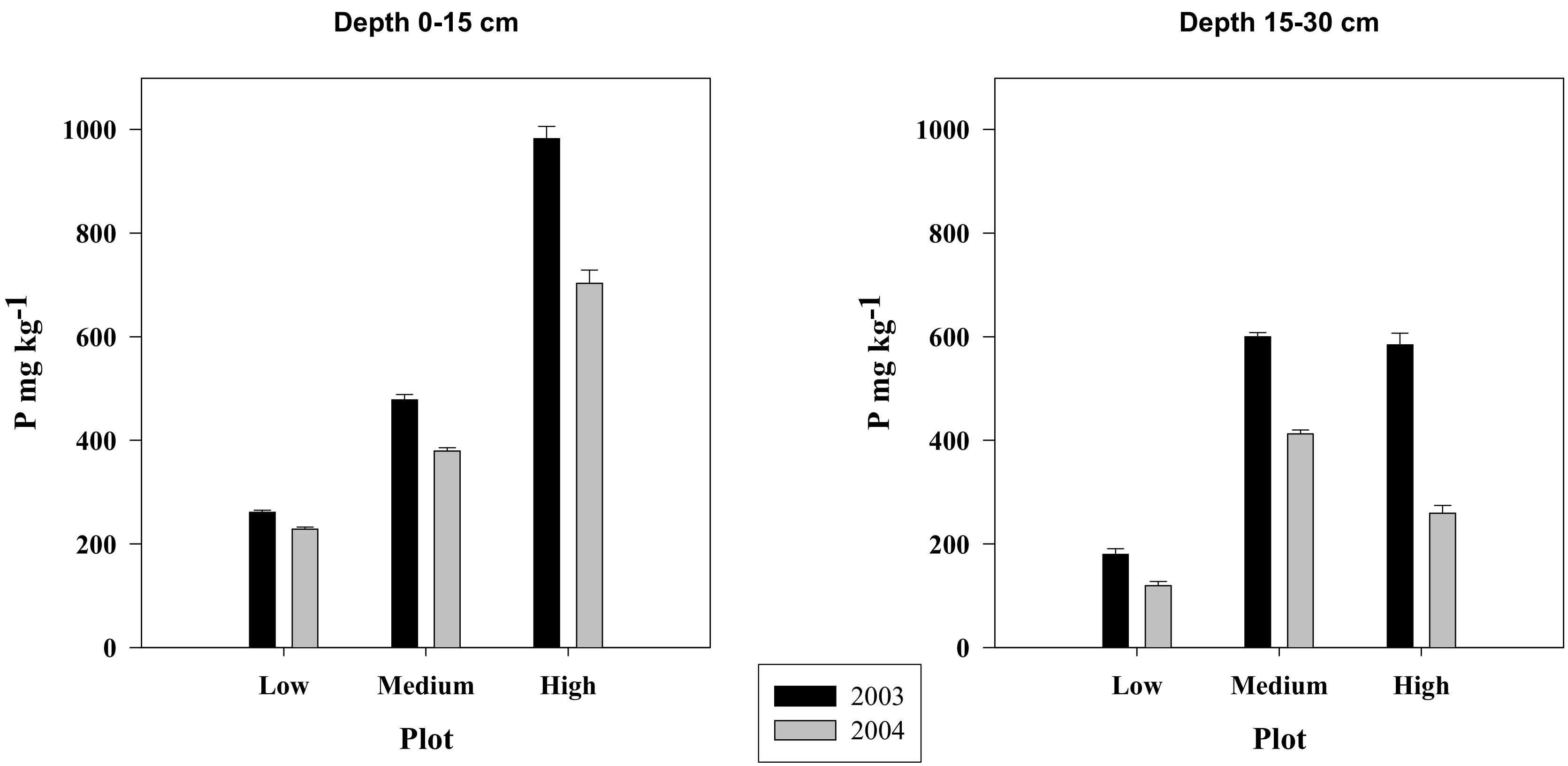


Table 6: Mean decrease (± standard error) in soil composition from spring 2003 to fall 2004 at 0 to 15 cm depth.

Field	pH	P	K	Ca	Mg	pH	P	K	Ca	Mg
		Decrease (mg kg^{-1})				Percent Decrease (%)				
Low	**0.6 ± 0.02**	**33 ± 2**	**6 ± 5**	**220 ± 9**	**32 ± 2**	**9.2±0.003**	**12.6±0.01**	**1.9±0.02**	**24.5±0.01**	**27.4±0.01**
Medium	**0.4 ± 0.02**	**92 ± 9**	**38 ± 6**	**149 ± 10**	**16 ± 1**	**6.3±0.004**	**18.5±0.02**	**15.8±0.02**	**25.0±0.02**	**27.3±0.02**
High	**0.3 ± 0.03**	**280 ± 15**	**40 ± 6**	**475 ± 25**	**48 ± 3**	**4.9±0.01**	**28.7±0.01**	**16.7±0.03**	**31.4±0.02**	**35.7±0.02**

Table 7: Mean decrease (± standard error) in soil composition from spring 2003 to fall 2004 at 15 to 30 cm depth.

Field	pH	P	K	Ca	Mg	pH	P	K	Ca	Mg
		Decrease (mg kg^{-1})				Percent Decrease (%)				
Low	**0.9 ± 0.02**	**55 ± 9**	**-9 ± 4**	**186 ± 17**	**21 ± 1**	**13.8±0.003**	**25.2±0.05**	**-8.6±0.03**	**27.7±0.02**	**24.0±0.01**
Medium	**0.5 ± 0.05**	**189 ± 12**	**34 ± 4**	**134 ± 14**	**11 ± 1**	**9.3±0.01**	**30.8±0.02**	**18.8±0.02**	**29.5±0.02**	**31.9±0.02**
High	**0.5 ± 0.04**	**325 ± 22**	**14 ± 7**	**384 ± 37**	**27 ± 3**	**7.5±0.01**	**54.1±0.03**	**7.6±0.03**	**53.5±0.03**	**31.9±0.03**

Low Field

For the Low field, all elements and pH at both depths decreased from year 2003 to 2004 except K in the subsurface soil (Table 6-7). The soil P had a mean reduction of 33 mg kg^{-1} of P (13%) in the surface and 55 mg kg^{-1} of P (25%) in the subsurface (Table 6-7).

The Low field had the largest percent decrease in pH. The mean pH changed from 6.3 to 5.7 in the upper depth and 6.2 to 5.3 in the lower depth. Such a large decrease in pH could have increased P loss in the system since P adsorption has been found to gradually decrease with decreasing pH near a soil pH≈5 (Arai et al., 2005). This may have been the case especially in the lower depth where the unit decrease in pH could have allowed the P to become more soluble and prone to leaching.

Medium Field

Similar to the other fields, the mean soil P in the Medium field decreased during the experiment by 229 mg P kg^{-1} (19%) in the upper depth of soil and 412 mg P kg^{-1} (31%) in the lower depth (Table 6-7). The decreases in percent soil P were higher in the Medium than the Low fields although the percent decreases were similar.

However, the relationship between the soil P concentrations in the surface and subsurface soils was different for the Medium field than the other fields. Unlike the Low and High fields in which the soil P was higher in the surface soil due to poultry litter application and decreased in the subsurface soil, the soil P in the Medium field was higher in the subsurface than the surface soils. Thus, at the start of the experiment, the surface soil P levels were lower for the Medium than the High fields but the subsurface P levels were not significantly different. However, by the end of the experiment, although the surface soil P concentration remained much lower for the Medium field than for the High field, the subsurface soil mean concentration was 150 mg P kg^{-1} higher in the Medium field than the High field. One potential cause for the high P of the subsurface soil relative to the surface soil in the Medium field could be related to the plowing of the field. If the soil was plowed using a moldboard plow, the surface soil could have been displaced in the subsurface depths relocating the P contained within the soil (Chaney, 2005).

High Field

The High field had the largest decrease in P during the 16 month experiment. In the upper depth, P decreased 280 mg kg^{-1} (29%) and in the lower depth P decreased 325 mg kg^{-1} (54 %) (Tables 6-7). In this experiment, the rate of decrease in soil test P was greater when soil P was higher. This trend in soil test P has been found in other studies where the rate of decrease in extractable soil P was reduced with decreasing soil P (Eghball et al., 2003). However, such a large reduction in soil test P is rarely seen in similar time periods with traditional agricultural practices (Eghball et al., 2003; McCollum, 1991).

One factor that could have contributed to the large decrease in soil P on the High field may have been grassy weeds growing within this field that were not present in the other two fields. These weeds were present despite the herbicide treatments. Although weeds were cut to reduce competition, they reached approximately 50 cm tall in a dense growth throughout the High field and the contribution to the decrease in soil P due to P uptake was possibly higher on the High field due to greater grass biomass. Studies conducted on the ability of grass species to remove soil P have found removal rates of

70.6, 22.4, 93 and 32.2 kg P ha^{-1} for Coastal Bermuda grass(*Cynodon dactylon*), Red clover (*Trifolium pretense* L.), Johnson grass (*Sorghum halepense* L.), and fescue (*Festuca* sp.) respectively (Novak, 2002). The grassy weeds could have removed similar amounts of P on the High field in this study contributing to the large decrease in soil P.

Tree Survival and Growth

Cuttings of two hybrid poplar clones were established on the experimental plots to determine the possibility that either clone could be utilized for P phytoremediation of high P soils commonly associated with poultry production on the Delmarva Peninsula. After the first growing season (2003), the hybrid poplars had high survival rates of 96%, 92% and 100% for Low, Medium and High fields, respectively. Survival rates higher than 80% indicate that the hybrid poplar clones are well adapted to survive in this environment (Kays, 2002). The majority of the trees that died during the first growing season did not become established and did not leaf out. Since the overall survival was high, the few tree mortalities were probably due to the initial condition of the cutting rather than treatment effects or variation in environmental conditions.

Following the first growing season (September 2003), the mean tree heights differed significantly among fields, clones, and their interaction (Table 8). In general, the OP-367 clones were taller than the DN-34 clones. However, the difference between the clone height means within a field was only significant for the Medium field, where the OP-367 trees had the greatest mean height (1.7 m) of any of the clone–field combinations. The tree height means were the next highest in the Low field where there was no significant difference between the clones. The shortest mean tree heights were the mean heights of the clones grown in the High field that were less than half the height of the OP-367 clones grown in the Medium field.

The mean heights of DN-34 and OP-367 in this study were similar to mean heights of a hybrid poplar clonal study (Kays, 2002) where after one year, clones OP-367 and DN-34 had mean heights of 1.31 m and 0.69 m, respectively. Height growth during the early stages of hybrid poplar development may be limited, as energy is preferentially allocated to rapidly-growing roots (Braatne et al., 1996). Once their root systems have become established, height growth becomes more rapid.

In each field, ten randomly selected hybrid poplar trees of each clone were coppiced 90 days after planting. These 60 trees were allowed to regrow in 2004 from their established root systems. The remaining 90 trees were not cut until the end of the second growing season. For 2003, the height and survival data were collected before any trees were coppiced. For 2004, the height and survival data coppiced and uncoppiced trees were statistically analyzed separately.

The survival rates of the remaining hybrid poplars at the end of second growing season (2004) were 90%, 94% and 98% for Low, Medium and High fields, respectively. All of the trees that died during the 2004 growing season had been coppiced in 2003 and did not regrow. While the survival rates were relatively good for both years, coppicing the trees decreased the probability of winter survival during the establishment year. Also, the survival rate for clone DN-34 was better than clone OP-367. In the Low and High fields, all of the trees that did not survive were clone OP-367. In the Medium field, five of the seven dead trees were clone OP-367.

Table 8: Hybrid Poplar height means and LSD values with analysis of variance to determine significant effects of field and clone on height for 2003 and 2004.

		2003	2004 Coppiced	2004 Uncoppiced
Field	Clone		Height (m)	
Low	**DN-34**	**1.1**	**2.0**	**2.7**
	OP-367	**1.4**	**1.9**	**3.1**
Medium	**DN-34**	**0.9**	**1.8**	**2.4**
	OP-367	**1.7**	**2.2**	**3.3**
High	**DN-34**	**0.7**	**1.5**	**2.1**
	OP-367	**0.8**	**1.5**	**2.5**
LSD		**0.3**	**0.5**	**0.4**
Source of Variation	df		F-Value	
Field	**2**	**22*** **	**4***	**17*****
Clone	**1**	**23*****	**NS**	**31*****
Field x Clone	**2**	**7****	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

The 2004 tree heights of the coppiced trees were significantly different among fields but differences between clones and the field by clone interaction were not significant (Table 8). In general, the year after the trees were coppiced, the trees in the Medium field had the highest mean height while the trees in the High field had the lowest mean height. The mean coppiced tree height in 2004 was almost double the height from 2003 since the trees were regrowing from an established root system.

The mean heights for the uncoppiced trees in 2004 were significantly different among fields and clones with no significant field by clone interaction (Table 8). The uncoppiced trees in the Low field had the highest mean height while the trees in the High field had the lowest mean height. The differences in growth among the fields may not be a result of the soil P effect but rather variations in the field's soil chemistry and competing vegetation. For all fields, uncoppiced trees of clone OP-367 had higher mean heights than clone DN-34.

In 2004, the average height of the coppiced trees was 1.8 m compared to 2.7 m for the uncoppiced trees. The uncoppiced trees had not been cut in 2003 and were expected to be taller than the coppiced trees. Clone OP-367 trees tended to be taller than DN-34 trees in 2003 and the uncoppiced trees in 2004. In the clonal study mentioned earlier (Kays, 2002), the mean height of two year old uncoppiced hybrid poplars was 3.0 m and 1.8 m for clones OP-367 and DN-34, respectively. In this study OP-367 was the superior performer of all the 11 clones tested. Another hybrid poplar field study utilizing clones from a *Populus* x *euramericans* and a *Populus tristis* x *Populus balsamifera* hybrid found similar mean growth rates to this study (2.5 m and 1.0 m, respectively) for the first year of growth (Dickmann et al., 1996). However, after two years of growth *Populus* x *euramericans* grew substantially taller with a mean height of 5.5 m. While clonal differences may be one reason for the differences in growth between these studies environmental effects may also have caused variation.

Tree Biomass

<u>Coppiced Trees 2003</u>

The leaf, stem and total tree biomass means for the coppiced hybrid poplars for 2003 are presented in Table 9. At the end of one growing season, the leaf, stem and total biomass (leaf biomass + stem biomass) means differed significantly among fields and clones but their interactions were not significant. The total biomass for the coppiced trees in 2003 was negatively correlated with the soil P (r= -0.38*). The total biomass mean across clones for the Low P field was almost three times higher than the total biomass mean for the High P field (22 g $tree^{-1}$ vs. 8 g $tree^{-1}$). Biomass values on the Low field were probably higher due to physical properties of the soil that were more conducive to hybrid poplar growth. Following the first year of growth, the biomass of the leaf and stem tissues each constituted about half of the total tree biomass. Clone OP-367 produced more leaf and stem tissue biomass than clone DN-34 but the survival rate of OP-367 the following season was lower than clone DN-34.

Coppiced 2004

After two seasons of growth, there were no significant differences among means for the leaf, stem or total biomass of the coppiced hybrid poplars (Table 9). The biomass results for 2004 were highly variable. The coefficients of variation for the biomass of the coppiced trees in 2003 ranged from 60-78% but were 78-90% for 2004. Overall, the biomass was higher in 2004 (44 g $tree^{-1}$) than 2003 (15 g $tree^{-1}$). While the coppiced tree mean leaf biomass was about the same from 2003 to 2004, the stem mass significantly increased the second year and comprised the majority of the total biomass.

Table 9: Coppiced hybrid poplar leaf, stem and total tree biomass means and LSD values with analysis of variance to determine significant effects of field and clone on biomass for 2003 and 2004.

		Leaf		Stem		Total		
		2003	2004	2003	2004	2003	2004	Both yrs
Field	Clone	Mass (g tree^{-1})						
Low	**DN-34**	**8**	**7**	**8**	**38**	**16**	**46**	**57**
	OP-367	**12**	**7**	**16**	**46**	**27**	**43**	**69**
Medium	**DN-34**	**6**	**11**	**6**	**44**	**12**	**55**	**65**
	OP-367	**8**	**9**	**12**	**53**	**20**	**62**	**89**
High	**DN-34**	**3**	**6**	**3**	**30**	**6**	**36**	**42**
	OP-367	**4**	**6**	**5**	**16**	**9**	**21**	**30**
LSD		**4**	**6**	**6**	**27**	**9**	**32**	**33**
Source of Variation	df	F-Value						
Field	**2**	**12*** **	**NS**	**8*** **	**NS**	**10*** **	**NS**	**4***
Clone	**1**	**5***	**NS**	**10****	**NS**	**8****	**NS**	**NS**
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively; NS = Not Significant at α = 0.05.**

Table 10: Uncoppiced hybrid poplar leaf, stem and total tree biomass means and LSD values with analysis of variance to determine significant effects of field and clone on biomass for 2003 and 2004.

		Leaf		Stem		Total		
		2003	2004	2003	2004	2003	2004	Both yrs
Field	Clone	Mass (g tree^{-1})						
Low	**DN-34**	**-**	**22**	**-**	**278**	**-**	**337**	**-**
	OP-367	**-**	**55**	**-**	**471**	**-**	**516**	**-**
Medium	**DN-34**	**-**	**17**	**-**	**115**	**-**	**132**	**-**
	OP-367	**-**	**71**	**-**	**473**	**-**	**544**	**-**
High	**DN-34**	**-**	**14**	**-**	**70**	**-**	**84**	**-**
	OP-367	**-**	**27**	**-**	**128**	**-**	**155**	**-**
LSD		**-**	**23**	**-**	**130**	**-**	**141**	**-**
Source of Variation	df	F-Value						
Field	**2**		**NS**		**10*** **		**11*** **	
Clone	**1**		**13*** **		**16*** **		**15*** **	
Field x Clone	**2**		**NS**		**NS**		**4***	

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively; NS = Not Significant at α = 0.05.**

Uncoppiced 2004

The leaf, stem and total biomass means of the uncoppiced trees for 2004 are presented in Table 10. The stem and the total biomass means were significantly different among the fields and the clones. The leaf biomass means were only significantly different between clones. The field x clone interaction was significant for the total biomass but not the leaf or stem biomass. The total biomass in 2004 for the uncoppiced trees was negatively correlated with both the initial soil test P results (r=-.58***) and the final soil test P results (r=-0.52**) across the fields. The High P field had the least total biomass with a mean of 120 g $tree^{-1}$ and stem tissue accounted for approximately 80% of the total biomass. The Low field had the highest mean total biomass of 449 g $tree^{-1}$ but was not significantly different from the Medium field which had a mean total biomass of 338 g $tree^{-1}$. The significant interaction for the total biomass can be explained because the mean total biomass for clone OP-367 was significantly more than the mean total biomass for clone DN-34 for the Low and Medium Fields, while the difference between the clones was not significant in the High P field. Based on the magnitude of the F-values, the main effects of Field and Clone were stronger than the interaction. Overall, clone OP-367 had a higher mean total biomass than clone DN-34, which was related to the increased mean height of the OP-367 trees.

Overall, the 2004 uncoppiced tree biomass was a great deal higher than the biomass of the coppiced trees for the same year as well as the coppiced trees combined 2-year biomass. While the High field had the lowest tree biomass of the three fields the uncoppiced biomass was about four times the coppiced tree biomass for 2004. The 2004 uncoppiced tree biomass for the Medium and Low fields was six and ten times the 2004 coppiced tree biomass for each field, respectively.

In this experiment, more biomass per year was accumulated by allowing the hybrid poplars two full seasons to grow without coppice. However, the regrowth of the coppiced trees could have been negatively affected by the relatively early harvest date. To achieve more vigorous regrowth for the coppiced trees, winter is the optimal time to harvest while the trees are dormant (St. John, 2001). However, for maximum phytoremediation, the removal of the whole plant including leaves maximizes P removal. Based on the P content of the leaves in this experiment from the coppiced trees, if the leaves were allowed to drop and not removed, they would have returned on average 29 mg P $tree^{-1}$ in 2003 and 47 mg $tree^{-1}$ in 2004. As the P content of the leaves increases with tree age, it would become increasingly important to remove the leaves from the site to prevent recycling of P into the soil. Even without annual coppicing of the trees, the leaves could be removed from the system by manual or mechanical means immediately following senescence for increased phytoremediation. While this may increase the overall cost of this type of management, phytoremediation will not work in a closed system. Plant material must be removed from the site in order to reduce soil P levels.

Regardless of coppice treatment, the hybrid poplars were shortest and had the least biomass on the High P field for both years. While this field had more than the optimum Mehlich-3 soil P required for most crops (Maguire and Sims, 2002), it had a limiting growth factor not exhibited in the Low or Medium fields. The soil in the High field was considerably compacted (> 2 MPa) as measured by a penetrometer. The High field was compacted (>2 MPa) at a shallow depth of 15 cm where as the Low and

Medium field reached the same level of pressure at much lower depths of 56 and 25 cm, respectively. The High field also presented evidence of compaction through difficult soil sampling and planting of the trees when compared to the other two fields. Extensive compaction occurred on this field because of the previous land use. The High field had not been tilled in 20 years and was the previous location of a chicken house. The high penetration resistance of this field may have limited root growth and subsequent aboveground production. While this field had the highest soil P levels, the poor poplar growth is most likely due to the compacted nature of the soil rather than the soil nutrient status. Had the soils been properly prepared and tilled penetrating the compacted zone prior to planting the trees on the High field would likely have had higher biomass values.

In 2004, the hybrid poplar trees were infested with web worm (*Hyphantria cunea*) in August which reduced the leaf and total biomass on all fields. The infestation was probably the cause of the excessive coefficients of variation for the 2004 leaf biomass, which were 90% and 73% for the coppiced and uncoppiced trees, respectively. Because the pests generally attack late in the season little permanent damage is done to most trees. Therefore, while the webs looked unsightly and defoliation occurred, the stem tissue was not affected by the worms and the trees future health was not in danger.

Hybrid poplar studies have found that the greatest amounts of biomass have been produced where soils were cultivated prior to planting and regrowth of herbaceous competition was eliminated by cultivation, herbicides, or combinations of both. (Czapowskyj and Safford, 1993) found that vegetation control greatly affected tree survival. On mowed fields, 83% of hybrid poplars survived ten years compared to a 57% ten year survival on unmowed fields.

Weeds were partially controlled in this experiment but were not eliminated which could have affected the biomass means. Although the tree shelters provided the trees some protection from competing vegetation, the shelters were not completely impervious. The weeds competed with the hybrid poplars for sunlight, water, nutrients and space which could have limited biomass production. Competing vegetation was especially vigorous on the High field. The hybrid poplars grew amongst thick grass on the High field whereas the Low and Medium fields had sparse weeds.

Tree Elemental Concentration

The hybrid poplar trees actively removed P from the soil and transported it to the aboveground leaf and stem tissue. In order for hybrid poplars to be a considered hyperaccumulators the combined leaf and stem tissue P concentration should be in tens of grams of P per kg of tree. While, the elemental P concentration differed among the trees depending on the clone, field and coppice effects, none of the trees accumulated exceptionally high concentrations of P.

<u>Coppiced 2003</u>

The elemental tissue concentration means for the coppiced trees in 2003 are presented in Tables 11-12. The elemental concentrations of the coppiced hybrid poplars in 2003 were significantly different among fields for all nutrients analyzed in the leaf tissue and for all nutrients except C in the stem tissue. The P concentration in the hybrid poplars ranged from 3.36-6.59 g P kg^{-1} in the leaf tissue and 1.49-2.82 g P kg^{-1} in the stem tissue. The P concentration of both the leaf and stem tissues were positively correlated with the surface soil P (r= 0.73*** and r= 0.76***, respectively). The Low field had the lowest P concentration in both the leaf and stem tissue and the High field had the highest P concentration in both the leaf and stem tissue.

Both leaf and stem P concentrations were dependent on the soil P concentration (Figure 2) indicating if planted on excess P soils the hybrid poplars may possess luxury consumption abilities. However, nutrient uptake model simulations and other observations report that P uptake in hybrid poplars is not solely a function of supply (Kelly and Ericsson, 2002).

The concentration of P of the coppiced trees was significantly different between clones in the stem tissue but not in the leaf tissue. Clone DN-34 had significantly higher P concentrations in the stem tissue than clone OP-367 for all fields in 2003.

Coppiced 2004

The elemental tissue concentration means for the coppiced trees in 2004 are presented in Tables 13-14. The elemental concentrations for the coppiced trees in 2004 were significantly different among fields for P, Ca and Mg in the leaf tissue and for P, Ca, Mg and N in the stem tissue. There was no significant leaf or stem P concentration differences among clones. Both coppiced leaf and stem elemental concentrations for 2004 followed the same trend as 2003 where P increased with increasing field soil P (Figure 3). The coppiced elemental leaf tissue P concentrations were also higher in 2004 than in 2003 however not always higher than uncoppiced trees with the same field and clone (Figure 4).

Table 11: Elemental concentration means and LSD values for coppiced hybrid poplar leaf tissue with analysis of variance to determine significant effects of field and clone on leaf tissue concentrations, 2003.

		P	K	Ca	Mg	C	N
Field	Clone	Elemental Leaf Concentration (g kg^{-1})					
Low	**DN-34**	**3.7**	**28.1**	**11.5**	**3.01**	**450**	**32.1**
	OP-367	**3.4**	**24.6**	**12.1**	**2.65**	**454**	**31.8**
Medium	**DN-34**	**4.4**	**28.5**	**13.7**	**2.90**	**442**	**29.7**
	OP-367	**3.8**	**24.0**	**13.4**	**2.69**	**453**	**26.4**
High	**DN-34**	**6.2**	**26.0**	**11.7**	**3.72**	**439**	**23.2**
	OP-367	**6.6**	**22.1**	**11.4**	**3.18**	**446**	**21.5**
LSD		**1.3**	**2.8**	**1.4**	**0.4**	**5.7**	**3.0**
Source of Variation	df	F-Value					
Field	**2**	**23*** **	**4***	**9*** **	**11*** **	**11*** **	**40*** **
Clone	**1**	**NS**	**25*** **	**NS**	**9****	**20*** **	**4***
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

Table 12: Elemental concentration means and LSD values for coppiced hybrid poplar stem tissue with analysis of variance to determine significant effects of field and clone on stem tissue concentrations, 2003.

		P	K	Ca	Mg	C	N
Field	Clone	Elemental Stem Concentration (g kg^{-1})					
Low	**DN-34**	**2.0**	**9.8**	**4.5**	**0.92**	**469**	**7.6**
	OP-367	**1.5**	**9.9**	**4.4**	**0.95**	**471**	**6.4**
Medium	**DN-34**	**2.5**	**10.3**	**4.9**	**0.87**	**468**	**7.9**
	OP-367	**2.1**	**9.8**	**4.5**	**0.80**	**471**	**6.2**
High	**DN-34**	**2.8**	**9.5**	**4.4**	**1.25**	**468**	**7.4**
	OP-367	**2.6**	**8.2**	**3.7**	**0.93**	**471**	**4.7**
LSD		**0.2**	**1.1**	**0.5**	**0.10**	**1.8**	**0.8**
Source of Variation	df	F-Value					
Field	**2**	**83*** **	**6***	**8*** **	**21*** **	**NS**	**8*** **
Clone	**1**	**31*** **	**NS**	**9****	**14*** **	**30*** **	**63*** **
Field x Clone	**2**	**NS**	**NS**	**NS**	**10*** **	**NS**	**4***

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

Table 13: Elemental concentration means and LSD values for coppiced hybrid poplar leaf tissue with analysis of variance to determine the significant effects of field and clone on leaf tissue concentrations, 2004.

		P	K	Ca	Mg	N
Field	Clone	Elemental Leaf Concentration (g kg^{-1})				
Low	**DN-34**	**5.0**	**25.8**	**16.1**	**3.43**	**21.0**
	OP-367	**4.1**	**24.4**	**14.8**	**3.47**	**22.3**
Medium	**DN-34**	**5.1**	**26.3**	**20.7**	**2.64**	**19.2**
	OP-367	**4.4**	**22.2**	**18.9**	**2.69**	**18.6**
High	**DN-34**	**9.5**	**25.6**	**16.5**	**4.06**	**21.5**
	OP-367	**7.4**	**22.3**	**14.4**	**3.61**	**19.9**
LSD		**2.1**	**3.9**	**2.5**	**0.5**	**2.8**
Source of Variation	df	F-Value				
Field	**2**	**9*** **	**NS**	**9*** **	**13*** **	**NS**
Clone	**1**	**NS**	**NS**	**NS**	**NS**	**NS**
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

Table 14: Elemental concentration means and LSD values for coppiced hybrid poplar stem tissue with analysis of variance to determine the significant effects of field and clone on stem tissue concentration, 2004.

		P	K	Ca	Mg	N
Field	Clone	Elemental Stem Concentration (g kg^{-1})				
Low	**DN-34**	**1.5**	**7.0**	**3.6**	**0.83**	**4.7**
	OP-367	**1.4**	**6.7**	**4.4**	**0.88**	**4.1**
Medium	**DN-34**	**1.8**	**7.2**	**4.8**	**0.85**	**5.9**
	OP-367	**1.5**	**5.8**	**5.3**	**0.78**	**4.7**
High	**DN-34**	**2.2**	**7.5**	**4.4**	**1.04**	**7.0**
	OP-367	**2.0**	**6.7**	**4.4**	**0.92**	**4.2**
LSD		**0.3**	**2.4**	**0.8**	**0.1**	**0.9**
Source of Variation	df	F-Value				
Field	**2**	**14*** **	**NS**	**3* **	**6** **	**4* **
Clone	**1**	**NS**	**NS**	**NS**	**NS**	**17*** **
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**3* **

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

Figure 2: Regression of coppiced hybrid poplar leaf and stem P concentration on initial soil-test P, 2003.

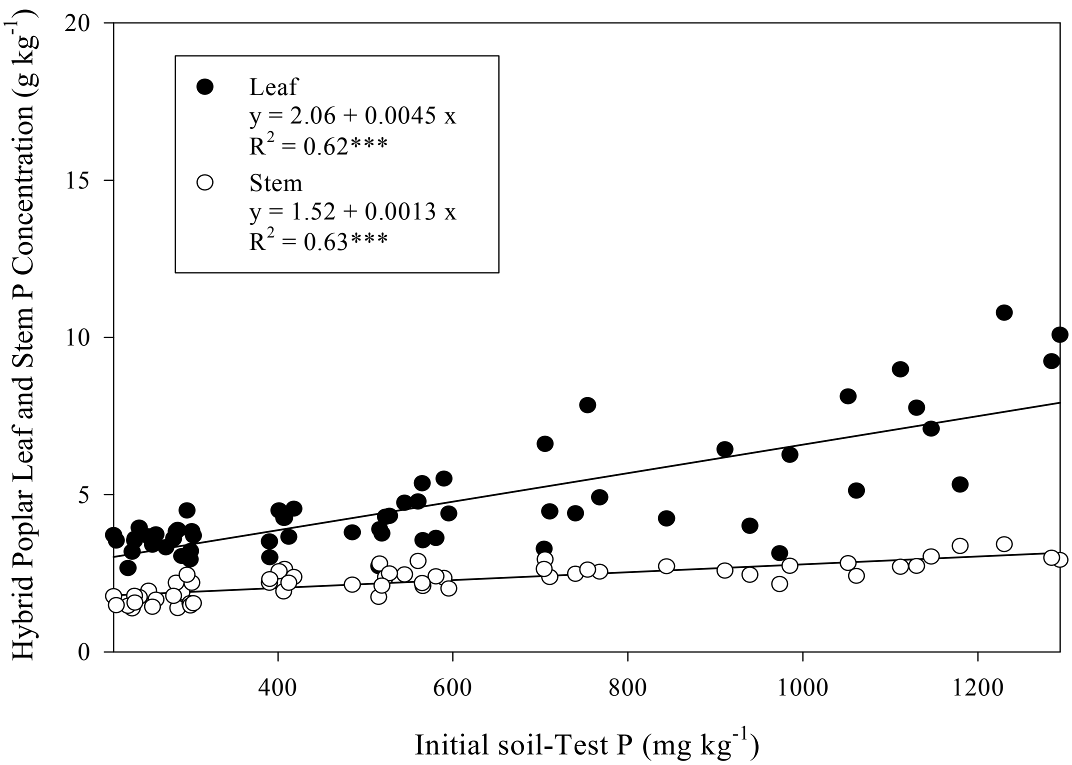


Figure 3: Regression of coppiced hybrid poplar leaf and stem P concentration on initial soil-test P, 2004.

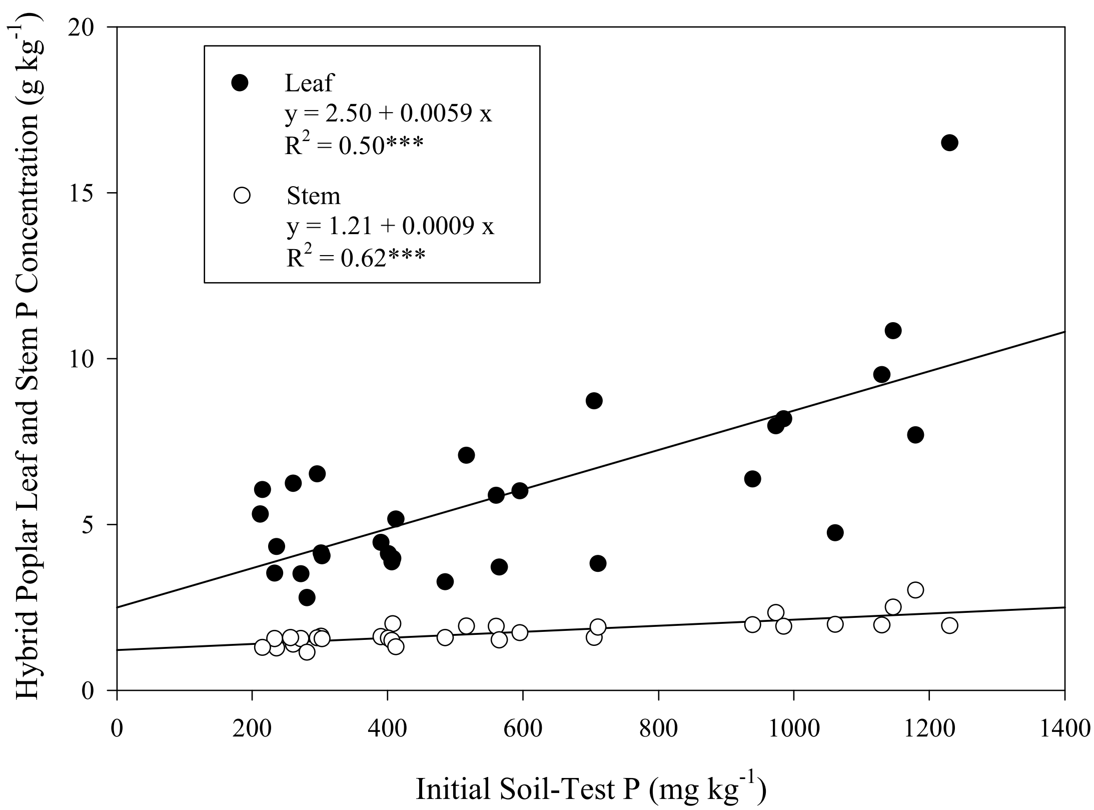


Figure 4: Hybrid poplar elemental leaf P concentration of coppiced (C) and uncoppiced (UC) trees in 2003 and 2004 for clones DN-34 and OP-367 with standard errors.

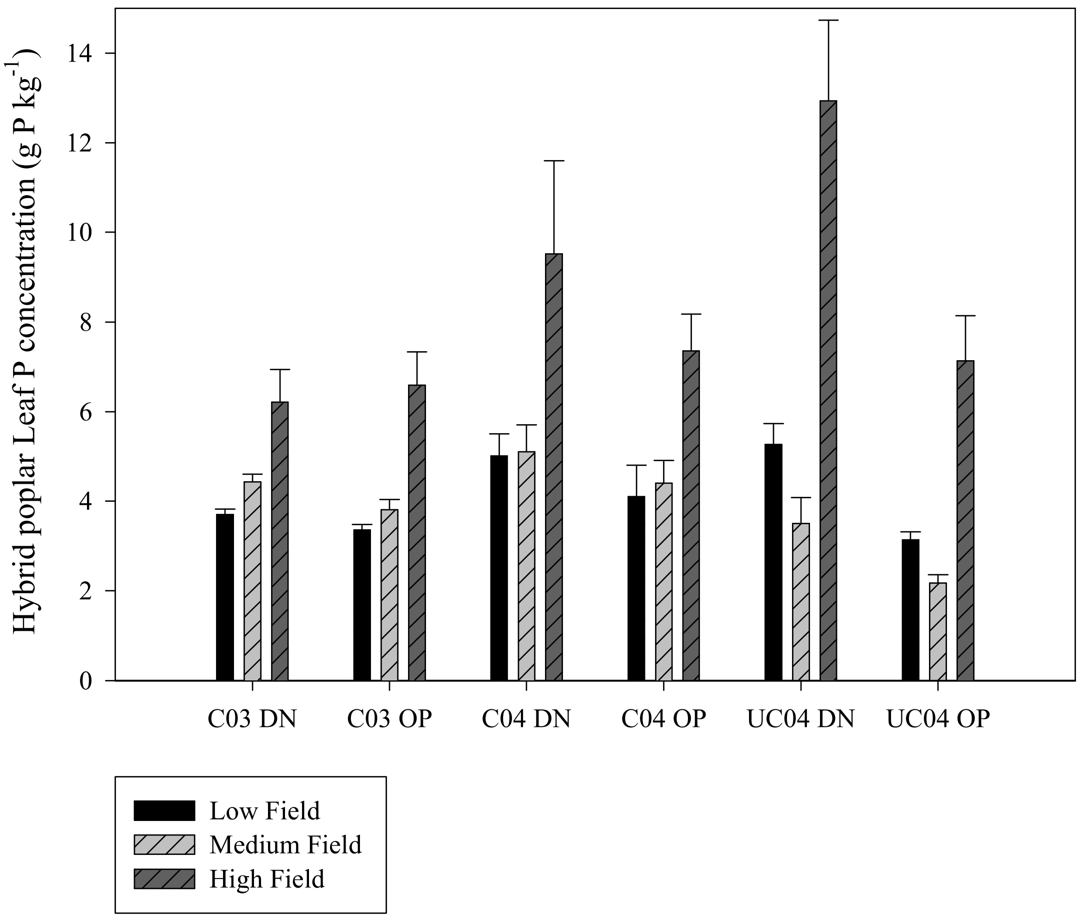
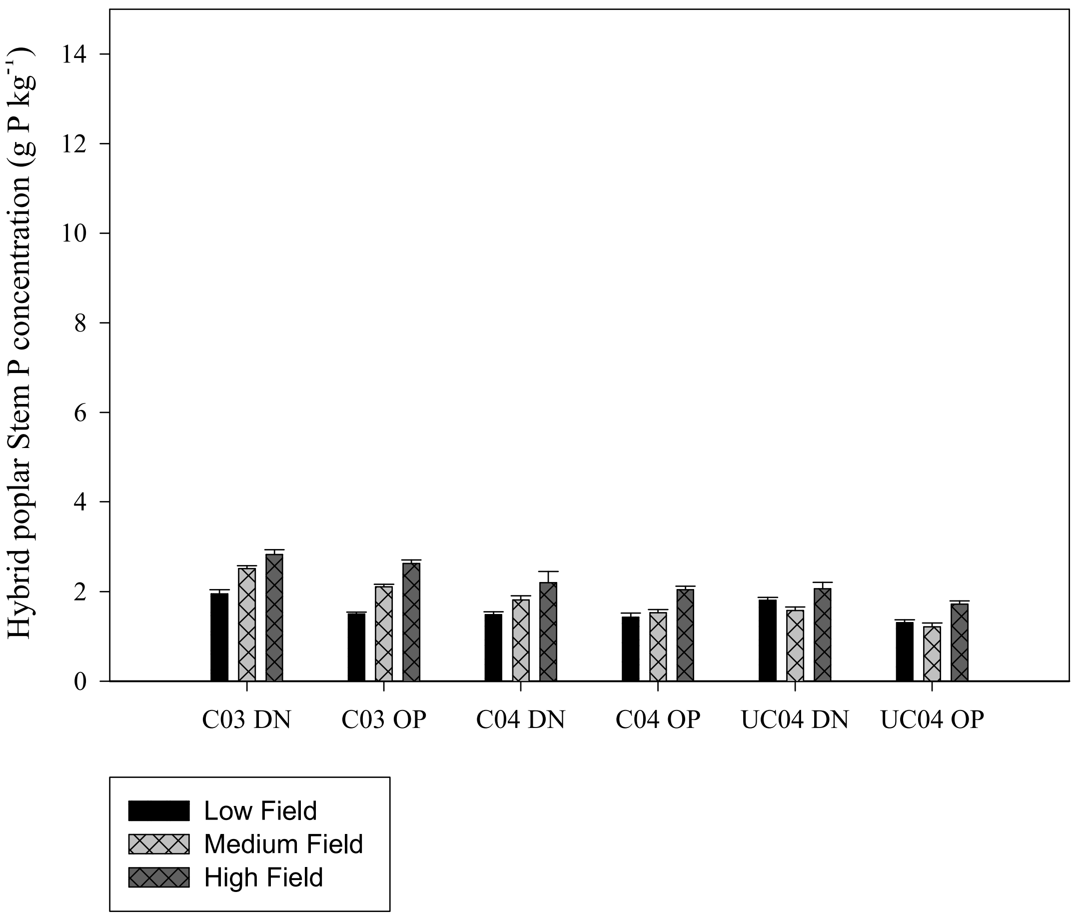


Figure 5: Hybrid poplar elemental stem P concentration of coppiced (C) and uncoppiced (UC) trees in 2003 and 2004 for clones DN-34 and OP-367 with standard errors.



Uncoppiced 2004

The elemental tissue concentration means for the uncoppiced trees in 2004 are also presented in Tables 15-16. The elemental concentrations for the uncoppiced trees in 2004 were significantly different among fields for P, Ca and Mg in the leaf tissue and for P, Ca, and N in the stem tissue. The mean elemental P concentration for both leaf and stem tissue was the highest in the trees on the High field. Unlike the coppiced trees for both years, the uncoppiced trees had the lowest leaf and stem elemental P concentration on the Medium field. However, the elemental P concentration was not significantly different between the Low and Medium field for both uncoppiced leaf and stem tissue in 2004. Regardless of field, clone or coppice the leaf tissue had significantly more elemental P than the stem tissue.

Both leaf and stem tissue for the uncoppiced trees had significantly different elemental P concentrations among clones. The uncoppiced Clone DN-34 had higher elemental P concentrations in both leaf and stem tissue than clone OP-367. The mean elemental leaf P concentration for clone DN-34 ranged from 3.50 - 12.90 g P kg^{-1} and 2.17 - 7.10 g P kg^{-1} for clone OP-367 and the mean elemental stem P concentration for clone DN-34 ranged from 1.58 – 2.06 g P kg^{-1} and 1.21 – 1.72 g P kg^{-1} for clone OP-367. Of the two clones based on leaf and stem elemental P concentration alone, DN-34 demonstrated better accumulation of P coppiced and uncoppiced.

Similar to the coppiced trees, the leaf and stem tissue P concentration for the uncoppiced trees was also dependent on the soil P concentration (Figure 6). However, the uncoppiced leaf tissue P was more responsive to soil P than the coppiced leaf tissue P represented by a higher regression slope (Figure 2, 3, and 6). The stem tissue P was more responsive to the soil P in the coppiced trees rather than the uncoppiced trees. This is possibly due to the larger biomass of the stem tissue in the uncoppiced trees compared to the coppiced trees. Overall, the clone and field with higher biomass values had lower P concentrations for coppiced and uncoppiced leaf and stem tissue. Since the P uptake in hybrid poplars may not be solely based on P supply, the increased biomass may have had a dilution effect on the P tissue concentration.

Table 15: Elemental concentration means and LSD values for uncoppiced hybrid poplar leaf tissue with analysis of variance to determine the significant effects of field and clone on leaf tissue concentrations, 2004.

		P	K	Ca	Mg	N
Field	Clone	Elemental Leaf Concentration (g kg^{-1})				
Low	**DN-34**	**5.3**	**24.1**	**12.5**	**3.50**	**26.2**
	OP-367	**3.1**	**17.3**	**13.5**	**3.36**	**17.2**
Medium	**DN-34**	**3.5**	**21.7**	**19.0**	**3.18**	**20.1**
	OP-367	**2.2**	**17.5**	**16.1**	**2.95**	**22.6**
High	**DN-34**	**12.9**	**22.1**	**17.7**	**5.01**	**18.4**
	OP-367	**7.1**	**20.1**	**15.5**	**4.34**	**16.0**
LSD		**1.8**	**3.7**	**3.2**	**0.5**	**5.3**
Source of Variation	df	F-Value				
Field	**2**	**41***	**NS**	**6**	**23***	**NS**
Clone	**1**	**21***	**10**	**NS**	**NS**	**NS**
Field x Clone	**2**	**4***	**NS**	**NS**	**NS**	**NS**

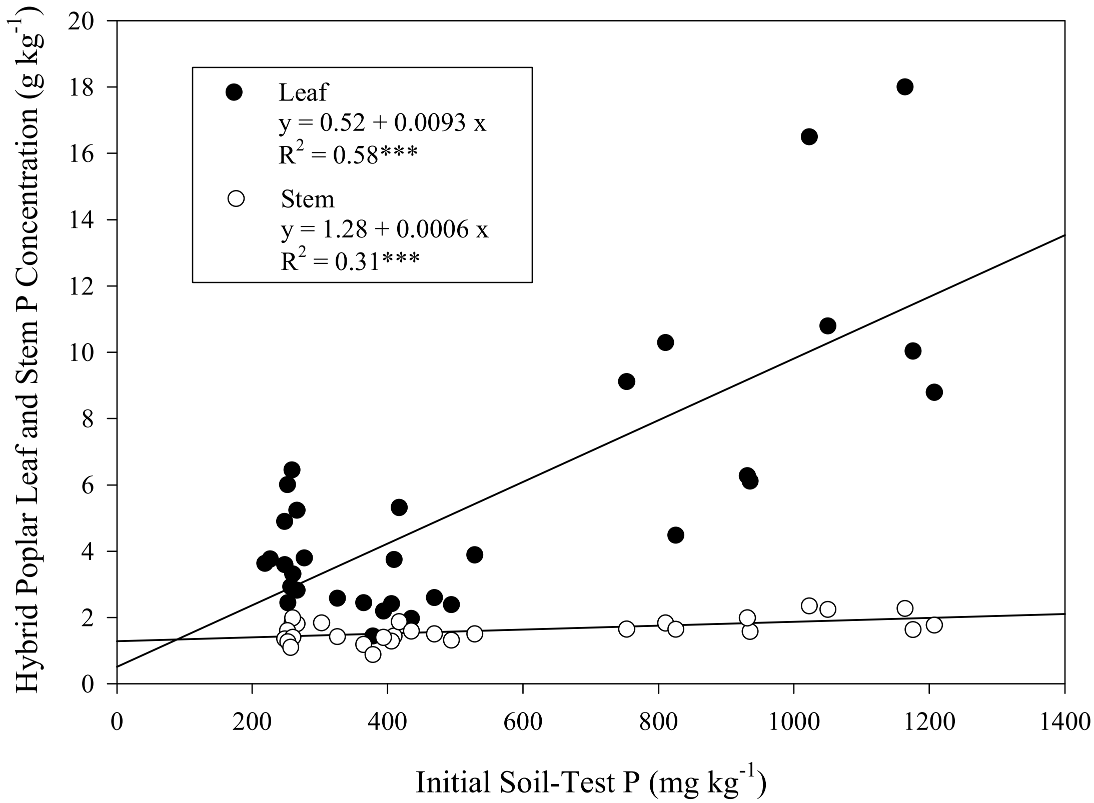
***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

Table 16: Elemental concentration means and LSD values for uncoppiced hybrid poplar stem tissue with analysis of variance to determine the significant effects of field and clone on stem tissue concentrations, 2004.

		P	K	Ca	Mg	N
Field	Clone	Elemental Stem Concentration (g kg^{-1})				
Low	**DN-34**	**1.8**	**6.7**	**4.6**	**1.21**	**5.8**
	OP-367	**1.3**	**4.8**	**4.3**	**1.10**	**4.2**
Medium	**DN-34**	**1.6**	**5.7**	**5.4**	**1.04**	**7.3**
	OP-367	**1.2**	**4.9**	**5.7**	**0.96**	**5.2**
High	**DN-34**	**2.1**	**6.0**	**4.2**	**1.16**	**6.8**
	OP-367	**1.7**	**4.7**	**3.8**	**1.11**	**5.2**
LSD		**0.2**	**0.8**	**0.4**	**0.1**	**1.0**
Source of Variation	df	F-Value				
Field	**2**	**17***	**NS**	**41***	**NS**	**4***
Clone	**1**	**32***	**17***	**NS**	**NS**	**20***
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

Figure 6: Regression of uncoppiced hybrid poplar leaf and stem P concentration on initial soil-test P, 2004.



Tree P Content

The P content of the hybrid poplar trees was calculated on an individual tree basis for the leaf tissue, stem tissue and whole tree by the multiplication of the biomass with the elemental P concentration of the plant tissue. The P content was used to measure P uptake and translocation on an individual tree basis. Because the P content was calculated from the P concentration and the tree biomass, it was highly variable and ranged from 157-503 mg P $tree^{-1}$ after two growing seasons.

<u>Coppiced 2003</u>

The 2003 and 2004 mean leaf, stem, and total aboveground P content for the coppiced trees are presented in Table 17. The only significant differences for the P content of the 2003 coppiced trees were the effects of field and clone for stem P content and the effect of clone for total aboveground P content. Thus, there were no significant differences in mean leaf P content. For stem P content and total tree P content, the OP-367 mean P content was more than the DN-34 P content. The P content of the coppiced trees in 2003 was strongly correlated with the biomass of the trees (r= 0.95***) but not the P concentration (r= -0.16 NS). Thus the P uptake was mainly a function of biomass.

<u>Coppiced 2004</u>

In 2004, the year following the coppicing, there were no significant differences in P content for leaf, stem, or total tree (Table 17). As in the previous year, P content was highly correlated with the total tree biomass (r= 0.90***). The large tree to tree variation for biomass, led to statistical tests that lacked power, which could have resulted in the inability to detect significant differences.

<u>Uncoppiced 2004</u>

The 2004 P content means of the uncoppiced trees are presented in Table 18. The uncoppiced tree P content was significantly different among the fields. The Low field had the highest P content per tree of the three fields with a mean of 714 mg P $tree^{-1}$, which was twice the mean P content in the High field of 361 mg P $tree^{-1}$ for the uncoppiced trees. The Medium field mean of 477 mg P $tree^{-1}$ was not significantly different from the High field mean. Similar to the coppiced trees, the total P content of the uncoppiced trees was strongly correlated with the total biomass (r= 0.92***) but was weakly negatively correlated with the 2003 and 2004 soil P (r= -0.41* and r= -0.37*, respectively).

Consistent with the coppiced trees, the higher biomass, clone OP-367 had higher total P content than clone DN-34.

Table 17: Coppiced hybrid poplar leaf, stem and total tree P Content means and LSD values with analysis of variance to determine significant effects of field and clone on P content, 2003 and 2004.

		Leaf		Stem		Total		
		2003	2004	2003	2004	2003	2004	Both yrs
Field	Clone	P Content (mg tree^{-1})						
Low	**DN-34**	**29**	**40**	**18**	**57**	**49**	**99**	**153**
	OP-367	**40**	**30**	**24**	**71**	**63**	**83**	**143**
Medium	**DN-34**	**26**	**64**	**14**	**82**	**40**	**147**	**179**
	OP-367	**32**	**40**	**25**	**78**	**57**	**117**	**196**
High	**DN-34**	**18**	**66**	**8**	**59**	**26**	**125**	**155**
	OP-367	**29**	**44**	**14**	**31**	**43**	**75**	**110**
LSD		**17**	**40**	**11**	**45**	**27**	**82**	**88**
Source of Variation	df	F-Value						
Field	**2**	**NS**	**4***	**NS**	**NS**	**NS**	**NS**	**NS**
Clone	**1**	**NS**	**6****	**4***	**NS**	**NS**	**NS**	**NS**
Field x Clone	**2**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively; NS = Not Significant at α = 0.05.**

Table 18: Uncoppiced hybrid poplar leaf, stem and total tree P Content means and LSD values with analysis of variance to determine significant effects of field and clone on P content, 2004.

		Leaf		Stem		Total		
		2003	2004	2003	2004	2003	2004	Both yrs
Field	Clone	P Content (mg tree^{-1})						
Low	**DN-34**	**-**	**108**	**-**	**497**	**-**	**677**	**-**
	OP-367	**-**	**170**	**-**	**604**	**-**	**736**	**-**
Medium	**DN-34**	**-**	**56**	**-**	**181**	**-**	**237**	**-**
	OP-367	**-**	**149**	**-**	**569**	**-**	**717**	**-**
High	**DN-34**	**-**	**171**	**-**	**145**	**-**	**316**	**-**
	OP-367	**-**	**188**	**-**	**217**	**-**	**406**	**-**
LSD			**81**		**186**		**210**	
Source of Variation	df	F-Value						
Field	**2**		**NS**		**8****		**5****	
Clone	**1**		**NS**		**7***		**6***	
Field x Clone	**2**		**NS**		**NS**		**NS**	

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively; NS = Not Significant at α = 0.05.**

Tree P Uptake

The field means for each clone for total P removed on a per hectare basis during the two year study is presented in Table 19. The coppiced trees combined mean uptake for years 2003 and 2004 was only 0.7 kg P ha^{-1} compared to 2.3 kg P ha^{-1} for the uncoppiced trees mean uptake for year 2004. The P accumulation for the uncoppiced trees was significantly different for the three fields. The Low field had the highest mean uptake of 3.1 kg P ha^{-1}. The High field had the lowest mean uptake of 1.6 kg P ha^{-1} but did not differ significantly from the Medium field which had a mean uptake of 2.1 kg P ha^{-1}. The uncoppiced trees had a significant clonal difference in P uptake with clone OP-367 removing more P per hectare than clone DN-34.

Table 19: Total P uptake means and LSD values for hybrid poplars with analysis of variance to determine significant effects of field and clone on P uptake, 2003-2004.

		Coppiced	Uncoppiced
Field	Clone	P accumulation (kg ha^{-1})	
Low	**DN-34**	**0.68**	**2.98**
	OP-367	**0.63**	**3.24**
Medium	**DN-34**	**0.79**	**1.04**
	OP-367	**0.86**	**3.16**
High	**DN-34**	**0.68**	**1.39**
	OP-367	**0.48**	**1.79**
LSD		**0.4**	**0.9**
Source of Variation	df	F-Value	
Field	**2**	**NS**	**5****
Clone	**1**	**NS**	**6***
Field x Clone	**2**	**NS**	**NS**

***, **, *** denotes significance at α =0.05, 0.01 and 0.001 respectively;**
NS = Not Significant at α = 0.05.

While the hybrid poplar trees had similar elemental P tissue concentrations to traditional agronomic crops the P removal per area was notably less because of the low density planting. One study conducted on the P removal of high P soils found P uptake values of 85 and 95 kg ha^{-1} for Indian mustard (*Brassica napus*) and corn (*Zea mays*), respectively (Delorme et al., 2000). The P uptake per area for these crops was considerably higher than the uptake of the hybrid poplars. However, the elemental concentration for the Indian mustard and corn is similar at 4.6 g kg^{-1} and 4.9 g kg^{-1}, respectively.

Planting the hybrid poplar trees at a higher density would increase the quantity of biomass per area and therefore increase P uptake. For peak production at 7-10 years trees should be planted on a 2.4 m^2 area (Netzer et al., 2002) however, this could be decreased for a shorter production period. Doubling the planting density used in this experiment, spacing on 0.56 m^2 centers rather than 2.25 m^2 centers, with all other variables held constant, the P uptake would increase by 400%. At this density the hybrid poplar uptake after two seasons would be approximately 12 kg P ha^{-1} which is still much lower than the maximum P removal of crops like Indian mustard and corn, mentioned above. Several factors should be considered when determining the spacing of trees such as the market size of the desired product, rotation length, design for weed control and harvesting (Heilman et al., 1996).

Eghball et al., (2003) reported in another study that the grain of various corn hybrids averaged across treatments removed 24.3 to 35.0 kg P ha^{-1} and 16.6 to 25.7 kg P ha^{-1} in two consecutive years. The elemental concentration was again similar to the hybrid poplars however, the grain yields under adequate irrigation ranged from 8.1 to

10.4 Mg ha^{-1} and 7.9 to 9.6 Mg ha^{-1} in the two years. The mean yield of the uncoppiced hybrid poplars in this study was 1.3 Mg ha^{-1} after two seasons. The annual yield of traditional agricultural crops is much greater than 1 and/or 2 years of hybrid poplar yield experienced in this study.

In this experiment, the biomass of the hybrid poplars is likely driving the P uptake. Therefore in order to increase the quantities of P removed from the soil the biomass must be increased. The growth and biomass of hybrid poplar trees is expected to increase with age. One study found that annual biomass increments for hybrid poplars planted on weed controlled fields in eastern Maine surpassed 1 Mg ha^{-1} within 3 years and 3 to 5 Mg ha^{-1} by year five (Czapowskyj and Safford, 1993). According to this growth rate, hybrid poplars could more than triple the P uptake in two additional years, assuming the P tissue concentrations did not decrease with increased biomass.

The annual biomass production of the hybrid poplars planted on 2.4 m^2 centers has been found to increase yearly peaking at 6-9 dry Mg per hectare per year at year 7-10 after which production will continue but at declining rates of increase (Netzer et al., 2002). Allowing the hybrid poplars to increase biomass with age will increase the uptake however; recycling of P back into the soil will occur as the trees drop their leaves yearly. To prevent this while still giving the trees adequate time to grow, the leaves could be collected annually just after senescence using a vacuum method followed by mulching; thus removing them from the system along with the P contained within. Without removal, the leaves in this study would have returned an average of 29, 47 and 140 mg P per tree for coppiced 2003, coppiced 2004 and uncoppiced 2004 hybrid poplars, respectively.

The biomass of the hybrid poplars in year-2 was much greater without coppice in this study and subsequently the P uptake was higher. Since the biomass of hybrid poplars will continue to increase with age, only collecting the leaves yearly rather than harvesting the entire tree is likely to have increased P uptake in the long run.

Based on an average established growth rate of hybrid poplar trees of 6-9 dry Mg per hectare per year at year 7-10 (Netzer et al., 2002) and a yearly increase in tissue P concentrations proportional to that observed in this study with all other elements constant a prediction model was created to roughly estimate future P uptake (Table 20). According to this model, harvest at year 9 would remove approximately 70 kg P ha^{-1} (not including the annual removal in the harvest of the leaves alone).

Prediction model: Maximum potential P removal (kg ha^{-1}) at years 5, 7 and 9 =

(P Leaf g kg^{-1})*(0.12* Total Yield kg ha^{-1}) + (P Stem g kg^{-1})*(0.88* Total Yield kg ha^{-1});

where 0.12 and 0.88 are the proportions of total biomass for leaf and stem, respectively

Table 20: Potential P removal of hybrid poplars age 2-9 based on predicted values.

Year	P Leaf*	P Stem*	Leaf Yield*	Stem Yield*	Total Yield*	Maximum potential P removal
	(g kg^{-1})			(kg ha^{-1})		
2	6	2	150	1100	1250	3
5	10	4	480	3520	4000	19
7	12	6	720	5280	6000	40
9	14	8	960	7040	8000	70

*Years 5-9 predicted values

Heilman (1996) found that 4-year old hybrid poplars produced aboveground leafless biomass ranging from 44-111 mg ha^{-1}. While this is not a considerably large amount of biomass the trees removed considerably more P from the soil at rates ranging from 41-105 kg ha^{-1}. Heilman's study shows an increased concentration of total tissue P in the tree at age 4. If the concentration of P in the tree tissue increases significantly with age, the P removal could be greater than that predicted in table 20 and therefore give the hybrid poplars more potential for P phytoremediation.

P Removal

The P removed by the hybrid poplars during this experiment does not alone account for the reduction in soil P (Table 21). Other variables present could have contributed to the decrease in soil P. Some possible variables being P uptake by other plants and weeds present on the fields, plant and soil chemical changes in P form, and P carried in water transported off site through surface and subsurface pathways.

If the P was taken up by other plants that were present in the field, the P was only temporarily removed from the soil since those plants were not removed from the site. The P could have been recycled back into the soil when those plants died after final soil samples were taken. Repeating the soil sampling on the fields some time after the final samples were taken would provide insight into this area as well as possible change in P form. The plant available P could have entered into a different soil P pool at the time of final sampling resulting in low Mehlich-3 P extraction however, total P may not have decreased. To quantify such changes in chemical form of P more thorough soil analysis would be needed like measurement of water extractable P as well as total P.

Table 21: Soil-test P decrease with standard errors and hybrid poplar maximum potential P uptake with LSD, 2003-2004.

Field	Clone	P decrease surface soil	P decrease subsurface soil	Hybrid Poplar maximum P uptake
		Mg kg^{-1}		kg ha^{-1}
Low	DN-34	33±2	55±9	2.98
	OP-367			3.24
Medium	DN-34	92±9	189±12	1.04
	OP-367			3.16
High	DN-34	280±15	325±22	1.39
	OP-367			1.79
LSD				0.9

CHAPTER 4: CONCLUSION

This thesis examined the possibility of using hybrid poplar trees to phytoremediate P from over enriched soils. Initial soil-test results indicated the fields used in this study had excessive P concentrations such that P-based nutrient management must be implemented. During the two growing seasons of this study reductions in soil-test P concentrations were observed at the surface and subsurface soil on fields planted with hybrid poplar. There was a positive relationship between the initial soil-test P and the magnitude of the P reduction. While the final soil-test P concentrations still exceeded the agronomic optimum concentration required for crop growth the levels where significantly lowered in 16 months. But, due to the design of the experiment we can not determine how much of the decrease in soil-test P was attributable to hybrid poplar production.

By measuring and comparing the overall growth and tissue P concentrations of two hybrid poplar clones, this thesis evaluated the capacity of these hybrid poplar clones for P phytoremediation. The high survival rates of the trees indicate that they are well adapted to survive in eastern Maryland. It was observed that the hybrid poplar growth, indicated by the total biomass, was negatively correlated with the soil P concentrations. However, due to various experimental variations in the fields such as compaction and competing vegetation, the soil P alone does not explain the biomass results. Of the two clones, OP-367 had higher overall height and biomass. The biomass of the uncoppiced trees in 2004 was greater than the combined 2003 and 2004 biomass of the coppiced trees.

The hybrid poplar trees actively transported P from the soil into the leaf and stem tissue. The magnitude of the P concentration in the tree tissue was dependent on the soil P. The highest P concentration was observed in the leaf and stem tissue of coppiced and uncoppiced trees planted on the High field in 2003 and 2004. While none of the trees had exceptionally high tissue P, the relationship with the soil P indicates that the trees are capable of taking up luxury quantities of P. Overall, the P in the leaf tissue was greater than the stem tissue and clone DN-34 had higher tissue P than clone OP-367.

The P content of the hybrid poplar trees was used to measure P uptake and removal from the soil. The P content had a strong positive correlation with the biomass of the trees. In general, due to its higher biomass, clone OP-367 had higher mean P content than clone DN-34. The total P removed from the soil by the hybrid poplars was based on the P content of the trees per unit area. Therefore the P removal was mainly a function of biomass. The uncoppiced trees removed more P in 2004 than the coppiced trees combined 2003 and 2004 uptake.

The P that the hybrid poplars removed in this study was minimal. Neither clone exhibited foliar P uptake that would be considered hyperaccumulation. Compared to other agricultural crops the hybrid poplars biomass per unit area is much lower. Since P uptake in hybrid poplars is a function of biomass, to maximize P phytoremediation the biomass of the trees must be increased.

Increasing the density of planting is a possible option that would increase the total biomass of hybrid poplar trees per unit area and thereupon increase the P removal. In this study since the uncoppiced trees removed more P from the soil than the coppiced trees after two seasons coppicing annually is not recommended. Extending the length of time between harvests and allowing the hybrid poplars to increase biomass with age will also heighten the accumulation of P. Harvesting only the leaves of the trees after senescence will also increase the P removal. Since hybrid poplars are a perennial crop they will continue to remove P from the soil at an increasing rate each year with little associated cost and maintenance. Whereas traditional agricultural crops require additional costs and maintenance associated with annual site preparation, planting, and harvest.

Since the decrease in soil P was larger than the P removed by the hybrid poplar trees the complete cause of the reduction in soil test P is not clear. It may be due to a combination of factors including hybrid poplar uptake, other plant uptake, drainage water transport, and change in chemical form. Thus illustrating the importance of the type of soil test used for P and other elements of P movement that should be further examined in future studies of this type.

Policy Implications

P pollution in the Chesapeake Bay region has neither a single cause nor a single solution. The complexity of the issue has led to ongoing debates among scientists, legislators, citizens, and other stakeholders about its importance and most effective solutions. The sensitive element of over-application of P to agricultural lands addressed in this study has been particularly targeted in these debates. While, based on this research, utilizing hybrid poplar trees to phytoremediate soils with excess P can not be presented as a sole remedy , elements of it may be beneficial in refining current policy.

Current nutrient management policy entails soil and water conservation plans (see above section) that include a variety of BMPs. Several BMPs such as riparian buffer strips and wetlands implement the use of trees and other vegetation to filter and trap nutrients in runoff. Although these practices are somewhat effective in preventing nutrients from entering water bodies, the plant species composition within the vegetative structures may need to be tailored for maximum P uptake and removal. Currently the policy surrounding this type of BMP mandates the use of native plant species due to fear that nonnative species may become invasive. However, it should be noted that not all nonnative species are invasive and therefore policy should focus on which plant species are best suited to remove nutrients. Hybrid poplar trees have been over-looked as candidates for vegetation BMPs because they are not native to the Chesapeake Bay region. But clones of hybrid poplar trees are sterile and pose no threat of becoming invasive. This research suggests that hybrid poplars have potential to remove excess P from soils and should be considered for use in forested buffer strips to maximize P removal.

Resource conserving cover programs like CRP (see above section) are another aspect of nutrient management policy that is currently under discussion. Through this type of program landowners enter contracts with the government to establish permanent areas of grass and trees on land that needs protection in order to improve water quality. The contracts last between 10 and 15 years during which the land must remain undisturbed. While this ensures no additional nutrients are added to the land during the time within the program, with no removal of grasses or harvest of trees it also implies no nutrient removal. Therefore, land that is high in soil P before entering into CRP will remain high in soil P at the end of the program if current policy is not altered. Reserve programs promote a closed system in which the P in the soil is continually recycled through the vegetation and back to the soil.

This thesis found that most of the P within the hybrid poplar tree was contained within the leaves. Based on data from this study, if the leaves drop and are left on the soil, they would be returning 29 mg P per tree after one year and 47-140 mg P per tree after two years. The rate of P return to the soil may continue to increase with age. Annually removing the tree leaves after senescence will help mine down the P within the soils without disturbing the land. Hybrid poplar trees should also be specifically considered for this type of program because of their ability to regrow after being cut to the ground. If the entire tree was to be harvested to remove the P from the system, the tree would regrow without the need to disturb the soil with replanting. Therefore, while setting aside land through conservation programs is beneficial, it could be improved if policy was modified to allow the use of nonnative species and minimal maintenance of the vegetated land.

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