THE EFFECT OF HYDROCARBON CONTAMINATION AND MYCORRHIZAL INOCULATION ON POPLAR FINE ROOT DYNAMICS

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ABSTRACT

Quantifying the effects of hydrocarbon contamination on hybrid poplar fine root dynamics provides information about how well these trees tolerate the adverse conditions imposed by the presence of petroleum in the soil. Infection by ectomycorrhizal (ECM) fungi may benefit hybrid poplar growing in contaminated soils by providing greater access to water and nutrients and possibly inducing greater contaminant degradation. The overall objectives of this research were to: 1) investigate the relationship between the varying concentrations of total petroleum hydrocarbons (TPH) and nutrients across a hydrocarbon-contaminated site, as well as interactions between these contaminants and physical and chemical soil properties; 2) quantify the effects of these properties on the spatial and temporal patterns of fine root production for Griffin hybrid poplar (*P. deltoides x P. petrowskyana* c.v. Griffin); and (3) quantify the effect of ectomycorrhizal colonization on hybrid poplar fine root dynamics and N and P uptake when grown in diesel contaminated soil under controlled conditions. A minirhizotron camera provides a nondestructive approach for viewing roots *in situ.* This camera was used in both the field and growth chamber experiments to provide the data necessary for estimating fine root production. The field study was conducted at Hendon, SK, Canada. Twelve minirhizotron tubes were distributed across the field site and facilitated quantification of fine root production in areas of varying contamination levels. Residual hydrocarbon contamination was positively correlated with soil total C and N, which may suggest that the hydrocarbons remaining in the soil are associated with organic forms of these nutrients or increased microbial biomass. Total fine root production at the site was greater in the 0- to 20-cm depth (1.27 Mg ha^{-1}) than the 20- to 40-cm depth (0.51 Mg ha^{-1}) in 2004. Fine root production was stimulated by small amounts of hydrocarbon contamination at the field site. Nonlinear regression described fine root production as increasing linearly up to approximately 500 mg kg^{-1} TPH, then remaining constant as contamination increased. This trend was most pronounced in the 0- to 20-cm soil layer, with a (r^2 = 0.915). Stimulation of fine root production in the presence of hydrocarbons has significant implications for phytoremediation. If hybrid poplar can maintain increased root production in hydrocarbon contaminated soils, the

rhizosphere effect will be exaggerated and increased degradation of contaminants is likely to occur. Under controlled conditions, colonization of hybrid poplar roots by the ectomycorrhizal fungus *Pisolithus tinctorius* increased fine root production in a diesel contaminated soil (5000 mg diesel fuel kg $soil^{-1}$) compared to non-colonized trees growing in the same soil. Fine root production was 56.6 g m^{-2} in the colonized treatment and 22.6 g m^{-2} in the non-colonized treatment. In diesel contaminated/ECM colonized treatment, hybrid poplar leaf N and P concentrations after 12 wk were 23.1 and 3.6 g kg^{-1}, respectively. In diesel contaminated/non-colonized treatment, N and P concentrations were 15.7 and 2.7 g kg^{-1}, respectively. After 12 wk, 5.0% of the initial concentration of diesel fuel remained in the soil of the non-colonized treatment and 6.7% remained in the colonized treatment. Both treatments removed more contaminants from the soil than an unplanted control, which contained 8.9% of the initial diesel fuel concentration after 12 wk. Significantly more hydrocarbons were found sequestered in hybrid poplar roots from the colonized treatment (354.1 mg kg^{-1}) than in the non-colonized treatment (102.2 mg kg^{-1}). The results of this study indicate that hybrid poplar may be good candidates for use in phytoremediation of petroleum hydrocarbons because of the stimulation of fine root production at low levels of hydrocarbon contamination. However, colonization of hybrid poplar growing in diesel contaminated soil by *P. tinctorius* inhibited remediation of diesel fuel.

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1. GENERAL INTRODUCTION

The rapid industrialization that has occurred in the past century, and is still occurring around the globe today, has led to an ever increasing demand for petroleum and other organic chemical-based products. World oil consumption has soared to 13 billion L d^{-1} in 2004, while world pesticide application in 2000 was roughly 1.7 million kg d^{-1} (U.S. EIA). Given this vast consumption, the contamination of soils with petroleum, as well as the other organic chemicals associated with industry, has become a global concern. This contamination is usually a result of leakage, spillage, or in cases involving pesticides and the like, over-application and drift. In the more recent past, the consequences of this resultant contamination have become fully realized, and efforts to remediate these sites have significantly increased in number.

Remediation of organic chemical-contaminated soils has traditionally involved removal and subsequent "washing" with solutions containing surfactants and/or mild solvents or land filling. *In situ* containment techniques such as vitrification and air stripping also have been used in the past (Riser-Roberts, 1998). These methods are usually only viable where a small area is concerned, and in some instances cost has been prohibitive (Pierzynski et al., 2000). The actual applicability and effectiveness of many *in situ* methods also has been called into question. Air stripping, for example, is only applicable to cases involving volatile compounds, while *in situ* soil washing can deteriorate the soil and produce large amounts of contaminated elutriate (Riser-Roberts, 1998).

Phytoremediation, the use of green plants and associated microorganisms to treat contaminated soil *in situ*, takes advantage of the fact that many plants can tolerate the adverse growing conditions resulting from organic chemical contamination (Cunningham et al. 1996). Many factors can affect the ability of plants to grow on contaminated soils. Soil pH is often significantly reduced due to organic wastes containing toxic heavy metals (Riser-Roberts, 1998). This reduced pH can have a substantial effect on plant growth as well as microbial activity. Soil bulk densities can

be greatly increased in hydrocarbon-contaminated soils, therefore negatively affecting water infiltration (Blakely et al., 2002). Nitrogen dynamics can be altered by contamination as well, resulting in a large proportion of immobilized N. On the other hand, requisite microbial communities and ectomycorrhizal fungi associated with certain tree species have been shown to play an important role in degradation of xenobiotic chemicals (Riser-Roberts, 1998).

The ability of plants to withstand the toxicity and resulting alteration of nutrient status associated with organic chemical-contaminated soils often have been evaluated in terms of aboveground production (Biernacki et al., 1995; Chaîneau et al., 1997). Therefore, given the fact that fine roots have been shown to be important in understanding resource allocation as well as soil microbe distribution, assessing the response of fine roots to contamination could provide interesting insights into plant survival strategies.

Ectomycorrhizal fungi are obligate biotrophs which are known to enter into symbiotic relationships with many tree species (Smith and Read, 1997). These fungi play an important role in the nutrient access capabilities of their hosts due to the hyphal network considerably increasing the volume of soil contacted. In fact, hyphal length of ectomycorrhizal fungi has been shown to reach 2.2 m^{-2} g^{-1} soil (Tisdall et al., 1997). Concomitantly, many ectomycorrhizal fungi have been proven capable of actively degrading hydrocarbons, and infection rates remain stable in hydrocarbon contaminated environments (Nicolotti and Egli, 1998; Gramss et al. 1999).

Considering the above, the overall objectives of this research are to: (1) quantify the effect of residual hydrocarbon contamination on hybrid poplar fine root dynamics, as well as identify relationships between residual contamination and various soil properties, thereby assessing the potential of hybrid poplar for phytoremediation applications, and (2) to determine the effect of ectomycorrhizal infection on poplar fine root dynamics growing in hydrocarbon-contaminated soil. These fungi may become important in nutrient access when poplars are grown in organic chemical contaminated soil, and may also provide a buffer in areas of fungal proliferation, due to direct hydrocarbon degradation.

2. LITERATURE REVIEW

2.1 Petroleum hydrocarbons

Unrefined petroleum, or crude oil, is a complex substance found in deposits throughout the world. In a natural state this material consists of hundreds of thousands of distinct hydrocarbon compounds (Riser-Roberts, 1998). All chemical compounds known as hydrocarbons are composed of various arrangements of carbon and hydrogen atoms, but those constituting crude oil can be divided into three main groups: saturated and unsaturated aliphatics and aromatic hydrocarbons (Huesemann and Moore, 1993).

Saturated hydrocarbons (also known as alkanes) have the maximum number of hydrogen atoms possible and therefore no double bonds. Alkanes can be linear, branched or cyclic structures (Riser-Roberts, 1998). Unsaturated compounds do not possess the full complement of hydrogen atoms, but instead have formed either one double carbon bond (alkenes), two double carbon bonds (dienes), or at least one triple carbon bond (alkynes). Aromatic hydrocarbons consist of an aromatic ring, which contains six carbon atoms connected with three alternating double bonds (Hornback, 1998). The BTEX compounds, namely benzene, toluene, ethylbenzene, and xylene, are examples of aromatic hydrocarbons. Aromatic molecules which contain two or more fused aromatic rings are referred to as polycyclic aromatic hydrocarbons, or PAHs (Frick et al., 1999).

Refined petroleum products are common to daily life, but still remain highly complex compounds. Kerosene, for example, contains as many as 10,000 distinct hydrocarbons (Riser-Roberts, 1998). The composition of these products is dependent on their level of refinement. Diesel fuels contain roughly 60 to 70% C_{10}-C_{19} aliphatic alkanes and cycloalkanes, with the majority of the remaining components being aromatic. Diesel fuels contain 5% or less PAHs (U.S. EPA).

Given the complexity of these substances, the degradation of hydrocarbons that have entered the soil environment depends on the metabolism of a vast array of compounds by microorganisms and plants. In general the relative degradability of

hydrocarbons is known to decrease with increased aromaticity and branching (Riser-Roberts, 1998) (Fig. 2.1).

Compounds such as PAHs and cycloalkanes are more resistant to microbial attack due to their high toxicity and increasing weight (Atlas, 1981). This increase in molecular weight leads to lessened bioavailability resulting from sorption or partitioning onto the soil colloid or organic matter (Young and Mulligan, 2004). These forces can have substantial influence on the effectiveness of remediation. Therefore, in order to maximize the potential of any bio/phytoremediation strategy, it is necessary to be acutely aware of sorption and partitioning characteristics as well as other factors affecting the bioavailability of hydrocarbons (Riser-Roberts, 1998).

2.1.1 Bioavailability and interactions with the soil

The bioavailability of organic contaminants for phytoremediation is affected by the physiochemical properties of the compound, as well as the various physical and chemical properties of the soil and environment. The characteristics that determine a compound's distribution between the vapor, liquid, solid, and adsorbed phase are, in turn, going to be the characteristics that determine the availability of this compound for degradation (Cunningham et al., 1996).

Volatilization can be responsible for the movement of gaseous-phase xenobiotics from the soil water to the atmosphere. Vapor pressure determines the extent and speed of volatilization of a chemical, with increased vapor pressure resulting in increased volatilization. The tendency to volatilize can be described by K_h, the Henry's Law constant. This value can be calculated using the vapor pressure and solubility of a compound, and characterizes the ability of the compound to move between the aqueous and gaseous phases (Cunningham et al., 1996). The higher the constant the more likely volatilization will occur. For example, the monocyclic aromatic benzene, with a Henry's law constant of 2.28×10^{-1} (dimensionless), will volatilize from the soil much faster than the polycyclic aromatic flouranthene, with a value of 6.60×10^{-4} (U.S. EPA). Along with vapor pressure and water solubility, adsorption rate also has an effect on a chemical's tendency to volatilize. Contaminants held tightly in the soil as a result of covalent bonding will be less likely to desorb, which could facilitate volatilization

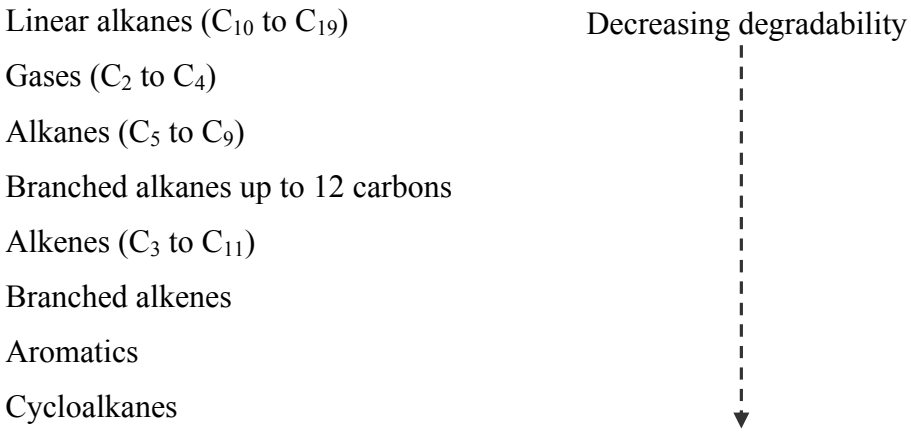


Figure 2.1 Relative degradability of hydrocarbon compounds. Modified from Riser-Roberts (1998).

(Cunningham et al., 1996). Increased volatilization rates result in decreased soil biodegradation due to limited residence time in the soil.

One of the most important factors influencing bioavailability of a contaminant is sorption (Cunningham and Ow, 1996). In general, high organic carbon contents (>5%) usually lead to increased adsorption while lower concentrations of organic matter in the soil can mean less area for sorption of hydrocarbons, although binding to inorganic soil fractions is also known to occur (Otten et al., 1997).

Once in the soil, a contaminant can bind to the organic and inorganic soil fractions through a variety of sorptive forces, at which point the contaminant may become unavailable for plant uptake and microbial breakdown. Charged pollutants are capable of forming electrostatic bonds with the negatively charged soil colloid, as well as with organic matter exchange sites, which are often pH-dependent (Novak et al., 1995). However, many common organic contaminants in the soil have no charge, and these chemicals have been shown to be sorbed by mechanisms such as van der Waals forces and the partitioning of lipophilic contaminants onto the solid phase of the soil (Novak et al., 1995; Cunningham et al., 1996). Organic contaminants and their polymers (resulting from enzymatic or abiotic action) also can become covalently bonded to the soil. These covalently bonded substances will be bound with much greater force than those bound by partitioning forces and will not readily desorb (Novak et al., 1995).

Soil humus is often the most important sorbent material in regards to organic compounds. Humic substances in the soil occur as the result of the decomposition of organic matter and can be defined as heterogeneous organic substances which are yellow to black in color and possess high molecular weights (Klavins and Serzane, 2000). Along with direct decomposition, humus can be synthesized through chemical reactions (abiotic condensation) of low molecular weight monomeric molecules (amino acids, lipids) that have been formed by the degradation of larger molecules such as polysaccharides or proteins (Tremblay et al., 2004).

As stated above, van der Waals forces and hydrophobic partitioning are thought to be the most important mechanism involved in contaminant binding with humic substances. Partitioning of an organic is directly related to its water solubility, and can be described by the octanol-water coefficient (log K_{ow}) (Burken and Schnoor, 1998;

Pierzynski et al., 2000). The octanol-water coefficient refers to the equilibirum concentration of a chemical between octanol and water, which indicates the affinity of the chemical for aqueous as opposed to organic phases (Schwab et al., 1998). The more lipophilic the contaminant, the more likely it will be sorbed to organic matter, whereas more hydrophilic compounds may be dissolved in the soil solution. Organic contaminants that are hydrophobic in nature will tend to sorb with increased strength to humic molecules of increased aromaticity. Increased aromaticity translates to a greater number of carbon-carbon double bonds within the molecule, and therefore increased strength of the van der Waals forces (Klavins and Serzane, 2000). Hydrophobic contaminants show the greatest bonding affinity to the hydrophobic acid fraction of the humus. In these instances of humic acid-xenobiotic interaction, aggregation between hydrophobic contaminants and humic acids can occur in such a way that the contaminants partition off into the hydrophobic interior of the humic molecule, considerably reducing the bioavailability of the contaminant (Novak et al., 1995). In fact, Ke et al. (2003) found that adding humic acid to soil contaminated with pyrene reduced overall loss of this contaminant from the system, suggesting that the chemical became bound to the acid and therefore was made less bioavailable.

Polymerization and incorporation of certain pollutants into the soil humus can also occur as a result of enzymatic action or abiotic reactions catalyzed by the humus itself (Cunningham et al., 1996; Klavins and Serzane, 2000). This process involves oxidation/reduction of the contaminant and subsequent covalent bonding of the contaminant with soil organic matter (Klavins and Serzane, 2000). Redox reactions involving the contaminant give rise to free radicals which react with compounds in close proximity. This free-radical induced reaction produces contaminant residue-organic matter polymers. Complexes resulting from this process are extremely persistent in the soil and are often referred to as “bound residues” (Novak et al., 1995).

The degree of residue formation between xenobiotics and humic substances is significant. In the case of pesticides, Khan (1982) found that anywhere from 7 to 90% of those applied become bound in this manner. Large compounds such as PAHs partition off quickly onto humic substances as well as soil particles, after which they may undergo further incorporation into the humus (Novak et al., 1995). Given their greater

hydrophobicity and low water solubility, PAHs tend to be some of the most persistent organic contaminants in the soil (Tremblay et al., 2004).

As the residence times of these compounds in the soil lengthens, they tend to become even more resistant to desorption and degradation. This is commonly referred to as weathering or aging of the compounds (Cunningham et al., 1996). In fact, Capriel et al. (1985) found that 83% of radiolabeled atrazine initially supplied to a field soil was still present after 9 yr. Reasons for this weathering phenomenon may be that the strength of chemical bonds increase with time, microbes become physically excluded from adsorption sites, and incorporation into humus becomes more pronounced (Calderbank, 1989; Scow and Hutson, 1992). Diffusion of the more hydrophilic humic substances, together with their sorbed organics may result in transfer of these compounds into sites that are even more restrictive, such as soil micropores, clay interlayers, or deeper into humic matrixes (Riser-Roberts, 1998). Therefore, as contaminants age and become more diffused into isolated micropores, they become less available for biodegradation.

If an organic molecule becomes sorbed to the soil, overall bioavailability for a plant depends on how readily the molecule will desorb back into solution. Contaminants sorbed to humic substances due to partitioning effects will be more readily desorbed, and therefore plant available, than those fractionated and bound through polymerization (Novak et al., 1995). In addition, the more aged a residue becomes the more resistant it will be to desorption and microbial attack (Pignatello, 1989). Still, microbes may utilize humic substances as a nutrient source and consequently release tightly sorbed contaminants in the process (Hsu and Bartha, 1976).

As mentioned above, the amount and type of clay in a soil can also affect the bioavailability of organic contaminants (Riser-Roberts, 1998). Clay particles have a high surface area in comparison to other soil particles, and therefore possess a greater area for adsorption of xenobiotics. The small particle size of clay also can lead to decreased diffusion of contaminants due to increased tortuosity, and therefore lower bioavailability (Cunningham et al., 1996). Clay particles can interact with humic substances as well, which stabilizes organic matter in soils. These clay-humic complexes usually appear as organic matter coatings on clay surfaces and can have significant effects on the adsorption properties of mineral solids (Tremblay et al., 2004).

Wang and Xing (2005) found that prepared montmorillonite clay-humic complexes sorbed over four times more phenanthrene as the clay alone.

In soils with low clay and organic matter concentrations the bioavailability of contaminants may be increased. Carmichael and Pfaender (1997) found that sandy soils had greater PAH degradation rates than clayey soils. Still, other factors may play a role in governing bioavailability in sandy soils. For example, Löser et al. (1999) reported that even in sandy soils residual phenanthrene pollution was detected, due to low bioavailability. This low bioavailability was, in turn, shown by BET analysis to be an effect of micropores on the soil surface. These pores enlarged the surface area by 120 times, resulting in increased adsorption of phenanthrene to the soil by physical bonding.

2.1.2 Effect on plant and microbial communities

The ability of plant species to tolerate hydrocarbon contamination varies greatly. At high concentrations these contaminants can kill or severely impede plant growth and/or germination. Udo and Fayemi (1975) demonstrated that hydrocarbon additions to the soil at concentrations of 10.6% (w/w) impeded germination of maize (*Zea mays* L.) plants. Seeds did germinate at concentrations of 8.5%, but yield was reduced by 95%. In fact, the authors observed a reduction in yield at concentrations as low as 1%. This poor yield response was attributed to the exclusion of air from the soil due to the contaminants interfering with soil/water relationships, and also to the depletion of oxygen by microbial degradation of the contaminants.

The effect of fuel oil contamination on germination and growth of various cultivated plants was studied by Chaîneau et al. (1997). The LC_{50} values (concentration of a xenobiotoic at which 50% of the exposed organisms die or do not grow) for germination in the presence of hydrocarbon contamination ranged from 0.3% (fuel oil in sand w/w) for lettuce (*Lactuca sativa* L.) up to 7% (fuel oil in sand w/w) for sunflower (*Helianthus annuus* L.). Reduction in aerial biomass of wheat and bean was as much as 80% in both the 0.6 and 1% fuel oil treatments. On the other hand, the authors found that maize faired better when grown on contaminated soils, with only a 30% reduction in biomass in the 1.2% treatment and no significant difference between the 0.6% treatment and the control.

Kirk et al. (2002) also studied germination rates and root growth of some legumes and wild grasses grown on hydrocarbon contaminated soils, in order to identify the best suited species for phytoremediation. Of the four plants tested, the authors found that perennial ryegrass (*Lolium perenne* var. Affinity) had the highest germination rates in all contamination treatments, followed by alfalfa (*Medicago sativa*), crown vetch (*Coronilla varia*) and little bluestem (*Schizachyrum scoparium*). Root growth of perennial ryegrass, little bluestem and crown vetch was significantly reduced in all treatments, while alfalfa root growth was actually stimulated at low levels of contamination. Hormesis, which refers to stimulated growth in response to small amounts of toxicity, was observed in alfalfa in the 1 and 1.5% treatments. This phenomenon was also documented by Salanitro et al. (1997), who found 40 to 70% growth stimulation in corn plants grown in crude oil amended soils over the control.

Hydrocarbon contamination can have a significant effect on soil microbial populations (Löser et al., 1998; Riser-Roberts, 1998). Many heterotrophic bacteria are able to utilize hydrocarbons as a source of C and energy, and most ecosystems support indigenous communities of microbes that are capable of substantial hydrocarbon degradation (Nichols et al., 1996). Heterotrophic soil bacteria such as *Pseudomonas* and *Arthrobacter* are capable of breaking down hydrocarbons through various metabolic pathways. Pseudomonads also maintain catabolic pathways which facilitate the degradation of several aromatic compounds, such as toluene and naphthalene, with a limited suite of enzymes (Morelli et al., 2005). Given this fact, it is not surprising that hydrocarbon contamination can increase microbial activity in soil, an effect that can lead to rapid degradation but can come at the expense of species diversity. Hydrocarbon degraders usually are present in the soil at a ratio of approximately 0.1% of the population. As contamination increases these microbes may eventually make up 100% of the population, as a result of either the inability of other species to utilize the contaminants as a substrate or outcompetition by better adapted degraders (Atlas, 1981). In fact, Morelli et al. (2005) found that while counts of both hydrocarbon-degrading and heterotrophic bacteria were greater in all sludge-soil mixtures tested (2.50, 5.00 and 10.00 g API [American Petroleum Institute] sludge kg^{-1} dry soil), diversity decreased with increasing contamination. The addition of sludge to the soil was found to

selectively enrich bacteria of the genus *Pseudomonas*, as well as the Gram-negative bacterial population in general. Del Panno et al. (2005), using the same API sludge concentrations, also found increased activity of heterotrophic and hydrocarbon-degrading microbes in the 2.50 and 5.00 g treatments, but saw a slight decline in the amount of hydrocarbon degraders in the 10.00 g treatment. This indicates that at high enough concentrations, microbial activity can be limited by hydrocarbon concentration. The authors also observed decreased diversity as contamination increased. In the 2.50 and 5.00 g treatments microbial community structure did return to a relatively stable and diverse state after 180 d, while the community in the 10.00 g treatment still reflected a disturbed state even after 360 d.

Some species of microbes appear to be limited in their ability to utilize hydrocarbons, while others show a greater amount of diversification. For example, bacteria of the genus *Mycobacterium* mineralizes pyrene and carbazole, as well as benzo[*a*]pyrene, while the genus *Xanthomonas* has proven capable of degrading only carbazole (Grosser et al., 1991). On the other hand, the activity of bacteria such as *Acinetobacter* decreases significantly in hydrocarbon contaminated soils, which is most likely due to these species inability to utilize the contaminants as a growth substrate (Del Panno et al., 2005). Heavier PAHs, usually consisting of five rings or more, often require cometabolism by different species of microorganisms in order to be degraded. Therefore, more extensive degradation and less long-term community disturbance will occur if diversity can be maintain in contaminated soils (Riser-Roberts, 1998).

Fungi, filamentous and unicellular, make up a large part of the microbial biomass in soil (Riser-Roberts, 1998). These organisms are important as hydrocarbon degraders, and produce nonspecific enzymes capable of breaking down aromatic structures. The extracellular enzymes can be beneficial as a substrate for bacterial growth, and facilitate xenobiotic cometabolism (Gramss et al., 1999). Many fungi, such as *Cunningham elegans*, *Saccharomyces cervisiae* and *Aspergillus ochraceus* degrade PAHs (Sutherland, 1991). Further information regarding mycorrhizal species of fungi will be discussed later.

2.2 Nutrient status of hydrocarbon contaminated soils

The effectiveness of phytoremediation, and bioremediation in general, is contingent upon available nutrients for use by plants and microorganisms. Nitrogen and phosphorus are the nutrients that most frequently limit bioremediation (Riser-Roberts, 1998). The addition of petroleum products to the soil can widen the C:N ratio, therefore limiting available N for degradation processes (Frick et al., 1999). In addition, microbes able to metabolize hydrocarbons will quickly immobilize the mineral N that is available, leaving unfavorable conditions for other microorganisms and growing plants (Newman et al., 2004). For example, Xu and Johnson (1997) found that when barley was grown on hydrocarbon-contaminated soils, plant N decreased as hydrocarbon contamination increased, while microbial N increased along with contamination. This increase in microbial N reduced available mineral N for plant uptake. It should be noted that this enlarged microbial N pool, resulting from selection and proliferation of hydrocarbon degrading microorganisms, can prove beneficial in that it acts as an N sink which will slowly remineralize and become plant available (Riser-Roberts, 1998).

This absence of sufficient N in the soil will, in turn, slow the degradation process resulting from plant and microbe metabolism (Riser-Roberts, 1998). Therefore, adding nutrients in the form of either organic or inorganic fertilizers can stimulate contaminant degradation (Huesemann & Moore, 1993). Hutchinson et al. (2001) found increased petroleum sludge degradation in fertilized treatments, in comparison to unfertilized treatments. However, this effect was more noticeable in the last 6 mo of the 12 mo study and overall was not as substantial as expected. This may have been due to sufficient N being supplied by the sludge itself. Amadi and Ue Bari (1992) also found that nutrient supplementation reduced the adverse effects of crude oil on maize performance, but results varied greatly depending on the type of supplement. Their research indicated that organic supplements increased germination rates, plant height, leaf area and dry matter yield when compared to inorganic supplements. Of the organic materials tested, poultry manure performed the best as a nutrient source, while sawdust was shown to exacerbate the adverse effects of crude oil in regards to all parameters other than germination rates. This was mostly likely due to a widening of the C:N ratio in the sawdust treatments.

Optimal fertilization rates are dependent on what plant species are used and what

contaminants are present. According to Brown et al. (1983), microbial degradation of oily sludge occurs most rapidly when N is added to obtain a C:N ratio of 9:1. On the other hand, Hutchinson et al. (2001) found no significant response to N additions in unplanted treatments of petroleum sludge, which could have been due to adequate concentrations of organic nutrients in the waste itself. The authors did, however, find an optimal C:N:P rate in planted treatments of 100:2:0.2.

2.3 Phytoremediation of hydrocarbon contaminated soils

Many types of plants phytoremediate soil contaminated with petroleum hydrocarbons. For example, Günther et al. (1996) found that the initial concentration of a hydrocarbon mixture in soil planted with ryegrass (*Lolium perenne* L.) was reduced by 97% after 3 wk, as opposed to 82% in unplanted controls. Aprill and Sims (1990) observed consistently higher rates of PAH degradation in soils planted with a medley of grasses, as opposed to unplanted soil. Poplar trees (*Populus deltoids* x *Wettsteinii*) also removed 60% of initial soil diesel fuel concentrations after 30 d (Palmroth et al., 2002).

Plants remediate hydrocarbon contaminated soil through either uptake and direct degradation and subsequent sequestration, adsorption onto roots, or indirect degradation through rhizosphere stimulation (Cunningham et al., 1996; Burken, 2003). These mechanisms are not mutually exclusive and usually coincide when contamination is present (Frick et al., 1999).

2.3.1 Plant uptake of hydrocarbons

The main pathway by which organic contaminants enter into a plant system is the translocation of water into the root with the transpiration stream. Once a contaminant comes into contact with the root surface, uptake is governed primarily by the chemicals relative hydrophobicity, as measured by the octanol-water partition coefficient (log K_{ow}) (Burken, 2003).

Extremely hydrophobic chemicals (log K_{ow}>4) will not be transported through the plant due to their affinity for lipids and therefore their tendency to be sorbed to the root surface (Fig. 2.2) (Schnoor et al., 1995; Burken and Schnoor, 1998). Several studies have found that plants grown in sludge amended soil contain significant amounts of

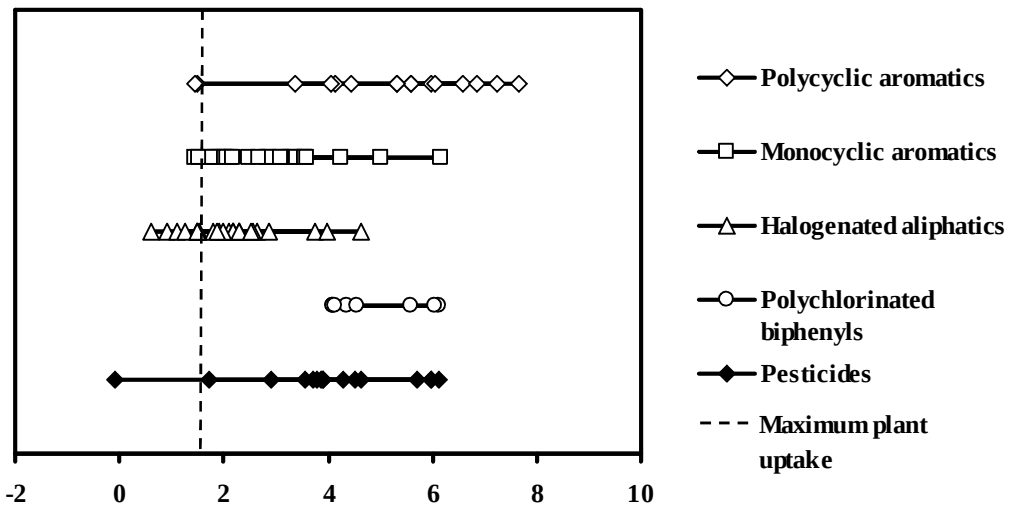


Figure 2.2 Log K_{ow} values for selected organic contaminants. Adapted from Ryan et al. (1988). Maximum uptake value based on Briggs et al. (1982).

highly hydrophobic PAHs in the roots, while none have entered the shoots (Gao and Zhu, 2004; Parrish et al., 2005). Root crops such as carrots also have been found to take up and accumulate PAHs in their peels, which is due to the high log K_{ow} values of the PAHs, and therefore, their partitioning off into the high lipid content carrot peels (Cunningham and Ow, 1996).

Extremely hydrophilic chemicals (log K_{ow}<1), on the other hand, will not be sorbed to plant roots, but neither can they pass through the endodermis of the root, due to low root permeability (Schnoor et al., 1995). Chaîneau et al. (1997) found no uptake of fuel oil (characterized here as a "complex mixture of low log K_{ow} hydrocarbons") by maize grown in concentrations up to 1% (w/w).

According to Schwab et al. (1998) contaminants with log K_{ow} values >1.5 are more likely to adsorb onto the roots of a plant than to be absorbed and translocated within the plant. Briggs et al. (1982) reported that the optimum lipophilicity for translocation of organic compounds to shoots occurs at log K_{ow} = 1.8. Chemicals with a log K_{ow} < 1.8 are relatively hydrophilic and can move easily through the apoplast, but will not diffuse through the organic membranes into the symplast of the endodermis, therefore limiting transport into the xylem. On the other hand, chemicals with a log K_{ow} > 1.8 can enter the epidermis, but are more likely to be adsorbed before passing into the xylem. This study shows a correlation between log K_{ow} values and the transpiration stream concentration factor (TSCF). The TSCF is calculated as the concentration of a compound in the transpiration stream divided by the concentration in the soil solution in contact with the root. Octanol-water partition coefficient values either greater or less than 1.8 corresponded with low TSCF values due to binding and/or exclusion of the chemical at the root interface (Briggs et al., 1982).

Adsorption of hydrocarbons onto roots can be significant, and can be seen as an accumulation process, given that the contaminants may be sequestered at the root interface in their initial form (Gao and Zhu, 2004). In a study which quantified the adsorption of naphthalene onto roots, Schwab et al. (1998) found that alfalfa adsorbed the contaminant at twice the rate of tall fescue (*Festuca arundiacea* Schreber). This was reported to be due to the lipid content of alfalfa, which was approximately twice as high as that of fescue (10 g lipid kg^{-1} dry root for alfalfa compared to 4.5 g kg^{-1} for fescue).

Adsorption can be affected by the age of the plant. Older roots tend to have less surface area for adsorption, as well as a diminished capability for water uptake and assimilation (Schwab et al., 1998).

Briggs et al. (1982) conducted experiments involving the adsorption of non-ionized organic compounds onto barley roots, as well as their subsequent uptake to the shoots. The series of chemicals used were *O*-methylcarbamoyloximes and substituted phenylureas. The researchers used the root concentration factor (RCF) to assess the amount of root uptake. The RCF is defined as:

RCF = Concentration in roots/Concentration in external solution [2.1]

The RCF has a positive linear relationship with log K_{ow}; as the lipophilicity of a compound increased, the amount in the root increased as well. Sorption, on the other hand, fell as lipophilicity increased, indicating that the observed RCF values for more lipophilic compounds were due almost exclusively to partitioning.

Several studies have shown that plant uptake of hydrocarbons to shoots is negligible (Schwab and Banks, 1994; Reilley et al., 1996). As discussed above, due to partitioning, hydrocarbons often become strongly sorbed to soil organic matter or roots and therefore are unavailable for plant uptake to shoots. Despite these facts, small amounts of petrogenic hydrocarbon uptake does occur. Parrish et al. (2005) found that zucchini (*Cucurbita pepo* ssp. *pepo*), cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo* ssp. *ovifera*) all took up PAHs, resulting in mean shoot concentrations of 0.825, 0.188 and 0.667 µg respectively, over four trials. Gao and Zhu (2004) reported uptake of pyrene and phenanthrene by 12 plant species. Shoot concentrations ranged from 0.10 to 0.31 mg kg^{-1} for phenanthrene and 0.23 to 7.37 mg kg^{-1} for pyrene when plants were grown in soils spiked with 133 mg kg^{-1} phenanthrene and 172 mg kg^{-1} pyrene. It should be noted that in unspiked control soils, shoot concentrations of phenanthrene ranged from 0.04 to 0.26 mg kg^{-1}, while for pyrene the range was 0.06 to 1.05 mg kg^{-1}. This indicates uptake of these PAHs from the air at the leaf surface.

2.3.2 Plant metabolism of organic contaminants

Plant metabolism of organic chemicals proceeds in three general steps: transformation, conjugation and sequestration (Fig. 2.3). Transformation occurs within the symplast and refers to the initial alteration of the compound as a result of the addition of various functional groups (Burken, 2003). Hydroxyl (-OH), Amino ($-NH_2$) and Sulfhydryl (-SH) groups are commonly introduced via transformation reactions (Trapp and McFarlane, 1995). Frequently, transformations of organic chemicals occur as a result of oxidation reactions, which are of utmost importance in increasing the solubility of lipophilic compounds (Burken, 2003). Hybrid poplar, for example, has been reported to oxidize trichloroethylene (TCE) upon uptake to metabolites such as trichloroacetic acid (Newman et al., 1997).

The most common oxidation reaction appears to be hydroxylation. This reaction results in the addition of an –OH functional group, which provides an appropriate site for subsequent conjugation (Burken, 2003). In pesticides and herbicides the major enzyme family responsible for catalyzing hydroxylation is the cytochrome P-450 oxygenases. *N*- and *O*-dealkylation are contingent upon cytochrome P-450 activity, while demethylation of pesticides in wheat and sorghum are catalyzed by this enzyme (Trapp and McFarlane, 1995). Less information is available regarding hydrocarbons, although hydroxylation is still deemed the most prevalent transformation reaction. Benzene and toluene conversion by plants is a result of hydroxylation due to aromatic ring cleavage (Ugrekhelidze et al., 1996). The primary products of this reaction are nonvolatile organic acids, with an appreciable amount of the resultant C atoms being incorporated into plant amino acids. Durmishidze (1977) reported that benzene, toluene, and xylene move into the transpiration stream relatively easily and were metabolized by cereal grasses within 2 to 3 d, corn within 4 to 5 d and root crops within 5 to 6 d. The author also noted that the products of enzymatic oxidation in plants carried out by copper-containing enzymes such as p-diphenol oxidase initiate nonenzymatic oxidation of organic contaminants.

Conjugation is the second step in the metabolism of xenobiotics, although it should be noted that if conditions allow, some contaminants may undergo conjugation reactions without any precursory transformation (Burken, 2003). In this step,

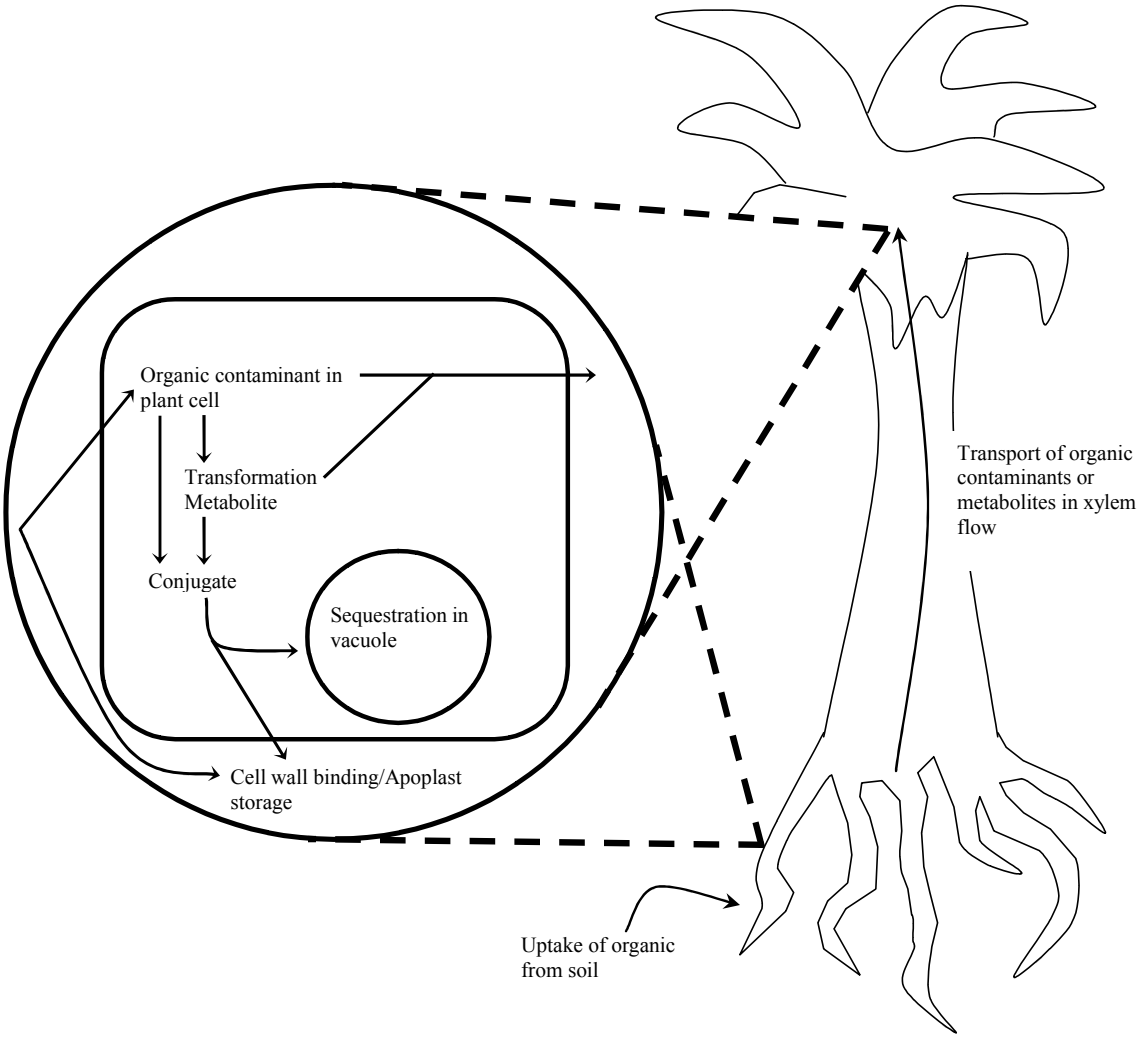


Figure 2.3 Basic mechanisms of organic contaminant uptake and metabolism in plant cells.

metabolites resulting from transformation reactions become covalently joined with an endogenous hydrophilic amino acid, peptide or any number of other molecules, therefore forming a water-soluble product. Ugrekhelidze et al. (1999) found that phenols taken up by mung bean (*Phaseolus aureus*) and wheat (*Triticum vulgare*) conjugated primarily with low-molecular-weight peptides.

The tripeptide glutathione is known to be an important conjugate of organic contaminants in plant and animal systems and is considered extremely important in the detoxification of organics (Coleman et al., 1997). The enzyme glutathione *S*-tranferase acts as catalyst for conjugation between xenobiotics and glutathione by utilizing functional groups either originally present or added in transformation (Burken, 2003). Other conjugates also can be formed. Glucosidation, for example, is catalyzed by glucosyltransferase enzymes and results in soluble glucosides, which are molecules that can be hydrolyzed to a simple sugar and compounds such as aldehydes, phenols or esters (Trapp and McFarlane, 1995). Conjugation generally works to further increase the solubility of the compound, facilitating removal from metabolic tissues into vacuoles. Initial conjugation of metabolites to cell wall can also occur and result in bound, or unextractable, residues within the plant (Burken, 2003).

The third step in organic chemical metabolism is sequestration, which can be seen as the mechanism by which plants remove xenobiotics from their metabolic tissues. Conjugates formed in previous metabolic reactions will in this step either be stored in cell vacuoles, stored in the apoplast or become covalently bound to cell walls (Burken, 2003). Once in a plant cell, storage of a xenobiotic conjugate will require passage through the tonoplast or the plasma membrane. Given that at the pH of the cytosol most conjugates assume a net negative charge active transport is necessary to transfer the compounds into the vacuole/apoplast (Coleman et al., 1997). As mentioned above, the glutathione conjugation pathway is a primary method of xenobiotic detoxification in plants. In the case of this pathway, transmembrane transport is governed by glutathione pumps, which target the requisite molecules resulting from conjugation and move them out of the cytosol. This permanently compartmentalizes the compounds away from metabolic pathways due to inhibited diffusion (Burken, 2003).

Bound residues form as a result of covalent bonding of xenobiotics, or their

metabolites, to lignin or other structural components such as pectin, cellulose and hemicellulose. As is suggested by their name these residues are basically nonextractable, and therefore their composition is difficult to evaluate. Still, methods have been developed to fractionate these residues to the point at which the initial individual compounds can be discerned. Castro et al. (2001) identified lignification (the binding of compounds to lignin) of the pesticide triazole in sunflowers. The incorporation of xenobiotics into lignin and other plant constituents of this nature have been shown to lead to residues so strongly bonded that they are effectively unavailable in the strictest sense. Khan et al. (1987) demonstrated that rats fed root material containing labeled, bound pesticide residues passed the xenobiotics through their digestive system along with the indigestible portion of the plant material.

2.3.3 Hydrocarbon degradation in the rhizosphere

Plant root growth influences degradation of organic chemicals in many ways. The exploration of the soil by roots can help to liberate contaminants trapped in micropores, therefore making them available for microbial attack. Ultimately though, the most important aspect of root impact on organic contaminant degradation is that of the rhizosphere phenomenon (Hutchinson et al., 2003). The rhizosphere is a zone of increased microbial activity at the soil-root interface. It is directly under the influence of the root, which distinguishes it from the bulk soil (Anderson et al., 1993; Frick et al., 1999). Microbial activity in the rhizosphere is usually described as the ratio of the number of microorganisms in the rhizosphere soil to the number in the bulk soil (the R/S ratio). This ratio can range from 5 to as high as 100. This surge in activity is often referred to as the "rhizosphere effect", and is responsible for increased metabolic degradation of organic contaminants (Anderson et al., 1993). The importance of the rhizosphere in phytoremediation was shown by Nichols et al. (1996), where soil contaminated with a mixture of organic chemicals had a greater proportion of organic C degrading bacteria in the rhizosphere soil of alfalfa and alpine bluegrass (*Poa alpina* L.) than in the bulk soil.

Root growth can stimulate microbial activity by encouraging the development of soil structure and therefore increasing water and oxygen infiltration, but the rhizosphere

effect can be attributed mainly to root exudates (Hutchinson et al., 2003). Root exudates of sugars, alcohols and acids can amount to 10 to 20% of photosynthesis annually (Schnoor et al., 1995). These exudates help to build up organic carbon in the rhizosphere, which can sustain large microbial communities (Anderson et al., 1993). The deposition of root exudates can be quite substantial, for example Jordahl et al. (1997) found that hybrid poplar (*Populus deltoids* x *nigra* DN-34, Imperial Carolina) grown under controlled conditions release 0.25% of their biomass as root exudates. The decay of fine roots along with the rhizodeposition of root cap cells, which can be sloughed off at a rate of 10,000 plant d^{-1}, also provide a carbon substrate for microbial growth (Anderson et al., 1993). Plants control the make up of their requisite microbial community depending on the type of exudate released (Anderson et al., 1993). BTX (benzene, toluene and xylene) degraders were five times more common in the rhizosphere of poplar trees grown without contamination than in the bulk soil (Jordahl et al. 1997). Plants also can release specific compounds in response to a contaminant being present to selectively enhance the growth of a certain degrading microbe (Nichols et al., 1996).

The rhizosphere effect becomes highly significant when dealing with hydrophobic contaminants such as PAHs. These compounds are not readily taken up by plants due to their tendency to partition off into soil organic matter, therefore making rhizosphere degradation the main detoxification pathway (Hutchinson et al., 2003). Several studies have shown the importance of the rhizosphere in PAH degradation. Siciliano et al. (2002) found an increased level of catabolic genes involved in hydrocarbon degradation (*ndoB*, *alkB* and *xylE*), as well as increased removal of naphthalene, in the rhizosphere soil of tall fescue. Miya and Firestone (2001) demonstrated that the addition of slender oat (*Avena barbata* Pott ex Link) exudates to phenanthrene contaminated soils helped to maintain greater populations of phenanthrene degraders for a longer period of time, and also showed increased degradation over the control. Banks et al. (1999) also found that 56% of ^{14}C-benzo[*a*]pyrene in soil planted with tall fescue was degraded, as opposed to 47% in an unplanted control. Plant uptake accounted for only 0.13% of the recovered ^{14}C label, therefore indicating a substantial rhizosphere effect. At the end of the experiment the authors noted that roughly 90% of

the ^{14}C remained in the soil mainly as degradation by-products, but also as residual benzo[*a*]pyrene. This reveals that the compound and its by-products have a propensity for adsorption onto and within humic fractions of the soil.

As stated above, the increase in microbial activity resulting from the rhizosphere effect can positively affect the degradation of organic contaminants. This degradation occurs as a result of microbes using the contaminant as a substrate for growth (Frick et al., 1999). Organic chemicals, such as hydrocarbons, provide carbon sources as well as energy for microorganisms, although sometimes a compound cannot support microbial growth alone. When this occurs the compound can still be degraded by the process of cometabolism. Cometabolism results from the introduction of a second growth supporting substrate that can provide an alternative energy source for microbes breaking down contaminants (Cunningham and Berti, 1993). Plant phenolics such as catechin and coumarin, for example, were shown by Donnelly et al. (1994) to serve as co-metabolites in the degradation of PCBs. Heavy hydrocarbons, such as PAHs also tend to be cometabolized (Frick et al., 1999).

Enzymes exuded from plant roots can play a role in the degradation of organic contaminants. Schnoor et al. (1995) stated that nitroreductase and laccase enzymes, which are known to be produced by plants such as parrot feather and poplar, can break down ammunition wastes such as TNT. Dehalogenase was also been found to degrade TCE, while Chekol et al. (2004) found high levels of dehydrogenase activity corresponded with greater rates of PCB degradation.

2.3.4 Ectomycorrhizal fungi and phytoremediation

Ectomycorrhizal (ECM) fungi are capable of degrading hydrocarbons. Only one out of 21 species tested by Gramss et al. (1999) was unable to degrade at least one PAH, while the species *Lactarius deliciosus* was able to degrade 32 and 39% of phenanthrene and fluoranthrene, respectively. There is evidence that ECM fungi produce a wide range of enzymes responsible for the degradation and metabolism of not only organic compounds in the soil, but synthetic organic contaminants as well. Phenol oxidizing enzymes such as laccase and catechol oxidase are produced by certain ectomycorrhizas in axenic culture. Several other phenol oxidizing enzymes are associated with ECM

fungi in unsterile soil, however at this point it is difficult to differentiate whether these enzymes where produced by the fungi themselves or are a result of fungi induced alteration of the rhizosphere (Meharg and Cairney, 2000).

The mycorrhizosphere effect, defined as alteration of the soil environment due to the interaction between ECM fungi and various other microorganisms, may have important implications in hydrocarbon degradation (Meharg and Cairney, 2000). Sarand et al. (1998) demonstrated that when Scots pine (*Pinus sylvestris)* was grown in association with the fungus *Paxillus involutus* in contaminated soil, the surfaces of some hyphal segments displayed bacterial biofilms containing genes for hydrocarbon degradation.

Ectomycorrhizas can greatly increase nutrients available to the host plant due to the greater forage area covered by their hyphae, yielding total mycelial lengths up to 8000 times greater than host roots alone, when associated with pine and willow (Meharg and Cairney, 2000). Some ECMs are capable of utilizing organic N, and subsequently translocating it to the host roots (Smith and Read, 1997). In fact Abuzinadah and Read (1986) showed that seedlings inoculated with ectomycorrhizal fungi could grow with protein as the sole N source. The authors of this study speculated that trees colonized with ECM fungi may have access to N earlier than those that are non-colonized, suggesting benefits to the ecosystem such as reduced losses of N and decreased adsorption and immobilization of N in organic complexes.

Ectomycorrhizal fungi also play an important role in P dynamics. Harley and McCready (1950) demonstrated that the uptake of P by colonized roots of beech is significantly greater than non-colonized. Mean ^{32}P (as phosphate) uptake by excised roots over a 2 hr period was 3.65 for colonized roots and 0.676 for non-colonized (units are absorption per unit area). However, 90% of the absorbed P in the excised roots was retained by the fungal sheath. Colonized roots are therefore barred from maximum uptake by the sheath, and P is released to the root at a slower rate over a longer period of time as compared with non-colonized roots. Storage of P in the sheath is beneficial to the host in that it results in the ability of the fungus to release stored P to the roots when supply in the soil is low (Harley and McCready, 1952).

The capacity of ectomycorrhizae to extend a host's physical access to nutrients

and also to utilize forms of these nutrients which may be plant unavailable is notable when considering phytoremediation. Given that hydrocarbon contaminated soils often have nutrient limitations or availability issues, inoculating tree species with ectomycorrhizae could prove highly beneficial in terms of nutrient acquisition and plant tolerance to the conditions (Meharg and Cairney, 2000). In fact Sarand et al. (1999) found that a Scots pine-*Suillus bovinus* association tolerated the presence of 2% w/v toluene with no reduction in biomass. Scots pine-*Suillus bovinus* and Scots pine-*Paxillus involutus* associations grown in petroleum hydrocarbon-contaminated soil also showed no depressive effects in terms of plant development.

On the other hand, several studies have shown that although ectomycorrhizal fungi can improve host nutrition and fitness in organic chemical-contaminated soils, these fungi can impede organic chemical degradation due to their outcompeting other soil microorganisms for available nutrients. Gadgil and Gadgil (1971) demonstrated that ectomycorrhizal fungi in forest soils slowed the degradation rate of humified organic matter, substances which have a similar structural composition to PAHs. Several ectomycorrhizal fungi were found by Olsson et al. (1996) to reduce bacterial activity in soil when in association with Lodgepole pine (*Pinus contorta)* seedlings. Thymidine incorporation (a measurement of bacterial activity) in soil containing a lodgepole pine-*P. involutus* association was reduced by 25% in comparison to the control, while the ectomycorrhizal fungi *Laccaria proxima* and *Hebeloma crusuliniforme* reduced incorporation by 50%. This decreased activity could translate to suppressed degradation of organic-chemicals. Joner et al. (2005) found that PAH degradation in spiked soil was lower in the rhizosphere of Scots pine inoculated with *S. bovinus* than in the rhizosphere of non-inoculated trees. The degradation of PAHs in soil is retarded when N and P are limited (Carmichael and Pfaender, 1997). The authors therefore attributed lower degradation rates in inoculated treatments to depletion of mineral nutrients by the fungus, which restricted microbial breakdown of the contaminants.

2.4 Poplar fine root dynamics

The definition of what constitutes a fine root is somewhat debatable. Commonly, fine roots are designated as roots anywhere from < 1 to < 5 mm (Fogel, 1983; Vogt,

1996; Li et al., 2003), while Ruess et al. (2005) claim that a large portion of fine root biomass is actually attributable to very fine roots with diameters < 0.350 mm. Despite these inconsistencies, a maximum diameter of 2 mm is commonly accepted as characteristic of fine roots. Still, it is important to realize when working with fine roots that not all roots within a classification will behave in the same manner. In the designation of arbitrary categories, morphological and functional differences may be overlooked (Hendrick and Pregitzer, 1996). Fitter (1996) relates that fine roots with diameters differing as little as 1 mm can evince contrasting functionality. Regardless of the variability in definition, fine root dynamics are integral to understanding the physiology of a tree and constitute a significant fraction in terms of resource allocation. Some studies even have shown that more than one-half of annual net primary production is allocated belowground in certain forests (Keyes and Grier, 1981).

Fine root biomass is a measurement of the standing root crop at a given period in time and varies throughout the growing season. The accumulation of fine root biomass often occurs in conjunction with leaf production, increasing from the spring into summer and declining with the onset of fall (Davis et al., 2004). Fine root biomass, however, does not have a definite relationship with the aboveground fraction, though it does decrease as total root biomass increases. Therefore, since coarse root and aboveground biomass have been shown to be allometrically correlated, a decline in fine root biomass would be a corollary of increasing aboveground biomass (Li et al., 2003).

The actual contribution, however, of fine roots to total biomass is quite small. Vogt et al. (1996), in an extensive literature review of root dynamics of various climatic zones, revealed that fine roots accounted for only an average of 4% of total tree biomass, with a range of 0.4% to 13%. In two hardwood ecosystems in Michigan, Hendrick and Pregitzer (1993) found soil core fine root biomass values to be 7.90 Mg ha^{-1} at the more southerly site and 6.89 Mg ha^{-1} at the northern site. This corresponded to 3.11% and 2.36% of total biomass production respectively. Ruess et al. (1996) studied fine root dynamics in various hardwood and coniferous forests of Alaska. The researchers found soil core fine root biomass values ranging from 2.21 Mg ha^{-1} for a floodplain black spruce (*Picea mariana*) forest to 8.32 Mg ha^{-1} for an upland birch (*Betula papyrifera*)/aspen (*Populus tremuloides*) forest. White spruce (*Picea glauca*)

communities showed the lowest ratio of fine root biomass to total biomass, with only 1.88%. Black spruce, on the other hand showed the highest ratio, with 5.82% of total biomass as fine roots. Hardwood communities ranged from 3.32% for balsam poplar (*Populus balsamifera*) to 4.6% for birch/aspen.

It may be tempting to assume that because fine roots constitute a relatively small portion of total biomass, allocation of resources to fine root production is minimal. In fact fine root production can account for 25 to 60% of total tree net primary production (NPP) (Fig. 2.4) (Bernier and Robitaille, 2004). Steele et al. (1997) reported minirhizotron fine root biomass values of 1.68 Mg ha^{-1} for jack pine (*Pinus banksiana*), 1.33 Mg ha^{-1} for black spruce and 0.43 Mg ha^{-1} for aspen, while fine root NPP was found to be 2.09 Mg ha^{-1} yr^{-1}, 2.35 Mg ha^{-1} yr^{-1} and 0.58 Mg ha^{-1} yr^{-1} respectively. In the previously mentioned study by Hendricks and Pregitzer (1993) where the root dynamics of two Michigan hardwood forests were assessed, fine root NPP was found to be 8.08 Mg ha^{-1} yr^{-1} for the southern site and 7.30 Mg ha^{-1} yr^{-1} for the northern site. This corresponds to 60% and 58% of total tree NPP respectively. This phenomenon, of low ratio of fine root biomass to total biomass corresponding with higher ratios for production, is due to the ephemeral nature of the fine root fraction. Root mortality and turnover have been shown to increase along with fine root NPP, evincing an ongoing cycle of production and senescence. In fact, Fogel et al. (1983) suggested that 40 to 92% of the standing root biomass turns over annually, adding an estimated 18 to 58% more N to soils than litterfall in some forests, as well as substantial carbon inputs (Vogt et al., 1986).

Production and mortality are known to occur simultaneously in time, but relative rates are not constant and may fluctuate greatly. This fluctuation does not necessarily correspond to seasonal changes and it is not thoroughly understood. Research has shown that fine root biomass is correlated with climate and forest type as well as soil order (Vogt et al., 1996). Heilman et al. (1994) found that the distribution of fine roots in hybrid poplar (*Populus trichocarpa x Populus deltoides*) was positively correlated with Kjeldahl N and organic matter. In environments where nutrient supply is abundant, root mass will likely be minimal, given the ease of access, but in environments which are nutrient limited, the plant may invest more in its root system (Pregitzer et al., 1993). The

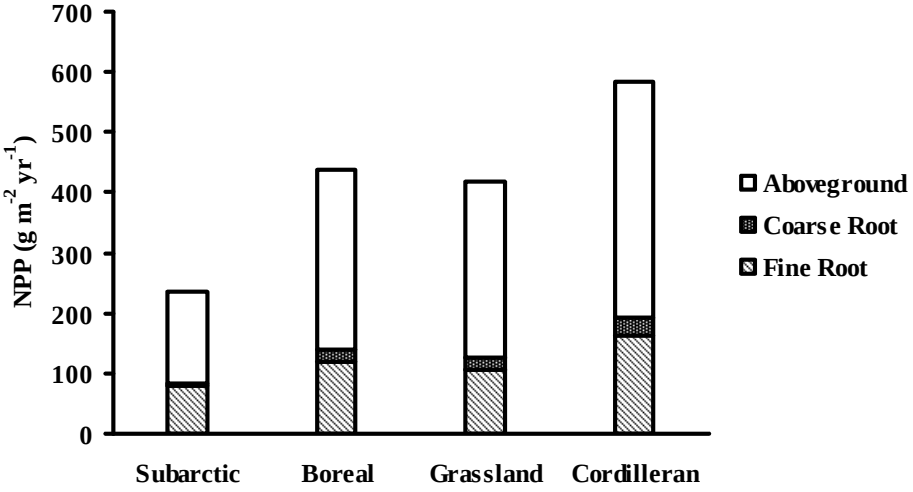


Figure 2.4 Fine and coarse roots and aboveground proportions of total NPP for forests of four Canadian ecoregions. Adapted from Li et al. (2003).

allocation of more resources belowground by the plant would allow access to more nutrients and/or water (Pregitzer et al., 1993; Vogt et al., 1986). On the other hand, Finér et al. (1997) found that there was a clear tendency for less root production in older, less fertile sites than in younger, more fertile sites when root dynamics of mixed conifer and broadleaf forests were studied. Overall, soil temperature will exhibit an influence on root production in governing the time of initial root flush in the spring and the final discontinuance of production in the fall. Tryon and Chapin (1983) positively correlated fine root growth of boreal trees to soil temperature, while Steele et al. (1997) found positive exponential relationships between daily root growth and soil temperature. Soil moisture may have an impact on fine root dynamics, albeit a slightly more inchoate one. Research has shown that increased soil moisture may actually decrease fine root NPP. Comeau and Kimmins (1989) found that tree communities on xeric sites exhibited higher rates of production than those on mesic sites. Burton et al. (2000) also found that root production was not increased in a sugar maple dominated forest in times of drought, but in fact was reduced.

Longevity of fine roots has been addressed by several studies, with a wide variety of results. Hendrick and Pregitzer (1992) found that approximately 50% of fine roots in a northern hardwood forests at depths < 110 cm survived longer than 346 d, while Black et al. (1998) reported that in cherry trees fewer than 60% of fine roots survived after 14 d. The reasons for different longevity times of fine roots are widely speculated upon. Pregitzer et al. (1995) found decreased longevity for roots in more fertile soils, while Black et al. (1998) reported greater root longevity in soil with greater N availability. This suggests that roots are maintained as long as the benefit they provide, in the form of efficient nutrient uptake, outweighs the C cost to the tree to keep them alive. Mycorrhizal colonization has also been reported to increase the longevity of fine roots. Treseder et al. (2004) reported the longevity of ectomycorrhizal roots to range from 1 to 6 yr, which was in keeping with previous research, and shows a significant increase in root longevity over non-colonized roots.

2.4.1 Minirhizotrons as a tool to evaluate fine root dynamics

Minirhizotrons provide a nondestructive approach for viewing roots *in situ.* The

system consists of a clear tube of varying material which is inserted into the ground; a camera is then inserted into the tube to capture fine root images (Johnson et al., 2001). These images can be downloaded to a computer for analysis. The quality of data gathered from minirhizotrons depends on several different factors.

Tube installation may cause soil disturbance and therefore may effect root growth. Coring for installation may sever roots, stimulating subsequent proliferation (Joslin and Wolfe, 1999). Soil voids and condensation may also be present at the tube interface, providing excess oxygen and water which may also cause increased root growth at the tube surface (Volkmar, 1993). Light also can stimulate root growth at the tube interface (Smit et al., 2000). It is important that minirhizotron images represent root growth typical of bulk soil. Therefore, after installation a period of time, roughly up to 1 yr, must elapse before conditions typical of undisturbed soils exist (Majdi, 1996).

The angle at which a tube is installed is important for obtaining quality results. Although placement has varied from horizontal to vertical, it seems angled tubes provide better results. Bragg et al. (1983) found that vertical tube placement was undesirable because it caused roots to grow downward along the tube, while angled tubes gave better estimates of root distribution when compared to soil cores.

Image collection frequency can substantially affect the gathered data. This is because of the possibility of root turnover occurring between sampling dates. Dubach and Russelle (1995) found that at a sampling rate of 2 or 3 wk root turnover ratios of alfalfa were estimated with 97% accuracy. If the sampling interval was increased to 8 wk, however, accuracy fell to 75%. The sampling interval should be chosen with an idea of the root longevity of the plant species, as well as taking into account the length of the study. In general, Johnson et al. (2001) reported that in most cases the shorter the sampling time the more accurate the results.

Minirhizotrons can be used to evaluate discrete values such as root biomass and root length density (RLD), as well as dynamic values such as root production, longevity and turnover (Smit et al., 2000). Minirhizotron estimates of RLD were positively correlated with soil coring (Ephrath et al., 1999), while Samson and Sinclair (1994) found that estimates between the two methods varied significantly. Transforming RLD of minirhizotrons to productivity estimates has proven problematic because of the

assumptions related to soil volume sampled and the arbitrary nature of assigning root mortality. For example, assigning a root to a "dead" category can sometimes be difficult given subjective interpretations of morphological changes.

Bernier and Robitaille (2004) have proposed a plane intersect method for finding root biomass and productivity in which the main variables are date of first sighting and root diameter. These easily observable variables do away with uncertainties resulting from last sighting estimates and the possibility of skewed volumetric occupation data which can result from root/tube contact. The method takes a line intersect method developed by Van Wagner (1968) to estimate the volume of woody debris on the forest floor and applies it to fine roots as observed by a minirhizotron camera. Imagine a root growing through the soil. If the soil (and the root it contains) was then sliced into an infinite amount of layers the root would be observed as an infinite series of elliptical cross sections. The diameter of the root would then govern the length of its short axis and its angle of approach would govern the length of its long axis. If we imagine that the point of root/tube contact is a 2-D plane containing an elliptical root cross section, then it can be expected that the long axis of the root is $\pi/\sqrt{2}$ times greater than its diameter. In this manner the plane intersect method uses diameter to produce biomass and productivity estimates in g m^{-2}.

2.5 Poplar and associated ectomycorrhizae

The use of poplar in association with ECM fungi in this study results from a review of literature indicating the potential for phytoremediation of the species as individual organisms, as well as the known ability of ECMs to colonize trees of the *Populus* family. As expressed above, poplars have the ability to metabolize organic chemicals (Newman et al., 1997), as well as release enzymes capable of chemical degradation (Schnoor et al., 1995). They have been shown to support a microbial community in the rhizosphere that has a naturally high percentage of BTX degraders (Jordahl et al., 1997), and found capable of degrading diesel fuel (Palmroth et al., 2002).

Ectomycorrhizal fungi have been shown to readily colonize hybrid poplar. Khasa et al. (2002) reported that ECMs colonized all 29 selected clones in their study, at rates ranging from 35 to 90%. Poplar shoot lengths have been shown by Baum et al. (2002) to

be significantly increased when inoculated with the ECM *Laccaria laccata*, and Smith and Read (1997) also report a positive correlation between ECM colonization and height and basal diameter. Also, colonization of ECMs does not seem to be greatly affected in hydrocarbon contaminated soil. Nicolotti and Egli (1998) found no difference in infection rates on poplar at contamination rates ranging from 0.1 to 50 g kg^{-1}.

3. FINE ROOT DYNAMICS OF HYBRID POPLAR GROWING AT A HYDROCARBON CONTAMINATED FIELD SITE

3.1 Introduction

Fine root dynamics are integral to understanding the resource allocation strategy of trees, but are often overlooked by researchers. The actual contribution of fine roots to total biomass is somewhat small given their defined size of < 2mm in diameter and their ephemeral nature (Hendrick and Pregitzer, 1993). However, the importance of these roots in nutrient and water access cannot be ignored. This fraction accounts for roughly 50% of total root surface area and 60% of total tree net primary production (NPP) (Keyes and Grier, 1981; Hendrick and Pregitzer, 1993; Bernier and Robitaille, 2004). The high turnover rate of fine roots reveals a constant cycle of overlapping production and senescence, with as much as 92% of the standing fine root crop being lost each year (Fogel, 1983). The spatial and temporal distribution of fine roots is influenced by factors such as soil temperature, soil available moisture and nutrient availability (Pregitzer et al., 1993; King et al., 1999). For example, Heilman et al. (1994) found that fine root allocation was positively correlated with Kjeldahl N and organic matter, and Tryon and Chapin (1983) positively correlated fine root growth of boreal trees with soil temperature.

At high concentrations, the addition of petroleum hydrocarbons to soil creates an environment that can be toxic to living organisms. These additions can adversely affect the nutrient status of the soil (Riser-Roberts, 1998). A widening of the C:N ratio is common in hydrocarbon contaminated soils, due to the N-deficient nature of the contaminants (Xu and Johnson, 1997). Microbial activity may be stimulated in hydrocarbon-contaminated soils, leading to a reduction in mineral N due to immobilization (Riser-Roberts, 1998). A larger amount of organic C present in the soil also can lead to lessened bioavailability of hydrocarbons for phytoremediation (Weissenfels et al., 1992).

Poplar trees are good candidates for use in phytoremediation because they root

deeply, cycle large amounts of water, and grow rapidly (Newman et al., 1997). Several studies have confirmed the phytoremediation capability of poplar (Newman et al, 1997; Palmroth et al., 2002; Wittig et al., 2003). Jordahl et al. (1997) demonstrated that BTX (benzene, toluene and xylene) degrading microorganisms were five times more common in the rhizosphere of poplar trees than in the bulk soil when grown in uncontaminated soil. No previous studies, however, have quantified the direct effect of petroleum hydrocarbon contamination on poplar fine root dynamics or the relative effects on soil properties.

Given that phytoremediation of hydrocarbon contaminated sites is dependent on the availability of nutrients for plant growth and the availability of contaminants for microbial or plant degradation, the objectives of this study were to: 1) investigate the relationship between the varying concentrations of total petroleum hydrocarbons (TPH) and nutrients across a hydrocarbon-contaminated site, as well as interactions between these contaminants and physical and chemical soil properties, and 2) quantify the effects of these properties on the spatial and temporal patterns of fine root production for Griffin hybrid poplar (*P. deltoides x P. petrowskyana* cv. Griffin).

3.2 Materials and methods

3.2.1 Site description

The field site is located at Hendon, in eastern Saskatchewan (SW 17 36 13 W2), within the aspen parkland ecoregion. This region is characterized by a subhumid continental climate with a frost-free period of approximately 100 to 120 d. Mean daily temperatures range from -17.0º C in January to 17.7º C in July, and precipitation averages 414.5 mm yr^{-1} (Environment Canada, 2004). The site covers approximately 0.15 ha, and the soil consists of a granular fill approximately 0.15 to 0.30 m in depth overlying a Gleyed Black Chernozem of the Yorkton Association (Stonehouse and Ellis, 1983). This site was previously used for tank storage of gasoline and diesel fuel, which gave rise to hydrocarbon contamination of the soil.

The site was originally planted with Walker hybrid poplar (*P. deltoides* x (*P. laurifolia* x *P. nigra*) c.v. Walker) and two types of willow (species unknown) in the summer of 2000 for the purpose of phytoremediation of the hydrocarbon contamination.

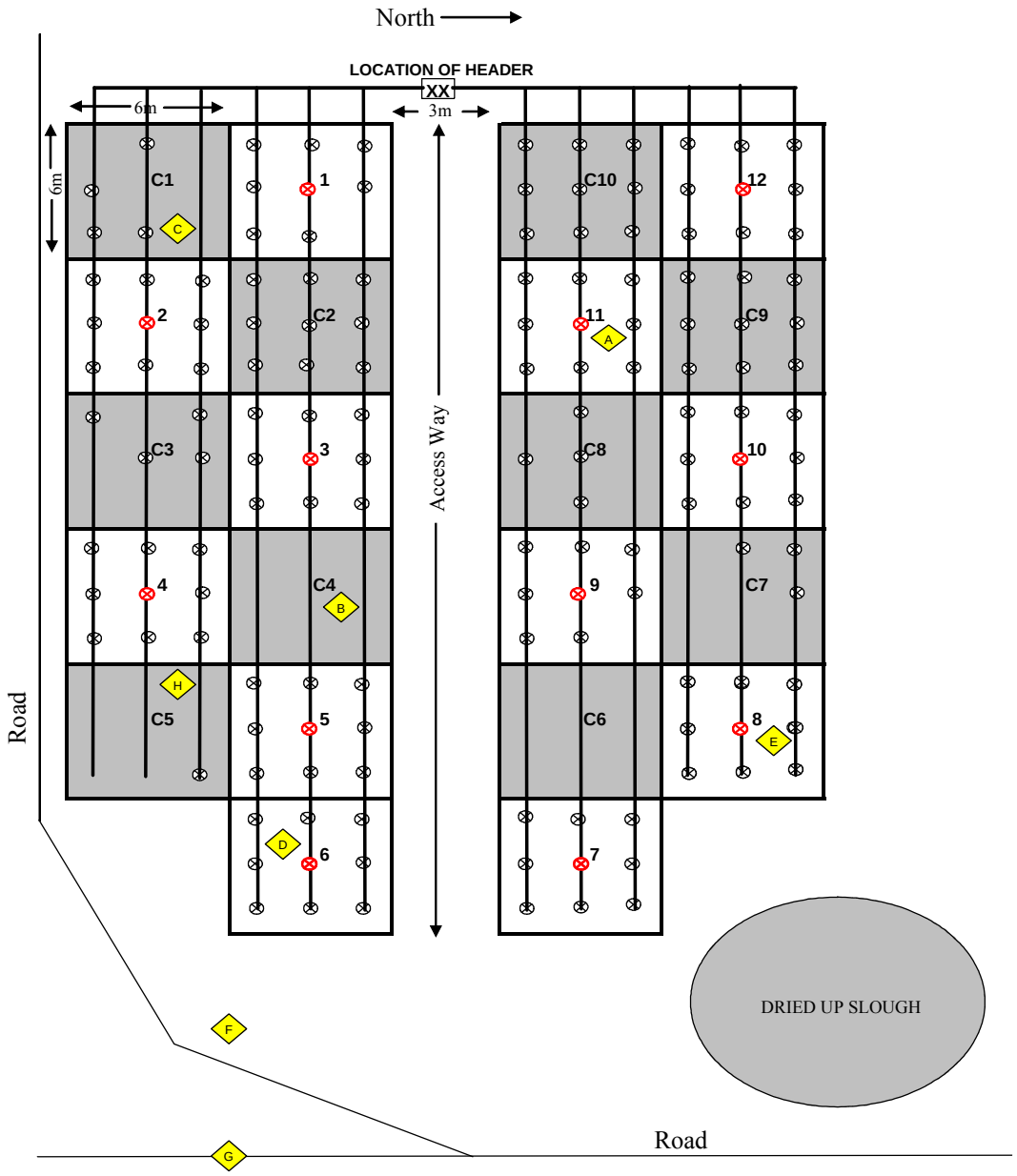
Because of high mortality of the willows, tree re-establishment was performed in summer 2001. In 2002 re-establishment was again necessary, but at this time the site was planted with 1-yr-old Griffin hybrid poplar and *Caragana arborescens* in alternating blocks of nine trees (Fig. 3.1). A drip irrigation system was installed in 2002 and was set to begin watering at 1:00 a.m. for 3 hr, providing trees with 7.6 L hr^{-1} of water and approximately 23 L $tree^{-1}$ wk^{-1} from approximately mid-June to mid-September. The irrigation system functioned in 2003 and 2004 as well, but was not needed in 2005 due to adequate rainfall.

In their 1999 environmental assessment, AGRA Earth and Environment Ltd. found hydrocarbon contamination to be greatest at the south eastern edge of the site, with hydrocarbon vapor levels exceeding 10,000 mg kg^{-1} for test drillings B, D and F (Fig. 3.1) (AGRA, 1999). Benzene was also found at concentrations of 6.2 mg kg^{-1}, exceeding SERM's *Non-Potable Groundwater* criteria of 1.9 mg kg^{-1}, in monitoring wells located at test drillings B and D (AGRA, 1999).

3.2.2 Soil sampling and analysis

Three soil samples were randomly collected on 1 July 2005 within each hybrid poplar block for the 0- to 20- and 20- to 40-cm layers. Sampling was performed with a 6-cm-diam. Edelman auger. The depth of the gravel fill was recorded for each block at the time of sampling. A sample in each layer was collected in each block using a 5-cm-diam. soil probe in order to calculate bulk density. Only one bulk density sample was taken due to difficulties in penetrating the gravel layer. All soil samples were stored at -4º C until analysis.

Bulk density was determined by calculating the volume of the intact cores for each layer and subsequently oven drying the samples at 105º C for 48 hr to determine weight. Three randomly selected soil samples from each layer were used to determine whole-site soil texture, utilizing the Bouyoucos hydrometer method (Tan, 1996). Prior to analysis, air-dried soil was passed through a 2-mm sieve to remove the coarse fraction and 50 g was used for analysis.



Legend:

⊗ = Live tree as of August 2005

◆ = Test Hole —— = Irrigation drip lines

Shaded Block = Caragana Trees White Block = Griffin Poplar Trees

Poplar block numbers correspond to tube numbers. Tubes are positioned under middle tree.

Caragana block numbers denoted C1-C10

Figure 3.1 Hendon site diagram showing tree location and placement of minirhizotron tubes.

Soil pH was determined by combining 20 g of dry soil with 20 mL of distilled water. This mixture was stirred frequently for 5 min and left to settle for 10 min. The solution was then measured for pH using a Fisher Scientific Accumet Model 50 pH electrode (Fisher Scientific International, Hampton, NH, USA). Total C and N were analyzed by combustion using a LECO CNS-2000 analyzer (oven temperature 1100º C) (LECO Instruments Ltd., St. Joseph, MI, USA). Measurement of NO_3 was accomplished by 2M KCl extraction (Maynard and Kalra, 1993) and subsequent analysis by Technicon Autoanalyzer II (Labtronics Inc., Tarrytown, NY).

Soil TPH contents were extracted by a shaking method based on Schwab et al. (1999) where 10 mL of 50:50 hexane:acetone solvent was added to 2 g of soil. Five grams of anhydrous sodium sulfate was added to remove water. Three replicate samples from each block and layer were shaken at 120 cycles min^{-1} for 1 hr, and centrifuged for 10 min at 180 *g*. The extract was decanted, 10 mL of new solvent added and the process repeated. The two 10 mL extracts were combined with 1 mL of toluene and evaporated down to approximately 1 mL using a Pierce Reacti-Therm III heating unit with a Reacti-Vap III N (Pierce Biotechnology, Rockford, IL, USA) evaporating attachment. Samples were stored at 4º C until GC analysis.

When compared with the Soxhlet or accelerated solvent extraction methods, the shaking method described above is less time consuming. This method also increases contact between solvent and soil because the sample is agitated during extraction. However, depending on the solvent, the age of the contaminants and the moisture status of the soil the shaking method may not recover TPH concentrations which are comparable to the Soxhlet or accelerated solvent methods (Schwab et al., 1999). Therefore three replicate samples from three randomly selected blocks and layers were extracted by the accelerated solvent method (Richter, 2000) using a Soxtec 2050 Autoextraction unit (Rose Scientific Ltd., Edmonton, AB, CA) with a 50:50 hexane:acetone solvent to serve as a comparison between the two methods. After extraction the samples were concentrated by evaporating under vacuum (60º C water bath on a Rotary evaporator) to approximately 2 mL.

Each extract was then transferred to a 2 mL vial and loaded into a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA), with flame ionization detector

(FID) and cold on-column injection. A 0.2 µL portion of the sample was injected and analyzed for TPH (C_{10}-C_{50}). A Varian CP-SIL 5CB column having the dimensions 15 m x 0.25 mm i.d. with a stationary phase thickness of 0.25 µm was used for analytical separation. The carrier gas was hydrogen held at an initial pressure of 11.73 kPa for 1 min, then ramped to 38.64 kPa at 2.07 kPa min^{-1} and held for 9.3 min. Initial injector temperature was 60º C and was ramped to 300º C at 100º C min^{-1}, and held for 20.9 min. Detector temperature was 300º C. Initial oven temperature was 40º C, held for 1 min and ramped to 300º C at 20 C min^{-1} and held for 9.3 min.

The shaking extraction recovered 79.8% ± 3.5% of TPH extracted by the accelerated solvent method. Although the shaking extraction recovered significantly less TPH, its precision, as evidenced by the small deviation between the three replicates, was sufficient to warrant its use in this experiment.

3.2.3 Minirhizotron tube installation and image collection

Twelve clear acetate-butyrate minirhizotron tubes having a 5-cm o.d. were installed in the hybrid poplar blocks in August of 2003. The tubes were installed using a steel coring device with a reverse-taper bit driven by a PacePik model 2550 hydraulic concrete breaker. The coring device entered the soil at a 38º angle with respect to the ground, and was driven to various vertical depths ranging from 40 to 54 cm. The diameter of the core was 5.025 cm, slightly larger than that of the minirhizotron tubes, which facilitated the insertion of the minirhizotron tubes into the space left by the coring device. Approximately 10 to 20 cm of the minirhizotron tube was left protruding from the ground and was secured by driving rebar rods into the soil on either side of the tube and fastening the tube with plastic cinch ties to the rods. Securing the tube in this manner restricted any movement. Movement of the tube may have led to inconsistent image collection at discrete depth increments. The aboveground portions of the tubes were painted black and then white to exclude light and heat radiation, which could stimulate root proliferation along the tube surface (Smit et al., 2000). The tubes were capped with a rubber plug, covered with an aluminum can and sealed in a plastic bag when not in use.

Image collection with a minirhizotron camera was accomplished by inserting the

camera into the tubes and capturing an image at 1.1-cm increments up the wall of the tube. A small hole drilled approximately 5 cm from the aboveground end of the tube allowed the camera to be locked in place, and the handle of the camera was notched to facilitate incremental movement up the tube. These images were stored on a Sony Vaio (Sony Inc., Tokyo, JAP) laptop computer in the field using I-CAP image capture software (Bartz Technology Co., Santa Barbara, CA, USA).

Beginning on 29 May 2004 images were collected from within poplar blocks at the site with the minirhizotron camera. Thereafter image capture was repeated on 30 June, 29 July, 30 Aug. and 30 Sept. 2004. This practice was resumed in 2005 with images collected on 31 May, 1 July, 1 Aug., 31 Aug. and 30 Sept.

3.2.4 Analysis of minirhizotron images

Images were downloaded onto a computer and each root was traced for length and diameter using RooTracker™ software (Version 2.0, Duke University, NC, USA). When a root traced in a previous session was later observed to be inchoate, missing or when coloration became black, the root was labeled "dead/missing" in RooTracker™ and traced in red (all other roots were traced in yellow). If, when tracing images for the subsequent month, these roots were still missing, or in the case of black or obscured roots became missing, they were deleted. Only missing roots were deleted, black and obscured roots continued to be traced as accurately as possible until such time as they may have become missing. Data from RooTracker™ was saved in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheets and organized by tube, frame, root, date and root diameter. This data was then analyzed in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) using the plane intersect method module (Appendix A) to acquire fine root productivity and biomass estimates.

The plane intersect method was developed by Bernier and Robitaille (2004) and modifies the line intersect method of Van Wagner (1968) to apply it to a three-dimensional environment. Estimates of fine root biomass and NPP are obtained by taking into account only the diameter of the root and the angle at which the root comes into contact with the tube. Imagine a root growing through the soil and coming into contact with a minirhizotron tube. The point of root/tube contact is a 2-D plane

containing an elliptical cross-section of the root where the long axis of the root is $\pi/\sqrt{2}$ times greater than its short diameter. The total area of these cross sections is calculated as:

$$\Sigma A_e = \frac{\pi^2 \Sigma r^2}{\sqrt{2}} \quad [3.1]$$

where A_e refers to the sum of expected elliptical cross sections of observed roots (mm^2) that would cross the plane delineated by the minirhiztron tube, and can be seen as the area of the root cross sections. The variable *r* represents the radius of observed roots. Using this equation fine root productivity (g m^{-2}) is calculated as:

$$P_{fr} = 2 \text{ x} 10^6 \, \rho_{fr} \, (1 - F_C) \, \Sigma A_e \, \frac{\sin\alpha \, \cos\gamma}{W} \quad [3.2]$$

where P_{fr} is the fine root biomass density, ρ_{fr} is the specific root mass (g mm^{-3}), F_C is the coarse fraction of the soil, α is the tube angle with respect to the ground, γ is the slope of the ground and W is the width of the minirhizotron camera frame. The value 10^6 converts mm^2 of ground area to m^2. The "2" is used because we are only seeing half of the minirhizotron tube surface, and it is assumed that an equal amount of roots are in contact with the back of the tube.

Specific root mass for both sampling layers was calculated as a whole-site value from three 5-cm-diam. soil cores taken from random hybrid poplar blocks. Roots were washed using a plastic mesh screen, and 20 roots were then randomly selected from each core to serve as representative samples. Diameter and length were measured and the roots were dried at 70º C for 36 hr and weighed. Specific root mass was calculated by dividing the total dry weight of the roots by the total root volume. Specific root mass was estimated to be 0.46 ± 0.09 (g mm^{-3}).

Soil coarse fraction for each layer was estimated when performing soil texture analysis. Values were 12% ± 4% for the 0- to 20-cm layer and 7% ± 5% for the 20- to 40-cm layer.

3.2.5 Statistical analysis

Statistical analysis was performed using SPSS version 13 (SPSS Inc., Chicago, IL). All differences reported are significant at $\alpha = 0.05$.

Bulk density, NO_3–N, pH and TPH data sets in the 0- to 20-cm layer and total C, total N, pH, TPH and 2004 fine root production data sets in the 20- to 40-cm depth exhibited non-normal distributions. Therefore, when comparing these variables between layers a Mann-Whitney U test was used. Correlations involving non-parametric datasets were performed using the Spearman rank correlation, with Pearson's correlation used in all other cases.

Total C and residual TPH concentrations were fitted with an exponential curve with residual TPH as the dependent variable using nonlinear regression of the form:

$$y = y_0 + \alpha(1 - e^{\beta x}) \qquad [3.3]$$

where y_0 refers to the y-intercept, α to the amplitude of increase, and β to the rate of change.

Fine root production and biomass estimates produced by the plane intersect method were analyzed using mixed design ANOVA. Estimates of fine root production and biomass were related in time, which necessitated the use of repeated measures analysis concerning the within-subjects effects. The 2 yr of the study were analyzed separately to observe trends within the growing season. The 4 mo within each year represented the within-subjects variable, and the two layers were the between-subjects factor.

Due to the heterogeneity of variances of fine root production datasets, monthly estimates for each tube were log transformed for the analysis. Violation of sphericity in the 2005 fine root productivity model demanded the use of a corrected *F*-ratio for within-subjects effects (Field, 2000). Therefore, significance was assessed using the Greenhouse and Geisser correction in this year. Differences in production between years and within layers were assessed using the Mann-Whitney U test, due to the non-normal distributions of the datasets.

To relate soil properties to fine root production, correlations were performed

using fine root production estimates from 1 July 2005 (this is an estimate of production occurring from 31 May to 1 July), as this was the same day soil sampling for property analysis was done. Correlations involved non-normal datasets, therefore the Spearman rank correlation was used in these cases.

The relationship between soil TPH concentrations and fine root production was explained by nonlinear regression of the form:

$$y = y_0 + \alpha(1 - e^{-\beta x}) \qquad [3.4]$$

This equation fits a curve that can be described as an exponential rise to a maximum.

3.3 Results

3.3.1 Relationships between soil properties and hydrocarbon contamination

Total petroleum hydrocarbon concentrations throughout the site ranged from 51.9 mg kg^{-1} in the 0- to 20-cm layer at block 4 to 2021.0 mg kg^{-1} in the 20- to 40-cm layer at block 10 (Table 3.1). At the whole site level, TPH concentrations were not significantly different between layers (Table 3.1 and Appendix B.1), although when blocks were analyzed individually differences in layers were observed. Blocks 1 and 9 contained higher TPH concentrations in the 20- to 40-cm layer, while blocks 5, 8 and 10 contained higher concentrations in the 0- to 20-cm layer (Table 3.1).

Total C and total N concentrations were significantly greater in the 0- to 20- than the 20- to 40-cm layer across the site (Table 3.1), with mean total C values of 35.0 and 31.0 g kg^{-1} respectively and mean total N values of 1.8 and 1.5 g kg^{-1} respectively. Total C and total N were correlated in both layers (Table 3.2), and soil C:N ratios across the site ranged from 16:1 to 27:1 (Table 3.1). Overall, ratios were not significantly different between the two layers, and the highest ratios were observed in the 20- to 40-cm layer in blocks 7 and 12. Total C and N were correlated with TPH concentrations in both layers (Table 3.2).

The relationship between total C and residual TPH contamination in both layers was explained by nonlinear regression (Fig. 3.2). Residual TPH concentrations remained

Table 3.1 Chemical and physical soil property values for the 12 blocks at Hendon as measured 1 July 2005.

Block	Total C	Total N	C:N Ratio	NO_3–N	TPH	pH	Bulk Density
	———— g kg^{-1} ————			———— mg kg^{-1} ————			Mg m^{-3}
				0- to 20-cm Soil Layer			
1	39.0 ± 1.6†	1.7 ± 0.1	22:1	15.6 ± 0.2**	109.6 ± 16.4	7.2 ± 0.2	1.45
2	34.9 ± 1.1	1.5 ± 0.2**	23:1*	13.0 ± 0.3	145.6 ± 19.0	7.1 ± 0.3	1.43
3	34.2 ± 3.3	1.4 ± 0.1*	24:1	16.7 ± 1.0	177.5 ± 8.5	6.9 ± 0.2	1.47
4	27.7 ± 0.3	1.7 ± 0.2**	16:1	8.6 ± 0.6	51.9 ± 13.3	7.2 ± 05	1.44
5	35.7 ± 0.2**	2.3 ± 0.2**	16:1	15.4 ±.0.4	239.7 ± 15.2*	7.1 ± 0.3	1.47
6	28.5 ± 0.6	1.3 ± 0.1**	22:1	16.0 ± 0.5	99.6 ± 6.8	6.8 ± 0.3	1.46
7	35.5 ± 1.2	1.9 ± 0.2**	19:1	15.2 ± 0.3	130.9 ± 11.4	7.1 ± 0.5	1.43
8	48.2 ± 1.7**	2.3 ± 0.4*	21:1*	18.5 ± 0.8	504.7 ± 27.0**	6.7 ± 0.6	1.45
9	31.4 ± 0.1	1.7 ± 0.1	19:1	23.6 ± 1.4*	168.5 ± 13.1	6.9 ± 0.4	1.42
10	53.9 ± 0.8**	2.5 ± 0.5**	21:1*	16.7 ± 0.5	1237.0 ± 28.0**	7.3 ± 0.6	1.40
11	35.7 ± 1.9	1.0 ± 0.2	23:1	16.5 ± 0.3**	168.2 ± 14.3	7.1 ± 0.2	1.41
12	29.1 ± 0.2	1.9 ± 0.2	16:1	15.6 ± 0.2*	86.2 ± 11.7	6.9 ± 0.3	1.40
Mean	35.0 ± 3.1*	1.8 ± 0.2*	20 ± 1:1	15.9 ± 0.7*	259.9 ± 133.7	7.0 ± 0.1	1.44 ± 0.01**

Table 3.1 cont'd

Block	Total C	Total N	C:N Ratio	NO_3–N	TPH	pH	Bulk Density
	g kg^{-1}			mg kg^{-1}			Mg m^{-3}
			20- to 40-cm Soil Layer				
1	39.1 ± 0.8	1.8 ± 0.2	22:1	11.5 ± 0.5	230.8 ± 14.9*	7.4 ± 0.4	1.29
2	28.7 ± 0.7	2.2 ± 0.2	17:1	14.9 ± 0.5	136.7 ± 12.3	7.2 ± 0.6	1.31
3	27.0 ± 0.7	1.2 ± 0.5	23:1	18.7 ± 0.6	134.0 ± 19.2	7.1 ± 0.3	1.27
4	24.3 ± 0.4	1.1 ± 0.1	22:1*	8.1 ± 1.2	59.6 ± 23.6	7.6 ± 0.3	1.34
5	25.5 ± 0.3	1.8 ± 0.2	16:1	13.2 ± 0.8	117.2 ± 27.6	7.3 ± 0.2	1.32
6	26.3 ± 0.8	1.0 ± 0.4	26:1	14.0 ± 0.3	192.4 ± 20.5	7.1 ± 0.4	1.25
7	25.4 ± 0.8	0.9 ± 0.1	27:1*	15.0 ± 0.2	88.6 ± 19.7	7.4 ± 0.3	1.21
8	37.1 ± 0.4	2.1 ± 0.1	18:1	17.0 ± 0.4	856.7 ± 26.6	7.1 ± 0.5	1.23
9	55.2 ± 1.2*	2.3 ± 0.3*	23:1*	18.1 ± 0.3	2021.0 ± 12.4*	7.6 ± 0.6	1.26
10	20.0 ± 1.5	1.2 ± 0.2	17:1	15.6 ± 0.4	99.4 ± 20.9	7.6 ± 0.4	1.24
11	26.7 ± 1.4	1.2 ± 0.4	23:1	12.3 ± 0.6	127.4 ± 29.1	7.5 ± 0.2	1.22
12	27.3 ± 0.7	1.0 ± 0.3	27:1*	12.4 ± 0.8	176.2 ± 34.1	7.2 ± 0.2	1.22
Mean	30.98 ± 2.5	1.5 ± 0.2	22 ± 2:1	14.2 ± 1.3	353.4 ± 230.3	7.3 ± 0.1*	1.26 ± 0.02

† Mean ± standard error
* indicates significantly greater values at the specified depth as compared to the other depth at $p \leq 0.05$ according to Mann-Whitney U test
** indicates significantly greater values at the specified depth as compared to the other depth at $p \leq 0.01$ according to Mann-Whitney U test

Table 3.2 Correlation coefficients for soil properties in the 0- to 20-cm and 20- to 40-cm layers as measured on 1 July 2005 at Hendon, SK.

Soil Layer	Total N	Total C	TPH	Bulk Density	pH
0- to 20-cm					
Property					
NO_3–N	0.10	0.14	0.48**	-0.09	0.40*
Total N		0.76**†	0.40*	-0.14	0.20
Total C			0.59**	-0.06	0.17
TPH				-0.34*	-0.04
Bulk Density					-0.28
20- to 40-cm					
Property					
NO_3–N	0.27	0.22	0.30	-0.37*†	-0.23
Total N		0.73**	0.47**	0.40*	0.05
Total C			0.66**	0.22	-0.18
TPH				-0.11	-0.27
Bulk Density					0.09

* significant at $p \leq 0.05$
** significant at $p \leq 0.01$.
† refers to the use of Pearson's correlation coefficient. Spearman's rank correlation used for all others.

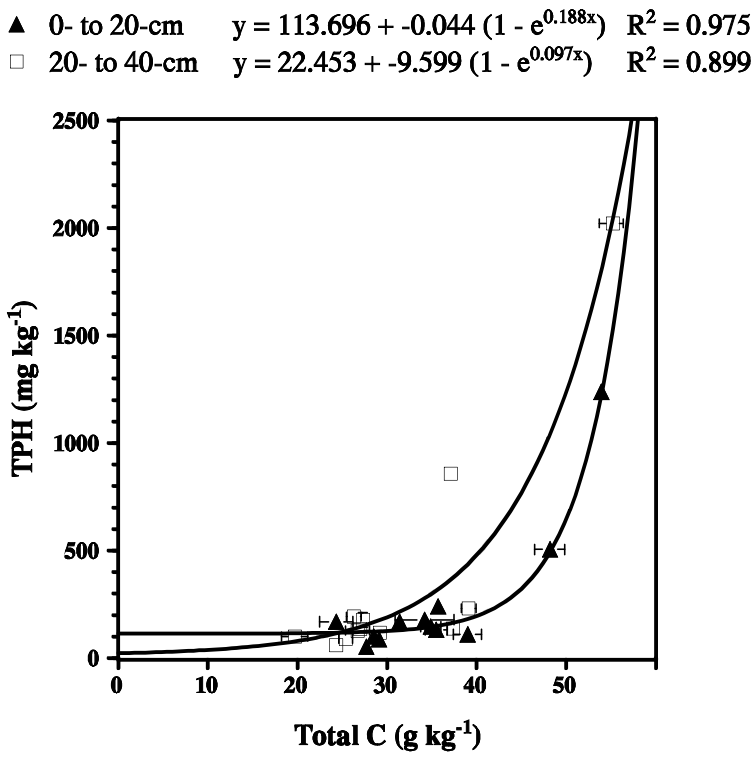


Figure 3.2 Nonlinear regression showing the relationship between soil total C and residual soil TPH concentrations at both depths for the twelve blocks at the site. Error bars represent SE of mean.

low with increasing total C levels up to approximately 35 to 40 g kg^{-1} C, at which point soil TPH levels increased dramatically. Coefficients of determination were 0.975 for the 0- to 20-cm layer and 0.899 for the 20- to 40-cm layer.

Nitrate-N concentrations were higher in the 0- to 20-cm layer than the 20- to 40-cm layer across the site, with mean values of 15.9 and 14.2 mg kg^{-1} (Table 3.1). Nitrate-N concentrations were greater in the 0- to 20-cm layer at blocks 1, 9, 11 and 12. Nitrate was also found to be positively correlated with TPH in the 0- to 20-cm layer (Table 3.2).

Bulk density was significantly higher in the 0- to 20-cm layer than the 20- to 40-cm layer across the site (Table 3.1). A weak negative correlation was observed between bulk density and TPH in the 0- to 20-cm layer. Soil texture also differed between the two layers. Soil texture of the 0- to 20-cm layer was a sandy clay loam, while the 20- to 40- cm layer was classified as clay.

Soil pH was higher in the 20- to 40-cm layer than the 0- to 20-cm layer across the site. No trends in soil pH among the blocks were observed.

3.3.2 Relationships between fine root production, soil properties and hydrocarbon contamination

Total fine root production for May to September 2004 ranged from virtually zero (0.00 Mg ha^{-1}) in the 20- to 40-cm layer at block 4 to 2.10 Mg ha^{-1} in the 0- to 20-cm layer at block 8 (Appendix C.1). In 2005 values ranged from 0.02 Mg ha^{-1} in the 20- to 40-cm layer at block 4 to 2.09 Mg ha^{-1} in the 0- to 20-cm layer at block 10 (Appendix C.2). No differences in total growing season production between years were observed within either layer (Appendix B.2). Mixed design ANOVA showed total overall fine root NPP to be significantly greater in the 0- to 20-cm layer in 2004 but not in 2005 (Table 3.3 and 3.4). A significant "month" effect was observed within both years which shows an influence of time on the pattern of fine root production. No "month x layer" interaction was seen in either year, indicating similar growth patterns between the two layers within both years. Further analysis regarding the influence of time on fine root productivity was carried out using orthogonal polynomial trend analysis (Table 3.3). This analysis is used to identify a proportional increase or decrease in group means in response to some factor, in this case the response of fine root production to time. A

Table 3.3 Mixed design ANOVA of hybrid poplar fine root production at Hendon, SK between the 0- to 20-cm and 20- to 40-cm layers, over a 4 mo sampling period in 2004 and 2005.

Year	df	Mean Square	F	Significance
2004				
Source of Variation				
Month	3	.545	9.400	.000
Linear†	1	1.624	29.335	.000
Layer	1	4.882	13.452	.001
Month x Layer	3	.018	.319	.811
2005				
Source of Variation				
Month	1.860	.565	3.832	.033
Quadratic†	1	.258	5.981	.023
Layer	1	1.594	2.747	.112
Month x Layer	1.860	.043	.291	.733

† Linear and quadratic refer to within-subjects trends according to orthogonal polynomial trend analysis

Table 3.4 Minirhizotron estimates of total yearly production at the Hendon site for May to September 2004 and 2005.

Soil Layer	Total Production	
	2004	2005
	Mg ha^{-1} yr^{-1}	
0- to 20-cm	1.27*a*†	1.09*a*
20- to 40-cm	0.51*b*	0.55*a*
0- to 40-cm	1.88 ± 0.60‡	1.59 ± 0.71

† Values in a column followed by the same letter were not different at $p \leq 0.05$ according to the Bonferroni method

‡ Mean ± standard deviation

significant negative linear trend in fine root production was observed for both layers in 2004, while in 2005 a significant quadratic trend was observed for both layers (Table 3.3 and Figure 3.3).

In this study some soil properties were highly correlated within each layer. Multiple regression is recommended against in cases of multicollinearity, and therefore was not used to explain the variation in fine root production measured on 1 July 2005. Correlations are discussed separately and possible interrelationships between soil properties in regards to fine root production are identified.

Fine root production was positively correlated with total C and N, NO_3–N, and TPH concentration in the 0- to 20-cm and 20- to 40-cm layer (Table 3.5). The relationship between fine root production and TPH concentration was explained by nonlinear regression (Fig. 3.4). Production increased linearly up to approximately 500 mg kg^{-1} TPH, at which point it began to level off and remained constant as contamination increased. This trend was most pronounced in the 0- to 20-cm layer, with a R^2 value of 0.915.

3.4 Discussion

3.4.1 Relationships between soil properties and hydrocarbon contamination

Soil nutrient status, as well as physical and chemical properties such as bulk density and pH, can be affected by hydrocarbon contamination (Riser-Roberts, 1998). These properties affect the ability of plants and microorganisms to survive in and remediate contaminated soils. Therefore, understanding the relationships between soil physical and chemical properties, nutrient status and TPH concentrations are integral to maximizing bioremediation potential.

The highest residual TPH concentration measured at Hendon in either layer was 2021.02 mg kg^{-1} (Table 3.1). Given that grasses such as tall fescue (*Festuca arundinacea*) and bermuda (*Cynodon dactylon*) tolerate TPH concentrations up to approximately 50,000 mg kg^{-1} (Hutchinson et al., 2001), and microbial activity has been

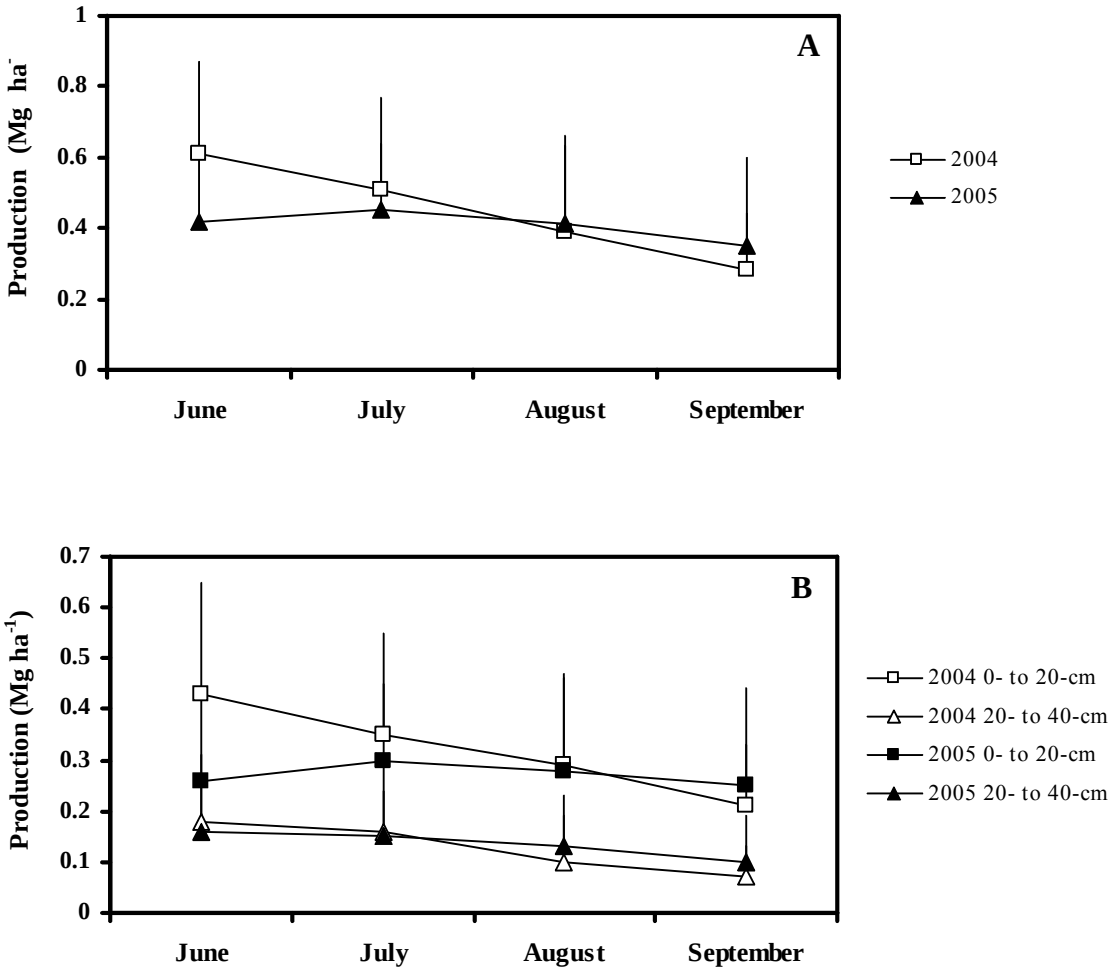


Figure 3.3 Patterns of fine root production at Hendon, SK over the two growing seasons for **A)** the entire 0- to 40-cm layer and **B)** the individual layers.

Table 3.5 Correlation coefficients for relationships between fine root productivity and various soil properties as measured on 1 July 2005.

Soil Layer	NO_3–N	Total N	Total C	TPH	Bulk density	pH
0- to 20-cm	0.39*	0.58**†	0.76**†	0.85**	-0.08	0.09
20- to 40-cm	0.58**	0.40*	0.38*	0.51**	-0.18	-0.24

* denotes a significant correlation at $p \leq 0.05$
** denotes a significant correlation at $p \leq 0.01$
† refers to the use of Pearson's correlation. Spearman's rank correlation used for all others.

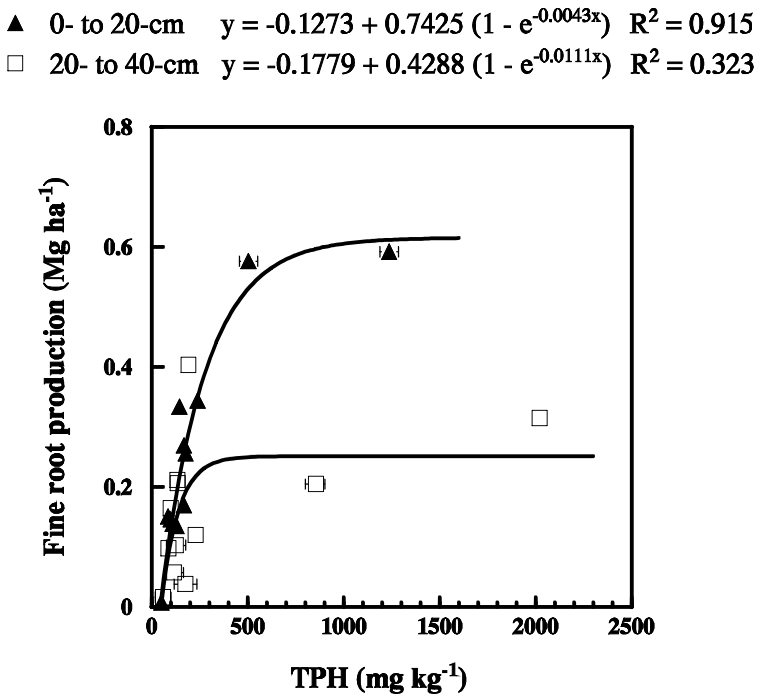


Figure 3.4 Relationship between residual soil TPH concentrations and fine root productivity in each block. Fine root production is the production as measured on 1 July 2005, which corresponds with the time of TPH sampling. Error bars indicate 1 SD.

reported to increase with TPH concentrations up to 10,000 mg kg^{-1} (Morelli et al., 2005), it can be assumed that toxic effects on living organisms are minor. However, even at relatively low residual concentrations, the variation in TPH concentration across the site provided an opportunity to observe the relationship between TPH concentration and the soil properties total C and N and NO_3, as well as bulk density and soil pH.

The distribution of TPH contamination at the Hendon site was not affected by depth on a whole site basis. Given the absence of any belowground storage tanks at the site, the equal amount of residual contamination in both soil layers is due to movement of contaminants from the soil surface deeper into the profile. Hydrocarbon compounds will move downward through the soil until the water table is reached, at which point movement will stop because these compounds are less dense than water (Young and Mulligan, 2004). This downward movement could cause increased contamination with depth. The fact that TPH concentrations do not increase with depth at Hendon suggests that some hydrocarbon compounds have become sorbed to soil fractions in the upper soil layer, therefore reducing mobility.

The varying texture of the soil influences the movement of hydrocarbon compounds. The upper soil profile (sandy clay loam) at the site was characterized by coarser particles than the lower profile (clay), which resulted from gravel fill being spread over the site to facilitate vehicular traffic. Hwang et al. (2002) found desorption of pyrene, and therefore leaching capability, to decrease with increasing clay content. In this study the possible effect of limited movement can be seen when looking at TPH levels for blocks 9 and 10 (Table 3.1). In block 10 a significantly greater amount of hydrocarbon contamination was measured in the 0- to 20-cm layer (1237.0 mg kg^{-1}) as opposed to the 20- to 40 cm (99.4 mg kg^{-1}). In block 9, the situation was reversed, with only 168.5 mg kg^{-1} measured at the 0- to 20-cm layer and 2021.0 mg kg^{-1} at the 20- to 40-cm layer. Given that the gravel fill was only 15 cm deep in block 10 and 26 cm deep in block 9 it can be theorized that the downward movement of the contaminant plume was disrupted at the surface of the clay layer.

Overall, the 0- to 20-cm soil layer had a higher bulk density than the 20- to 40-cm layer. This is in keeping with the coarser soil texture, but may also indicate more compaction in the 0- to 20-cm layer, although the value here (1.44 Mg m^{-3}) was not high

enough to suggest severe soil compaction. In the 0- to 20-cm layer a weak negative correlation was observed between bulk density and TPH (Table 3.2). It is assumed that this negative correlation is due to the fact that bulk density was not extremely high, because other research has indicated that increased contaminant retention will occur with increased bulk density (Jarsö et al., 1994; Hayden et al., 1996).

Along with texture, nutrient status and organic matter content are the important factors affecting petroleum hydrocarbon degradation in the soil (Riser-Roberts, 1998; Young and Mulligan, 2004). The addition of petroleum hydrocarbons to the soil generally increases the C:N ratio (Xu and Johnson, 1997). Xu and Johnson (1997) suggest that ratios less than 25:1 lead to mineralization and ratios greater than 30:1 lead to immobilization. Immobilization can severely limit bio- and phytodegradation of organic contaminants due to insufficient N being available for metabolism of contaminants (Riser-Roberts, 1998). Given that ratios measured at the Hendon site were all below 30:1 (Table 3.1) the mineral N content of the soil should be sufficient to facilitate microbial degradation of hydrocarbons. The highest ratio was found in blocks 7 and 12, which, surprisingly, corresponded with TPH concentrations that were low (Table 3.1). High C:N ratios in blocks 7 and 12 may result from increased rates of mineralization compared to more contaminated soils, and therefore increased leaching of excess inorganic N, effectively widening the C:N ratio. Low C:N ratios in blocks with higher TPH concentrations may be caused by microorganisms immobilizing mineral N, therefore limiting movement of N out of the system (Xu and Johnson, 1997). Incorporation of N in humic substances may also limit movement of N, maintaining a narrow C:N ratio.

The bioavailability of petroleum hydrocarbons in soils decreases as the organic C content of the soil increases (Weissenfels et al., 1992). In the presence of high amounts of organic C as soil organic matter the sorption of hydrocarbons becomes more pronounced, mainly because of hydrophobic partitioning of the compounds onto humic substances and diffusion of compounds into soil-humus matrices (Weber et al., 1998; Hwang et al., 2002). Murphy et al. (1990) reported increased sorption of hydrocarbons on clays coated with natural humic substances compared to uncoated particles. Conte (2000) demonstrated that the retention of PAHs by soil increased as humic acid content

increased. This sequestration of the contaminants can limit bioremediation.

In this study organic C was not analyzed, but an interesting relationship occurred between total C and TPH concentration (Fig 3.2). Murphy et al. (1990) reported that sorption of hydrophobic organics onto humic acid coated soil was nonlinear; rates were initially rapid but eventually reached equilibrium. This may be due to hysteresis (irreversible adsorption) or preferential humic acid adsorption and resultant exhaustion of hydrophobic sites. In the current study the relationship between total C and TPH was nonlinear but the inverse of the relationship reported by Murphy et al. (1990). The relationship was characterized by a gradual increase in residual TPH up to approximately 30 to 40 g kg^{-1} C, at which point TPH increased exponentially. The relationship is probably due to the association of TPH with the organic C fraction, and is probably related to time. Given that approximately 7 yr have passed since the last contamination event, hydrocarbon compounds in soils with low concentrations of organic C may have had greater opportunity for desorption and possible degradation than compounds in soils with high organic C concentrations. Concomitantly, larger humic molecules are likely to be associated with increased soil total C content. These molecules would increase the sorption potential of the soil and lead to greater concentrations of residual TPH. The rapid increase in TPH concentration beginning at approximately 50 g kg^{-1} C may be a result of this increased sorption potential. On the other hand, the relationship between total C and TPH may result from the C occurring in the form of microbial biomass, which are using the TPH as growth substrates (Morelli, 2005). The point where TPH concentration begins to increase at a greater rate than that of soil total C content may, in fact, indicate the maximum number of organisms supported by the TPH as a growth substrate.

The positive correlation between total N and total C and TPH in both soil layers is important to note. The higher correlation between total N and total C compared to total N and TPH in both layers demonstrates that these two variables are more strongly linked than total N and TPH. The relationship between total N and TPH could be more a result of the association of TPH with total C and the contemporaneous relationship of total C to total N.

The lack of correlation between total N and NO_3–N coupled with the positive

correlation between NO_3–N and TPH in the 0- to 20-cm layer is also interesting. The reason for this is not known, but it can be speculated that where TPH concentrations are high, degradation of hydrocarbons could lead to mineralization of associated organic N, and therefore seemingly random pools of increased NO_3.

Even though NO_3–N and TPH were positively correlated in the 0- to 20-cm layer, it should be noted that in blocks 1 and 9, where NO_3–N concentrations were observed to be greater in the 0- to 20-cm layer compared the 20- to 40-cm layer, significantly less contamination was found in the 0- to 20-cm layer compared to the 20- to 40-cm layer. Increased N mineralization and hydrocarbon degradation in the 0- to 20-cm layer compared to the 20- to 40-cm layer may be a result of the coarse texture of the upper soil profile enhancing microbial activity (Franzluebbers et al., 1996). Availability of substrates may also be greater in coarse textured soils, whereas clayey soils tend to be associated with more intensely humified organic substances (Carmichael and Pfaender, 1996). Coincidentally, microorganisms may be excluded from or within micropores in finer textured soils (Carmichael and Pfaender, 1996).

Hydrocarbon contaminated soils are often acidified to some degree (Riser-Roberts, 1998). In this case however, no correlation between soil pH and TPH was found (Table 3.2). Soil pH was found to be significantly lower in the 0- to 20-cm layer (7.0) than in the 20- to 40 cm layer (7.3).

3.4.2 Relationships between fine root production, soil properties and hydrocarbon contamination

Estimates of hybrid poplar fine root NPP reported as weight are not common in the literature. However, Block (2004) reported production values from minirhizotron cameras of 0.7 to 1.2 Mg ha^{-1} yr^{-1} for 2-yr-old hybrid poplar (*P. deltoides* x *P. petrowskyana*). The variability was due to stock type. Swamy et al. (2006), using a regression model, estimated fine root NPP of 4- to 6-yr-old hybrid poplar to vary from 0.20 to 0.50 Mg ha^{-1} yr^{-1} depending on the clone. More extensive research has been carried out on forest trees of the *Populus* genus. Steele et al. (1997) reported fine root NPP estimates of 0.88 and 0.58 Mg ha^{-1} yr^{-1} for trembling aspen (*Populus tremuloides*) in northern Manitoba and central Saskatchewan, respectively, using a minirhizotron

camera. Ruess et al. (1996), using a soil coring protocol, reported fine root NPP of Alaskan balsam poplar (*Populus balsamifera*) to be 4.39 Mg ha^{-1} yr^{-1}. Fine root NPP estimates in this study fell between the previously reported native and plantation values for various species of *Populus* (Table 3.4). The discrepancy between values of fine root NPP reported here and estimates from other hybrid poplar could result from tree age, climate, soil moisture or temperature, nutrient availability or hydrocarbon contamination (Comeau and Kimmins, 1989; Finér et al., 1997; Steele et al., 1997; Pregitzer et al., 2000).

In this study, fine root production was higher in the 0- to 20-cm layer compared to the 20- to 40-cm layer in 2004, but not in 2005 (Table 3.3 and 3.4). Greater fine root NPP is expected in the upper soil profile because available nutrients are usually most abundant at this depth, as was the case with NO_3–N, total N and total C in this study. In fact, 44.2% of fine root productivity occurred in the 0- to 20-cm layer in a northern hardwood forest (Hendrick and Pregitzer, 1996), and 50% of all fine root production and mortality occurred within 22 cm of the surface in a white oak (*Quercus alba*) forest (Joslin and Henderson, 1987). It has been reported that deeper rooting may be induced by periods of high water demand, in order to access moisture at depth (Hendrick and Pregitzer, 1996).

In 2005, no difference in fine root production was observed between the two layers (Table 3.4). This seems to be due to less production in the 0- to 20-cm layer rather than stimulated production lower in the profile (Fig. 3.3). Above normal precipitation was recorded in May and June 2005 (≈ 31.7 and 40.1 mm above average respectively). This, combined with the clay soil beneath the gravel fill and the close proximity of the site to a semi-permanent slough, may have suppressed root production for a period of time in the spring due to water-saturated conditions. When water supply is adequate there is less incentive for belowground allocation of resources due to ease of access. For example, root production in mesic sites is lower compared to xeric sites (Comeau and Kimmins, 1989). High water inputs also may mobilize contaminants and hydrocarbon vapors in the soil, therefore possibly inhibiting root production (Davis et al., 2005).

Regardless of the amount of root production, patterns of fine root NPP were

similar between layers within each year (Fig. 3.3). Patterns of fine root NPP typically follow two trends over the course of the growing season. A quadratic pattern occurs when production increases from spring to summer and decreases thereafter. A peak in production in August at a northern hardwood site in New York was observed by Burke and Raynal (1994). Ruess et al. (2003) also reported peak production of an Alaskan black spruce forest occurring in July. Other studies have reported a negative linear trend in fine root NPP over the course of the growing season. For example, fine root production decreased throughout the growing season in a hardwood forest (Hendrick and Pregitzer, 1996), and Steele et al. (1997) reported that more fine root growth occurred from June to July in a trembling aspen stand than at any other time of the year. In the current study, fine root NPP was observed to decrease linearly in 2004, while evincing a quadratic trend in 2005.

The different overall trends in fine root NPP between the 2 yr can be best explained in relation to climate and soil properties. The greatest monthly differences in fine root production between the 2 yr of this study occurred in June. The linear pattern of fine root production observed in 2004 may have resulted from greater initial flush of fine root production in this year compared with 2005. This early proliferation of fine roots may be an adaptive measure in climates subject to droughts during the growing season (Hendricks and Pregitzer, 1996). Very dry conditions in 2003 and below normal precipitation in several previous years may have exaggerated this phenomenon in the spring of 2004.

The relatively smaller fine root production values in June 2005 accounted for the quadratic pattern of production in 2005. Wetter conditions in 2005 could have suppressed mineralization and available N early in the growing season, or simply decreased the need for belowground production as discussed above. More moisture also may have induced the movement of hydrocarbon vapor into the upper soil profile. Davis et al. (2005) reported greater diffusion of vapors in soil contaminated by gasoline nonaqueous phase liquid in wet conditions. Under drier conditions the vapors were seen to retreat to the point of vertical O_2 diffusion. In the narrow zone where the upward movement of these vapors overlapped with the downward movement of O_2, most of the O_2 was consumed by aerobic degradation of the hydrocarbon vapors. This could have

briefly extended suppression of root growth in the present study after water had percolated through the profile.

Total C and TPH concentrations were positively correlated with each other in this study, and both also were positively correlated with fine root production in both layers (Table 3.5). Because of this relationship between total C and TPH, discerning which variable fine root NPP was more dependent on was difficult. The correlation between total soil C and fine root NPP was not unexpected. The soil is known to be a C sink, with greater accumulations of organic carbon associated with lower temperatures and precipitation. Northern forest ecosystems are known to sequester large amounts of carbon, due to colder temperatures limiting decomposition (Ruess et al., 2003). Fine roots are extremely important in soil C cycling because of their relatively fast decomposition rates in contrast to litterfall (Ruess et al., 2003). The greatest input of organic C to the soil comes from roots, and the depth of distribution is related to depth of rooting (Jobbágy and Jackson, 2000). Increased microbial activity in the presence of roots may also be a reason for the association between total C and root production (Anderson et al., 1993).

The positive correlation between TPH concentration and fine root NPP is more puzzling (Fig. 3.4). The xenobiotic nature of TPH would suggest a decrease in fine root production with increasing TPH concentrations. Poplar tolerates hydrocarbon contamination (Newman et al., 1997; Palmroth et al., 2002), and supports large populations of BTX degraders in its rhizosphere (Jordahl et al., 1996), but a stimulatory effect of contamination on root growth was still not expected. Fine root NPP rose with TPH concentration up to a point (≈ 500 mg kg^{-1}) and then leveled off. This could signify hormesis, or the stimulatory effect on organism performance in response to low levels of toxic compounds. This phenomenon has been seen in relation to hydrocarbon contamination before (Kirk et al., 2002). For example, corn (*Zea mays*) yields were 40 to 70% higher in oily soils as compared to soils that had been bioremediated for 8 to 11 mo (Salanitro et al., 1997). Soybean (*Glycine max*) yields also increased over 50% when grown in sandy peat soils contaminated with 7500 mg kg^{-1} oil compared to uncontaminated soil (Carr, 1919). On the other hand much literature is available reporting the inhibitory effect of hydrocarbons on plant growth (Udo and Fayemi, 1975;

Cunningham et al., 1996). In this study, residual TPH contamination was probably stabile in the soil and contamination was light. Thus a stimulatory effect is not all together surprising, especially because the effect seems to disappear at approximately 300 to 500 mg kg^{-1} TPH. It is assumed that at increasing levels of contamination an inhibitory effect on root NPP would be observable, although the absence of highly contaminated blocks in this study did not allow the effect of greater contaminant concentrations to be seen.

The effect of TPH on total fine root NPP was strongest in the 0- to 20-cm layer, and is probably related to the positive correlation between TPH concentrations and soil nutrients in this layer. In fact, if it is assumed that most residual TPH in the soil are recalcitrant, the relationship between TPH concentration and fine root NPP may actually be result of the correlation of TPH with total C, N and NO_3, and the contemporaneous correlation between these soil nutrients and fine root NPP (Table 3.2 and 3.5).

The correlation between fine root NPP and TPH was considerably lower in the 20- to 40-cm layer than in the 0- to 20-cm layer (Table 3.5). The fact that the correlation between fine root NPP and NO_3–N strengthens in this layer while the correlation between TPH and fine root NPP weakens seems to support the hypothesis that the relationship between TPH and fine root NPP is due mainly to the association of TPH with soil nutrients.

3.5 Conclusion

The relationship between residual soil TPH concentrations and soil properties was complex. Soil texture may have affected contaminant movement, as well as nutrient distribution. Approximately 7 yr after the last contamination event, it seems likely that the more bioavailable and easily degradable hydrocarbon compounds have been degraded, leaving behind more recalcitrant materials sorbed to organic matter or physically trapped within the organic or mineral fractions. Microbial activity may also be stimulated in the presence of hydrocarbons.

Total fine root NPP was not significantly different between the 2 yr of this study in either soil layer and was greater near the soil surface than at depth in 2004, but not in 2005. Fine root production in 2004 was characterized by a negative linear trend over the

growing season. The large flush of production that occurred early in the season may have been a drought-adaptive response by the hybrid poplar. Fine root production in 2005 was characterized by a quadratic trend that may have been a result of increased precipitation in this year coupled with low permeability of the subsoil.

An inhibitory effect of hydrocarbon contamination on fine root production was not seen in this study, approximately 7 yr after the last contamination event. In fact, root growth was stimulated in the presence of hydrocarbon contamination. This increased production in relation to increased hydrocarbon contamination could be explained by hormesis or, if contaminants have reduced bioavailability, as a side effect of the positive correlations between TPH concentrations and soil nutrients. For whatever reason, the positive correlation between TPH and fine root NPP has significant implications for phytoremediation because increased root production stimulates microbial activity due to the rhizosphere effect. Therefore, if hybrid poplar are able to maintain increased fine root production rates in hydrocarbon-contaminated soils, increased microbial degradation of contaminants is likely to occur.

4. ECTOMYCORRHIZAL COLONIZATION OF HYBRID POPLAR PLANTED IN DIESEL CONTAMINATED SOIL: IMPACT ON FINE ROOT PRODUCTION, ABOVEGROUND BIOMASS AND NUTRIENT UPTAKE

4.1 Introduction

Poplar trees are good candidates for use in phytoremediation because they root deeply, cycle large amounts of water, and grow rapidly (Newman et al., 1997). Several studies have confirmed the phytoremediation capability of poplar (Newman et al, 1997; Palmroth et al., 2002; Wittig et al., 2003). Jordahl et al. (1997) demonstrated that benzene, toluene and xylene degrading microorganisms were five times more common in the rhizosphere of poplar trees than in the bulk soil when grown in uncontaminated soil.

Ectomycorrhizal (ECM) fungi are able to degrade a host of organic chemicals. Of 42 ECM species screened, 33 degraded at least one class of organic contaminant, and only one out of 21 species could not degrade at least one polycyclic aromatic hydrocarbon (PAH) (Gramss et al., 1999). Sixteen of these 21 species were able to degrade all five PAHs tested. Enzymes capable of degrading organic compounds are present in soils colonized by ECM fungi, and some lignin and phenol degrading enzymes have been observed to be associated with these fungi in sterile conditions (Gramss et al., 1999). Furthermore, interactions between ECM fungi and other microorganisms may be important in hydrocarbon degradation. When *Paxillus involutus* was grown in association with *Pinus sylvestris* in petroleum hydrocarbon-contaminated soil bacterial bio-films involved in hydrocarbon degradation were found on the surface of some hyphae (Sarand et al. 1998).

The ectomycorrhizal fungus *P. tinctorius* was selected for use in this study because it is known to associate with trees of the *Populus* genus, as well as many others (Navratil and Rochon, 1981; Cripps and Miller, 1995; Cairney and Chambers, 1999). Due to its wide host range, this fungus is commonly found in commercial inoculum. Given that this study was focused on utilizing an easily obtainable commercial inoculum

that could be applied to a variety of phytoremediation applications, *P. tinctorius* was a good candidate. This fungus also is known to proliferate on marginal and industrially polluted soils (Marx and Artman, 1979; Agerer, 1987-2002).

Mineral forms of N are often limited in hydrocarbon-contaminated soils (Xu and Johnson, 1997). Ectomycorrhizal fungi are able to utilize organic forms of N and P in the soil and subsequently transfer these nutrients to the host. Seedlings of several tree species colonized by *Hebeloma crustuliniforme* grew with protein as the sole N source because of external proteases produced by the fungi (Abuzinadah and Read, 1986).

The overall objective of this study was to assess the phytoremediation potential of the hybrid poplar/*Pisolithus tinctorius* association. Specific objectives were to quantify the effect of colonization by the ECM fungus *Pisolithus tinctorius* on aboveground biomass and fine root production, and nutrient acquisition by hybrid poplar grown in soil contaminated with diesel fuel.

4.2 Materials and methods

4.2.1 Soil preparation

Soil was excavated to a depth of approximately 60 cm from an area adjacent to University of Saskatchewan experimental agroforestry plots near Meadow Lake, Saskatchewan (SW 31 57 19 W3). The soil is classified as a Brunisolic Gray Luvisol, with a loamy sand A horizon overlying a sandy clay loam B horizon (Table 4.1). After transport, soil was dried at room temperature for 5 d, crushed with a rolling pin and pushed through a 4-mm sieve to remove the stone fraction. Observable organic matter was removed.

Soil was contaminated with #2 diesel fuel obtained from Shell Canada. Spiking of the soil with diesel fuel was done by weighing 5 kg of soil, adding 25 g of diesel fuel to that soil and mixing thoroughly by hand with a garden trowel, resulting in a contamination rate of 5 g diesel kg^{-1} soil. This was repeated until 100 kg of soil were spiked. No carrier solvent was used because the aim of this procedure was to produce a spiked soil resembling an unintended contamination event in which distribution of contaminants in the soil is not completely uniform. However, uniformity between treatments was assured by mixing small amounts (5 kg) at a time.

Table 4.1 Selected properties of the soil collected near the University of Saskatchewan agroforestry plots in Meadow Lake, SK and used in the ECM fungi/hybrid poplar study.

Horizon	Total C	Organic C	Total N	NO_3–N	NH_4–N	Bulk Density
	——— g kg^{-1} ———			—— mg kg^{-1} ——		Mg m^{-3}
A	34.8	31.5	2.7	12.8	3.6	1.44
B	11.2	6.2	1.0	5.0	1.3	1.67

4.2.2 Assembly of a pot system designed to allow minirhizotron image collection

Pots were designed to allow minirhizotron observation of fine roots and constructed using schedule 40 polyvinyl chloride pipe with an inside diameter of 14.1 cm (Fig. 4.1). Three rows of eight pots were assembled by drilling two 5-cm holes, 19 cm from the top of the pot, opposite each other in the sides of the pot and inserting a 400-cm long acetate-butyrate minirhizotron tube horizontally through the pots. The minirhizotron tube was held in place by Mono Ultra® caulk (Tremco, Toronto, ON, CA) around the outer walls of the pots. Each pot was 28-cm deep and after the insertion of the minirhizotron tube had a volume of 4.05 L. The pots were then secured to a 5 x 56 x 400-cm piece of plywood. Any exposed tube surface was painted black to exclude light then silver to reflect radiation. Tubes were capped with an aluminum can when not in use.

4.2.3 Hybrid poplar stock

Walker hybrid poplar (*P. deltoides* x (*P. laurifolia* x *P. nigra*) c.v. Walker) rooted cuttings purchased from Smoky Lake Forest Nursery, Smoky Lake, AB were used in this experiment. Trees were approximately 6-mo-old, shipped from cold storage in a dormant state and kept at -2º C. Thirty-six hours prior to planting seedlings were removed from cold storage and placed in the growth chamber to allow for acclimation.

4.2.4 Mycorrhizal inoculum

Mycorrhizal inoculum (Mycogrow™ Tree Tabs) was purchased from Fungi Perfecti® LLC, Olympia, WA, USA. The inoculum was in tablet form containing spores of *Pisolithus tinctorius* and *Rhizopogon* spp. held together by an inert binder.

4.2.5 Experimental design and maintenance

Six replicates of four treatments were used in the experiment (Table 4.2). Two replicates of each treatment were randomly assigned within each row. At time of planting two Mycogrow™ Tree Tabs were dissolved in 1 L of water for each treatment

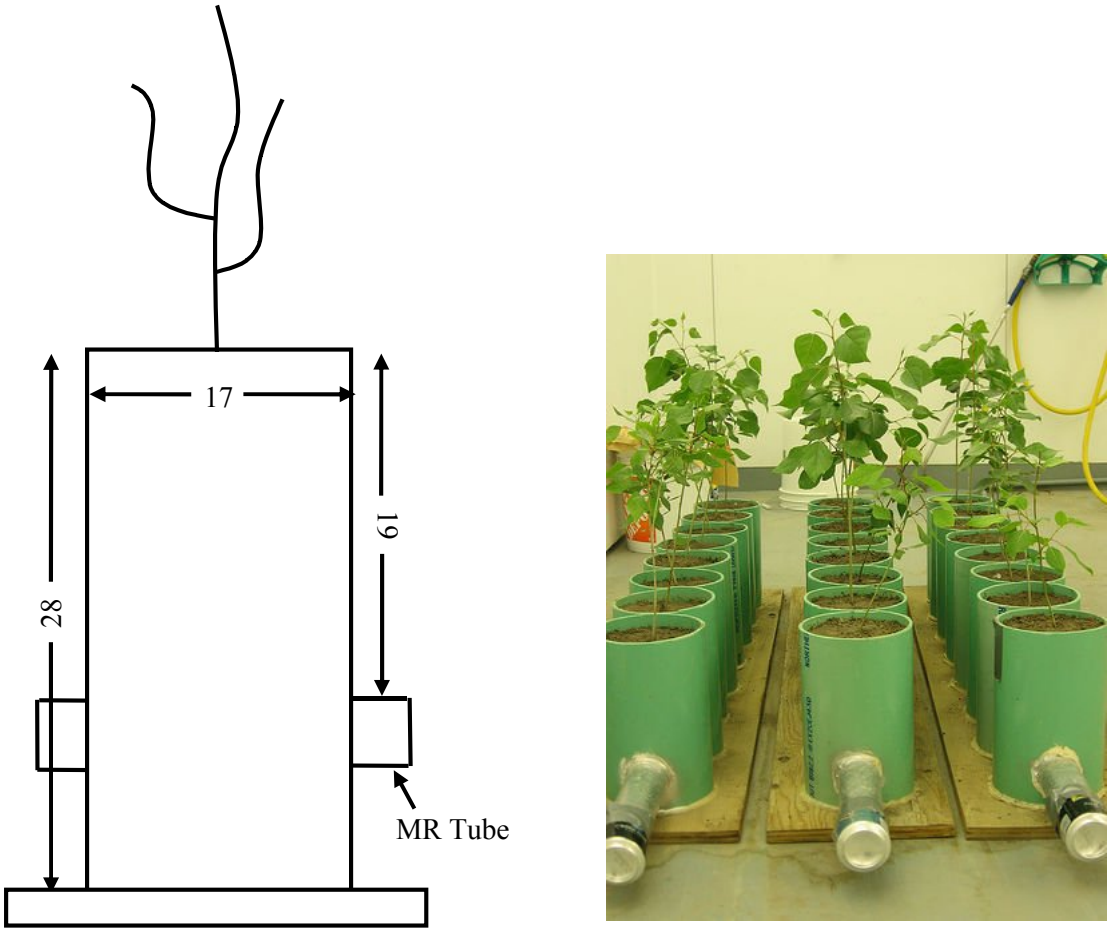


Figure 4.1 Pot design for the experiment. Measurements are cm.

Table 4.2. Treatment design.

HC	Diesel contaminated†
ECM-HC	Mycorrhizae inoculated-Diesel contaminated‡
ECM	Mycorrhizae inoculated
NA	No additions

† Soil was contaminated at 5000 mg diesel kg^{-1} soil
‡ Mycorrhizal inoculum contained *Pisolithus tinctorius* and *Rhizopogon* spp.

receiving inoculant. Trees were placed in pots and pots were filled with 16 cm of soil. Five hundred milliliters of the inoculant solution was applied in the requisite treatments around tree roots at this depth. Pots were then filled to capacity, lightly packed, and the remaining 500 mL of solution was added to the soil surface. Uninoculated treatments received 1 L of water with no inoculum in the same manner.

Trees were grown for 12 wk under a 16 hr light/8 hr dark cycle. Temperatures were maintained at 22º C and 16º C during light and dark periods, respectively. Throughout the study a HydroSense™ volumetric soil probe (Campbell Scientific Ltd., Townsville, QLD, AU) was used to measure water content every 2 d. Pots observed to have a volumetric water content of <10% at the time of measurement were watered to approximately 20% volumetric water content. Watering in this manner resulted in pots being filled to ≈ 85% of field capacity, which was determined to be 23% volumetric water content (Tan, 1996). Six replicates of unplanted contaminated soil were placed in plastic pots of the same volume as the planted pots to serve as a remediation control. The control soil was maintained at the same water contents as the planted treatments.

4.2.6 Data collection and processing

4.2.6.1 Minirhizotron image capture and analysis

Image collection with the minirhizotron camera was accomplished by inserting the camera into the tubes and capturing an image at 1.1-cm increments along the wall of the tube. A small hole drilled approximately 5 cm from the open end of the tube allowed the camera to be locked in place, and the handle of the camera was notched to facilitate incremental movement up the tube. These images were stored on a Sony Vaio (Sony Inc., Tokyo, JAP) laptop computer using I-CAP image capture software (Bartz Technology Co., Santa Barbara, CA, USA). Images were captured every 2 wk during the study.

Images were downloaded onto a computer and each root was traced for length and diameter using RooTracker™ software (Version 2.0, Duke University, NC, USA). When a root traced in a previous session was later observed to be inchoate, missing or when coloration became black, the root was labeled “dead/missing” in RooTracker™ and traced in red (all other roots were traced in yellow). If, when tracing images for the

subsequent 2 wk, these roots were still missing, or in the case of black or obscured roots became missing, they were deleted. Only missing roots were deleted, black and obscured roots continued to be traced as accurately as possible until such time as they may have become missing. Data from RooTracker™ was saved in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheets and organized by tube, frame, root, date and root diameter. This data was then analyzed in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) using the plane intersect method module (Appendix A) to acquire fine root productivity and biomass estimates.

The plane intersect method was developed by Bernier and Robitaille (2004) and modifies the line intersect method of Van Wagner (1968) to apply it to a three-dimensional environment. Estimates of fine root biomass and NPP are obtained by taking into account only the diameter of the root and the angle at which the root comes into contact with the tube. Imagine a root growing through the soil and coming into contact with a minirhizotron tube. The point of root/tube contact is a 2-D plane containing an elliptical cross-section of the root where the long axis of the root is $\pi/\sqrt{2}$ times greater than its short diameter. The total area of these cross sections is calculated as:

$$\Sigma A_e = \frac{\pi^2 \Sigma r^2}{\sqrt{2}} \qquad [4.1]$$

where A_e refers to the sum of expected elliptical cross sections of observed roots (mm^2) that would cross the plane delineated by the minirhiztron tube, and can be seen as the area of the root cross sections. The variable r represents the radius of observed roots. Using this equation fine root productivity ($g\ m^{-2}$) is calculated as:

$$P_{fr} = 2 \times 10^6\ \rho_{fr}\ (1 - F_C)\ \Sigma A_e\ \frac{\sin\alpha\ \cos\gamma}{W} \qquad [4.2]$$

where P_{fr} is the fine root biomass density, ρ_{fr} is the specific root mass ($g\ mm^{-3}$), F_C is the coarse fraction of the soil, α is the tube angle with respect to the ground, γ is the slope of the ground and W is the width of the minirhizotron camera frame. The value 106 converts mm^2 of ground area to m^2. The "2" is used because we are only seeing half of

the minirhizotron tube surface, and it is assumed that an equal amount of roots are in contact with the back of the tube.

At the end of the study, 20 roots (<2 mm in diameter) from each treatment were randomly selected from the excavated roots and used to calculate specific root mass. Values were 0.41, 0.37, 0.39 and 0.35 for the HC, ECM-HC, ECM and NA treatments, respectively. Soil coarse fraction was estimated as 3% (w/w) by passing a 5-kg sample of soil through a 2-mm sieve and weighing the particles which were retained.

A correction was applied to the plane intersect method due to the fact that the tubes were horizontally oriented with respect to the ground. This correction factor uses a tube angle of 90º and the value of *W* is set as the width of the pot. Initial productivity estimates at each time period are then multiplied by:

19 cm (depth to tube surface) / 1.1 cm (minirhizotron frame width) [4.3]

This scales up the productivity estimates to include the full 19 cm layer.

4.2.6.2 Harvest and root excavation

At the end of the experiment aboveground biomass was harvested, weighed and dried at 60º C for 4 d. Leaves and woody biomass were separated and ground to pass through a 1-mm sieve using a Thomas Wiley Laboratory Mill Model 4 (Thomas Scientific, Swedesboro, NJ, USA) for N and P analysis. Subsamples of three stems from each treatment, weighing 2 g each, were stored without drying for TPH analysis.

Roots were excavated from the soil by gentle washing over a 0.5-mm plastic mesh screen to catch any fragments. Excavated roots were then separated from the bole, enclosed in the mesh screen and hand washed thoroughly in warm water. Roots were blotted dry, weighed, root:shoot ratios determined, and stored at 4º C in plastic bags until TPH analysis.

Prior to root excavation one 1-cm diameter soil core was taken to the full depth of the pot from each replicate within the two contaminated treatments. These samples were used to assess residual diesel fuel concentrations in the soil, and were stored in plastic bags at -2º C until analysis.

4.2.6.3 Mycorrhizal infection

Mycorrhizal infection counts were performed by randomly selecting 15 roots from each replicate of all treatments. Presence of ectomycorrhizae was assessed by visual inspection under a dissection microscope at 30x magnification. Identification to species was done using dissection and compound light microscopy (up to 400x magnification) and was based on the morphological characteristics of ramification, mantle and rhizomorph type as described by Agerer (1987-2002; 1999).

4.2.6.4 Plant N and P concentrations

Plant leaves were acid digested following the method of Thomas et al. (1967). Subsequent analysis using the Technicon Autoanalyzer II (Labtronics Inc., Tarrytown, NY) yielded plant contents of N as NH_4 and P as PO_4.

4.2.6.5 TPH extraction

Total petroleum hydrocarbons were extracted from the soil samples by accelerated solvent extraction (Richter, 2000) using a Soxtec 2050 Autoextraction unit (Rose Scientific Ltd., Edmonton, AB, CA) with 50:50 hexane:acetone as a solvent. After extraction the samples were concentrated by evaporating under vacuum (60º C water bath on a Rotary evaporator) to approximately 2 mL.

Each extract was then transferred to a 2 mL vial and loaded into a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA), with flame ionization detector (FID), and cold on-column injection. A 0.2 µL portion of the sample was injected and analyzed for TPH (C_{10}-C_{50}). A Varian CP-SIL 5CB column having the dimensions 15 m x 0.25 mm i.d. with a stationary phase thickness of 0.25 µm was used for analytical separation. The carrier gas was hydrogen held at an initial pressure of 11.73 kPa for 1 min, then ramped to 38.64 kPa at 2.07 kPa min^{-1} and held for 9.3 min. Initial injector temperature was 60º C ramped to 300º C at 100º C min^{-1}, and held for 20.9 min. Detector temperature was 300º C. Initial oven temperature was 40º C, held for 1 min and ramped to 300º C at 20 C min^{-1} and held for 9.3 min.

Extraction of TPH from tree roots was done in the following manner. Ten grams of fresh roots from each replicate of contaminated soil treatments were placed in a

ceramic mortar. Liquid N was then added to the bowl and the roots or stems crushed with a pestle as finely as possible. This method was used to protect against any dissipation of hydrocarbons that may come about from drying and produced good results after some initial trial and error.

Five grams of roots were extracted by the accelerated solvent method and prior to GC analysis and treated to remove polar compounds. The sample was passed through a drying column containing 10 mL of anhydrous $NaSO_4$. Ten milliliters of hexane was initially used to condition the column. The sample was then passed through the column and collected. Ten milliliters of hexane were then passed through the column as a rinse and collected along with the sample. One milliliter of toluene was added and the sample was then evaporated down to 2 to 3 mL using a rotary evaporator. Next, the sample was run through a clean up column containing 5 g silica gel (baked overnight at 110º C) and 2 g of anhydrous $NaSO_4$ conditioned with 20 mL of 50:50 hexane:DCM. The column was rinsed with 50 to 75 mL of hexane:DCM. The sample was then evaporated down to 2 to 3 mL using the rotary evaporator and transferred to a GC vial for analysis.

Three replicate stem samples from each treatment were extracted in the same way as roots. Only three replicates from each treatment were analyzed because it was postulated that the amount of hydrocarbons present in the stem would be negligible, as was found to be the case. Gas chromatography for roots and stems was performed in the same manner as for the soil samples.

4.2.7 Statistical analysis

Statistical analysis was performed using SPSS version 13 (SPSS Inc., Chicago, IL). All differences reported are significant at $\alpha = 0.05$.

Analysis of fine root production was performed using mixed design ANOVA. The six biweekly estimates of production represented the within-subjects variables. Given that these variables were related in time it was necessary to use repeated measures analysis to determine effects. The four treatments represented the between-subjects factors.

Due to the heterogeneity of variances of fine root production datasets, estimates were log transformed. Even after log transformation the variances of some variables

remained heterogeneous. Therefore the significance of treatment effects was determined using the Games-Howell *post hoc* procedure, which does not require equal variances for accuracy. Violation of sphericity necessitated the use of the Greenhouse and Geisser corrected *F*-ratio when assessing significant within-subject effects (Field, 2000).

A significant "week x treatment" effect indicated that patterns of root production differed between treatments. Therefore, orthogonal polynomial trend analyses were run separately for each treatment to determine the effect of time on fine root production.

Significant differences between treatments in N and P contents, final aboveground biomass, and residual soil and root TPH levels were assessed using one-way ANOVA. Multiple comparisons were made using the Bonferroni test.

4.3 Results

4.3.1 Mycorrhizal infection

Mycorrhizal infection was 28% in the ECM treatment, 23% in the ECM-HC treatment and 4% in the NA treatment. No mycorrhizal associations were seen in the HC treatment. All mycorrhizae in the ECM and ECM-HC treatments were identified as *P. tinctorius* (Agerer, 1987-2002; 1999). Identification was based on a plectenchymatous mantle with a yellowish white to grey, mottled color possessing emanating hyphae, evincing irregularly pinnate ramification. Rhizomorphs were uncommon but two were observed, both of which were highly differentiated with thicker hyphae arranged centrally. Two of the four infected roots found in the NA treatment were not identified as *P. tinctorius*. Visual characteristics (yellow to orange color, monopodial-pyramidal ramification) suggested the species could have been *Lactarius controversus*, which is known to associate with *Populus* and was most likely present in the soil or associated with the poplar stock prior to the study.

4.3.2 Fine root production

Mixed design ANOVA showed a significant between-subjects effect (Table 4.3). *A posterior* testing revealed significantly greater fine root production in the NA and ECM treatments than the HC and ECM-HC treatments (Table 4.4) (Appendix D.1). The ECM-HC treatment showed significantly greater fine root production than the HC

Table 4.3 Mixed design ANOVA and orthogonal polynomial trend analysis of hybrid poplar fine root production in diesel contaminated soils as affected by ECM colonization.

Source of Variation	df	Mean Square	F	Significance
Within-subjects				
Week	5	.319	30.144	.000
Between-subjects				
Treatment	3	4.039	123.886	.000
Error	20	.033		
Week*Treatment	15	.451	42.685	.000
Orthogonal Polynomial Trend Analysis				
HC				
Linear	1	7.018	470.540	.000
ECM-HC				
Quadratic	1	.305	51.819	.001
ECM				
Quartic	1	.042	15.940	.010
NA				
Linear	1	.109	22.656	.005

Table 4.4 Minirhizotron estimates of hybrid poplar fine root production and aboveground biomass and root:shoot ratio as affected by diesel contaminated soil and ECM colonization.

Treatment	Total Fine Root Production	Mean Aboveground Biomass	Root:Shoot ratio
	——— g m^{-2} ———	——— g ———	
ECM	77.31*a*†	101.17*a*‡	0.33*bc*‡
NA	74.70*a*	82.83*b*	0.32*c*
ECM-HC	56.58*b*	48.83*c*	0.42*ab*
HC	22.58*c*	30.83*d*	0.45*a*

†Letters in this column denote significant differences ($p \leq 0.05$) according to the Games-Howell multiple comparison test.

‡ Letters in these columns denote significant differences ($p \leq 0.05$) according to the Bonferroni method

treatment. A significant within-subjects effect of "week" was observed, indicating an overall effect of time on fine root production (Table 4.3). Concomitantly, a "week x treatment" effect was also evident, denoting that the effect of time was different depending on the treatment.

Orthogonal polynomial trend analysis can be used to identify a proportional increase or decrease in group means in response to some factor, in this case the response of fine root production to time. This analysis showed a negative linear trend over time in the NA treatment and a positive linear trend over time for the HC treatment (Table 4.3 and Fig. 4.2). A quadratic trend was observed in the ECM-HC treatment, indicating that production initially increased with time and then decreased as the study progressed. A quartic trend was seen in the ECM treatment, which describes a more consistently fluctuating pattern of production over the study period.

4.3.3 Impact of ECM colonization on aboveground biomass, root:shoot ratios and nutrient status of poplar in contaminated soil

Aboveground biomass differed significantly between all treatments after 12 wk, with trees in the ECM treatment generating more biomass than any other, and trees in the HC treatment generating the least (Table 4.4) (Appendix E.3 and E.4).

Root:shoot ratios were significantly different between treatments. Ratios were smaller in the NA treatment than in the HC and ECM-HC treatment (Table 4.4) (Appendix E.3 and E.4). The root:shoot ratio in the ECM treatment was significantly less than the HC treatment, but did not differ from the ECM-HC or the NA treatments.

Significantly less N was found in the leaves of hybrid poplar grown in the HC treatment than in the ECM-HC treatment and the ECM treatment (Table 4.5) (Appendix E.1 and E.2). Leaves of trees from the NA treatment contained significantly less N than those from the ECM treatment, but did not differ from the ECM-HC or the HC treatments.

The pattern of leaf P concentrations between the treatments was the same as that of N (Table 4.5) (Appendix E.1 and E.2). Significantly less P was found in the HC

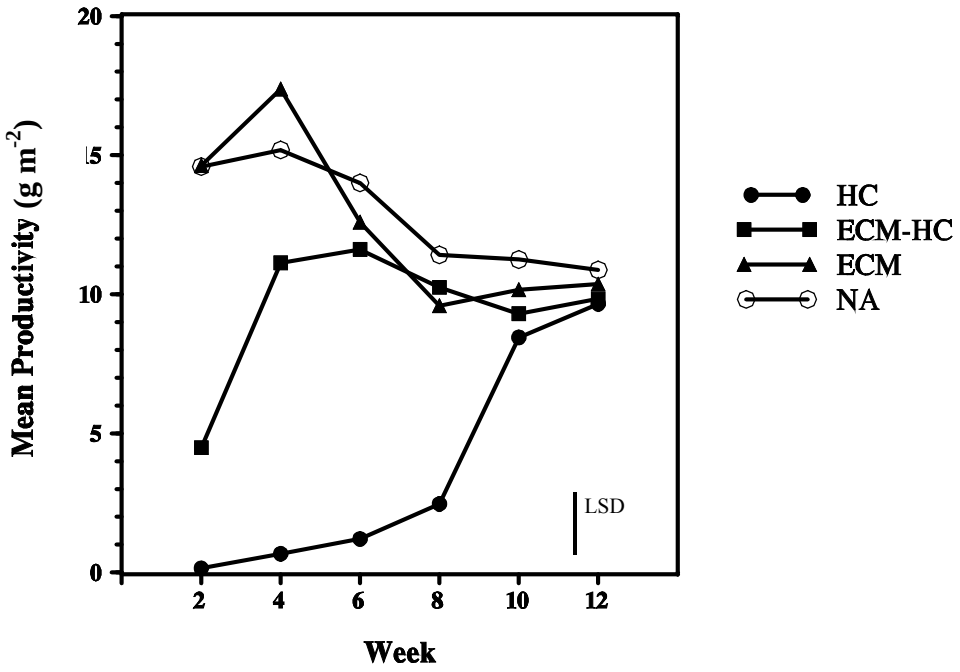


Figure 4.2 Pattern of hybrid poplar fine root production in diesel contaminated soils as affected by ECM inoculation over 12 wk. LSD = 3.53 and applies to the entire dataset.

Table 4.5 Mean N and P content of hybrid poplar leaves as affected by ECM-colonization and diesel contaminated soil.

Treatment	N	P
	——— g kg^{-1} ———	
ECM	25.3*a*‡	4.1*a*
ECM-HC	23.1*ab*	3.6*ab*
NA	18.9*bc*	3.1*bc*
HC	15.7*c*	2.7*c*

† Letters denote significant within-column differences at $p \leq 0.05$ according to the Bonferroni method.

treatment than the ECM-HC and ECM treatments. Leaves from the NA treatment contained significantly less P than those from the ECM treatment, but did not differ from the ECM-HC or the HC treatments.

4.3.4 Hydrocarbon concentrations remaining in the soil

More hydrocarbons remained in the soil of the ECM-HC treatment than the HC treatment after 12 wk (Table 4.6) (Appendix E.6). In the ECM-HC treatment 6.7% of the initial concentration of diesel fuel remained in the soil after 12 wk, and 5.0% remained in the HC treatment. In the unplanted-contaminated treatment 8.9% of the diesel fuel remained. Significantly more hydrocarbons were found sequestered in roots in the ECM-HC treatment than in the HC treatment (Table 4.6) (Appendix E.6). Stem TPH concentrations between the treatments were not different (Appendix E.6).

4.4 Discussion

4.4.1 Mycorrhizal infection

Host infection by ECM fungi is a rapid process, with the initial colonization event occurring within 24 to 48 h of first contact (Smith and Read, 1997). Research indicates that colonization may increase with tree age (Smith and Read, 1997). Although no values for % infection of *Populus* by *P. tinctorius* could be found in the literature, Khasa et al. (2002) found unidentified mycorrhizal infection of sampled roots to be 35 to 90% for selected 5-yr-old hybrid poplar clones at an Alberta field site. Aguillon and Garbaye (1989) reported only 20% infection of *P. involutus* in 5-mo-old hybrid poplar. Baum et al. (2002) reported 18 and 20% infection of 6-mo-old black cottonwood (*P. trichocarpa*) by *Laccaria laccata* in a pot and field experiment, respectively. The rates of infection for 3-mo-old hybrid poplar in the present study were 23% for the ECM-HC treatment and 28% for the ECM treatment, which are similar to, and even slightly greater than, the aforementioned pot studies.

Nicolotti and Egli (1998) found infection of black poplar (*P. nigra*) by a suite of mycorrhizae to range from ≈ 58 to 70% after 4 mo. That study was mainly concerned with assessing the effects of soil crude oil contamination on ECM and AMF fungi infection. The researchers reported that increasing concentrations of crude oil (up to 50

Table 4.6 Mean TPH concentrations recovered from soil and roots of the diesel contaminated treatments after 12 wk. Soil initially contained 5000 mg diesel kg^{-1}.

Treatment	Soil TPH	Root TPH
	mg kg^{-1}	
Unplanted-Contaminated	420.7*a*†	X‡
ECM-HC	336.5*b*	354.1*a*
HC	253.3*c*	102.2*b*

† Letters denote significant within-column differences at $p \leq 0.05$ according to the Bonferroni method.

‡ No sample

g kg^{-1}) did not affect ECM infection. With contamination of 5 g kg^{-1} (the same as used in the present study) infection rates where still approximately 45 to 65%. Although infection in the present study was not as high as those found by Nicolotti and Egli (1998), the effect of diesel fuel contamination on infection was negligible.

4.4.2 Fine root production

Many studies have reported enhanced host growth along with ectomycorrhizal colonization resulting from photosynthetic stimulation (Smith and Read, 1997), increased access to and uptake of soil nutrients (Vogt et al., 1991), and the ability of ECM fungi to use organic forms of these nutrients (Abuzinadah and Read, 1986). In this study, however, no difference in fine root production was observed between the ECM and NA treatments (Table 4.4). Baum et al. (2002) also observed no significant difference between root weights of poplar inoculated with *L. laccata* and those not inoculated after 6 mo; however, Navratil and Rochon (1981) did observe a stimulatory effect of *P. tinctorius* inoculum on total root length on the hybrid poplar clone *P* x euroamericana after 48 d.

The absence of a stimulatory effect with regards to fine root production is not surprising given that the C cost to the tree of the ECM association can be great. This increased C cost may, in fact, lead to reduced fine root production in favor of aboveground production, in order to maximize rates of photosynthesis (Rygiewicz and Anderson, 1994; Smith and Read, 1997). Lessened root production may also be due to the greater nutrient absorption by mycorrhizal plants, thus reducing the need for belowground resource allocation (Smith and Read, 1997).

Although mean fine root production in the ECM and NA treatments was not significantly different, the trends of production over time were dissimilar (Table 4.3 and Fig. 4.2). Fine root production in the NA treatment showed a negative linear trend, indicating that an initial flush of fine roots occurred in this treatment and that production decreased thereafter. A negative linear trend in fine root production of trees over the growing season is commonly reported in the literature (Hendrick and Pregitzer, 1996; Steele et al., 1997). Given that this study spanned 3 mo, or the equivalent of a short growing season, a negative linear trend may be a reflection of more or less normal

growing conditions in this treatment. This behavior may be due to the fact that trees coming out of a dormant phase need a strong initial flush of root production to access soil nutrients.

The quartic trend observed in the ECM treatment describes a fluctuating pattern of growth over the study period (Table 4.3 and Fig. 4.2). Production in this treatment was initially very similar to the NA treatment, but increased at a greater rate between 2 and 4 wk. Production then declined quite drastically from approximately 17 g m^{-2} to 9 g m^{-2} from 4 to 8 wk, at which time rates increased slightly up to 12 wk. Trees associated with ECM fungi in a natural setting can evince a linear trend in fine root production; however, in the present case of the ECM treatment the quartic trend is most likely a product of the fluctuating nutrient requirements of the fungi and the hybrid poplar.

Although ECM fungi enzymatically derive C from organic sources within the soil, the main supply of C to the fungus is derived from photosynthetic activity by the host plant (Read, 1999). This can amount to a large portion of the plant acquired C (Smith and Read, 1997). In the ECM treatment, the initial flush of root production that occurred up to 4 wk most likely coincided with mycorrhizal colonization. Subsequent to colonization, the C cost to the tree may have intensified, leading to a decrease in fine root production in favor of aboveground production over the following 4 wk. Allocation of nutrients in the plant was most likely directed into the aboveground biomass at this time to maximize photosynthetic potential and therefore C acquisition. The slight rebound in production from 8 to 12 wk could be seen as a compensation for the exhaustion of nutrients from this heightened aboveground allocation.

Mixed design ANOVA indicated that ECM inoculation increased fine root production in the ECM-HC treatment as compared to the HC treatment, but not to equivalence with the non-contaminated treatments (Table 4.4). This increase in fine root production in the ECM-HC treatment compared to the HC treatment may be due to the fact that many ECM fungi are capable of degrading hydrocarbons and other organic xenobiotics (Gramss et al., 1999). The ability of *P. tinctorius* to degrade hydrocarbons has not been assessed specifically, although it was demonstrated to degrade 2,4,6-trinitrotoluene (TNT) (Meharg and Cairney, 2000). Still, the fungus may create a buffer around the root system as a result of metabolizing hydrocarbons, or by inducing

increased bacterial degradation of these compounds. Ectomycorrhizal fungi have been shown to stimulate bacterial activity in their vicinity, a phenomenon known as the mycorrhizosphere effect (Meharg and Cairney, 2000). Some researchers have attributed increased hydrocarbon degradation in mycorrhizal associations to this effect, rather than the fungi themselves (Meharg and Cairney, 2000). Another reason for increased fine root production in the ECM-HC treatment over the HC treatment may be better access to nutrients in the soil as a result of fungal scavenging of organic substrates and subsequent nutrient transfer to the host plant (Smith and Read, 1997).

A quadratic trend in fine root production was observed over time in the ECM-HC treatment, while in the HC treatment there was a positive linear trend in production (Table 4.3 and Fig. 4.2). Fine root production rates in the HC treatment remained quite low until 8 wk. Between 8 and 10 wk, fine root production in this treatment increased by approximately three times, at which point rates were similar to that of the other treatments. This lag in fine root production was most likely due to the inhibitory effects on plant growth resulting from diesel contamination of the soil. After 8 wk, however, it seems that this effect was mitigated by the removal of the contaminants from the system by processes such as microbial degradation, plant uptake, root adsorption, volatilization or reduced bioavailability. In the ECM-HC treatment, on the other hand, the contamination effect seen in the HC treatment was alleviated more rapidly. At 2 wk, fine root production rates in the ECM-HC treatment were low. This may be due to inadequate colonization by the ECM fungi to exact an effect at this early stage in the study. However, the jump in productivity between 2 and 4 wk can only be seen as a result of the ECM association stimulating nutrient access and/or contaminant detoxification. By 6 wk, rates of production in the ECM-HC treatment had reached levels comparable to those in the ECM and NA treatments.

An inhibitory effect of diesel contamination on fine root production was still seen in the ECM-HC treatment as compared to the non-contaminated treatments. This is most likely due to the xenobiotic nature of the contaminants and nutrient limitations resulting from hydrocarbon contamination (Xu and Johnson, 1997).

4.4.3 Impact of ECM colonization on aboveground biomass, root:shoot ratios and nutrient status of hybrid poplar in contaminated soil

Aboveground biomass at the end of 12 wk was greatest in the ECM treatment (Table 4.4). A stimulatory effect of ECM colonization on host plant growth has been documented in the literature, an observation that was supported in this study (Smith and Read, 1997). Both diesel contaminated treatments produced significantly less biomass than the non-contaminated treatments, but plants in the ECM-HC treatment did perform better than the HC treatment. Again, it is likely that this is a result of direct fungal degradation of contaminants, a mycorrhizosphere effect or a host response to the increased C needs of the fungal symbiont.

Root:shoot ratios were similar to other studies involving young hybrid poplar (Proe et al., 2002; Glynn et al., 2003), and were greater in the diesel contaminated treatments than in the NA treatment (Table 4.4). Typically, root:shoot ratios decrease with ECM association due to increased nutrient uptake capability, although some researchers have found increased ratios because of the added weight of the fungal biomass (Alexander, 1981; Smith and Read,1997). In this study, diesel contamination was the most important factor governing root:shoot ratios, given that higher ratios were induced in contaminated treatments even though overall fine root production was suppressed. Increased root:shoot ratios in plants grown in contaminated soils are most likely an indication of hormesis due to contact with xenobiotic compounds or a response to stress resulting from nutrient deficiencies. Hormesis has been documented in other plant species in hydrocarbon contaminated soils (Salanitro et al., 1997; Kirk et al., 2002). Pregitzer et al. (1993) demonstrated that fine root production is stimulated in nutrient limited conditions as a means to access a larger volume of soil.

In this study, ECM inoculation increased leaf N and P content. Leaves from both the ECM and ECM-HC treatments contained more N and P than leaves from the HC treatment, while the ECM treatment contained greater concentrations than the NA as well (Table 4.5). It is well known that ECM fungi are important in nutrient acquisition, due to their ability to utilize organic N sources and greatly increase plant P uptake (Harley and McCready, 1950; Abuzinadah and Read, 1986). Therefore, the greater leaf concentrations of N and P in the ECM treatment as opposed to the NA and HC

treatments are not surprising. On the other hand, the fact that leaf concentrations in the ECM-HC treatment were boosted to the same level as the ECM and NA treatments is noteworthy. These similar nutrient concentrations demonstrate an important potential benefit for phytoremediation applications; ECM colonization provides the host tree with increased nutrient access over non-mycorrhizal trees in diesel contaminated soils, therefore increasing productivity and possibly degradation. The fact that no significant difference was observed in N and P concentrations between the NA and HC treatments also confirms the importance of ECM colonization. It demonstrates that uncolonized trees growing in a non-contaminated soil were no better at accessing N and P than uncolonized trees in a contaminated environment, where nutrient deficiencies are common.

4.4.4 Hydrocarbon concentrations remaining in the soil

The HC treatment had the least amount of remaining diesel fuel after 12 wk, with only 5.0% of the initial concentration remaining (Table 4.6). The ECM-HC treatment had 6.7% remaining and the unplanted control had 8.9% of the initial concentration remaining after 12 wk. Even though ECM colonization increased fine root production and aboveground biomass in hybrid poplar grown in contaminated soils, actual phytoremediation capability was reduced. Research has shown that ECM fungi degrade hydrocarbons in pure culture and help to initiate cometabolism in the soil (Gramss et al., 1999). Some ECM fungi also induce the formation of bacterial biofilms containing hydrocarbon degrading genes on the surface of external hyphae (Sarand et al., 1998). Given this information, the findings of the present study may seem somewhat surprising. However, ECM fungi can reduce bacterial activity in the soil due to outcompetition for water and nutrients, as well as by diverting C that would enter the soil from root exudates into fungal biomass (Olsson et al., 1996). This may decrease bioremediation by suppressing microbial degradation of contaminants. The production of antibacterial substances by ECM fungi also has been documented (Rasanayagam and Jeffries, 1992). Joner et al. (2005) observed lower PAH degradation rates in phytoremediation experiments with ECM fungi, which the researchers attributed to depletion of mineral N and P by the fungi.

Even though differences between treatments were significant, relative recovery percentages were all <10%. Degradation of diesel fuel was only 2.2% less in the unplanted control than in the ECM treatment. This indicates that the majority of the hydrocarbons present in the unplanted control were either lost from the system by volatilization, leaching or degraded by microbial action, while the added benefits of poplar/ECM were not tremendous. The reason for this may be that diesel fuel is composed of relatively light hydrocarbons, which are less toxic and more easily degraded than heavier compounds, and may remain more mobile and accessible for remediation.

Total petroleum hydrocarbon concentrations extracted from the roots of hybrid poplar were approximately three times greater in the ECM-HC treatment than in the HC treatment. In fact 7.6% of the initial concentration of diesel fuel was sequestered in the roots of the ECM treatment, as compared to only 2.2% in the HC treatment. Hydrophobins exist within the mycelial cell walls of certain species of ECM fungi (Bücking et al., 2002). These water-repellent complexes lower the ion permeability of the fungal sheath and also may serve as a sorption site for hydrocarbons in the soil. *P. tinctorius* forms a hydrophobic mycelium (Bücking et al., 2002), so the increased TPH content of the ECM colonized roots is expected. Root sequestration of contaminants by partitioning onto the fungal sheath is in itself a phytoremediation process, due to the fact that the contaminants have been removed from the soil. However, this phenomenon may only be temporary given that hydrocarbons not degraded at the root surface could reenter the soil upon root senescence.

4.5 Conclusion

The colonization of hybrid poplar by the ECM fungus *P. tinctorius* increased fine root production and aboveground biomass in a diesel contaminated soil. However, trees in the ECM-HC treatment did not perform as well as trees in the non-contaminated treatments. The stimulated fine root production in the ECM-HC treatment compared with the HC treatment is probably due to the increased nutrient access and absorption that results from ECM associations. In fact, concentrations of N and P in the leaves of the colonized treatments were higher than the non-colonized treatments. Greater

fluctuation in fine root production was observed in the ECM treatment than in other treatments, which may indicate temporally shifting nutrient demands of the host and fungus. Increased fine root production and aboveground biomass in the ECM-HC treatment over the HC treatment also may have resulted from less contact with the diesel fuel because of the sequestration of these contaminants in the hydrophobic fungal sheath of *P. tinctorius*.

While ECM fungi may help hybrid poplar to better tolerate hydrocarbon contaminated soils, their usefulness in phytoremediation may be limited over the short term. Smaller concentrations of hydrocarbons remained in the soil after 12 wk in the DC treatment compared to the ECM-DC treatment, although compared to unplanted controls all of the planted treatments reduced contamination levels. A goal of many phytoremediation strategies is simply establishing plants on a contaminated site. Over the long-term the stimulation of root and shoot growth achieved by ECM fungal inoculation may improve establishment, weed competition and long-term productivity on these contaminated sites.

It should be noted that ≈ 90 to 95% of the compounds disappeared from the soil within 12 wk in all contaminated treatments. This is probably due to the fact that diesel fuel is comprised of mainly light aliphatic hydrocarbon compounds that are more mobile and easily degraded than heavier PAH compounds. Number-two diesel fuel contains 5% or less PAHs. It can therefore be assumed that the 5 to 10% of the compounds remaining in the soil at the end of this experiment coincided with the recalcitrant PAH fraction of the diesel fuel, which may have persisted for an extended period of time in all treatments if the study had been prolonged.

5. GENERAL CONCLUSION

Modern chemistry has generated an array of products which have at best helped to improve our quality of life and at worst have threatened not only our health directly, but also the health of the earth that supports us. Petroleum products have fostered extensive industrial progress and have become an immense industry in and of themselves. Still, many of these products are known to be toxic to living organisms, and with vast global dependence it is inevitable that sizable quantities of these products will find a way into the soil, either by honest mistake or carelessness. It is then our responsibility to correct these mistakes and attempt to leave not a toxic environment, but a salubrious and functional one.

The soil is a complex and varied system, and the fate of petroleum hydrocarbons entering this system is mutable. These compounds can become bound to soil organic matter, and to a lesser extent mineral fractions, through Van der Waals and partitioning forces (Young and Mulligan, 2004). Incorporation within humic materials and soil micropores can limit bioavailability and therefore biological degradation. Biological degradation is the primary method of removal of hydrocarbons from the soil and results from enzymatic reactions mediated by microorganisms, which bring about changes in the chemical structure of these contaminants (Riser-Roberts, 1998). Furthermore, phytoremediation applications seek to stimulate this microbial degradation via the "rhizosphere effect", while some plants also have the capability to take up and metabolize hydrocarbons themselves (Hutchinson et al., 1999). The usefulness of this method depends on many things, such as plant tolerance and contaminant bioavailability (Cunningham et al., 1996).

The aim of this study was three-fold. The first objective was to determine the nature of the relationship of residual hydrocarbon contamination and soil nutrients and physical properties. Soil nutrient distribution and availability are of utmost importance in determining the extent and speed of hydrocarbon degradation. These nutrients may be limited in contaminated environments due to the inherent nutrient deficiencies of the contaminants and immobilization by microorganisms (Xu and Johnson, 1997).

The second objective was to evaluate the effect of hydrocarbon contaminants on hybrid poplar fine root dynamics. Hybrid poplars are known to be capable phytoremediators, which is at least in part due to their rapid growth and deep rooting. The fine root fraction of these trees is extremely important in nutrient and water uptake, and it is this portion of the plant that comes directly in contact with toxic substances in the soil. Yet very little research as to the effects of this contamination on fine roots has been carried out.

The third objective of this study was to assess whether of not ECM fungi colonization of hybrid poplar increased phytoremediation ability. The association of trees of the genus *Populus* with ECM fungi is also an important consideration when dealing with phytoremediation applications. These fungi are capable of degrading hydrocarbons in pure culture and their contribution to increased degradation in symbiosis has also been recognized (Gramss et al., 1999). These fungi are also able to utilize organic forms of nutrients such as C and N for growth (Abuzinadah and Read, 1986). However some studies have found that these fungi limit hydrocarbon degradation by suppressing microbial activity in their presence (Olsson et al., 1996). This could result from outcompetition for nutrients, or the production of antibacterial substances by the fungus.

Residual concentrations of TPH at the Hendon site were observed to be associated with total C and N. This suggests association of TPH with organic forms of these nutrients as a result of sorption. It would be expected that, given site decommission was 7 yr previous to this study, the majority of the residual contamination would be associated with organic matter and therefore somewhat recalcitrant. On the other hand, these positive correlations between TPH and total C and N may result from these nutrients occurring as microbial biomass, and therefore indicate increased microbial activity in the presence of hydrocarbons. The correlation between NO_3 and TPH in the upper soil profile may also be indicative of increased microbial activity.

Hybrid poplar fine root production was observed to follow a different pattern of growth in each year of the study, which was most likely due to climatic conditions. Fine root production was also observed to increase with TPH concentration up to

approximately 400 to 500 mg kg^{-1}, and then reach equilibrium. It can be surmised that at concentrations higher than those present in this study fine root production would begin to decrease; however, this indicates that in small amounts, hydrocarbons actually stimulate root growth. This may be an instance of the phenomenon known as hormesis, or stimulated growth in response to small amounts of toxic substances. Conversely, if the contaminants have limited bioavailability, as may be expected so long after the contamination event, stimulation of root production could simply be due to the association of TPH with organic and relatively mineralizable N sources.

Regardless of the reason for this increased productivity, it has significant implication for phytoremediation. If hybrid poplar can maintain increased root production in hydrocarbon contaminated soils, the rhizosphere effect will be exaggerated and therefore increased degradation of contaminants is likely to occur.

Colonization of hybrid poplar growing in diesel contaminated soils by the ECM fungus *P. tinctorius* increased total aboveground and fine root production over non-colonized trees growing in the same soil. This is probably due to better access to nutrients by the trees in the ECM-HC treatment, which is evidenced by the fact that greater concentrations of N and P were found in the leaves of this treatment. However, removal of diesel fuel from the soil was significantly greater in the HC treatment than in the ECM-HC treatment. This may be due to suppression of microbial activity by the fungus. The amount of hydrocarbons sequestered in the roots of trees from the ECM-HC treatment was greater than the HC treatment. This was expected due to the hydrophobic nature of the mycelium and sheath produced by *P. tinctorius*. While this root sequestration does effectively remove the hydrocarbons from the soil for the time being, upon root senescence the contaminants may return to the soil.

This study indicates that ECM colonization of hybrid poplar growing in diesel contaminated soils improves tolerance and therefore plant performance, although there is little phytoremediation benefit. Still, the ECM-HC treatment did remove significantly more diesel fuel from the soil than an unplanted control.

Even though total fine root production was greater in the ECM-HC treatment, rates of fine root production were similar between the HC and ECM-HC treatments after 12 wk. This indicates that as contaminants degrade or become more recalcitrant the

stimulatory effect of ECM colonization is less pronounced. Given that the hydrocarbons at Hendon most likely have reduced bioavailability there would likely be no increase in fine root production if inoculation of the trees at this site was performed. Even if a stimulatory effect of ECM colonization on fine root production at the Hendon site occurred, the ECM colonization portion of this study indicates that degradation of contaminants would be suppressed.

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APPENDIX A

SAS script for the plane intersect method

/*This procedure is designed to process minirhizotron data in order to obtain fine root productivity in g of dry mass per m2 of horizontal ground surface. It follows "Method #2"of Bernier and Robitaille, (Plant and Soil, in press as of march 2004). For any question or comments, please contact Pierre Bernier (pbernier@cfl.forestry.ca)

The first infile is the main fine root data file and is read into TMP. Each line must be a single observation of one particular root at one particular date. This infile must be an EXCEL file and it must have the following column names: Tube, Frame, Root, Date and RtDiam.

Tube, frame and root columns must contain sequential integers only(no letters); Date must be in a Julian or day-of-year format; RtDiam must be in mm.

The second infile contains the tube and site descriptors and is read into TubeProp. It must also be an EXCEL file and have the following column names: Tube, TubeAngle, Slope, StonFrac, as well as parameter values for A0, A1 and A2 for describing the specific mass of the fine roots.

Tube is the tube number as in the first infile. TubeAngle is the angle of the tube (in degrees) with respect to the ground. Slope is the slope angle (in degrees) with respect to the horizontal. StonFrac is the fraction of coarse (D>2mm) particles in the soil (0<=stonFrac<=1). Parameters A0, A1 and A2 describe the specific mass of the roots (in g/cm3).
A0 is an average specific mass.
A0 is given a value >0 only if an average specific mass value is used.
A0 is set to 0 if a diameter-dependant function is used to describe the specific mass of roots
A1 and A2 are the two parameters of a Poisson function "A1*(1-exp(-A2*RootDiam)"
A1 and A2 are adjusted to field data supporting the diameter dependency of specific mass
A1 and A2 are set to 0 if only a mean specific mass (i.e. A0>0) is used

The computation of productivity assumes that W, the width of the minirhizotron frames is equal to 11mm

The results are written to an EXCEL file for which the user must provide appropriate directory coordinates in the last procedure of this program. This final output files produces for each observation date (except the first one) and for each tube the following variables: Mass_T0, Mass_T1 and productivity. Mass_T1 is the actual mass of roots observed at the date indicated on the line. Mass_T0 is the mass of roots seen at the previous date, but without those roots that will have disappeared at T2 (see Bernier and Robitaille for details). Productivity is the difference between these two numbers. Productivity cannot be a negative value. All masses are given in g/m2 of horizontal ground surface*/

```
/*First infile: enter the root measurements*/
PROC IMPORT OUT= Tmp
        DATAFILE= "C:\Documents and Settings\Jeff\My
Documents\sasrootinputs.xls"
        DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;
/*Second infile: Enter the site and tube properties*/
PROC IMPORT OUT= TubeProp
        DATAFILE= "C:\Documents and Settings\Jeff\My Documents\tubeprops.xls"
                        DBMS=EXCEL2000 REPLACE;
    GETNAMES=YES;
RUN;
/**********************START DATA
PREPARATION**********************/
/*Creates file TMP1; creates a specific ID for each observation
that is made up or the root identifiers and the  date*/
DATA Tmp1;
        SET Tmp;
        ID = COMPBL(Tube||Frame||Root);
RUN;
/*Sorts TMP1 by root ID and date*/
PROC SORT DATA = Tmp1;
        BY ID DATE;
RUN;
/*In TMP1, creates three new columns called MaxDiam ADDNEW and DECreasing*/
DATA Tmp1;
        SET Tmp1;
        MaxDiam = RtDiam;
        ADDNEW = '       ';
        DEC = '       ';
RUN;
/*Creates table DATE_ALL that contains the list observation dates*/
PROC SQL;
        CREATE TABLE DATE_ALL AS
                SELECT DISTINCT DATE
                FROM Tmp
                ORDER BY DATE;
/*Creates table Rt_FL that identifies the first and last measurement date for each root*/
        CREATE TABLE Rt_FL AS
                SELECT ID, MIN(DATE) AS DATEF, MAX(DATE) AS DATEL
                FROM Tmp1
                GROUP BY ID
                ORDER BY ID;
```

```
/*In Rt  FL, numbers all lines sequentially in the variable "NUM"*/
DATA Rt_FL;
        SET RT_FL;
        Num = _N_;
RUN;
/*Merge Tmp1 and Rt_FL by root ID: this assigns first and last dates to all root
observations*/
PROC SQL;
        CREATE TABLE Tmp1 AS
                SELECT a.*, b.* /*b.Num*/
                FROM Tmp1 as a, Rt_fl as b
                WHERE a.ID = b.ID;
/*Counts the number of roots*/
PROC SQL;
        CREATE TABLE NBROOT AS
        SELECT COUNT(*) AS NBROOT
        FROM RT_FL;
/*Calls up macro nbroot using nbroot as input*/
DATA nbRoot;
        SET nbRoot;
        CALL symput("nbRoot",nbRoot);
RUN;
/*This macro checks root by root (unique ID) if there are missing observations within a
sequence of observations of a given root, and if diameter of that root is decreasing.  If
there are missing observations, it adds a new line that contains the same RtDiam as the
line above.  If the diameter is decreasing, it maintains in column MaxDiam the
maximum diameter ever measured for that root. Analysis of productivity will be done
using this column.  Labels "adding" and "decrease" are added to the temporary file to
identify lines that were either added or modified.  The macro creates a new file called
"ALL" that contains the following columns: id Date  MaxDiam ADDNEW dec.  See
Bernier and Robitaille for further explanation on the necessity of this procedure*/
%MACRO nbroot;
        /*Loop through all roots*/
        %DO i=1 %TO &nbRoot %BY 1;
                /*Add the root number to the list of dates*/
                DATA date;
                        SET Rt_fl (where = (Num = &i));
                RUN;

                PROC SQL;
                        CREATE TABLE Dates AS
                                SELECT a.*, b.*
                                FROM date AS a, date_All AS b;
                DATA Dates;
                        SET Dates;
```

```
        IF date < datef OR date > datel THEN DELETE;
    RUN;
    DATA root;
        SET Tmp1 (where = (Num = &i));
    RUN;
    DATA Roots;
        MERGE Root Dates;
        BY Date;
    RUN;

    PROC IML;
        USE roots;
        READ ALL;
        matSize = nRow(MaxDiam);
        row = 1;
        DO WHILE (row < matSize);
            MaxDiam_p = MaxDiam[row];
            id_p = ID[row];
            Tube_p = Tube[row];
            row1 = row+1;
            MaxDiam_n = MaxDiam[row1];
            IF MaxDiam_n = . THEN DO;
                MaxDiam[row1] = MaxDiam_p;
                id[row1] = id_p;
                Tube[row1] = Tube_p;
                ADDNEW[row1] = 'adding';
            END;
            IF (MaxDiam_n < MaxDiam_p & MaxDiam_n > .)
        THEN DO;
            MaxDiam[row1] = MaxDiam_p;
            dec[row1] = 'decrease';
            END;
            row = row + 1;
            END;
        CREATE rep VAR{id Tube Date RtDiam MaxDiam ADDNEW
        dec};
        APPEND;
        CLOSE rep;

        PROC APPEND BASE=All;
        RUN;
%END;
%MEND;
%nbroot;
```

```
/******************** END DATA PREPARATION **********************/

/************************* START INCREMENT **********************/
/*In this section, fine root productivity is computed as in the Method 2 of
Bernier and Robitaille (2004), using their equations 1, 2 and 4.  The variables are:
rho: the specific mass, g/cm3
TubeAngle: the angle of the tube w/r to the ground , degrees
Slope: the angle of the ground with respect to the horizontal, degrees
StonFrac: the fraction of coarse material in the soil
A0 is the average specific mass (g/cm3) if only an average is used
A0 is set to 0 is values are provided for parameters A1 and A2
A1 is the first parameter of a two-parameter Poisson function
A2 is the second parameter of a two-parameter Poisson function
A1 and A2 are set to 0 if a value of A0 is provided


The computation assumes that W, the width of the minirhizotron frames
is equal to 11mm

This section computes the volume per unit area of ground for each individual root
All the roots are within file "ALL", a file created above in the macro*/

DATA Tubeprop2;
        SET Tubeprop;
        sinAlpha=sin(3.1416*(TubeAngle)/180);
        cosBeta=cos(3.1416*(slope)/180);
RUN;
data a;
merge all tubeprop2;
by tube;
run;

PROC SQL;
        CREATE TABLE All1 AS
                SELECT a.*, b.sinAlpha, b.cosBeta, b.StonFrac, b.A0, b.A1, b.A2
                FROM All AS a left join Tubeprop2 AS b
                ON a.Tube = b.Tube;
/* Computes the volume of each root as in eqs 1 and 2 of Bernier and Robitaille
        Make sure that the units of Rho, the specific mass, are g/cm3 and that
        of the root diameters is mm*/
DATA Tmp2;
        SET All1;
        rho = A0+A1*(1-exp(-A2*maxDiam));
        W=11;
        Ae=3.1416**2*(maxDiam/2)**2/sqrt(2);
```

```
        P = 2*10**6*(rho/1000)*(1-StonFrac)*Ae * sinAlpha*cosBeta/W;
RUN;
PROC DATASETS;
        DELETE ALL;
RUN;
/*Identifies the last date of measurement in the main file Tmp2 within a new
        file T2*/
PROC SQL;
        CREATE TABLE T2 AS
                SELECT a.*, b.num
                FROM  Tmp2 as a left join rt_fl as b
                ON a.ID = b.ID AND a.Date = b.Datel;
/*In new file T3_START, for a particular date, selects all roots that are not
                at their last date of measurement*/
PROC SQL;
        CREATE TABLE T3_START AS
                SELECT Tube, Date, sum(P) as Sum_P
                FROM  T2
                WHERE Num =.
                GROUP BY Tube, DATE
                ORDER BY Tube, DATE;
/*In new file T3_end, for a particular date, selects all roots, even those at
                their last date of measurement*/
        CREATE TABLE T3_END AS
                SELECT Tube, Date, sum(P) as Sum_P
                FROM  T2
                GROUP BY Tube, DATE
                ORDER BY Tube, DATE;
DATA T3_START;
        SET T3_START;
        ID_ = COMPBL(TUBE || DATE);
RUN;
DATA T3_END;
        SET T3_END;
        ID_ = COMPBL(TUBE || DATE);
RUN;
/*Add the volume computed from eq. 2 to T3_START, grouped by date*/
PROC SQL;
        CREATE TABLE T3_START AS
                SELECT b.Tube, b.Date, a.Sum_P
                FROM  T3_START as a RIGHT JOIN T3_END as b
                ON a.ID_ = b.ID_;
/*Adds a label number*/
DATA T3_START;
        SET T3_START;
        Num = _N_ ;
```

```
        IF Sum_P = . THEN Sum_P = 0;
RUN;
/*Labels the end dates sequentially*/
DATA T3_END;
        SET T3_END;
        Num = _N_ - 1;
        IF Sum_P = . THEN Sum_P = 0;
RUN;
/*Creates table "Diff" as the difference in volumes between dates "Start" and
        "END" and the resulting change in mass is attributed to the last date of
        the date pair in "b.date"*/
PROC SQL;
CREATE TABLE Diff AS
                SELECT b.Tube, b.Date, a.Sum_P AS Mass_t0, b.Sum_P AS Mass_t1,
Mass_t1 - Mass_t0 AS Increment
                FROM  T3_START as a , T3_END as b
                WHERE a.Num = b.Num AND a.Sum_P ne 0;

/**************************END INCREMENT************************/
PROC EXPORT DATA= Diff
        OUTFILE= "C:\Documents and Settings\Jeff\My Documents\sasoutput.xls"
        DBMS=EXCEL2000 REPLACE;
RUN;
```

APPENDIX B

Chapter three statistical analysis

Table B.1 Soil property concentrations as affected by depth of soil layer at Hendon, SK using the Mann-Whitney U test. Separate tests were run for each property.

Soil Property	Soil Layer	Mean Rank	Sum of Ranks	Mann-Whitney U	Significance
NO_3–N	0- to 20-cm	42.03	1513.00	449.000	0.025
	20- to 40-cm	30.97	1115.00		
Total N	0- to 20-cm	42.47	1529.00	433.000	0.015
	20- to 40-cm	30.53	1099.00		
Total C	0- to 20-cm	42.51	1530.50	431.500	0.015
	20- to 40-cm	30.49	1097.50		
TPH	0- to 20-cm	36.50	1314.00	648.000	1.000
	20- to 40-cm	36.50	1314.00		
Bulk Density	0- to 20-cm	54.50	1962.00	0.000	0.000
	20- to 40-cm	18.50	666.00		
pH	0- to 20-cm	22.38	805.50	139.500	0.000
	20- to 40-cm	50.63	1822.50		

Table B.2 Statistical analysis of mean fine root production within each soil layer as affected by year at Hendon, SK using the Mann-Whitney U test.

Soil Layer	Year	Mean Rank	Sum of Ranks	Mann-Whitney U	Significance
0- to 20-cm					
FR Production	2004	13.50	162.00	60.000	0.514
	2005	11.50	138.00		
20- to 40-cm					
FR Production	2004	11.83	142.00	64.000	0.671
	2005	13.17	158.00		
0- to 40-cm					
FR Production	2004	13.42	161.00	61.000	0.551
	2005	11.58	139.00		

APPENDIX C

Chapter three fine root production and biomass estimates for individual blocks

Table C.1 Fine root production estimates for each block as measured for the two soil layers over the 4-mo sampling period in 2004.

Block	June	July	August	September	Total Year
	Mg ha^{-1}				
	0- to 20-cm Soil Layer				
1	0.19	0.25	0.27	0.24	0.94
2	0.44	0.20	0.05	0.06	0.76
3	0.53	0.67	0.43	0.14	1.77
4	0.11	0.08	0.09	0.08	0.36
5	0.46	0.38	0.40	0.12	1.37
6	0.15	0.21	0.30	0.15	0.81
7	0.74	0.35	0.10	0.13	1.32
8	0.56	0.54	0.60	0.40	2.10
9	0.34	0.39	0.41	0.17	1.31
10	0.79	0.54	0.35	0.40	2.07
11	0.53	0.52	0.41	0.34	1.80
12	0.32	0.04	0.06	0.25	0.66
Mean	0.43 (0.22)†	0.35 (0.20)	0.29 (0.18)	0.21 (0.12)	1.2 (0.58)
	20- to 40-cm Soil Layer				
1	0.12	0.12	0.13	0.10	0.47
2	0.18	0.03	0.09	0.05	0.35
3	0.37	0.28	0.26	0.06	0.97
4	0	0	0	0	0
5	0.10	0.29	0.24	0.03	0.66
6	0.18	0.07	0.01	0.10	0.36
7	0.16	0.14	0.07	0.01	0.38
8	0.25	0.35	0.17	0.23	0.99
9	0.11	0.13	0.13	0.04	0.41
10	0.06	0.16	0.05	0.03	0.30
11	0.14	0.05	0.03	0.07	0.29
12	0.46	0.37	0.04	0.07	0.94
Mean	0.18 (0.13)	0.16 (0.13)	0.10 (0.09)	0.07 (0.06)	0.51 (0.31)

† Mean (standard deviation)

Table C.2 Fine root production estimates for each block as measured for the two soil layers over the 4-mo sampling period in 2005.

Block	June	July	August	September	Total Year
	Mg ha^{-1}				
	0- to 20-cm Soil Layer				
1	0.14	0.31	0.48	0.40	1.32
2	0.33	0.25	0.12	0.21	0.92
3	0.26	0.14	0.28	0.53	1.21
4	0.01	0.12	0.00	0.00	0.13
5	0.34	0.39	0.25	0.16	1.14
6	0.15	0.13	0.00	0.00	0.27
7	0.13	0.37	0.35	0.34	1.20
8	0.58	0.56	0.47	0.30	1.91
9	0.17	0.21	0.16	0.00	0.54
10	0.59	0.57	0.47	0.47	2.09
11	0.27	0.32	0.43	0.39	1.40
12	0.15	0.24	0.37	0.17	0.92
Mean	0.26 (0.18)†	0.30 (0.15)	0.28 (0.18)	0.25 (0.19)	1.09 (0.59)
	20- to 40-cm Soil Layer				
1	0.12	0.05	0.10	0.11	0.38
2	0.21	0.22	0.19	0.14	0.76
3	0.21	0.29	0.14	0.09	0.73
4	0.02	0.01	0.00	0.00	0.02
5	0.06	0.13	0.11	0.10	0.40
6	0.40	0.19	0.08	0.00	0.67
7	0.10	0.07	0.06	0.01	0.24
8	0.20	0.27	0.35	0.22	1.04
9	0.31	0.21	0.02	0.04	0.58
10	0.16	0.19	0.18	0.30	0.84
11	0.10	0.17	0.23	0.11	0.61
12	0.04	0.05	0.14	0.07	0.30
Mean	0.16 (0.11)	0.15 (0.09)	0.13 (0.10)	0.10 (0.09)	0.55 (0.29)

† Mean (standard deviation)

Table C.3 Fine root biomass estimates for each block as measured for the two soil layers over the 4-mo sampling period in 2005.

Block	June	July	August	September	Monthly Mean
	Mg ha^{-1}				
	0- to 20-cm Soil Layer				
1	0.61	0.68	0.82	0.82	0.73
2	0.81	0.91	0.63	0.63	0.75
3	2.10	2.64	2.78	2.50	2.51
4	0.57	0.55	0.59	0.41	0.53
5	1.15	1.53	1.77	1.73	1.55
6	0.61	0.78	1.28	1.41	1.02
7	1.45	1.46	1.07	1.12	1.27
8	1.41	2.05	2.86	2.53	2.21
9	0.97	1.36	1.65	1.79	1.44
10	1.40	1.93	1.74	1.67	1.68
11	1.07	1.97	1.88	2.17	1.77
12	1.29	0.48	0.52	0.68	0.74
Mean	1.12 (0.45)	1.36 (0.69)	1.47 (0.80)	1.46 (0.73)	1.35 (0.63)
	20- to 40-cm Soil Layer				
1	0.42	0.52	0.74	0.66	0.58
2	0.56	0.37	0.48	0.48	0.47
3	1.29	1.30	1.54	1.12	1.31
4	0.02	0.02	0.02	0.02	0.02
5	0.50	0.82	1.06	0.96	0.84
6	0.28	0.27	0.16	0.24	0.24
7	0.27	0.25	0.24	0.20	0.24
8	0.73	1.05	0.84	0.91	0.88
9	0.22	0.35	0.46	0.48	0.38
10	0.14	0.33	0.31	0.34	0.28
11	0.27	0.28	0.29	0.35	0.30
12	0.79	0.92	0.71	0.65	0.77
Mean	0.46 (0.35)	0.54 (0.39)	0.57 (0.43)	0.53 (0.34)	0.53 (0.36)

† Mean (standard deviation)

Table C.4 Fine root biomass estimates for each block as measured for the two soil layers over the 4-mo sampling period in 2005.

Block	June	July	August	September	Monthly Mean
	Mg ha^{-1}				
	0- to 20-cm Soil Layer				
1	0.78	0.70	0.60	0.46	0.63
2	0.77	0.86	0.76	0.73	0.78
3	1.52	1.74	1.73	1.23	1.56
4	0.18	0.43	0.27	0.23	0.28
5	0.88	0.94	0.82	0.70	0.84
6	0.88	1.53	1.44	0.97	1.21
7	0.33	0.72	0.74	0.73	0.63
8	1.26	1.23	1.15	0.83	1.12
9	0.88	1.03	1.02	0.69	0.91
10	1.12	1.07	1.13	1.19	1.13
11	1.08	1.56	1.81	1.55	1.50
12	0.27	0.34	0.32	0.35	0.32
Mean	0.83 (0.41)	1.01 (0.44)	0.98 (0.50)	0.81 (0.38)	0.91 (0.41)
	20- to 40-cm Soil Layer				
1	0.44	0.36	0.31	0.26	0.34
2	0.84	0.98	0.80	0.54	0.79
3	0.66	0.67	0.43	0.43	0.55
4	0.02	0.03	0.02	0.02	0.02
5	0.71	0.98	1.05	0.84	0.89
6	0.19	0.74	0.68	0.59	0.55
7	0.21	0.17	0.14	0.05	0.14
8	0.05	0.89	0.75	0.54	0.56
9	0.76	0.55	0.61	0.46	0.59
10	0.30	0.62	0.62	0.66	0.55
11	0.28	0.38	0.34	0.36	0.34
12	0.37	0.29	0.22	0.26	0.29
Mean	0.40 (0.28)	0.56 (0.32)	0.50 (0.30)	0.42 (0.24)	0.47 (0.25)

† Mean (standard deviation)

APPENDIX D

Chapter four fine root production and biomass estimates for individual treatments

Table D.1 Fine root productivity estimates for hybrid poplar over the 12 week study, as affected by ECM colonization and diesel contaminated soil.

Treatment	Replicate	Week						Total
		2	4	6	8	10	12	
		g m^{-2}						
HC	1	0.2	1.1	0.3	3.2	10.1	11.3	26.1
	2	0.2	0.8	3.0	2.2	7.0	9.6	22.8
	3	0	0	0	2.0	8.2	8.0	18.3
	4	0.3	1.0	1.4	3.0	7.9	9.1	22.6
	5	0.1	0.6	1.9	2.1	9.4	10.9	25.1
	6	0	0.5	0.8	2.3	8.0	9.0	20.7
	Mean	0.2 (0.1)	0.7 (0.4)	1.2 (1.1)	2.5 (0.5)	8.5 (1.1)	9.7 (1.2)	22.6 (2.9)
ECM-HC	1	5.7	6.9	12.5	11.4	9.4	9.8	55.7
	2	7.9	14.2	11.5	10.9	8.1	10.1	62.7
	3	4.0	12.4	10.8	9.8	10.3	9.5	56.9
	4	3.5	13.2	11.5	10.0	9.3	9.5	57.0
	5	2.7	8.3	11.9	9.5	8.9	10.0	51.4
	6	3.0	11.8	11.5	9.8	9.7	10.1	55.8
	Mean	4.5 (2.0)	11.1 (2.9)	11.6 (0.6)	10.2 (0.7)	9.3 (0.7)	9.8 (0.3)	56.6 (3.6)
ECM	1	19.7	20.5	17.3	11.0	10.9	17.5	96.9
	2	12.8	12.2	5.9	9.2	10.3	10.7	61.1
	3	11.6	19.1	14.3	8.4	9.4	11.3	74.1
	4	11.0	13.6	15.3	8.3	11.3	5.8	64.6
	5	15.1	19.3	10.8	10.4	10.2	7.7	73.4
	6	17.5	19.7	11.9	10.3	8.9	9.8	78.1
	Mean	14.6	17.38 (3.5)	12.6 (4.0)	9.59 (1.1)	10.2 (0.9)	10.4 (4.2)	74.7 (12.6)

Table D.1 cont'd.

Treatment	Replicate	Week 2	4	6	8	10	12	Total
		g m^{-2}						
NA	1	16.7	15.1	20.7	15.7	15.5	16.1	99.8
	2	16.1	14.1	10.5	12.6	8.9	8.0	70.3
	3	10.7	16.5	10.8	5.8	9.5	8.7	62.2
	4	17.1	18.3	18.3	14.5	13.1	12.8	94.1
	5	14.1	12.8	10.8	8.5	9.9	10.1	66.1
	6	12.7	14.3	12.9	11.4	10.5	9.5	71.3
	Mean	14.6 (2.5)	15.2 (2.0)	14.0 (4.4)	11.4 (3.8)	11.2 (2.5)	10.9 (3.1)	77.3 (15.7)

† Mean (standard deviation)

Table D.2 Fine root biomass estimates (g m^{-2}) for hybrid poplar over the 12 week study, as affected by ECM colonization and diesel contaminated soil.

Treatment	Replicate	Week						Mean
		2	4	6	8	10	12	
		g m^{-2}						
HC	1	0.2	0.7	1.1	3.8	12.8	20.4	6.5
	2	0.2	1.5	2.6	4.5	9.0	16.7	5.7
	3	0.0	0.0	0.0	2.0	9.7	14.4	4.4
	4	0.3	1.0	2.1	2.8	11.2	19.4	6.12
	5	0.1	0.5	0.8	3.8	9.5	16.2	5.2
	6	0.0	0.8	0.8	3.7	10.7	15.8	5.3
	Mean	0.2 (0.1)†	0.8 (0.5)	1.2 (1.0)	3.4 (0.9)	10.5 (1.4)	17.1 (2.3)	5.5 (0.8)
ECM-HC	1	5.7	7.0	15.8	19.8	34.3	27.6	18.4
	2	7.9	12.1	19.5	28.7	31.4	42.3	23.7
	3	4.0	7.9	18.4	25.9	34.0	36.4	21.1
	4	3.5	8.3	16.2	28.0	31.8	33.0	20.1
	5	2.7	10.0	17.1	21.3	33.8	37.8	20.5
	6	3.0	7.5	19.8	21.9	34.1	35.1	20.2
	Mean	4.5 (2.0)	8.8 (1.9)	17.8 (1.7)	24.3 (3.8)	33.2 (1.3)	35.4 (4.9)	20.7 (1.7)
ECM	1	19.7	31.3	56.0	60.9	66.3	74.9	51.5
	2	12.8	19.5	14.2	18.6	23.8	31.1	20.0
	3	11.6	24.2	31.2	36.7	44.4	44.7	32.2
	4	11.0	28.4	39.8	44.8	47.3	57.9	38.2
	5	15.1	23.7	30.1	35.8	41.1	49.1	32.5
	6	17.5	23.2	32.2	35.5	43.0	44.0	32.5
	Mean	14.6 (3.5)	25.1 (4.2)	33.9 (13.7)	38.7 (13.9)	44.3 (13.6)	50.3 (14.9)	34.5 (10.3)

Table D.2 cont'd.

Treatment	Replicate	Week 2	4	6	8	10	12	Total
		g m^{-2}						
NA	1	16.7	25.7	44.8	56.1	66.8	76.1	47.7
	2	16.1	32.6	35.2	43.6	49.9	55.4	38.8
	3	10.7	26.6	24.0	25.9	32.2	39.6	26.5
	4	17.1	26.1	29.3	34.8	54.3	63.3	37.5
	5	14.1	29.5	37.3	44.8	48.6	52.5	37.8
	6	12.7	29.6	37.6	46.2	47.2	55.6	38.2
	Mean	14.6 (2.5)	28.3 (2.7)	34.7 (7.2)	41.9 (10.4)	49.8 (11.2)	57.1 (12.1)	37.7 (6.7)

† Mean (standard deviation)

APPENDIX E

Chapter four statistical analyses

Table E.1 One-way ANOVAs for N and P contents of hybrid poplar as affected by ECM colonization and diesel contaminated soil. Separate tests were performed for each parameter.

Source of Variation	df	Mean Square	F	Significance
$N\text{-}NH_4$	3	962.972	18.028	0.001
$P\text{-}PO_4$	3	3.140	20.150	0.000

Table E.2 One-way ANOVAs for aboveground biomass and root:shoot ratio of hybrid poplar as affected by ECM colonization and diesel contaminated soil. Separate tests were performed for each parameter.

Source of Variation	df	Mean Square	F	Significance
Aboveground biomass	3	6102.833	63.002	0.000
Root:shoot ratios	3	0.024	9.641	0.000

Table E.3 Bonferroni multiple comparisons for N and P concentrations of hybrid poplar as affected by ECM colonization and diesel contaminated soil.

Parameter	Treatment (I)	Treatment (J)	Mean Difference (I-J)	SE	Significance
$N\text{-}NH_4$	HC	ECM-HC	-33.333*	5.967	0.003
		ECM	-38.333*	5.967	0.001
		NA	-12.666	5.967	0.399
	ECM-HC	ECM	-5.000	5.967	1.000
		NA	20.666	5.967	0.051
	ECM	NA	25.666*	5.967	0.016
$P\text{-}PO_4$	HC	ECM-HC	-1.366*	.322	0.017
		ECM	-2.266*	.322	0.001
		NA	-0.366	.322	1.000
	ECM-HC	ECM	-0.900	.322	0.141
		NA	1.000	.322	0.088
	ECM	NA	1.900*	.322	0.002

Table E.4 Bonferroni multiple comparisons for aboveground biomass and roots:shoot ratios of hybrid poplar as affected by ECM colonization and diesel contaminated soil.

Parameter	(I) Treatment	(J) Treatment	Mean Difference (I-J)	SE	Significance
Aboveground Biomass	HC	ECM-HC	-18.000*	5.682	0.029
		ECM	-70.333*	5.682	0.000
		NA	-52.000*	5.682	0.000
	ECM-HC	ECM	-52.333*	5.682	0.000
		NA	-34.000*	5.682	0.000
	ECM	NA	18.333*	5.682	0.025
Root:shoot ratios	HC	ECM-HC	0.028	.028	1.000
		ECM	0.111*	.028	0.006
		NA	0.130*	.028	0.001
	ECM-HC	ECM	0.083	.028	0.053
		NA	0.101*	.028	0.012
	ECM	NA	0.018	.028	1.000

Table E.5 One-way ANOVAs for residual TPH as affected by ECM colonization and diesel contaminated soil. Separate tests were performed for soil, roots and stems.

Source of Variation	df	Mean Square	F	Significance
Soil†	2	83888.528	40.010	0.000
Roots‡	1	39491.213	11.699	0.007
Stems§	3	561.307	4.066	0.577

† Compares soil from the HC and ECM-HC treatments with an unplanted-contaminated treatment

‡ Compares roots from the HC and ECM-HC treatments

§ Compares stems from HC, ECM-HC, ECM and NA treatments

Table E.6 Bonferroni multiple comparisons for residual soil TPH concentrations of the diesel contaminated treatments.

Treatment (I)	Treatment (J)	Mean Difference (I-J)	SE	Significance
HC	ECM	-152.778*	30.716	0.001
	Unplanted	-274.772*	30.716	0.000
ECM	Unplanted	-121.994*	28.959	0.004