

Petroleum hydrocarbon contamination in boreal forest soils: a mycorrhizal ecosystems perspective

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ABSTRACT

The importance of developing multi-disciplinary approaches to solving problems relating to anthropogenic pollution is now clearly appreciated by the scientific community, and this is especially evident in boreal ecosystems exposed to escalating threats of petroleum hydrocarbon (PHC) contamination through expanded natural resource extraction activities. This review aims to synthesize information regarding the fate and behaviour of PHCs in boreal forest soils in both ecological and sustainable management contexts. From this, we hope to evaluate potential management strategies, identify gaps in knowledge and guide future research. Our central premise is that mycorrhizal systems, the ubiquitous root symbiotic fungi and associated food-web communities, occupy the structural and functional interface between decomposition and primary production in northern forest ecosystems (i.e. underpin survival and productivity of the ecosystem as a whole), and, as such, are an appropriate focal point for such a synthesis. We provide pertinent basic information about mycorrhizas, followed by insights into the ecology of ecto- and ericoid mycorrhizal systems. Next, we review the fate and behaviour of PHCs in forest soils, with an emphasis on interactions with mycorrhizal fungi and associated bacteria. Finally, we summarize implications for ecosystem management. Although we have gained tremendous insights into understanding linkages between ecosystem functions and the various aspects of mycorrhizal diversity, very little is known regarding rhizosphere communities in PHC-contaminated soils. This makes it difficult to translate ecological knowledge into environmental management strategies. Further research is required to determine which fungal symbionts are likely to survive and compete in various ecosystems, whether certain fungal - plant associations gain in ecological importance following contamination events, and how PHC contamination may interfere with processes of nutrient acquisition and exchange and metabolic processes. Research is also needed to assess whether the metabolic capacity for intrinsic decomposition exists in these ecosystems, taking into account ecological variables such as presence of other organisms (and their involvement in syntrophic biodegradation), bioavailability and toxicity of mixtures of PHCs, and physical changes to the soil environment.

Key words: ectomycorrhiza, ericoid mycorrhiza, mycorrhizal ecosystems, boreal forest soils, ecosystem processes, petroleum hydrocarbons, soil pollution, biodegradation, bioremediation.

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I. INTRODUCTION

Boreal and sub-boreal forest ecosystems include arctic, sub-arctic and northern mid-latitude forest regions that are dominated by a cold climate and are able to support only a few coniferous and broadleaf tree genera (Burton *et al.*, 2003). Petroleum hydrocarbons (PHCs) are complex mixtures of aliphatic, alicyclic and aromatic compounds (Miller & Herman, 1997; Potter & Simmons, 1998) plus constituents that contain N, S or O in addition to H and C. PHCs may find their way into terrestrial ecosystems by surface spills or leaks from pipelines or storage tanks. The microbial ecology of boreal forest ecosystems, with or without PHCs, is incompletely understood. It is known, however, that symbiotic fungi colonize and extend beyond the roots of dominant plant species, thereby forming an intimately interwoven belowground mycorrhizal system. Mycorrhizal fungi account for most of the microbial biomass in organic soil horizons (Lundström, van Breemen & Bain, 2000; Dahlberg, 2001). The traditional role of individual symbioses involves the exchange of soil nutrients for carbohydrates fixed through plant photosynthesis (Smith & Read, 1997). Nutrients are obtained from inorganic sources inaccessible to plants or accessible, but more readily obtained, by the mycobiont. However, some mycorrhizal systems appear to possess well-developed saprotrophic capabilities (i.e. oxidative and hydrolytic enzyme systems) that mobilize nutrients from organic sources. Such capabilities may have developed through selection in ecosystems characterized by slow decomposition and retention of nutrients in organic polymers (Hibbett, Gilbert & Donoghue, 2000; Burke & Cairney, 2002; Cairney & Meharg, 2002; Read & Perez-Moreno, 2003). Mycorrhizal

systems capable of metabolizing exogenous organic compounds therefore may be candidates for use in remediation of soils contaminated with PHCs.

Mycorrhizal fungal mycelia and surrounding soil (i.e. mycorrhizosphere) provide suitable habitats for diverse communities of microorganisms due to increased availability of high-energy metabolic substrates and surfaces for colonization (Sarand *et al.*, 2000; Sen, 2003; Heinonsalo, Hurme & Sen, 2004). This enhances bacterial decomposition of plant materials because mycelia provide a path, together with associated water films, through which bacteria can migrate to substrates in micropores. Metabolic synergism between fungal and bacterial members of soil communities ensures that virtually all organic compounds are subject to biotransformation (if available to decomposer organisms) and that nutrients and energy-rich compounds are exchanged between plants and the soil environment *via* mycorrhizal fungal networks (Simard *et al.*, 1997; Read & Perez-Moreno, 2003; Diaz, 2004; Heinonsalo *et al.*, 2004). Consequently, in addition to their direct transformation of organic compounds, mycorrhizal systems may indirectly enhance degradation of PHCs in soil by modifying the structure of associated bacterial communities (Cairney & Meharg, 2002).

Oil extraction, refinement and transportation activities in boreal regions have resulted in surface and near-subsurface soil contamination with PHCs including crude (or synthetic crude) oil, gasoline, diesel and creosote (Kanaly & Harayama, 2000). The current standard against which environmental impacts are evaluated is sustainability (maintenance of ecological integrity) using various ecological indicators as measures. Sustainability requires management strategies for large areas and long periods of time that satisfy diverse environmental, social and economic needs

(Burton *et al.*, 2003). The relationship between soil microbial communities and ecosystem processes (e.g. decomposition and biogeochemical cycling) provides insights into how communities and ecosystems respond to environmental change. Microbial diversity (the variety of taxonomic, genetic and functional characteristics of organisms) helps sustain terrestrial ecosystems by conferring ecosystem stability (the ability to withstand change), resilience (the ability to recover from change) and resistance (the inherent capacity to withstand disturbance) (Andr n & Balandreau, 1999; Tiedje *et al.*, 1999; Nannipieri *et al.*, 2003; Swaminathan, 2003; Fitter *et al.*, 2005). Lower diversity or higher specialization occurs in disturbed soil systems due to: (1) extinction of populations that lack sufficient tolerance to the change imposed, and/or (2) selective enrichment of populations that tolerate or thrive under the new conditions (D az, 2004; Hofman,  vih lek & Holoubek, 2004). To understand the basis of community differences associated with changes in environmental conditions, it is necessary to integrate the functional properties and environmental requirements or tolerances of communities with processes at an ecosystem level (Bengtsson, 1998; Cairney, 1999; Dahlberg, 2001; Read & Perez-Moreno, 2003).

The potential toxicity of some PHCs to human, plant and animal receptors is used in managing contaminated sites, but the physical, chemical and biological impacts on soil microbial communities are less extensively studied and used (Miller & Herman, 1997; Nicolotti & Egli, 1998). Controlled experiments have provided valuable information regarding the toxicological impacts of chemicals on test organisms, which forms the scientific basis for current remediation standards. In soils, toxicity of PHCs to soil organisms including plants occurs concurrently with physical and chemical changes to the soil habitat following PHC contamination (Tarradellas & Bitton, 1997; Blakely, Neher & Spongberg, 2002; Trofimov & Rozanova, 2003). Is it possible to separate the effects of chemical toxicity from habitat changes such as hydrophobicity, lowered redox potential or reduced nutrient supply in PHC-contaminated soils? Are methods available for assessing the fate and behaviour of PHCs in forest soils that include bioavailability and indicators for ecological integrity that also complement measures for plant productivity? In addition, many PHCs are structurally analogous to organic compounds naturally found in the soil environment and appear to be degraded by soil microbial communities using the same biochemical pathways (McGill, Rowell & Westlake, 1981; Siciliano & Germida, 1998). Can functional aspects of microbial populations and communities (e.g. exocellular enzymes) be manipulated for bioremediation of contaminated soil?

Numerous reviews have addressed various aspects of mycorrhizal systems (e.g. Meharg & Cairney, 2000; Dahlberg, 2001; Burke & Cairney, 2002; Allen *et al.*, 2003; Read & Perez-Moreno, 2003; Fitter *et al.*, 2005) or of PHC behaviour and biodegradation in soil (McGill *et al.*, 1981; Riser-Roberts, 1998; Alexander, 1999, 2000; Prince & Drake, 1999; D az, 2004; Stokes, Paton & Semple, 2005; R mbke, J ansch & Scroggins, 2006). How might we advance the understanding of the fate and behaviour of

PHCs in boreal forest soils in both ecological and sustainable management contexts? We aim to do so by synthesizing information regarding the interactions between mycorrhizal communities and PHC contaminants in boreal soils. From this, we hope to evaluate potential management strategies, identify gaps in knowledge and guide future research. Our central premise is that mycorrhizal systems occupy the structural and functional interface between decomposition and primary production in northern forest ecosystems and as such are an appropriate focal point for such a synthesis. Information in this synthesis should be useful to professionals ranging from ecologists to engineers involved in the management and remediation of contaminated boreal forest soils. Our approach is first to provide pertinent basic information about mycorrhizas, followed by insights into the ecology of ecto- and ericoid mycorrhizal systems. Next we review the fate and behaviour of petroleum hydrocarbons in forest soils, with an emphasis on interactions with mycorrhizal fungi and associated bacteria. Finally, we summarize implications for ecosystem management.

II. MYCORRHIZAS

(1) Classification and structure

Mycorrhizas are symbioses between plant roots and an array of soil-inhabiting, filamentous fungi. These associations are virtually ubiquitous and generally considered mutualisms (i.e. reciprocally increase the fitness of both partners) as they are based on a bidirectional exchange of nutrients that is essential to the growth and survival of both partners (Smith & Read, 1997; Peterson & Massicotte, 2004; Sapp, 2004). The fungal partner acquires nitrogen (N), phosphorus (P) and other nutrients from the soil environment and exchanges them with the plant partner for photosynthetically derived carbon (C) compounds that fuel fungal metabolism. The structural attributes of mycorrhizas are related to their primary function of nutrient exchange and provide the basis for broad classification into seven currently recognized groups: ectomycorrhizas, ericoid mycorrhizas, ectendomycorrhizas, arbuscular mycorrhizas, arbutoid mycorrhizas, monotropoid mycorrhizas and orchid mycorrhizas (Peterson, Massicotte & Melville, 2004). In boreal forest ecosystems, most trees typically form ectomycorrhizal (ECM) symbioses, whereas the major constituents of the understorey vegetation often form arbuscular (AM), ericoid (ERM) or arbutoid (ARM) mycorrhizas. The ECM and ERM groups will be considered herein in the greatest detail.

Ectomycorrhizas, the associations between ECM fungi and the roots of woody plants, are characterized by three structural components: the mantle, the Hartig net and the extraradical mycelium (Smith & Read, 1997). The mantle is a sheath of fungal tissue that covers the highly active tips of the lateral roots of the plant and forms the boundary between the root and the soil environment. Its compact, but also variable, morphological nature provides a buffering

capacity that helps to prevent root cell dehydration or penetration by pathogenic organisms (Brundrett, 1991). Fungal cells (hyphae) emanate from the outer mantle as extraradical mycelia and grow into the surrounding soil where they reach micropore areas and absorb nutrients that may otherwise be inaccessible, both physically and biochemically (i.e. enzymatic processing of organic compounds), to roots (Söderström, 1992; Perez-Moreno & Read, 2000). Some ECM fungi also form rhizomorphs, which are thick linear aggregates of hyphae that are specialized for long-distance translocation of nutrients and water (Agerer, 2001). Lipids, phenolic compounds, proteins and polyphosphates may accumulate in the hyphae of the outer mantle, which may also bind heavy metals and thereby prevent their uptake into roots (Peterson *et al.*, 2004). The inner mantle consists of repeatedly branched hyphae, suggesting a role in nutrient exchange such as enabling absorption of glucose and fructose from the root and conversion to fungal sugars (e.g. trehalose, mannitol or glycogen) (Peterson *et al.*, 2004). At the interface of nutrient exchange is a highly branched structure known as the Hartig net, which is formed by multidigitate growth of fungal hyphae between epidermal and cortical cells of the root, and is the probable site for exchange of resources between symbionts (Peterson *et al.*, 2004). Subtle variations in morphological attributes viewed using light microscopy are often used to distinguish between ECM fungal taxa; development and differentiation of extraradical mycelia may provide predictive features relevant to the ecological classification of ECMs (Agerer, 1987-2002, 2001).

The common feature of plants that form ericoid mycorrhizas is the formation of very fine lateral roots that are composed of a vascular cylinder, one or two rows of cortical cells and an epidermal layer of enlarged cells (Peterson *et al.*, 2004). ERM fungi do not form mantles or Hartig nets, but rather colonize the epidermal cells of these fine roots and develop intracellular hyphal coils that are specialized for nutrient exchange (Peterson *et al.*, 2004). The intracellular fungal symbiont is separated from the plant cytoplasm by a plant-derived membrane, which invaginates to follow fungal growth and coil formation (Perotto, Girlanda & Martino, 2002). ERM fungal taxa cannot be distinguished by morphological characters using light microscopy. From molecular studies, it appears that ERM roots are composite structures that house multiple fungal symbionts, which implies that epidermal root cells may potentially function as separate units colonized by a variety of fungi (Perotto *et al.*, 2002).

(2) Diversity

Mycorrhizal symbioses have been an important force in evolution (Pirozynski & Malloch, 1975; Blackwell, 2000; Cairney, 2000; Sapp, 2004). Based on reconstructions of evolutionary lineages (phylogenies) from fungal DNA and the fossil record, it is currently accepted that the first mycorrhizal associations were pivotal in allowing plants to colonize the terrestrial environment about 600 million years ago and they form the evolutionary basis of present plant

communities (Pirozynski & Malloch, 1975; Blackwell, 2000). Redecker, Kodner & Graham (2000) reported fossilized fungal hyphae and spores found from the Ordovician of Wisconsin (about 460 million years old) that strongly resemble modern Glomales-like AM fungi. Modern AM fungal species persist in most extant plant species and form a single monophyletic group descended from these first mycorrhizas (Cairney, 2000). The AM group represents four orders (Archaeosporales, Paraglomerales, Diversisporales and Glomerales) of fungi within the phylum Glomeromycota (Smith & Read, 1997).

ECM fungal diversity appears to have arisen about 200 million years ago, corresponding to changes in climate that allowed for colonization of the land with trees and increased organic matter content of some ancient soils (Cairney, 2000). Although ECM plant partners (phytobionts) represent only about 8000 species (mostly in the families Pinaceae, Betulaceae, Fagaceae, Dipterocarpaceae, Salicaceae and Myrtaceae), these species are of global importance because of their disproportionate occupancy and domination of terrestrial ecosystems in boreal, temperate and subtropical forests (Smith & Read, 1997). It has been estimated that 5000-6000 species of fungi (of the subdivisions Basidiomycotina, Ascomycotina and Zygomycotina) form ECM symbioses (Molina, Massicotte & Trappe, 1992; Horton & Bruns, 2001), but these numbers are expected to rise as more regions are progressively explored in detail (Cairney, 2000). Phylogenetic analyses reveal that ECM fungi have originated from several independent lineages and that symbiosis with plants has been convergently derived (and perhaps lost) many times over millions of years (Hibbett *et al.*, 2000). Some ECM taxa are closely related to, or descended from, wood-rot fungi and some are related to other saprotrophic fungal taxa (Tanesaka, Masuda & Kinugawa, 1993; Hibbett *et al.*, 2000). This variation in the ability to degrade wood may have helped drive fungal speciation to avoid competition between closely related species that would otherwise use the same resources and occupy the same niche (Tanesaka *et al.*, 1993; Bruns, 1995; Martin, Perotto & Bonfante, 2000). The ability to degrade the complex aromatic chemical structures of lignin in wood may also confer an ability to transform similar structures in PHCs.

ERMs evolved about 100 million years ago, as sclerophyllous vegetation (i.e. plants with small, tough foliage and tissues that are rich in lignin and cellulose, but deficient in N and P) emerged in nutrient-poor soils (Cairney, 2000). Many plants of the family Ericaceae (e.g. *Vaccinium*, *Rhododendron*, *Gaultheria*, *Ledum* species) are common components of the understorey vegetation in northern forests and usually form typical ERM (Vrålstad, Schumacher & Taylor, 2002b). In the Southern hemisphere, plant species of the family Epacridaceae form ERM (Cairney & Ashford, 2002). ERM fungi were thought to belong to the Ascomycotina, of which fungal strains in the *Rhizoscyphus ericae* – *Scytalidium vaccinii* species complex (Helotiaceae, Helotiales, Ascomycota) are most commonly studied and reported (Vrålstad, Myhre & Schumacher, 2002a; Zhang & Zhuang, 2004). In addition, ERM fungi identified as *Oidiodendron* (anamorphs of the ascomycete family

Myxotricaceae) as well as a broad range of sterile mycelia with divergent morphologies and unknown identifications have been described (Vrålstad *et al.*, 2002a). Recent morphological (clamped hyphae and dolipore septae forming typical ERM coils on *Vaccinium*, *Rhododendron* and *Gaultheria* species) and molecular (rDNA sequences) evidence indicates that some ERM fungi may belong to the Basidiomycotina (Berch, Allen & Berbee, 2002; Perotto *et al.*, 2002). It has become increasingly apparent that a wider spectrum of taxa is involved in the ERM symbiosis than had been previously imagined.

ECM communities appear to consist of large numbers of fungal species (i.e. exhibit high species richness), even within small areas with little heterogeneity in plant communities, soil properties, climate and disturbance patterns (Bruns, 1995; Kranabetter, Hayden & Wright, 1999; Taylor, Martin & Read, 2000; Mah *et al.*, 2001; Robertson *et al.*, 2006). ERM fungal communities also appear to exhibit high richness. For example, Monreal, Berch & Berbee (1999) isolated 20 fungi (five of which formed ERM *in vitro*) from sixty segments (each 3 mm long) of fine roots from an 8-cm-long salal (*Gaultheria shallon* Pursh) rhizome. This ERM fungal richness is consistent with other reports of species-rich communities of mycorrhizal and non-mycorrhizal endophytes in individual root systems of other ericaceous [e.g. *Calluna vulgaris* (L.) Hull] and epacridaceous [e.g. *Woolfsia pungens* (Cav.) F. Muell.] plants. All groups of ericoid fungi reported globally have been found associated with salal from a single site on Vancouver Island (British Columbia, Canada) and all ERM groups reported on salal have been found associated with other plant species elsewhere in the world (Berch *et al.*, 2002). It is currently hypothesized that sterile mycelia with ERM behaviour represent a heterogeneous group of fungal taxa that are mostly unidentified and appear to include a variety of unculturable mycobionts (Berch *et al.*, 2002; Perotto *et al.*, 2002). High species richness and abundance may represent ecological adaptation to local environmental heterogeneity and is thought to provide forests with a range of strategies to maintain efficient functioning under an array of environmental conditions (Cairney, 1999; Nannipieri *et al.*, 2003).

Establishing whether diversity is important for ecosystem processes has become a central issue in ecology (Leake, 2001). In general, soil microbial communities appear to comprise groups of organisms that fulfil broadly similar ecosystem functions (i.e. exhibit functional redundancy) (Yin *et al.*, 2000). Functional diversity represents the value and range of capabilities that are possessed by organisms present in a given ecosystem and are relevant to ecosystem processes (Allen *et al.*, 2003; Sobek & Zak, 2003). There is a growing body of evidence suggesting that the functional characteristics of component taxa are at least as important as species richness for maintaining essential ecosystem processes (Naeem, 2002; Nannipieri *et al.*, 2003). Knowledge of the individual roles of mycorrhizal fungal species, or of their distribution either in relation to each other or to the physical and chemical environments of the soil, is limited (Goodman & Trofymow, 1998; Rosling *et al.*, 2003) and insufficient for determination of community needs and responses by building up from the species level. Moreover,

in mycorrhizal ecosystems, we hypothesize that the functional significance of individual taxa is overshadowed by the integrated functional capability of the community, which is likely not an additive function of the independent capabilities of component species. The tendency to generalize ecological functions from a few fungal isolates reveals little information about the intrinsic physiological potential of most taxa (Cairney, 1999; Cairney & Meharg, 2003) or of the community. Current evidence suggests that ongoing parallel evolution of plant and fungal partners in response to environmental change on local and global scales may most readily explain extant patterns of mycorrhizal diversity and specificity (Cairney, 2000). Although functional redundancy almost certainly exists within mycorrhizal communities, high taxonomic and genetic diversity of ECM (and probably ERM) fungi may indicate that they also exhibit a high level of functional heterogeneity (Cairney, 1999).

Are all the pieces of an ecosystem essential for restoration? Following a disturbance, should the management target be to maintain (or reintroduce) the original species richness at all costs, or, alternatively, to nurture the survivors (stress-resistors) so that they can contribute to the restoration of habitats in a future (altered) state? Is it likely that resistant organisms will modify the environment in ways that favour only themselves (i.e. preserving a specialized community), or do modifications lead eventually to succession by organisms that are incapable of tolerating the initial conditions (as suggested by most concepts of ecological succession)? Species richness may be a critical aspect of ecosystem resilience and functioning, but within a restoration context, more emphasis should perhaps be devoted to the resistant biota and their contribution in restoring pre-contamination conditions. Community specialization may indicate environmental stress, but we hypothesize specialization may also be a desirable response to stress, and a useful characteristic in allowing stressed ecosystems to achieve long-term stability and diversity.

III. ECOLOGY OF ECTO- AND ERICOID MYCORRHIZAL SYSTEMS

(1) Soil habitat

Soils are living, open, dynamic systems. They contain structured and heterogeneous matrices, generally store nutrients and energy, and support high microbial diversity and biomass (Nannipieri *et al.*, 2003). To thrive, soil microorganisms must mobilize energy and nutrients stored in soil. Soil structure provides a complex and variable set of microbial habitats ranging from energy-rich to barren, or aerobic to anaerobic, over micrometre distances. Soil structure is determined by soil aggregation, which occurs when soil particles within aggregates cohere more strongly to each other than to adjacent aggregates (Hartel, 1998). Aggregates are composed of sand, silt and clay particles that are held together by organic matter, precipitated inorganic materials, microorganisms and the products of their

metabolic activities (Griffiths & Caldwell, 1992; Hartel, 1998). Aggregates are dynamic, constantly forming and disintegrating. Organic substrates and plant residues are entrained and protected during aggregate formation and released during aggregate disintegration (Plante & McGill, 2002). The solid phase adsorbs important biological molecules (e.g. DNA, enzymes, etc.) and many soil reactions are catalyzed at the surfaces of soil minerals such as clays, Mn (III and IV) oxides and Fe (III) oxides (Nannipieri *et al.*, 2003). In addition, the zeta potential of charged mineral and organic surfaces generates a steep pH gradient around them. For example, McLaren & Skujins (1968) cite examples of the pH optima of enzymes being several units higher in colloidal systems than in solution, apparently due to the lower pH in the immediate environment of the enzyme, close to colloidal surfaces. Water occupies the aggregate pore spaces and forms a meniscus around a central pocket of air, which provides an aerobic and aqueous habitat suitable for supporting bacterial communities (Wardle, 2002). Pore water also retards gas exchange, thereby creating anaerobic microsites. Pore water also participates in hydrolysis and mediates other soil reactions (Hartel, 1998).

Boreal forest soils are typically acidic with seasonal or intermittent availability of mineral nutrients (N and P) and high C:N ratios due to the surface accumulation of recalcitrant organic matter resulting from incomplete oxidation of plant material (Prescott, Maynard & Laiho, 2000; Allen *et al.*, 2003). This organic layer (mor humus) stores nutrients and also contributes to moisture retention and soil structure (Prescott *et al.*, 2000). The forest floor is the most metabolically active fraction of these soils and is heavily colonized by ECM and ERM root systems of trees and understorey vegetation (Lundström *et al.*, 2000). Wallander *et al.* (2001) estimated the extraradical mycelia biomass of ECMs to represent about 820 kg ha⁻¹ in boreal forest soils. Fungal metabolic activities produce organic acids that percolate with rain water down through the soil profile and contribute to accelerated weathering of mineral soils (Griffiths & Caldwell, 1992; Heinonsalo *et al.*, 2004). Soluble complexes are formed between the organic acids and Fe and Al ions in the upper mineral soil, thereby fostering leaching of Fe and Al ions and creating a weathered, eluvial horizon (Lundström *et al.*, 2000). These complexes percolate further downward and precipitate, creating a characteristic rust-coloured illuvial B horizon overlying the parent material (Lundström *et al.*, 2000). These changes with depth in soil chemical and mineralogical properties create contrasting habitats for microorganisms. For example, Rosling *et al.* (2003) found that the species composition of the ECM community varied between organic and mineral horizons of boreal podzolic soils and that most taxa occurred in only one part of the soil profile.

Less than 5% of the soil volume is occupied by microorganisms, but these sites of increased biological activity are where the majority of soil reactions are mediated (Díaz, 2004). The availability and nutrient content of organic matter are key factors influencing microbial biomass and community composition (Tiquia

et al., 2002). Other major factors controlling the distribution and abundance of soil microbial communities include: (1) properties of the soil environment (e.g. pH, O₂ supply and availability of water and nutrients such as N, P, Fe); (2) factors affecting dispersal (e.g. soil structure, micro-aggregate stability and routes of dispersal); and, (3) the controls of population turnover (e.g. nematode or protozoan grazing, controls on lytic enzymes, protective soil matrices) (Tiedje *et al.*, 1999). Introduction of PHCs alters all three of these fundamental characteristics. For example, O₂ supply is often reduced, water movement is restricted and soil fauna including nematodes and protozoa are temporarily lost from the contaminated ecosystem.

Microbial growth in soils is typically resource-limited (most often energy-limited) and increases rapidly in response to addition of reduced C to provide energy for the large chemo-organotrophic biomass (Nannipieri *et al.*, 2003; Morgan, Bending & White, 2005). Actively growing roots leak or secrete (exude) soluble and insoluble organic compounds into the surrounding soil (rhizosphere) that provide most of the low molecular weight C available to microorganisms (Darrah, 1991; Garbaye, 1994). Rhizodeposition is concentrated at the root tips and at sites of lateral branch formation, which correspond to sites of greater microbial population density and community complexity compared to bulk soil (Linderman, 1988; Chanway, 1997; Sarand *et al.*, 2000; Söderberg *et al.*, 2004). Soluble forms of C (e.g. monosaccharides, amino acids and organic acids) are readily metabolized by microorganisms to CO₂ or converted to biomass; insoluble forms of C (e.g. mucilages, sloughed cortical cells and dead root hairs) are less readily metabolized (Darrah, 1991), but they may form new microbial habitats, which are eventually consumed. As with fungi, bacterial richness and functional redundancy are both high, at least at coarse scales. Using fatty acid methyl ester profiles (FAME analysis) and 16S rRNA gene sequences, Axelrood *et al.* (2002a, b) described immense bacterial richness (isolates representing 42 known bacterial genera and clones spanning nine divisions, respectively) in surface organic matter and mineral soil samples from forests in the central interior of British Columbia. Culture collections were well represented by *Pseudomonas*, *Bacillus*, *Paenibacillus* and *Arthrobacter* species (Axelrood *et al.*, 2002a), whereas molecular clones were represented by *Bradyrhizobium*, *Rhizobium*, *Pseudomonas* and *Burkholderia* species (Axelrood *et al.*, 2002b). These genera are considered common soil inhabitants and important components of rhizosphere communities with respect to nutrient cycling and transformation of minerals and complex organic substrates (Axelrood *et al.*, 2002b).

It has not yet been fully appreciated that the establishment of mycorrhizal symbioses substantially alters the morphology and physiology of plant roots (e.g. alters permeability of root membranes), which also changes root exudation patterns as well as the types of C substrates exuded (Linderman, 1988; Ingham & Molina, 1991; Rygielwicz & Andersen, 1994). The extraradical mycelia generate increased volumes of mycorrhizosphere soil compared to noncolonized roots and not only support microbial growth through exudation of energy-rich

substrates, but also provide surfaces for colonization and contribute to formation of soil structure (Griffiths & Caldwell, 1992). The presence of ECM mycelia alters bacterial community structure by stimulating proliferation of selected bacterial populations, among other mechanisms (Frey *et al.*, 1997; Heinonsalo *et al.*, 2000). Fluorescent pseudomonads isolated from the mycorrhizosphere of Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco] appear preferentially to use trehalose, a carbohydrate derived from fungal metabolism (Frey *et al.*, 1997). Fluorescent pseudomonads and actinomycetes have been observed around ECM roots of birch, closely associated with the mantle and in proximity to fungal exudates (Ingham & Molina, 1991). There is also some evidence that diverse microbial communities may be selectively present in association with certain ECM mycelia (Garbaye, 1994; Read & Perez-Moreno, 2003). For example, Olsson & Wallander (1998) found that structure of soil bacterial communities, assessed using phospholipid fatty acid (PLFA) analyses, depended both on ECM fungal species and soil type. Fungal mycelial (mat) communities are unique soil habitats that contribute to maintenance of high richness of bacterial and fungal taxa within ecosystems (Griffiths & Caldwell, 1992).

In summary, conditions within soil habitats vary by orders of magnitude over micrometre distances, in response to physical (structure and aggregates), chemical (pH, O₂, soluble substances) and biological (microorganisms, soil fauna, plant roots) variables. Soil habitats may also be substrates (e.g. plant residues). Perhaps because of this almost infinite variety of habitats at the microbial-size scale, it is difficult to find any soil sample that is missing major genera of the known microbiota of terrestrial ecosystems. Molecular techniques continue to show increasingly large ranges of genetic material within soils (e.g. Axelrood *et al.*, 2002b; Prosser, 2002), with most (more than 99%) of the bacterial genotypes represented currently not culturable (Pace, 2005). With greater sampling effort, the number of known bacterial divisions has expanded substantially in recent years (Pace, 2005). From a management perspective, the genetic potential to mediate virtually any biogeochemical reaction and the habitat needed to support it appears to exist in most soils, with only specialized capabilities potentially missing.

(2) Community interactions

(a) Mycorrhizosphere bacteria

As mycorrhizal fungi constitute the most significant rhizosphere communities, they have immense potential for interactions with other soil organisms such as bacteria, fungi, protozoa, nematodes, arthropods and mammals, as well as with each other (Fitter & Garbaye, 1994; Read & Perez-Moreno, 2003; Cairney, 2005). The primary factors that influence the composition of associated communities are the quality and quantity of C compounds present, competitive interactions between mycorrhizal fungi and free-living microorganisms for mineral nutrients, and the beneficial, detrimental or neutral impacts of secondary metabolites produced by symbiotic or free-living organisms

(Siciliano & Germida, 1998; Cairney & Meharg, 2002). The outcomes of interactions between ECM, ERM and saprotrophic fungal mycelia may include mutual interference of growth (deadlock) or replacement of one taxon with another through competition (Cairney, 2005).

The interactions between ECM/ERM fungi and the heterotrophic bacterial community are important for accessing mineral nutrients (Burke & Cairney, 1998). Observations that enhanced decomposition of organic compounds occurs in (mycor)rhizosphere soils have been attributed to the greater metabolic activities associated with higher densities of microorganisms (Heinonsalo *et al.*, 2000). Enriched bacterial communities, often arranged as biofilms (organised systems consisting of layers of biologically active cells), have been noted at the surfaces of the ECM fungal mantle and extraradical mycelia, which are the sites of nutrient mobilization, uptake and translocation (Sen, 2003). The exposure of microbial biofilms to organic polymers such as cellulose and proteins appears to drive degradative secondary metabolism; this enables plant and microbial uptake of simple compounds (e.g. sugars, amino acids and mineral nutrients) that are released during the decomposition process (Sen, 2003).

From the germination of fungal propagules in soil to establishment of true symbiosis, mycorrhizal fungi experience a free-living stage during which they interact with bacteria (known as mycorrhizal helper bacteria, MHB) that appear to be beneficial to the colonization process *via* one or more of several proposed mechanisms (Garbaye, 1994). In axenic culture with nutrient limitation, MHB may act by direct trophic interactions (where bacteria provide C substrates or growth factors to the free-living fungi) or by metabolic detoxification of fungal metabolites (e.g. polyphenols, etc.) (Duponnois & Garbaye, 1990). Bacteria that are active at the time of mycorrhizal formation may facilitate recognition between the plant and mycorrhizal fungus, improve the receptivity of the root for fungal colonization, or stimulate fungal growth, thereby increasing encounters between roots and mycelia (Frey-Klett, Pierrat & Garbaye, 1997). MHB also appear to colonize fungal hyphae and stimulate initial mycorrhizal formation through production of vitamins, amino acids, phytohormones and/or cell wall hydrolytic enzymes, which may influence germination and growth rates of fungal structures, enhance root development and/or decrease susceptibility to infection (Martin *et al.*, 2000). Shishido *et al.* (1996) found that three strains of fluorescent pseudomonads enhanced spruce seedling growth through mechanisms unrelated to increased mycorrhizal colonization, but growth promotion of pine by two strains was facilitated by an interaction with mycorrhizas. Mycorrhizal root tips tended to support slightly higher populations of *Pseudomonas* spp. than non-mycorrhizal root tips and additional colonization sites or altered/enhanced exudation in the mycorrhizosphere were observed. Frey-Klett *et al.* (1997) found that high levels of bacterial inoculum (MHB *Pseudomonas fluorescens* BBc6) in the rhizosphere are not necessary for a helper effect to occur.

Another group of naturally occurring, free-living soil bacteria that colonize roots and enhance plant growth when added to seeds and roots are known as the plant growth

promoting rhizobacteria (PGPR) (Chanway & Holl, 1991). PGPR activity has been reported in *Azospirillum*, *Bacillus*, *Clostridium*, *Hydrogenophaga*, *Serratia*, *Staphylococcus*, *Streptomyces*, and *Microbacterium* species (Chanway, 1997). Holl & Chanway (1992) found that growth of mycorrhizal pine was stimulated by inoculating the rhizosphere with *Bacillus polymyxa* strain L6, which appeared to be a function of the size of the bacterial population. Plant growth promotion was not attributed to increased symbiosis by the ECM fungus *Wilcoxina*, and was also unlikely to be due to N fixation as this *Bacillus* strain contributed to only 4% of seedling foliar N. Rather, stimulation of pine growth may have been a result of bacterial production of plant growth substances such as indoleacetic acid. Other researchers have suggested that PGPR may, at least in the short term, improve the C supply to mycorrhizas by providing an increased supply of N (fixed from the atmosphere) to the plant (Ingham & Molina, 1991; Martin *et al.*, 2000). Microorganisms may directly stimulate plant growth by providing nutrients (e.g. N, P, S) or growth factors (e.g. auxin, cytokinin, gibberellin), increasing root permeability or inducing plant systemic resistance to pathogens. Indirectly, microorganisms may influence other rhizosphere components that influence plant growth, such as increasing legume or alder root nodule number and size, increasing colonization frequency of mycorrhizal fungi, or suppressing deleterious rhizobacteria (Chanway, 1997).

(b) Plant linkages

Plant communities in northern forest ecosystems are linked below ground *via* the extensive extraradical mycelial network of mycorrhizal fungi (Dahlberg, 2001; Simard & Durall, 2004). Host-specific fungi form intraspecific plant linkages, whereas fungi with more general host requirements may form interspecific linkages that allow for nutrient and C transfer between different tree species. In a microcosm experiment, radiolabelled C transfer through the soil mycelial network has been demonstrated between Sitka spruce [*Picea sitchensis* (Bong.) Carr.] and pine species (*Pinus contorta* Dougl. ex Loud. and *P. sylvestris* L.) (Finlay & Read, 1986). In the field, Simard *et al.* (1997) demonstrated bidirectional C transfer between Douglas-fir and paper birch (*Betula papyrifera* Marsh.) *via* a common mycelial network, with a significant net gain by the shaded Douglas-fir. Mycorrhizal networks appear to have the capacity to mediate significant N transfer among interconnected plants (*Casuarina* and *Eucalyptus* pairs); N gradients (between N-rich donors and N-limited receivers) may drive unidirectional N transfer (He *et al.*, 2005). Similarities in the composition of ECM communities associated with various host species in bioassays and field surveys indicate the potential for linkages between varieties of plant species (Kranabetter *et al.*, 1999; Massicotte *et al.*, 1999).

The coexistence of ECM and ERM plants in boreal forests provides many opportunities for sharing ECM and ERM fungi that link plants and translocate nutrients, although little research on this issue has been conducted (Perotto *et al.*, 2002). Vrålstad, Fossheim & Schumacher (2000) demonstrated that fungal strains derived from ECM

morphotype *Piceirhiza bicolorata* constituted assemblages of very close relatives to ERM type *Rhizoscyphus ericae*. Similarly, Monreal *et al.* (1999) showed sequence similarity (ITS2 region) between the ECM fungus *Phialophora finlandia* and *R. ericae*. In a resynthesis experiment using 12 *R. ericae* strains on ECM and ERM hosts, Vrålstad *et al.* (2002b) showed that genetically close relatives of the ERM fungus *R. ericae* are true ECM partners with conifer (spruce and pine) and angiosperm (birch) species, but no isolates tested formed both ECMs and ERMs. These studies indicate that ECM and ERM plants may share mycobionts of this species complex (known as the *R. ericae* aggregate) and, based on ITS phylogeny, the ability to form both ECM and ERM symbioses may have evolved with the *R. ericae* aggregate. Villarreal-Ruiz, Anderson & Alexander (2004) recently reported the ability of a fungus from the *R. ericae* aggregate to form simultaneously both ECMs and ERMs in culture with *Pinus sylvestris* and *Vaccinium myrtillus* seedlings, respectively, based on rDNA sequencing and microscopy.

Due to the complexity of the molecular mechanisms involved in establishment of a tight (host-specific) symbiosis, the type of fungal associations with different plant hosts may not be of great physiological importance under non-contaminated conditions, but may gain ecological importance under stressed environmental conditions (Perotto *et al.*, 2002). Mycelial linkages may influence fungal and plant ecology by providing a source of fungal inoculum to newly growing roots, allowing the C demands of the mycelium to be met by more than one plant and facilitating the transfer of C and mineral nutrients between neighbouring trees (Jones, Durall & Cairney, 2003). It has been proposed that ECM and ERM fungi may contribute to development of plant communities if the net transfer of C and nutrients is predominantly from a pioneer plant species to a late successional species, but a greater awareness of these processes is important for understanding the interactions between trees and understorey vegetation (Dahlberg, 2001). Kernaghan *et al.* (2003) demonstrated a positive correlation between ECM fungal richness and overstorey host tree richness that was explained by resource heterogeneity in combination with the preference (specificity) of ECM fungi for certain plant hosts. Recently, DeBellis *et al.* (2006) showed that the distributions of ECM fungi in southern mixed-wood boreal forests are influenced by the relative proportions of host tree species. Conservation of stand diversity should therefore support diverse fungal communities. Whether such communities are essential for ecosystem recovery following PHC contamination is still not known. Regardless, minimizing overstorey disruption increases the possibility of preserving the integrated below-ground mycelial network with its associated communities, and maximizes its potential to hasten site recovery.

(3) Ecosystem processes

Biogeochemical cycling of nutrients and energy through ecosystems is driven by ordering (e.g. photosynthesis, growth, humus formation) and dissipative (e.g. respiration, senescence, decomposition) processes (Addiscott, 1995).

Mycorrhizal systems form the functional interface between decomposition (release of carbon and nutrients from organic substrates) and primary production (formation of biomass) for both above-ground and below-ground communities. In the below-ground food web, chemo-organotrophic organisms (those that obtain energy and carbon from organic substrates) appear to be ultimately responsible for governing nutrient availability for plant productivity (Wardle, 2002; Wardle *et al.*, 2004). In reconstructed soil profiles (mini-ecosystems), the microflora (bacteria and fungi) were found to exert a greater influence on nutrient mobilization and tree growth than either the fungus-feeding mesofauna or predator trophic groups (Setälä, Haimi & Siira-Pietikäinen, 2000). Setälä *et al.* (2000) reported that although species composition of the trophic groups was important for system functioning, species richness within functional groups had a negligible impact on primary production. Soil fauna (nematodes, protozoa, enchytraeids, microarthropods, earthworms, termites, etc.) that feed on the microflora are also important in stimulation of primary production (Wardle, 2002) by preventing nutrient sequestration within inactive microbial biomass.

(a) Decomposition

Heterotrophic bacteria and fungi directly decompose complex carbohydrates (mainly cellulose and lignin) in plant detritus (Wardle, 2002). Cellulose is readily biodegradable as it consists of $\beta(1-4)$ linkages of D-glucose that form flat, linear chains H-bonded together to create microfibril sheets (Evans & Hedger, 2001). By contrast, lignin is a three-dimensional aromatic polymer consisting of $\beta(0-4)$ linkages of monomeric units of either cinnamyl alcohol: coumaryl alcohol (grasses), coniferyl alcohol (gymnosperms) or sinapyl alcohol (angiosperms) that surround the microfibrils and provide rigidity to plant cell walls (Evans & Hedger, 2001). Due to its complex and uniquely heterogeneous structure (i.e. hydrophobicity and thermodynamic stability), lignin is highly resistant to degradation (i.e. recalcitrant) and inhibits decomposition by up to a few years by limiting access of microorganisms or enzymes to substrates (Prescott *et al.*, 2000; Steffen, 2003).

In the early stages of decomposition, soluble compounds and cellulose are rapidly metabolized under conditions where C is available and N is usually limiting (Prescott *et al.*, 2000). Decomposition rates slow over time due to changes in substrate compounds (increased lignin fraction) and succession of microorganisms able to compete for various substrates (Berg, 2000). In the later stages of decomposition, there is a net loss of lignin and N is mineralized from humus (Prescott *et al.*, 2000). Growth may become N-limited in habitats with high C:N ratio substrates, whereas in habitats with low C:N ratio (<30:1) substrates, decomposition of organic matter may result in C limitation (Tiquia *et al.*, 2002). With the loss of cellulose, relative lignin and N concentrations increase and the higher N concentration can slow the decomposition rate. Such slowing may be due to low molecular mass N-containing compounds reacting with lignin residues during humification (Prescott *et al.*, 2000), creating more recalcitrant aromatic compounds; or mineral

N may repress synthesis of lignin-degrading enzymes in a wide range of soil organisms (Gallo *et al.*, 2004); or a combination of these (Magill & Aber, 1998). Higher initial N concentration (lower C:N ratio) leads to lower decomposition extent (i.e. lower mass loss) and more stabilized organic matter in forest soils (Berg, 2000). The process of humus formation (i.e. humification) is thought to involve microbial modifications of lignin and condensation of proteins or amino acids into humus precursors, which polymerize into structurally intricate humus molecules (Prescott *et al.*, 2000). Compared to the original plant material, humus is low in carbohydrates (e.g. cellulose and hemicellulose) and high in polyphenolics (e.g. lignin constituents) and immobilized N, which is sparingly available to plants (Prescott *et al.*, 2000).

Although it is generally accepted that mycorrhizas play important roles in decomposition and cycling of C, N and P in ecosystems, details of their functions in nutrient dynamics and regulation of nutrient and energy flows are continuing to develop and be refined (Martin, 2001; Allen *et al.*, 2003). The traditional view of the role of mycorrhizas in obtaining limiting nutrients from forest soils involves fungal exploration for nutrients [e.g. amino acids, ammonium (NH_4^+), nitrate (NO_3^-) and inorganic P] that are released during decomposition of plant organic matter by heterotrophic fungal and bacterial communities or that are bound to the soil matrix (e.g. insoluble forms of Al and Ca phosphates) (Martin *et al.*, 2000). However, molecular studies have revealed that some fungal species, previously regarded as decomposers of woody debris (saprotrophs), are both frequent and abundant components of ECM communities (Hibbett *et al.*, 2000; Kõljalg *et al.*, 2000). This, along with other conceptual advances in biocomplexity theory, have led to re-evaluation of how mycorrhizas function within ecosystems and how interactions between multiple species of plants, mycorrhizal fungi and soil saprotrophs regulate community composition and ecosystem processes (Allen *et al.*, 2003).

It has been hypothesized that the distribution of the different mycorrhizal categories is related to specialization for nutrient acquisition in particular environments (Read & Perez-Moreno, 2003). In higher latitude and higher elevation forest ecosystems, where seasonally low temperatures and dry conditions result in very slow rates of decomposition, natural selection may have favoured ECM and ERM symbioses with the capacity to mobilize nutrients from organic material and provide them to plants (Read, 1991; Perotto *et al.*, 2002; Read & Perez-Moreno, 2003). Fungal specialization for N acquisition and utilization may be important for determining community structure, lending strong theoretical support to the idea that ECM diversity increases the effectiveness of nutrient acquisition from different spatial locations and different substrates in soil (Martin *et al.*, 2000; Leake, 2001).

Some ECM fungi appear to be directly involved in nutrient mobilization from organic compounds through production of a wide range of hydrolytic and oxidative enzymes such as polyphenol oxidases (e.g. laccase, catechol oxidase and tyrosinase) and endochitinases (Martin *et al.*, 2000; Burke & Cairney, 2002; Lindahl & Taylor, 2004).

Most ECM fungi so far investigated have demonstrated limited phenol-degrading activities, but few have been studied. By contrast, ERM fungi (e.g. *R. ericae*) appear to have well-developed saprotrophic abilities and degrade most polymeric components (e.g. polysaccharides, lignin, protein, chitin and pectin) of plant and fungal cell walls (Martin *et al.*, 2000). It is widely accepted that enzymatic degradation of organic polymers, through production of an array of hydrolytic enzymes in the extraradical mycelia and translocation of nutrients to the root, is the major benefit of ERM symbioses to plants (Perotto *et al.*, 2002). ERM plants may enhance their exploitation of complex soil substrates by broadening their metabolic capabilities through an association with several fungi endowed with different functional enzymes (Martin *et al.*, 2000). This implies that simultaneous associations with a variety of symbiotic fungi may be an important strategy to broaden the range of functions in the colonization of different substrates (Perotto *et al.*, 2002). Whereas many ECM and ERM fungi appear to have the ability to access N and P directly from organic compounds, the extent to which they contribute directly (*via* enzymatic catabolism) or indirectly (by influencing soil microbial community structure) to decomposition remains unclear (Cairney & Meharg, 2002; Heinonsalo *et al.*, 2004).

(b) Primary production

Regardless of the mechanisms involved, symbioses with mycorrhizal fungi improve plant nutrient acquisition by facilitating access to organic sources, potentially increasing the uptake of nutrients via extensive growth of the mycelia and circumventing nutrient depletion zones in the soil (Buscot *et al.*, 2000). Nutrients are absorbed across fungal membranes and are either retained by the fungi for biosynthesis and growth, or transported distances of centimeters to meters to the plant roots, where translocation to the host enhances the photosynthetic machinery of the plant (Allen *et al.*, 2003). Plant photosynthetic rates depend on the concentrations of N (for enzymes), P (for ATP and ADP), Fe and Mg (for chlorophyll), internal CO₂ and water (to keep stomata open to fix CO₂) (Buscot *et al.*, 2000). The resulting nutrient sink (root cells) allows for nutrient absorption and translocation through fungal cells that occurs more quickly than diffusion to the roots through soil (Martin *et al.*, 2000). It is thought that, as long as N or P is limiting in the soil environment, plants will support their fungal partners through continued allocation of C (Allen *et al.*, 2003).

By absorbing, assimilating and translocating nutrients (e.g. nitrate, ammonium and amino acids such as glutamate, glutamine and alanine), fungi create a C sink in the mycorrhizal roots (Allen *et al.*, 2003). Heinonsalo *et al.* (2004) recently reported equivalent ¹⁴C allocations to roots and ECMs in organic and mineral A and B horizons of a podzol, using a *Pinus sylvestris* mini-rhizotron system. The sink strength controls the rate of photosynthate production and the sugar supply appears to regulate some fungal gene expression (Buscot *et al.*, 2000). Carbon metabolism provides fungal mycelia and plant cells with the energy, reducing power and biomass required for synthesis of

various metabolites (e.g. amino acids) required for growth (Martin *et al.*, 2000). Martin *et al.* (2000) have suggested that differences in ECM morphological features (e.g. abundant emanating hyphae with increased metabolic activity *versus* few emanating hyphae with decreased activity) may reflect differences in the need for C between taxa. Mycorrhizal fungi acquire most or all C *via* host photosynthesis and translocation (average 10–20% of net photosynthetic yield), but may also obtain C through assimilation following biodegradation of organic polymers in the soil (Martin *et al.*, 2000; Allen *et al.*, 2003).

The ecological significance of using organic polymers as C sources, which may decrease the need for C from the host plant, is unknown (Martin *et al.*, 2000). A substantial amount of fungal C is allocated to the synthesis of recalcitrant compounds such as chitin (60% of the fungal cell wall) that can persist in the environment for years and increase soil aggregation, stability, C storage and water-holding capacity. It has been estimated that as much as 20% of N in boreal podzolic forest floors may be retained in chitin (β -1,4 linked N-acetylglucosamine units) present in dead and alive fungal mycelia (Lindahl & Taylor, 2004). The remaining C is respired (43–60%), accumulated as fungal storage sugars (e.g. mannitol and trehalose) or lipids, or deposited in the mycorrhizosphere as labile compounds (sugars, amino acids) that support the growth of bacterial communities (Allen *et al.*, 2003; Heinonsalo *et al.*, 2004). The release of microbial communities from C limitation provides the potential for them to play a major role in decomposition and nutrient mobilization (Read & Perez-Moreno, 2003). The C cost for maintaining the external mycelium is unknown, but the extent to which C is allocated from roots to mycelial systems may be intrinsically linked to growth and nutrient-foraging activities of ECM fungi. Using digital autoradiographic techniques, Leake *et al.* (2001) showed that patterns of C allocation within ECM mycelia are highly dynamic and responsive to changes in niche (caused by spatial variability in resource quality or interactions with other organisms). It has been hypothesized that coexistence among fungi may be explained by the differential partitioning of C resources among fungal species (Allen *et al.*, 2003). The key role of mycorrhizas in C cycling (particularly in the positive feedback loop between plant growth, decomposition and leaf litter quality) may have important consequences for the C gains and losses of ecosystems and thus for the C budget at local, regional and global scales (Read, 1991; Cornelissen *et al.*, 2001; Allen *et al.*, 2003).

(c) Summary

The influences of mycorrhizal fungi on plant populations and communities are not merely the sum of effects on the individuals within populations (Dahlberg, 2001; Koide & Dickie, 2002). Due to their long history and multiple evolutionary events, different plants and fungi bring independent characteristics to the symbiosis, resulting in extensive physiological variation among mycorrhizas (Allen *et al.*, 2003). For example, some fungal species, previously regarded as saprotrophs, are both frequent and abundant

components of ECM communities (Hibbett *et al.*, 2000; Kõljalg *et al.*, 2000). The spatial scales within which individual mycelia operate as physically or physiologically integrated entities in nature are also not clear (Cairney, 2005). For example, all groups of ERM fungi reported globally have been found associated with salal from a single site (Berch *et al.*, 2002). Whereas a distinct suite of functions can be assigned to a single mycorrhiza, the many genomic combinations (genetic diversity) of symbionts, environmental heterogeneity and the extensive connectedness of mycorrhizal root systems result in a complex suite of ways that mycorrhizas can function in ecosystems (Cairney, 1999; Allen *et al.*, 2003). For example, certain species of mycorrhizal fungi, rhizosphere organisms and plants may interact such that there is a net immobilization of nutrients, which results in slower rates of decomposition (Allen *et al.*, 2003). Other combinations of organisms (guilds) may not influence the equilibrium of either nutrient cycling or decomposition, or may interact to increase nutrient quality of litter and decomposition rates.

High species richness and abundance may represent ecological adaptation to local environmental heterogeneity and is thought to provide forests with a range of strategies to maintain efficient functioning under an array of environmental conditions (Cairney, 1999; Nannipieri *et al.*, 2003). From a management perspective, a key challenge is to discover if modifications of the environment send mycorrhizal ecosystems in predictable directions. Or, alternatively, is it the combination of fungal and plant species that directs the trajectory? Current evidence suggests a vast range of genetic potential in most mycorrhizal ecosystems ready to respond to changing environmental conditions. Consequently, the hypothesis that environment is predominant in determining the outcome warrants testing.

IV. PETROLEUM HYDROCARBON CONTAMINATION OF FOREST SOILS

(1) Disturbance

Analysis of changes to the structure and taxonomic diversity of soil communities following disturbance can provide clues to genetic and functional diversity by revealing some features of the surviving organisms. Although rarely considered in this way, discrete PHC contamination events such as oil spills are disturbances that disrupt ecosystem, community or population structure and alter the physical environment and resource or substrate availability (White & Pickett, 1985). Forest soil contamination is usually from a point source (i.e. involving discrete, localized and often readily measurable discharge of chemicals) and often results in rapid surface contamination, sometimes with large quantities of PHCs. For economic and toxicological reasons, the fate and behaviour of organic pollutants in soils has been the subject of intense research, with special interest focused on those chemicals that can be taken up or transformed by living organisms (Alexander, 1999; Semple, Morriss & Paton, 2003). However, the capacity of soil

microorganisms to biodegrade organic pollutants not only depends on whether they have the necessary metabolic pathways, but also on whether the chemicals inhibit the microorganisms or are biologically available. Short- and long-term changes to soil microbial communities may result if some populations are susceptible to chemical toxicity (i.e. inhibition of cellular metabolic processes) or to changes to their physical (soil) and chemical (substrates/ inhibitors) habitat. Whether these changes alter community functions depends on the degree of redundancy within soil communities.

(a) Chemical toxicity

Petroleum products are complex mixtures that can contain numerous aliphatic (linear and branched chains), alicyclic (unsubstituted and alkyl substituted structures) and aromatic (unsubstituted and alkyl substituted structures with at least one unsaturated ring) compounds (Miller & Herman, 1997; Potter & Simmons, 1998). Numerous methods are available for analysing petroleum hydrocarbon mixtures in environmental media (Weisman, 1998). Natural gases are generally composed of methane, ethane and small amounts of higher molecular mass hydrocarbons whereas most crude oils contain compounds such as paraffins, aromatics, naphthenics and asphaltenes that are present in varying proportions (McGill *et al.*, 1981; Potter & Simmons, 1998). Gasoline typically contains compounds in the nC4 to nC12 range, while diesel compounds are in the nC8 to nC21 range (Potter & Simmons, 1998). Light paraffinic crude oils are dominated by molecules with C numbers less than 16 and consisting mainly of alkanes (paraffins) and cycloalkanes (naphthenes). These chemicals are generally first metabolized by microorganisms (Westlake *et al.*, 1973; Riser-Roberts, 1998). The heavier oils have greater proportions of aromatic hydrocarbons and heterocyclic NSO compounds (i.e. containing N, S, or O) with C numbers usually above 20 (McGill *et al.*, 1981; Delille, Coulon & Pelletier, 2004).

Using chromatographic techniques, crude oils can be resolved into four categories of compounds: asphaltenes, saturates, aromatics and polars (eg. NSO compounds) (McGill *et al.*, 1981; Pollard *et al.*, 1992; Weisman, 1998). Asphaltenes are a mixture of pentane-insoluble, colloidal compounds including polyaromatic and alicyclic molecules with some alkyl substitutes (usually methyl groups) that vary in molecular mass between 500 and several thousand (McGill *et al.*, 1981). The structures of these compounds, particularly those with higher mass, share characteristics with proposed structures of humic acids (Prescott *et al.*, 2000). Saturated and aromatic hydrocarbons (mainly n-alkanes, branched alkanes, mono-, bi-, and polycyclic alkanes and mono-, bi- and polyaromatics) usually account for 75% of the mass of crude oils (McGill *et al.*, 1981; Potter & Simmons, 1998). Up to 25% of the total mass may be n-alkanes, with cyclic hydrocarbons accounting for 30-60% of the total mass. Monocyclic aromatic compounds (e.g. toluene, benzene and xylene) and bicyclic types (e.g. naphthalene, biphenol) represent 1-2%; polycyclic aromatics (usually methylated derivatives of fluorene, phenanthrene, anthracene, chrysene, benzofluorene and pyrene) are

present in lower amounts (McGill *et al.*, 1981). The NSO fraction contains polar compounds such as naphthenic acids, mercaptans, thiophenes and pyridines. Most of the N in crude oil is contained in the distillate residue as part of the asphalt and resin fraction and usually accounts for less than 0.2% (rarely exceeds 1%) by mass. The S content varies between 0.3 and 3% whereas the O content usually does not exceed 3% (McGill *et al.*, 1981).

Aliphatic compounds are generally less toxic than aromatics, and toxicity has been found to vary with compound size (McGill *et al.*, 1981; Edwards *et al.*, 1998). The quantity and composition of polycyclic aromatic hydrocarbons (PAHs) are major considerations in the evaluation of toxicity of PHC mixtures (Miller & Herman, 1997). PAHs with four or more benzene rings are known to be genotoxic to humans and other ecological receptors; in general, as relative molecular mass and polarity (i.e. degree of oxidation) of PAHs increase, carcinogenicity also increases and acute toxicity decreases. This is due to the metabolic production of highly reactive electrophilic intermediates that can access biological molecules such as DNA, RNA and proteins and react to form adducts or lesions (Landis & Yu, 1995). Little is known about PHC toxicity to plant and microbial communities in forest soils as the majority of studies have excluded the complex interactions between combinations of chemicals, interacting communities and the soil environment that may exert synergistic, potentiative or antagonistic effects (Landis & Yu, 1995; Evans & Hedger, 2001; Koivula *et al.*, 2004). It is likely that toxicity varies with the type of pollutant, the extent of pollution and the general condition (i.e. extent of obvious signs of stress or disease) of the ecosystem prior to chemical disturbance (Seghers *et al.*, 2003).

In agricultural clay soils, the maximum toxicity of crude oil (indicated by worm survival, seed germination, bacterial bioluminescence and photosynthesis inhibition) was highest immediately after introduction of oil (Chaineau *et al.*, 2003). An initial decrease in microbial density has often been observed immediately following the addition of PHCs to soil. For example, the addition of 10% (volume/mass) toluene to soil resulted in survival of only about 1% of the indigenous bacteria, which eventually recolonized the soil to reach a high cell density (Huertas *et al.*, 2000). Biotransformations of PHC substrates may also lead to the release of an array of potentially toxic metabolites into the surrounding environment (McGill *et al.*, 1981; Riser-Roberts, 1998). For bacteria, toxicity resulting from PHC contamination has been inferred from decreases in enzyme (hydrogenase and invertase) activity (Suleimanov, Gabbasova & Sitdikov, 2005), although reduced enzyme activities may also result from competition for limiting nutrients following PHC contamination.

The toxicity of non-ionized organic contaminants for microorganisms is primarily due to a nonspecific mode of action that involves partitioning of organic chemicals into the hydrophobic (lipophilic) layer of the cell membrane and disruption of membrane integrity (i.e. increased membrane permeability) (Miller & Herman, 1997). Kirk *et al.* (2005) suggested that interference with fungal membranes may explain the reduced extraradical hyphal growth of AM

fungi (*Glomus* species) in PHC medium with soil. In general, fungi are considered to be more tolerant of high concentrations of polluting chemicals than bacteria, possibly due to differences in cell wall structure (Blakely *et al.*, 2002). Some yeasts (e.g. *Saccharomyces cerevisiae*) have been found to alter their membranes (i.e. increase hydrophilicity) to exclude hydrophobic contaminants (Park, Chang & Kim, 1988). It is possible that similar compensation mechanisms occur in other fungi as well. Long-term resistance to PHCs could also be due to the ability of ECM fungi to produce spores that resist environmental stress factors and germinate when the concentration of toxicants associated with PHC contamination has decreased sufficiently over time (Nicolotti & Egli, 1998).

PHCs may directly kill plants on contact, slow their growth, inhibit seed germination, create nutrient-deficient conditions or, at lower concentrations, stimulate plant growth (McGill *et al.*, 1981). Nicolotti & Egli (1998) have suggested that crude oil has a caustic or lethal effect on plants only when it comes into direct contact with tissues and that reduced growth and biomass may be manifestations of changes to soil communities. In general, the taller the trees and the deeper their roots, the greater their tolerance to increased PHC concentrations in soil (Trofimov & Rozanova, 2003).

(b) Soil properties and processes

Blakely *et al.* (2002) found that creosote impacted soil food webs and decomposition processes more by altering the habitat of microinvertebrates and their prey (i.e. fungi and bacteria) than *via* direct chemical toxicity. Kirk *et al.* (2005) suggested that PHCs may interfere with plant-fungus communication by altering root exudation patterns or changing the soil environment such that migration of diffusible chemical signals (e.g. flavonoids, auxins, etc.) is prevented. Many of the major impacts of PHCs on soil biota and plants in forest ecosystems appear to be associated with disturbances to water, nutrient and oxygen supplies related to the hydrophobicity and fluidity of oily products (Tarradellas & Bitton, 1997; Trofimov & Rozanova, 2003).

The disturbance caused by PHC contamination leads to considerable changes in physical and chemical properties that are not typical of unpolluted soils. PHC constituents may be found in mobile form, fixed in the soil pores and fissures, adsorbed on the surface of organic and mineral soil constituents or forming a continuous cover on the soil surface (Trofimov & Rozanova, 2003). Morphological changes in PHC-contaminated podzolic soils exhibit fragmentary patterns resulting from the unevenness of chemical distribution in the soil mass, increased amounts of iron in the upper horizons, and increased amounts of cemented soil aggregates (Trofimov & Rozanova, 2003). The extent of physical movement in the soil profile depends on temperature, PHC viscosity, moisture content, soil structure and soil texture (McGill *et al.*, 1981). Greater lateral spread of PHCs occurs in cold conditions; in hot and dry soil conditions, vertical movement into the water-unsaturated zone may occur more frequently (McGill *et al.*, 1981). In sandy soils, frontal migration of PHCs down the

soil profile along the paths of roots and fissures altered the soil profile to a depth of greater than 1 m (Trofimov & Rozanova, 2003). In gray forest soils, heavy fractions of PHCs were retained in the upper layer and filled the largest infiltration, aeration and drainage pores; lighter fractions were largely retained in illuvial horizons and filled fine water retention pores (Suleimanov *et al.*, 2005). This can lead to waterlogging and reducing conditions in the soil profile, both of which inhibit decomposition processes (Trofimov & Rozanova, 2003). Large amounts of oily material in soils may also indirectly increase soil temperature (by 1–10°C) if there is loss of surface vegetation. In some cases, more damage to the soil may occur due to the high osmotic potential of associated brine water (salinity 40,000–45,000 µg mL⁻¹) than to the presence of PHCs alone (McGill *et al.*, 1981).

Water insolubility, hydrophobicity and soil sorptive properties increase with increasing size (number of aromatic rings) and complexity (molecule topology or pattern of ring linkages) of chemicals; PAHs with three or more rings tend to be strongly sorbed to the soil (Reilley, Banks & Schwab, 1996; Alexander, 2000; Kanaly & Harayama, 2000; Cerniglia & Sutherland, 2001; Chaîneau *et al.*, 2003). Chemical persistence in soil also depends on several environmental factors, including the type and quality of clay particles (as well as cation exchange capacity), the type and concentration of solutes in surrounding solution, soil organic matter (SOM) content and composition, pH and temperature (Alexander, 1999; Semple *et al.*, 2003). Organic chemicals may be sorbed to and retained by soil particles by adsorption or partitioning. Adsorption entails chemical processes (ion exchange) or, more often, physical forces (H bonding or van der Waals forces) to surfaces of organic polymers or the external surfaces of 1:1 clay minerals and the external and internal surfaces of 2:1 (expanding) clays (Alexander, 1999; Miller & Herman, 1997; Ellerbrock *et al.*, 2005). Sorption to minerals must compete with water and may be very low for nonionized organics in hydrated systems. Sorption to organic solids may occur *via* physical binding, which concentrates chemicals on outer surfaces or within the pores of a solid. Partitioning of organic chemicals into the SOM is a process of transfer from the bulk state of one phase to the bulk state of another by mechanisms analogous to dissolution and leads to a distribution of molecules within a portion or the entire volume of the organic matter (Alexander, 1999; Chaîneau *et al.*, 2003). The extent of chemical retention in the SOM fraction is directly correlated with the octanol-water partition coefficient of the substance (K_{ow} , measure of chemical hydrophobicity), the amount of SOM in the solid phase and its degree of oxidation or polarity (Xing, McGill & Dudas, 1994; Alexander, 1999; Wang, Sato & Xing, 2005). In forest soils, the sequestration of organic pollutants in SOM (i.e. sorbed inside soil aggregates or at inactive particle surfaces) may decrease toxicity of chemicals through physical separation from biological receptors, which also decreases substrate bioavailability for enzymatic degradation (Alexander, 2000; Ellerbrock *et al.*, 2005). Toxicity of hydrophobic organic contaminants has been found to be less severe for organisms in soils with high humus content (Salminen & Haimi, 1997).

PHC-polluted soils are characterized by lower values of hygroscopic moisture, hydraulic conductivity and water retention capacity (i.e. wettability) compared to unpolluted soils (Trofimov & Rozanova, 2003; Suleimanov *et al.*, 2005). This is related to the spatial arrangement of hydrophobic components within SOM (Roy & McGill, 2000). Higher molecular mass components and their degradation products remain near the soil surface and form crusts that decrease water availability and limit water and gas exchanges between the soil and the atmosphere. The creation of discrete and continuous water-repellent fronts parallel to the soil surface is also recorded in post-fire forest soils (Certini, 2005). Hydrophobic films on the exterior surfaces of soil aggregates reduce the wettability of the soil and increase structure stability (McGill *et al.*, 1981; Certini, 2005). Many PHC-contaminated soils eventually take up water and remain wet; however, long-term (years) hydrophobicity of crude oil contaminated agricultural soils has been documented in western Canada (Roy, McGill & Rawluk, 1999).

The longer some chemicals remain in soil, the more they appear to resist desorption and biodegradation. Weathered (aged) chemical residues have considerable time to interact with the physical and chemical components of soil. Interactions may entail: (1) sorption, most likely *via* partitioning; or (2) irreversible incorporation into soil organic matter (*via* humification) by the catalytic activity of a variety of oxidative enzymes present in the soil matrix (Miller & Herman, 1997; Alexander, 1999). PHC pollution has been found to substantially increase the organic C (humic acid) content of soils (Trofimov & Rozanova, 2003). Humification of PHC constituents is explicit to transformation processes. Covalent bonding between organic chemicals and humic polymers (humin, fulvic acid and humic acid) in soil can form stable linkages to dialkylphthalates, alkanes and fatty acids that are resistant to microbial degradation and are not readily extractable with many organic solvents (McGill *et al.*, 1981; Alexander, 1999). Petroleum residues (as indicated by dichloromethane extraction) are associated with soil organic matter (Roy *et al.*, 2003). Sorption of volatile PHCs from adjacent soil has generated hydrophobicity in soils not directly contaminated with PHCs (Roy & McGill, 2000). They may be either directly incorporated through H bonding of phenolic and benzene carboxylic acids into the molecular structure of soil humic materials or adsorbed to the surface of the molecule (McGill *et al.*, 1981). They are not entirely associated with the humic component, however, because exhaustive extraction with NaOH did not eliminate hydrophobicity of soils (Roy & McGill, 2000). It is not known whether complexes between hazardous organic chemicals and soil humic materials are cleaved in nature to give detectable levels of the original compound, whether these complexes are assimilated by animals and plants, or whether they pose problems of present or future toxicological significance (Alexander, 1999).

In PHC-contaminated soils, the C (energy) supply increases, which promotes metabolic activity on the part of all the microorganisms not directly inhibited by the PHCs and concurrently the C:N ratio tends to increase.

The carrying capacity of a soil is the maximum level of microbial activity that can be supported under existing environmental conditions, which depends on the size of the population, availability of O₂ or nutrients, temperature and water availability. The carrying capacity of soils may be exceeded as a result of large inputs of C from PHCs (Miller & Herman, 1997). The intensive growth of PHC-oxidizing microorganisms in response to increased C availability is accompanied by consumption of soil nutrients, resulting in decreased nutrient availability for plants (Xu & Johnson, 1997; Tiquia *et al.*, 2002; Trofimov & Rozanova, 2003). A decrease in available N may also be partly due to the inhibition of nitrification and ammonification processes or from loss of nitrates (McGill *et al.*, 1981; Suleimanov *et al.*, 2005). Some studies have shown that nitrifying bacteria were not found in freshly contaminated soils, but that nitrification processes were eventually regained; stable organic matter and total N content have increased significantly following PHC contamination (McGill *et al.*, 1981). An increase in total N has been attributed to increased atmospheric N₂ fixation during PHC biodegradation (McGill *et al.*, 1981). Thus, as the immobilization of mineral N present in the soil increases, the amount of available N decreases such that organisms benefiting from N₂ fixation, or consortia capable of recycling N from microbial biomass, are the only organisms that can thrive under these conditions (i.e. selection of PHC-tolerant species) (Nicolotti & Egli, 1998). Oxidative degradation may also alter the composition of soil bacterial communities, so that aerobic cellulolytic and proteolytic species decrease and anaerobic N-fixing species increase (Nicolotti & Egli, 1998). Under disturbed water and extreme O₂ limitation, P may be reduced and escape to the atmosphere as hydrogen phosphide (Suleimanov *et al.*, 2005), although this is a very small loss mechanism.

It does not appear that a single addition of PHCs limits microbial communities in the long term. However, several studies indicate that although total microbial numbers tend to increase over time, species richness often decreases, which may or may not have deleterious impacts on ecosystem functions (McGill *et al.*, 1981; Hofman *et al.*, 2004). In general, PHC contamination is expected to lead to an initial loss of richness, followed by rapid proliferation of metabolically competent members of communities inhabiting the new environmental conditions imposed by the chemical contaminants (Gramss, Günther & Fritsche, 1998; Seghers *et al.*, 2003; Díaz, 2004). Some studies have reported drastic reductions in overall ECM biomass and colonization potential in soil following a spill, whereas some fungi appeared resistant to the PHCs and may have benefited from their presence (Nicolotti & Egli, 1998). In greenhouse experiments, spruce seedlings grown in crude-oil-contaminated soils exhibited shifts in ECM community structure in response to increased contaminant concentrations (Nicolotti & Egli, 1998). Nitrogen availability may be a major factor structuring ECM fungal communities; mineral N and foliar nutrient ratios (N:P, P:Al) were found to be excellent predictors of fungal taxonomic richness in organic horizons and organic nitrate availability was a good predictor of their relative abundance (Lilleskov *et al.*, 2002).

From studies of impacts of acid rain on soil communities, changes to soil chemical status and functions of the decomposer community have been suggested to lead to imbalances in nutrient cycling and ecosystem productivity (Pennanen *et al.*, 1998).

(2) Biodegradation

The ability of heterotrophic bacteria and fungi to degrade organic pollutants appears to be inherent in most natural microbial communities and it is generally accepted that biological processes eventually degrade or transform most bioavailable (i.e. accessible by organisms or their enzyme systems) organic compounds (McGill *et al.*, 1981; Sarand *et al.*, 2000; Nannipieri *et al.*, 2003; Delille *et al.*, 2004; Díaz, 2004). Many xenobiotic chemical constituents (e.g. PHCs) are structurally analogous to compounds naturally found in the soil environment (e.g. plant material, fungal and root exudates and allelopathic chemicals) and appear to be biodegraded through the same biochemical pathways (Miller & Herman, 1997; Siciliano & Germida, 1998). In addition to accidental releases, low levels of PHCs may also enter soils from natural seepages or *via* atmospheric deposition after burning of fossil fuels (Knox *et al.*, 1999; Kanaly & Harayama, 2000; Trofimov & Rozanova, 2003; Certini, 2005). Biodegradative potential does not appear to be a distinguishing taxonomic character as metabolic ability (*via* different genes and biochemical pathways) is widespread among many species of ubiquitous genera of bacteria and fungi (Siciliano *et al.*, 2003; Chaillan *et al.*, 2004).

The complete catabolic conversion (mineralization) of organic substrates to inorganic products (H₂O and CO₂) and use of nutrient constituents (C, N, P, S and other elements) for synthesis of cellular components is known as growth-linked biodegradation (Alexander, 1999). Biodegradation of some organic pollutants appears to result from transformations by microbial populations that are unable to use the substrate as a source of energy (even if the conversion is an energy-releasing oxidation reaction) or as an essential nutrient (i.e. not significant sources for biosynthesis) and is known as cometabolism (Miller & Herman, 1997; Alexander, 1999; Sarand *et al.*, 2000). Cometabolism is usually attributed to the activity of enzymes with relaxed specificities that act on structurally related substrates (Hickey, 1998). Organic substrates may be transformed to products that are not typical intermediates of central metabolism and the organism may not possess enzymes to convert further the compound into metabolic intermediates for biosynthesis or energy production. Alternatively, products may inhibit enzymes for subsequent metabolic conversions, suppress the growth of the organism, or the organism may require a second substrate (cofactor) to bring about a particular reaction (Alexander, 1999). Cometabolic reactions also have impacts in nature that are different from growth-linked biodegradation. Whereas the rate of growth-linked biodegradation characteristically increases with time as populations that are able to use the substrate as a source of energy and nutrients multiply, the environmental consequences of a population's inability to

grow at the expense of the substrate are slow rates of biotransformation (due to small microbial biomass) and accumulation of organic products that tend to persist in the environment (Alexander, 1999). The potential for substantial biodegradation of PHCs in soil (*via* growth-linked and cometabolic pathways) results in release of a nearly limitless array of metabolites into the soil environment. Some metabolites may be toxic to the soil biota; some may react with soil constituents or may be quickly degraded by other microorganisms present (McGill *et al.*, 1981; Riser-Roberts, 1998).

In a community context, biodegradation involves synergism. Syntrophic biodegradation occurs when two or more populations carry out transformations that one population alone cannot perform or performs slowly (Alexander, 1999). Thus, even if a particular population can metabolize only a small number of the chemical substrates available, other populations occupying the same habitat may possess complementary degradative enzyme capabilities that may ultimately result in complete chemical mineralization. Studies of mixed populations (*i.e.* communities) of bacteria have revealed more complex and rapid biodegradation than was previously believed possible based on studies of pure cultures (McGill *et al.*, 1981). Little is known concerning syntrophic biodegradation by specific guilds of organisms. If functional redundancy is the norm in most ecosystems, is the degradation of a substrate in soil dictated by specific guilds of organisms, or are the properties of the substrate and soil environment more important?

(a) Bacterial pathways

Because of their metabolic versatility, bacteria are able to obtain energy from virtually every organic compound (Romantschuk *et al.*, 2000; Díaz, 2004). The most common electron acceptor for microbial respiration is O₂ and aerobic processes provide the highest amount of energy to cells. Oxygen is not only the electron acceptor, but also participates in activation of the substrate *via* oxygenation reactions (Díaz, 2004). Anaerobic (anoxic) conditions are prevalent in aquifers, aquatic sediments and waterlogged soils. Here, biodegradation is carried out by strict anaerobes or facultative organisms using alternative electron acceptors such as nitrate (*e.g.* denitrifying organisms such as *Pseudomonas*, *Alcaligenes* and *Flavobacterium*), sulphate (*e.g.* sulphate reducers such as *Desulfobacterium*), Fe(III) (*e.g.* ferric iron reducers such as *Geobacter*), CO₂ (*e.g.* methanogens such as *Methanospirillum*) or others such as chlorate, Mn or Cr (McGill *et al.*, 1981; Díaz, 2004). Use of alternative acceptors depends on their availability as well as competition between different microorganisms for electron donors. The energy obtained using Fe(III) or nitrate is almost as efficient as using O₂, but less energy is generated by sulphate reducers and methanogens. Fermentative strains may be energy-limited and restricted to syntrophic existence, requiring other populations to consume the potentially toxic endproducts of fermentation. Photosynthetic organisms use energy from the sun to degrade aromatics anaerobically to acetyl-CoA, which is subsequently used in biosynthetic reactions (Díaz, 2004). In both

aerobic and anaerobic degradation, structurally diverse compounds are degraded through many different peripheral pathways to a few intermediates that are further channelled *via* biochemical pathways (*i.e.* reactions leading to the formation of Krebs cycle intermediates) to the cell's central metabolism (Díaz, 2004).

Many microorganisms can use the aliphatic compounds present in PHCs as C sources. The mid-size straight-chain n-alkanes (nC10 to nC18) appear to be metabolized more readily than n-alkanes with shorter or longer chains, and saturated (single C bonds) are degraded more readily than unsaturated (double C bonds) compounds (Miller & Herman, 1997; Delille *et al.*, 2004). The extent and location of hydrocarbon sidechain branching or halogen substitution slows the biodegradation of the compound (Miller & Herman, 1997). The most common aerobic biochemical pathway involves direct incorporation of one atom of O₂ into the alkane by a mixed function oxidase or mono-oxygenase enzyme, but both O₂ atoms can also be incorporated. In either case, a primary fatty acid is formed that is subjected to consecutive removal of two-carbon fragments (β -oxidation), which are converted to acetyl-CoA. This intermediate enters the Krebs cycle where complete mineralization to CO₂ and H₂O occurs (Miller & Herman, 1997). For alkenes, the first step is attack at the terminal or subterminal methyl group or at the double bond to yield an alcohol or epoxide that can be further oxidized to a primary fatty acid and enter β -oxidation. Degradation of alicyclic hydrocarbons (which are major components of crude oil, constituting 20-67% by volume) is thought to occur primarily *via* cometabolic reactions to open rings and subsequently cleave linearized products for entry into the Krebs cycle (Miller & Herman, 1997).

Under anaerobic conditions, however, oxygenation of hydrocarbons using O₂ is not possible. Aromatic ring structures may be activated under anaerobic conditions using a reductive rather than oxidative process. There is growing evidence that under anaerobic conditions some microbial communities are able to use O from H₂O or CO₂ for ring cleavage of aromatics or to prepare for ring cleavage of aromatics. For example, Schink, Brune & Schnell (1992) propose H₂O as the O source for ring cleavage during benzoate metabolism by fermenting and denitrifying bacteria, and CO₂ as the O source for carboxylation in preparation for ring cleavage of aniline by a sulphate-reducing bacterium.

Westlake *et al.* (1973) found that the ability of mixed populations of bacteria to use crude oil as a sole C source depended on the composition and amount of n-saturates, asphaltenes and NSO compounds and also that the aromatic fraction of crude oil was capable of supporting bacterial growth. It seems that PHC biodegradation is more closely related to the intrinsic biodegradability of chemicals (and their bioavailability) than to the particular enzymatic capacities of the microorganisms involved (Chaillan *et al.*, 2004).

Bacteria such as *Pseudomonas*, *Mycobacterium*, *Rhodococcus*, *Flavobacterium*, *Acinetobacter*, *Arthrobacter*, *Bacillus* and *Nocardia* are considered the primary degraders of polycyclic aromatic hydrocarbons (PAHs) in soil (Kanaly & Harayama, 2000;

Chaillan *et al.*, 2004). *Pseudomonas* has been the most extensively studied, owing to its ability to degrade so many different contaminants and its ubiquity in soils containing PHCs (McGill *et al.*, 1981; Axelrood *et al.*, 2002a; Delille *et al.*, 2004; Díaz, 2004). Most aerobic peripheral pathways involve oxygenation reactions carried out by monooxygenases and/or hydroxylating dioxygenases that incorporate one or two atom(s) of O₂ into the aromatic ring structure to generate dihydroxy aromatic intermediates (e.g. catechol, protocatechuate, gentisate, hydroxyquinol and hydroquinone) (Miller & Herman, 1997; Siciliano & Germida, 1998; Cerniglia & Sutherland, 2001; Watanabe, 2002; Díaz, 2004). These compounds are the substrates of ring-cleavage enzymes that use molecular oxygen to open the aromatic ring between the two hydroxyl groups (*ortho* cleavage, catalyzed by intradiol dioxygenases) or proximal to one of the two hydroxyl groups (*meta* cleavage, catalyzed by extradiol dioxygenases). After several subsequent steps, the linearized products are incorporated into the Krebs cycle. Anaerobic peripheral pathways usually converge to benzoyl-CoA, which is dearomatized by specific multicomponent reductases and consumes ATP (Díaz, 2004).

The initial hydroxylation step is considered to be rate limiting and the enzymes involved generally determine the substrate range of microorganisms, although other factors (e.g. substrate specificity of transcriptional regulators and membrane transporters) may also contribute (Watanabe, 2002). In a recent study of biodegradation by indigenous microbial communities in sub-antarctic soils, PAHs with greater than three rings were generally not degraded (Delille *et al.*, 2004). There is little evidence that microbial growth can be sustained with PAHs with four or more rings as a sole substrate (although they may be degraded by syntrophic cometabolism) and there is very limited information regarding bacterial degradation of PAHs with five or more rings (Reilley *et al.*, 1996; Kanaly & Harayama, 2000). However, bacterial degradation of pyrene (a pericondensed four-ring PAH) has been reported from sediment near a hydrocarbon source, and a *Mycobacterium* species isolated from that sediment has been shown to degrade pyrene using an inducible enzyme system (Kanaly & Harayama, 2000).

The metabolic flexibility of bacteria is related to their genetic adaptability. For example, Siciliano *et al.* (2003) found that a substantial decrease of aged PHCs in soil was related to a greater presence of catabolic genes (i.e. *alkB*, alkane monooxygenase; *ndoB*, naphthalenedioxygenase; *xylE*, catechol-2,3-dioxygenase) in bulk and rhizosphere soil. However, it was unclear whether the number of organisms containing these genes increased, or if the number of genetic elements present in the community increased. The genes responsible for aromatic biodegradation pathways are usually arranged in clusters (operons) in mobile genetic elements (e.g. plasmids or transposons). Gene clusters contain catabolic genes (encode enzymes for catabolic pathways), transport genes (responsible for active uptake of the compound) and regulatory genes (adjust expression of the catabolic and transport genes to the presence of the compound to be degraded) (Díaz, 2004). For example, the catabolic genes of the *ortho* and *meta* pathways

are organized as operons with flanking transposon elements on the TOL (toluene) plasmid (Sarand *et al.*, 1998). This facilitates horizontal transfer of the respective genes and rapid adaptation of microorganisms to the presence of new substrates (Díaz, 2004). Conjugation (transfer of genetic material from one microorganism to another) appears to be important in the dissemination of catabolic genes in the indigenous environment (Sarand *et al.*, 2000; Siciliano *et al.*, 2003).

Depending on chemical structure, contaminant concentration and environmental conditions, the onset of PHC biodegradation generally follows a period of acclimation in which no chemical degradation is evident (Alexander, 1999). Adaptation most commonly occurs by induction of the enzymes necessary for biodegradation, followed by increases in populations of biodegrading organisms (Miller & Herman, 1997). Chronic exposure to PHC substrates (e.g. near natural seepages or in areas where frequent spills occur) results in shorter acclimation periods (due to maintenance of biodegradation pathways within adapted communities) and subsequently increased transformation rates (Miller & Herman, 1997; Alexander, 1999). This pollution-induced community tolerance appears to increase proportionally with increased exposures (Seghers *et al.*, 2003). The end of this period is marked with a rise in respiration and increase in density (varying from slight to several orders of magnitude) that reflects growth of hydrocarbon-degrading populations as well as increased growth of organisms such as protozoa that graze microflora or decompose necrotic tissue (McGill *et al.*, 1981). Subsequent declines in microbial respiration may occur due to complete degradation of labile fractions or to limiting availability of N and P (McGill *et al.*, 1981). High abundance, rapid growth and the ability to transfer genes horizontally allow for rapid microbial adaptation to changes in environmental conditions (Romantschuk *et al.*, 2000; Díaz, 2004).

Genetic changes such as mutations (i.e. appearance of new genotypes) may occur when communities are faced with chemicals that do not have natural chemical analogues (Miller & Herman, 1997). Such events occur at low frequency; however, if new genotypes possess physiological characteristics that provide a selective advantage (e.g. new metabolic capacities), they may multiply (*via* horizontal gene transfer) within the surviving community (Alexander, 1999). The length of time required for a genetic change or for selection and development of an adapted community is not yet predictable (Miller & Herman, 1997). However, given enough time and favourable environmental conditions, the capacity to degrade almost any organic compound is likely to evolve in or immigrate to a contaminated site (Romantschuk *et al.*, 2000).

(b) Fungal cytochrome P450 and ligninolytic systems

Many fungi (e.g. *Aspergillus*, *Penicillium*, *Fusarium*) isolated from PHC-contaminated soils and cultured on PHC-containing medium have been found to use crude oil as a sole C and energy source (Chaillan *et al.*, 2004). In general, eukaryotic organisms oxidize aromatic compounds

to water-soluble products *via* a cytochrome P450 mono-oxygenase reaction, incorporating one atom of molecular O₂ into the aromatic ring to form a transient arene oxide and reducing the second atom of O₂ to H₂O. The arene oxide is immediately hydrated by an epoxide hydrolase to yield a trans-dihydrodiol or, alternatively, is non-enzymatically isomerized to form phenols that can conjugate with sulphate, glucuronic acid or glutathione (Miller & Herman, 1997; Cerniglia & Sutherland, 2001). These reactions increase both the water solubility and bioavailability of chemical substrates; soil conditions that favour fungal activity may initially increase the toxicity of the parent chemicals (Reilley *et al.*, 1996). Whereas complete mineralization results in innocuous endproducts (CO₂ and H₂O), partial biodegradation can produce intermediate metabolites with unchanged, reduced or increased chemical toxicity. Toxic chemical intermediates with increased water solubility are of particular concern as this can result in the transport and spread of contaminants through the environment (Miller & Herman, 1997).

The ligninolytic enzyme system of white rot fungi (WRF) has been extensively studied due to structural analogies between lignin and PAHs as metabolic substrates (Scheel *et al.*, 2000). Although lignin is a much larger and more heterogeneous polymer than the fused benzene ring structures of PAHs, it is also hydrophobic and insoluble, thereby posing similar problems for enzyme catalysis (Harvey & Thurston, 2001). WRF degrade lignin using a complex nonspecific enzyme system, often while simultaneously obtaining C from cellulose and hemicellulose (*i.e.* cometabolism) (Scheel *et al.*, 2000; Steffen, 2003). As with the cytochrome P450 system, the oxidizing enzymes of the ligninolytic system increase the bioavailability, solubility and redox status of the chemical substrates for subsequent metabolism (Harvey & Thurston, 2001). Different fungi appear to possess different combinations of oxidizing enzymes (Harvey & Thurston, 2001).

The initial hydroxylation step of the pathway is accomplished with small, diffusible oxidizing agents (highly reactive radicals) generated by three groups of extracellular enzymes: lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases (Collins & Dobson, 1997; Harvey & Thurston, 2001). LiP (EC 1.11.1.14) and MnP (EC 1.11.1.13) are heme-containing enzymes that function at low pH and catalyze the oxidation of lignin, humic substances and many organopollutants (Schlosser & Höfer, 2002). Both enzymes require H₂O₂, which is generated through fungal glucose oxidase, glyoxal oxidase and arylalcohol oxidase reactions (LiP and MnP) or oxidation of organic acids (MnP only) (Evans & Hedger, 2001). In the white rot basidiomycete *Phanerochaete chrysosporium*, PAHs with ionization potentials at or below about 7.55eV are substrates for direct one-electron oxidation by LiP, whereas those with ionization potentials above this threshold appear to be acted upon by radical species formed during MnP-dependent lipid peroxidation reactions (Bogan, Schoenike & Lamar, 1996). For LiP, radical cations are produced from one-electron oxidations of non-phenolic compounds, which act as non-specific redox mediators and extend the substrate range and redox capacity of LiP (Harvey & Thurston,

2001). During the catalytic cycle of MnP, the active centre is oxidized by H₂O₂. Reduction of the resting enzyme is achieved by two successive one-electron transfers that oxidize Mn²⁺ to Mn³⁺, which is facilitated by fungal organic acids (*e.g.* oxalate or malonate) upon chelation of the highly reactive Mn³⁺ state. Schlosser & Höfer (2002) found evidence supporting a physiological role of laccase-catalyzed Mn²⁺ oxidation in providing H₂O₂ for extracellular oxidation reactions and demonstrated a novel type of laccase-MnP cooperation relevant to biodegradation of lignin and organic pollutants.

Laccases (benzodiol:oxygen oxidoreductase, EC 1.10.3.2) are (blue) multicopper enzymes (glycosylated polyphenoloxidases) that are an essential component of a complex nonspecific enzyme system secreted by different kinds of fungi that have been shown to oxidize lignin and various organic contaminants (Schlosser & Höfer, 2002; González *et al.*, 2003; Hoegger *et al.*, 2004). In addition to lignin depolymerization and polyphenol degradation, laccases are thought to be involved in the release of N from insoluble protein-tannin complexes, mycelial pigmentation, humus formation, fruiting body formation and detoxification of phenolic compounds, which protects fungi against soil pollutants and host defense compounds (Kanunfre & Zancan, 1998; Burke & Cairney, 2002; Hoegger *et al.*, 2004). Most reports refer to laccase activity as extracellular, but some WRF may also have intracellular laccases (Burke & Cairney, 2002). Laccases catalyze the reduction of O₂ to H₂O (4 electron reduction without formation of free reduced oxygen species) using a range of phenolic compounds as hydrogen donors (Burke & Cairney, 2002; Schlosser & Höfer, 2002). An electron is removed from the phenolic hydroxyl groups of lignin to form free phenoxy radicals, which are further oxidized to quinines (Hoegger *et al.*, 2004).

(c) Metabolic potential of ECM/ERM fungi

As some ECM and ERM fungi are closely related to WRF, some researchers have suggested that they may have retained some ability to degrade organic substrates, including PHCs (Hibbett *et al.*, 2000; Meharg & Cairney, 2000; Meharg, 2001). Braun-Lüllemann, Huttermann & Majcherczyk (1999) reported on the ability of 16 species (27 strains) of ECM fungi that were isolated from middle European forests to degrade PAHs (1500 ppm of phenanthrene, pyrene, chrysene and benzo[*a*]pyrene) in pure, liquid culture. The slow but efficient metabolism of benzo[*a*]pyrene by ECM fungi was comparable to results of experiments with WRF (Braun-Lüllemann *et al.*, 1999). A study in which 58 fungal isolates from different physiological groups were exposed to a range of PAHs showed that all fungal groups could degrade PAHs, but that ECM fungi were 19% as efficient as WRF (Gramss *et al.*, 1999). Similarly, Meharg & Cairney (2000) tested 42 ECM fungal species with several types of persistent organic pollutants and found that 33 species were able to degrade one or more classes of chemicals. Only one of 21 ECM fungal species could not degrade at least one PAH; degrading species seemed to prefer chemicals with four to five rings. In each

case, the direct oxidative activities correlated with production of extracellular enzymes that appeared to metabolize aromatic rings (Braun-Lüllemann *et al.*, 1999; Gramss *et al.*, 1999). Green *et al.* (1999) reported that the ECM fungus *Tylospora fibrillosa* degraded 4-fluorobiphenyl to significant extents *via* sequential hydroxylation reactions.

A variety of ECM and ERM fungi are known to produce polyphenol oxidases (e.g. laccase, catechol oxidase and tyrosinase) in culture conditions, but there is little evidence for production of extracellular peroxidases (e.g. LiP and MnP) (Cairney & Burke, 1998; Burke & Cairney, 2002). Timonen & Sen (1998) assayed macerated fungal mycelia from several regions of a microcosm system and found that levels of enzyme activity in the environment were lower for ECM fungi than for saprotrophic fungi, possibly due to avoidance of competition or preferential exploitation of substances of a particular quality. Gramss *et al.* (1998) reported that most cultured ECM fungi (from sporocarps collected from previously contaminated forest plots and industrial sites) exhibited oxidase activity: high extracellular enzyme activities were found for *Lactarius* and *Russula* species and high intracellular enzyme activities were found for *Suillus*, *Hebeloma*, *Leccinum* and *Tricholoma* species. Donnelly & Entry (1999) found extracellular enzyme activity at the advancing hyphal front of ECM fungi and suggested the possibility of a lack of complete dependence for C on the plant partners.

Few studies have considered mycorrhizal fungi in symbiosis with a plant for the degradation of organic pollutants (Koivula *et al.*, 2004). Meharg, Cairney & Maguire (1997) found that the mineralization rate of ^{14}C -labelled 2,4-dichlorophenol by ECM fungi (*Suillus* and *Paxillus* species) in symbiotic culture with *P. sylvestris* seedlings was increased by 50% and 250% compared to respective rates of those ECM fungi in axenic culture. As mineralization was extremely slow in vermiculite (i.e. no bacteria present), these data were interpreted to suggest that fungal patch differentiation led to greater enrichment and stability of bacterial communities at the fungal-soil interface. It is unknown how the C contributions of the phytobiont influenced fungal responses or how synergistic or antagonistic interactions between mycorrhizas and other microorganisms altered their ability to mineralize or degrade organic pollutants.

(d) Genetic controls

Ligninolytic enzymes are typically produced by WRF as multiple isoenzymes. This biochemical diversity had been attributed to post-transcriptional modifications of a single gene product, but characterization of several laccase gene families suggests that at least part of this diversity could be due to the multiplicity of laccase genes in fungal genomes (González *et al.*, 2003). For example, Hoegger *et al.* (2004) found that *Coprinopsis cinerea* has at least eight different laccase genes within the haploid genome, which is the largest laccase gene family reported so far from a single haploid fungus. Bogan *et al.* (1996) found that the genome of *Phanerochaete chrysosporium* contains at least 10 structurally related genes (*lipA* through *lipJ*) encoding LiP proteins and at least three MnP genes (*mnp1*, *mnp2*, *mnp3*).

Screening genomes for genes that encode laccases and peroxidases may represent a reliable means of identifying potential enzymatic activities in ECM and ERM fungi (Burke & Cairney, 2002). As DNA sequences for laccase and peroxidase genes are now available for several saprotrophic fungi, opportunities exist to design molecular probes or primers for identification of similar genes and/ or mRNA transcripts in mycorrhizal fungi (D'Souza, Boominathan & Reddy, 1996; Burke & Cairney, 2002). For example, Chen *et al.* (2003) used laccase gene primers to screen ECM basidiomycetes for laccase-like genes, which were amplified from *Lactarius*, *Russula*, *Piloderma* and *Tylospora* species. Timonen & Sen (1998) examined gene expression in identified functional components of *P. sylvestris* mycorrhizal systems and found expression of isozymes (i.e. polyphenol oxidase and acid phosphatase) was increased in hyphal fronts of *Paxillus involutus* and *Suillus bovinus* ECM systems as they advanced in the humus. Through gene amplification and sequencing, Chambers *et al.* (1999) reported evidence for MnP genes and peroxidase activity in cultured *Tylospora fibrillosa*. Chen *et al.* (2001) extracted DNA from dried basidiomes or fungal cultures and amplified and sequenced genes for LiP and MnP using primers based on *Phanerochaete chrysosporium* genes. Although they reported the presence of LiP genes in a broad range of ECM fungal taxa and MnP genes in some ECM fungal taxa (three Atheliaceae taxa), Cairney, Taylor & Burke (2003) recently attempted to repeat these experiments and reported a lack of evidence to support the presence of peroxidase genes in ECM fungi.

Extracellular laccase is constitutively produced in small amounts by several fungi, but enzyme expression is considerably enhanced by a wide range of substances, including a variety of different aromatic compounds (Burke & Cairney, 2002; González *et al.*, 2003). Regulation of laccase production appears to be complex and vary between taxa. For example, in the WRF *Pycnoporus cinnabarinus*, laccase activity is increased with an increase in C:N ratio whereas in *Phanerochaete chrysosporium*, laccase activity is repressed by glucose regardless of N content and increased in the presence of cellulose when N is also increased (Burke & Cairney, 2002). From assays of liquid cultures of the ECM fungus *Thelephora terrestris*, Kanunfre & Zancan (1998) found increased secretion of extracellular laccase with a decreased C:N ratio. At the molecular level, Collins & Dobson (1997) demonstrated that laccase gene (*lc*) transcription was activated by copper and nutrient N and that induction occurred at the level of gene transcription in the presence of two aromatic compounds. Chen *et al.* (2003) reported that some laccase-like genes amplified from *Lactarius*, *Russula*, *Piloderma*, and *Tylospora* species appeared to be regulated at the transcriptional level, with transcription enhanced by higher N content.

V. IMPLICATIONS FOR MANAGEMENT

Two broad objectives dominate management goals for contaminated forest sites: (1) reduction of risk; and, (2) long-term forest sustainability. In Canada, as in many jurisdictions,

contaminated site remediation is required if there is risk to human or environmental health; this is not well defined for forest ecosystems and has led to situations of either over- or under-management. Here we wish to reflect on both these objectives and show how they converge.

Risk arises from the conjunction of three conditions: a contaminant (toxicant), a pathway and a receptor. Mycorrhizal ecosystems in contaminated sites may attend to the toxicant by metabolizing and removing or immobilizing it, or by transforming and altering its mobility and toxicity. Moreover, mycorrhizal ecosystems may also be among the critical receptors. Consequently, the concept of risk entails both remediation and ecotoxicology.

Bioremediation can be defined as the use of organisms to detoxify contaminants through immobilization, chemical transformation and mineralization processes (Díaz, 2004). Bioremediation efficacy is influenced by a variety of substrate and soil conditions, including: PHC composition; soil temperature, texture and structure; length of time the PHCs have been in the soil; and associated bioavailability, together with associated toxicants (Pollard, Hrudey & Fedorak, 1994). Use of microorganisms as management tools requires knowledge of which organisms (or functional guilds of organisms) are likely to be present in a particular ecosystem, how they respond to different types of physical and chemical disturbances, and methods for ascertaining whether organisms are actually healthy and not just surviving (Blakely *et al.*, 2002). From previous sections of this review, we can ask: (1) does the genetic potential exist (or is it likely to exist) to metabolize the array of substrates expected in the PHCs at a given site; (2) if so, what environmental conditions are likely to foster its expression and can these conditions be achieved; (3) is there need for added genetic potential through genetic engineering technologies; (4) if so, what constraints might limit its expression and what precautions might be needed; (5) is bioaugmentation needed to increase genetic potential; and, (6) if so, what precautions might be necessary, and how might its potential be best exploited? Although much remains unknown, considerable insights have been gained from recent and ongoing research regarding these questions.

Observations that virtually all organic substrates appear to be transformed by soil microorganisms if they are accessible (e.g. Simard *et al.*, 1997; Read & Perez-Moreno, 2003; Díaz, 2004; Heinonsalo *et al.*, 2004), combined with the continually increasing diversity of soil microbial communities revealed by molecular techniques (e.g. Axelrood *et al.*, 2002b; Berch *et al.*, 2002), the observation that catabolism of PHCs and plant residues share many common elements (Section IV.2 above), and the absence of reports of soils that lack the ability to metabolize PHCs, all point to the ubiquitous genetic potential by soil communities to transform and perhaps completely catabolise PHCs. Consequently, bioremediation appears to be a sensible and potentially feasible intervention and has been extensively used. The focus of bioremediation strategies tends to be on contaminant disappearance, but is based on a limited understanding of links between degradation and the basic nutritional needs of the responsible soil microbial community (Mills *et al.*, 2003). In addition, the optimum

environmental conditions to sustain communities that degrade PHCs are understood only in broad terms. Continued progress may be expected by careful attention to, and documentation of, the connection between the environmental conditions imposed on a site by PHC contamination, as well as catabolic response and environmental preference by persistent communities. In essence, PHCs impose their own environment, including varying concentrations of toxicants. Consequently, PHCs control the community or guild that survives, which in turn dictates the optimum conditions for its functioning. Based on first principles, and observations on a wide range of chemo-organotrophic microorganisms, it is reasonable to expect that a slightly acidic pH, well oxygenated and nutrient sufficient environment would favour functional guilds that would metabolize PHCs. Attaining such conditions, however, can be challenging in forest ecosystems without disrupting them.

Is there a need to use recombinant organisms? In the early development of bioremediation technology, investigators recognized the scope of environmental pollution and the diversity of chemical pollutants, and invested significant effort into metabolic engineering to manipulate specific catabolic pathways or particular host cells (Alexander, 1999). Metabolic engineering has created recombinant organisms with novel hybrid pathways of biodegradation and increased substrate ranges; it has completed incomplete pathways, created multiple pathways, and provided mechanisms that enhance chemical bioavailability (Díaz, 2004). Because of issues (e.g. biosafety or inability to compete for resources) associated with introducing recombinant bacteria to contaminated ecosystems, bioremediation (using recombinant bacteria) is often conducted *ex situ*, under relatively controlled conditions (Díaz, 2004). *Ex situ* bioremediation (with or without recombinant bacteria) is also used in situations where a high degree of control of environmental conditions is wanted (Riser-Roberts, 1998) and where added energy inputs such as in rotating bioreactors are desired. Recombinant organisms face public resistance due to the fear of their escape from the site, or transfer of genetic material to indigenous populations. Further, they may not always compete well with indigenous populations, or may require specialized environments. Consequently, they may be of limited potential for use on a large scale in forested ecosystems.

There are fewer biosafety issues associated with bioaugmentation, the introduction of exotic microorganisms isolated from unrelated sites, but adapted to contaminant biodegradation (Ward, Singh & Van Hamme, 2003) or to extreme soil conditions (Cunningham *et al.*, 2004; Stallwood *et al.*, 2005). Such additions can be made in a variety of ways, including industrial production and subsequent slurry applications. Soil samples from adjacent contaminated and remediated sites used as an inoculum would seem to be reasonable candidates as well. Although it can be readily used in the field, bioaugmentation lends itself better to highly engineered systems (e.g. slurry bio-reactors), to recalcitrant or novel contaminants for which the indigenous population may be ill-equipped, or for extreme environments (e.g. Cunningham *et al.*, 2004; Stallwood *et al.*, 2005).

Cost is also a factor from a management perspective. Leavitt & Brown (1994) reported on three case studies comparing bioaugmentation using a commercial supplement with stimulation of indigenous soil organisms for removal of PHCs in a bioreactor and a land-treatment facility, and acetone or *bis*-2-chloroethyl ether in a waste-water facility. Based on cost and efficacy, they concluded that bioaugmentation was not warranted in their situations and that biostimulation of indigenous organisms was the best choice. *In situ* bioremediation through enhancing contaminant biodegradation by indigenous soil populations and communities (i.e. syntrophic bioremediation) is considered less destructive and more cost-effective for remediating contaminated soils on large scales (Doelman & Breedveld, 1999; Delille *et al.*, 2004) and has proven successful when properly implemented (Nelson, Hicks & Andrews, 1994). *In situ* bioremediation is more likely to maintain the desired integrity of below ground mycelial networks (Section III.2b).

Phytoremediation refers to all plant-induced biological, chemical and physical processes that aid in the remediation of contaminants (Cunningham *et al.*, 1996). Traditionally, research in this area has focused on use of agricultural plant species for the remediation of agricultural or industrial soils. Reilley *et al.* (1996) found that the presence of vegetation significantly enhanced the dissipation (and likely biodegradation) of anthracene and pyrene in the soil environment. Others have reported that various grasses, legumes and woody plants facilitate the degradation of PHCs in soil (Aprill & Sims, 1990; Chaîneau, Morel & Oudot, 2000; Liste & Alexander, 2000; Palmroth, Pichel & Puhakka, 2002; Merkl, Schultae-Kraft & Infante, 2005). Plants and associated mycorrhizospheres may increase the activity of PHC-degrading organisms, either *via* general enhancement [i.e. (mycor)rhizosphere effect] or due to proliferation of specific microbial groups (i.e. altered functional component of the microbial community) (Siciliano *et al.*, 2003). They may also mediate desorption of contaminants bound to soil constituents by altering pH and redox potential, as well as concentration and types of organic compounds in the (mycor)rhizosphere. Research has shown that at sites containing plants and expected mycorrhizal associations experience more rapid reduction in toxicity of PHCs (Parrish, Banks & Schwab, 2005).

An increased awareness of the abundance and diversity of mycorrhizal systems in vegetated soils has led to their consideration for *in situ* bioremediation (Meharg, 2001). Where there is little risk to human or ecological health, the purposeful planting of trees inoculated with specific mycorrhizal fungi is expected to establish these mycelial systems in soil and allow gradual decontamination over a period of several years (Braun-Lülleman *et al.*, 1999; Meharg & Cairney, 2000). A related approach is to transplant plugs or sprigs of vegetation from non-contaminated soil, as is done in various restoration ecology projects (e.g. Fraser & Kindscher, 2005), into contaminated areas for the final stages of clean up. This approach allows for simultaneous remediation and revegetation of sites without further disruption to physical and chemical properties of the soil and provides an inoculum of a soil community adapted to the site. Although it may require several months

or years for tree root systems and associated mycorrhizal biomass to establish, mycelial systems would be expected to remain in a vital state for several decades, whereas other organisms (e.g. WRF) may complete their life cycles in a few days or weeks and then rest as spores (Gramss *et al.*, 1999). A more thorough knowledge of which fungal symbionts are likely to survive and compete in various ecosystems, as well as which fungi contribute directly (exhibit biodegradative capabilities) or indirectly (provide suitable habitat for other microorganisms that exhibit biodegradative capabilities) to bioremediation of contaminated sites is required as part of management strategies that adopt this approach. Other phenomena, such as fungal specificity to plant hosts, may also require consideration (Molina *et al.*, 1992). The spectrum of possible plant hosts that can be selected by a particular mycorrhizal fungus can vary from a few to many. At the same time, the host receptivity (the number of different fungi accepted by a particular plant) can also differ. Both may impact the ecological contribution made by the symbiosis. For example, alder, compared to Douglas-fir, is very selective, typically initiating symbioses with a very restricted number of fungal species. Some fungi may form symbioses with one plant genus, whereas other fungi are less selective, initiating symbioses with potentially hundreds of plant hosts. How this selective nature between fungi and plants impacts ecological functions in general, and bioremediation in particular, remains unknown.

Intrinsic bioremediation (contaminant biodegradation by adapted indigenous communities) may be an acceptable management strategy where risks to human or ecological health are low (Alexander, 1999). Nicolotti & Egli (1998) showed that some ECM fungi surviving in contaminated forest soil may metabolize chemicals in crude oil and suggested that crude oil spills in mixed agricultural and forest areas do not cause long-term environmental damage of the kind associated with coastal ecosystems, possibly due to intrinsic bioremediation by the soil community. Intrinsic bioremediation of PHC-polluted soils differs substantially between ecosystems and depends on the particular combination of soil-forming factors, soil properties, microbial communities and the content and composition of PHCs and their products (Trofimov & Rozanova, 2003). Studies of indigenous microbiota along with key physical and chemical parameters provide hope for the eventual ability to predict how natural attenuation will proceed at contaminated sites. Intrinsic bioremediation may be an appropriate management strategy in boreal forest ecosystems in some situations.

If external action to improve the bioremediation of a soil seems essential, environmental and ecological knowledge is required to choose the least destructive methods (Romantschuk *et al.*, 2000). Ideally, bioremediation strategies should be based on knowledge of the microorganisms present in polluted environments, their metabolic abilities and how they respond to changes in environmental conditions in an ecological context (Blakely *et al.*, 2002; Díaz, 2004). The current state of knowledge, however, does not permit predictions or management strategies to be built up from the species level. Studying the physiology, biochemistry and genetics of soil microorganisms is important for contaminated site bioremediation, as well as for biomonitoring

the impacts of chemicals as disturbance agents. Unfortunately, this is mostly unknown and current management is largely based on empirical rather than theory-based deductions. As demonstrated by the research described in this review, factors that alter the survival or activity of soil biota are important considerations for ecosystem management (Setälä *et al.*, 2000). However, the key question in terms of sustainability is how contamination events impact ecosystem functions in the near and long-term future.

Ecosystem health is not well defined, but has been described in terms of vigour (productivity), organization (diversity and mutual dependence) and resilience (maintenance of structures and patterns in the presence of environmental change) and interpreted by correlating biological indicators for processes that are considered critical for ecosystem function (Blakely *et al.*, 2002; Lu & Li, 2003). Traditional indicators for contaminated sites include impacts of chemical contaminants on plant condition and biomass, with more recent interest in specific organisms capable of biodegradation. Fewer studies have examined changes in soil properties that define its fertility and agro(eco)logical properties (Suleimanov *et al.*, 2005). It has been suggested that criteria for remediation of PHC-contaminated soils should be amended to include suppression or significant modification of the plant community, reduction in plant biomass, disturbance in functioning of soil biota, simplification of the soil community, decreases in the biological activity of the soil, and movement of PHCs into surface water or ground water (Trofimov & Rozanova, 2003). Soil quality guidelines in Canada have been developed using most of these criteria for agricultural, residential/ urban parkland, commercial and industrial lands, but not for forest ecosystems (Ouellet, Crommentuijn & Gaudet, 2002).

Many biological and chemical-physical approaches have been proposed to predict or measure the bioavailability of organic compounds for biodegradation or to ecological receptors (i.e. toxicity). Biological measures of bioavailable toxicants include seedling emergence and growth tests, along with various soil invertebrate tests of acute toxicity, chronic toxicity, behaviour and reproduction (Stephenson *et al.*, 2002). Römbke *et al.* (2006) recently identified potential invertebrate species and testing methodologies for assessing ecotoxicity of contaminants in boreal forest soils. Other biological measures include contaminant uptake and impact on organism biomolecules, and impacts on organism-mediated processes (e.g. nitrification) (Svendsen, Paton & Weeks, 2002). Chemical-physical approaches include: kinetics of PHC desorption, mild solvent extraction, solid phase extraction (SPE), supercritical fluid extraction and cyclodextrin extraction. SPE correlates well with biological measures of ecotoxicity (and bioavailability) and does not have the potential to disrupt the structure of soil organic matter phases as do chemical methods (Ehlers & Loibner, 2006).

VI. CONCLUSIONS

(1) The importance of developing multi-disciplinary approaches to solving problems relating to anthropogenic

pollution is now clearly appreciated by the scientific community, and this is especially evident in boreal ecosystems exposed to escalating threats to PHC contamination through expanded natural resource extraction activities. In this review, we have presented a mycorrhizal ecosystems perspective on PHC contamination in boreal forest soils in order to identify gaps in knowledge and to guide future research in both ecological and sustainable management contexts so that scientists, land and facilities managers, industrialists and government officials will be better prepared to manage the inevitable accidents that will occur.

(2) We know that the taxonomic, genetic and functional diversity of mycorrhizal ecosystems in boreal soils is immense and continues to expand with increased sampling effort. We also know that the functioning of these communities underpins survival and productivity of the ecosystem as a whole. It appears that redundancy in broad-scale biodegradative functions is essential for ecosystem recovery following PHC contamination, to account for the loss of community components that are unable to tolerate the altered physical and chemical conditions imposed by the PHCs. What remains to be determined are the details and the translation of this information into effective ecosystem management.

(3) The ubiquity and enormous biomass of extraradical mycelia of mycorrhizal fungi in forest soils implies a key role in forest ecosystem processes. Recent studies have highlighted the high taxonomic and genetic diversity of ECM or ERM fungal communities associated with certain plants in some ecosystems, though many potential hosts and types of ecosystems have not yet been surveyed.

(4) Although there is thought to be some relationship between high diversity (species richness) and ecosystem health due to some degree of redundancy, the functional basis of ECM and ERM fungal diversity is virtually unknown. The physiological mechanisms of nutrient exchange between fungal and plant partners are also not well understood, particularly with respect to nutrient acquisition from the soil environment and especially for PHC-contaminated soils. A more thorough knowledge of which fungal symbionts are likely to survive and compete in various ecosystems is required, as well as a better understanding of whether certain types of fungal associations with different plant hosts gain in ecological importance following disturbance events. Whereas community responses (e.g. shifts in community structure) to some types of disturbances (e.g. fire, forestry practices, etc.) have been described in the recent literature (although it is unknown if these are related to shifts in function), responses to PHC pollution are not well understood. In fact, very little is known regarding rhizosphere communities in forest soils subjected to PHC contamination.

(5) Studies of PHC contamination in forest soils are rare, as are the impacts on soil organisms and the intrinsic decomposition in these systems. The scientific basis for current remediation standards is based on information from experiments examining the toxicological impacts of PHC chemicals on test organisms. However, sequestration of organic pollutants in forest SOM may decrease the chemical toxicity of chemicals through physical separation from biological receptors, which also decreases substrate

bioavailability for enzymatic degradation. More research in this area is needed. Also necessary are improved methods for assessing the fate and behaviour of PHCs in forest soils, including determinations of bioavailability and development of a wider variety of indicators for ecological integrity than the traditional measures of plant productivity. Future research is needed to determine how toxicity varies with type of pollutant, mixtures of pollutants, extent of pollution, and the general condition of the ecosystem prior to chemical disturbance.

(6) Few studies have examined whether the coexistence of ECM and ERM plants in boreal forests provides opportunities for sharing ECM and ERM fungi that link plants and translocate nutrients, and virtually nothing is known of how PHC contamination may interfere with processes of nutrient acquisition and exchange.

(7) Recent studies have shown that some ECM and ERM fungi appear to play a direct role (*via* enzymatic catabolism) in biodegradation of complex organic substrates (including PHCs). However, few studies have examined various fungi in detail, or have examined mycorrhizal fungi in symbiosis with a plant. It is unknown as to how PHC contamination might interfere with fungal metabolic processes.

(8) Incomplete biodegradation can produce potentially toxic intermediate metabolites; toxic intermediates with increased water solubility are of particular concern as this can result in the transport and spread of contaminants through the environment. More research is required in this area.

(9) Few studies have considered the indirect role of ECM and ERM systems in biodegradation through their interactions with the mycorrhizosphere-associated bacterial communities and little is known regarding syntrophic biodegradation by different functional guilds of organisms. Most fungi have been examined in isolation from an ecosystem context, thereby excluding interactions of individual ECMs and ERMs with each other, their soil environment and other members of the plant and microbial communities. Thus, information gained from these studies may have little ecological relevance for understanding how forest ecosystems function or for informing bioremediation management strategies for contaminated soils.

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