forests

MDPI

Article

Distance from the Forest Edge Influences Soil Fungal Communities Colonizing a Reclaimed Soil Borrow Site in Boreal Mixedwood Forest

Tod Ramsfield [1,*], Philip-Edouard Shay [1,2], Tony Trofymow [2,3], Colin Myrholm [1], Bradley Tomm [1], Patrick Gagné [4] and Jean Bérubé [4]

1 Northern Forestry Centre, Canadian Forest Service, Natural Resources Canada, 5320-122nd Street NW, Edmonton, AB T6H 3S5, Canada
2 Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, 506 West Burnside Road, Victoria, BC V8Z 1M5, Canada
3 Department of Biology, University of Victoria, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada
4 Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, PO Box 10380 Stn Saint-Foy, Québec, QC G1V 4C7, Canada
* Correspondence: tod.ramsfield@canada.ca; Tel.: 825-510-1303

Received: 28 February 2020; Accepted: 2 April 2020; Published: 9 April 2020

Abstract: Soil fungi are important components of boreal forest ecosystems; for example, saprotrophic fungi regulate nutrient cycling, and mycorrhizal species facilitate nutrient uptake by plants. This study aimed to assess soil fungal communities in a reclaimed area and an adjacent natural mixedwood forest and to identify the distribution of taxa available for seedling colonization. Soil fungal microbiomes were assessed along three transects (from 10 m inside the interior of the undisturbed forest to 40 m inside the reclaimed area) and in the roots of small aspen within the natural forest. Using high-throughput deoxyribonucleic acid (DNA) sequencing of internal transcribed spacer amplicons, a total of 2796 unique fungal taxa were detected across fine roots, forest floor, and mineral soils collected along the transects, whereas 166 taxa were detected in the aspen roots from the natural forest. Within the interior of the forest, ectomycorrhizal fungi were more common, whereas in the reclaimed areas, arbuscular mycorrhizae and saprophytes were more common. This survey showed that natural areas of adjacent undisturbed forest can act as a source of ectomycorrhizal fungi for dispersal into reclaimed areas. Notably, soil fungal taxa colonizing the root systems of small aspen included species that are specifically associated with soils from the undisturbed forest (primarily ectomycorrhizae) or the reclaimed clearing (saprotrophs and plant pathogens).

Keywords: mycorrhizae; Illumina; land reclamation

1. Introduction

Complex relationships exist between soil fungi and plants, including symbiotic mycorrhizal relationships in which fungi colonize roots, leading to benefits for both organisms. Colonization of root systems by mycorrhizal fungi can improve plant growth and survival by increasing the availability of nutrients to plants in exchange for carbon compounds and protection from pathogenic fungi [1]. Several types of mycorrhizal relationships are known to exist, classified according to the structure of the interface between the host plant and the fungus [2]. The most common mycorrhizal association with woody plants takes the form of ectomycorrhizae, in which the fine roots are covered by a mantle of fungal hyphae [2]. The assemblage of ectomycorrhizal fungi is diverse and changes over time as trees age and stands mature [3,4]. Mycorrhizal fungi naturally disperse by aerial discharge of spores produced in fruiting structures [5,6], dispersal by fungivorus organisms [7,8], or belowground

through contact with existing mycorrhizal networks [9], or contact with fungal structures such as chlamydospores and sclerotia that are present in the soil propagule bank [6].

In Alberta, proven reserves of 165.4 billion barrels of oil are bound within oil sands deposits that lie under approximately 142,200 km^2 of the province; the area overlying the surface mineable oil sands that are up to 75 m deep is approximately 4,800 km^2 [10], while deeper deposits are accessed using in-situ methods. Access to surface mineable deposits is achieved by removing overburden material to expose the bitumen-bearing material, removing it and processing the oil from the material, replacing overburden material, and reclaiming the site [11]. To access deeper deposits, in-situ extraction uses steam injection to liquefy the bitumen belowground, allowing it to be brought to the surface for further refining [12]. Accessing bitumen deposits by both open-pit and in-situ methods has resulted in a highly disturbed and fragmented landscape due to open pit mines, and infrastructure, such as well pads, that must be built for in-situ operations. By provincial law, these disturbed sites must be reclaimed upon mine closure for restoration of ecosystem services [13]. In Alberta, reclamation following resource extraction requires that soil be placed and vegetation established, by planting and natural colonization [14], such that the site is on a trajectory for return to equivalent land capability, a condition in which the post-disturbance landscape can support pre-disturbance activities [15]. As such, land reclamation represents an area of intensive research, with studies focused on soil placement e.g., [16–18] and plant community establishment e.g., [14,19,20], including the potential for ectomycorrhizal fungi to promote seedling establishment e.g., [21–23]. In reclaimed areas where soils have been replaced after a period of stockpiling, the soil microbial community, including ectomycorrhizal fungi, is degraded relative to that in adjacent undisturbed areas [21,24]. Over time, the ectomycorrhizal community will likely recover, through long-distance dispersal from remnant undisturbed areas as has been observed in forestry harvest blocks [25]. A study of ~30yr old reclaimed spruce stands at Gateway Hill, a certified reclaimed area near Fort McMurray, Alberta, demonstrated that soil fungi, including ectomycorrhizal fungi, have become established in the reclaimed area, although the species richness was lower than an adjacent natural white spruce stand [26].

The current study investigated the rapid (approximately one year) natural dispersal of fungi from an undisturbed forest into an adjacent reclaimed area, and the hypothesis that undisturbed forests are a potential source of fungi that can disperse into a reclaimed area, enabling natural colonization of the reclaimed site by beneficial fungi. The study involved 1. determining the distribution of fungal taxa colonizing the roots of small trembling aspen (*Populus tremuloides* Michx.) present in the undisturbed forest interior and edge, and 2. assessing the composition and diversity of soil fungal communities in an undisturbed boreal mixedwood forest and an adjacent recently reclaimed area using high-throughput DNA sequencing.

2. Materials and Methods

2.1. Site Description, Transect Layout, and Sample Collection

The study site was located within the Cold Lake lease operated by the Imperial Oil Company where bitumen is extracted using in-situ methods, which require the construction of gravel well pads that must later be reclaimed, rather than large open-pit mines. This experimental site is located in the boreal plain ecoregion and was chosen as the site history and land reclamation techniques were typical of those used by industry and because it was adjacent to an undisturbed boreal mixedwood forest. After logging in 2008, topsoil at the D-East Borrow site (Universal Transverse Mercator (UTM) zone 12V, easting 538816, northing 6058237), was stripped and stockpiled in 2011, underlying gravel removed to use for construction on the mine lease in 2013, and topsoil reapplied and reclamation completed in 2015. Adjacent to the reclamation area were undisturbed natural mixed stands of aspen, white spruce (*Picea glauca* (Moench) Voss), and black spruce (*Picea mariana* (Mill.) BSP). Increment cores collected from a sample of mature trees indicated a maximum age of 157 years. Prior to harvest, the forest stand in the reclaimed area and adjacent forest was a 24 m tall moist white spruce (80%) and

aspen (20%) of ~1890 origin, with a 13 m tall wet black spruce stand (100%) of ~1900 origin in the NW corner of the reclaimed area and adjacent forest (Figure 1).

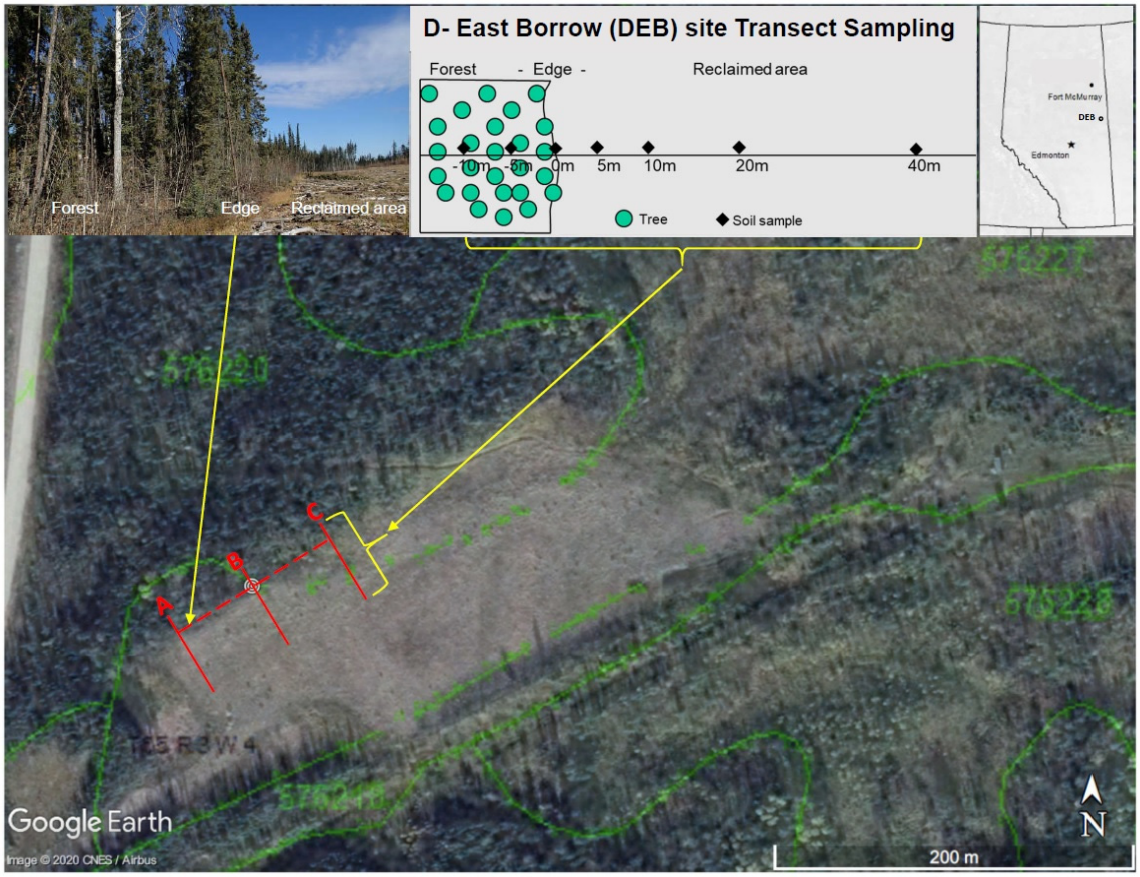

Figure 1. Satellite image in 2015 of the D- East Borrow (DEB) site (concentric circles symbol - UTM zone 12V, easting 538816, northing 6058237) in east central Alberta, Canada after completion of reclamation, with an overlay (green lines and numbers) showing forest cover types mapped in 1999 prior to the start of forest harvest and gravel mining. The three (**A**, **B**, **C**) 50 m transects (red lines) sampled in 2016, spanned the edge of the uncut forest and reclamation area, 10 m within the forest and 40 m in the reclaimed area. Soil was sampled at seven locations along each transect. Most of the DEB site had been within mixed white spruce and aspen forest (polygon 576227). However, the NW portion of the site (and part of transect C) had been within black-spruce-dominated forest (polygon 576220). See text for descriptions of the site history and forest types.

In October 2015, eleven small aspen (root collar diameter up to 2 cm and maximum height 2 m) were collected from the adjacent undisturbed forest, between the stand edge and up to 10 m inside the stand near the location where the transects would be installed in 2016. Aspen that were obvious suckers, as determined by examination of the root system, were not collected. Fungi were cultured, and DNA was extracted, from root tissue as outlined in Sections 2.2 and 2.3.

In September 2016, three parallel transects, 50 m apart and designated by letters A (western transect), B (middle transect), and C (eastern transect), were set up, spanning from 10 m inside the interior of the undisturbed forest, across the edge, to 40 m inside the reclaimed area (Figure 1). Seven sampling points were located along each transect. Two sampling points were located within the forest, at 10 m and 5 m from the edge (designated –10 m and –5 m, respectively); the third sampling point was located at the forest edge (designated 0 m); and the remaining four sampling points were located within the reclaimed area (at 5 m, 10 m, 20 m, and 40 m from the edge, designated accordingly) (Figure 1). Within the undisturbed forest, trees were counted in a 3.99 m radius plot; in addition,

at each sampling point, seedlings and ground cover were estimated within a 1 m radius plot to characterize the vegetation for that point. Forest floor (10 cm × 10 cm organic layer) and mineral soil (10 cm × 10 cm × 15 cm deep) samples were collected separately at each sampling point using a trowel, which was sterilized in bleach and rinsed with water between soil layers and sampling points to avoid cross-contamination. Organic and mineral soils were processed and DNA extracted as outlined in Section 2.4. The forest floor was very thin or not present in some areas (i.e., no forest floor samples at 5 m on transect A) and so deep in other areas that we did not reach the mineral layer (i.e., mineral soils not collected at –5 m, 0 m, and 5 m along transect C).

2.2. Culturing and DNA-Based Identification of Fungi from Aspen Roots

Fungi were cultured from fine roots by cutting 8 to 10 pieces of tissue from roots 2–5 mm in diameter. These root fragments were surface-sterilized in 30% hydrogen peroxide for 20 s, followed by two washes in sterile distilled water and then placed onto modified Melin–Norkrans (MMN) media amended with tetracycline (MMN-T) or tetracycline + benomyl (MMN-TB). Mycelial growth from root fragments was monitored, and any mycelium was sub-cultured onto fresh MMN media. All cultures were sorted on the basis of morphology, and representative samples of each morphotype were then identified from Sanger sequences of internal transcribed spacer (ITS) amplicons (ITS1-5.8S-ITS2) generated using polymerase chain reaction (PCR) primers ITS-1F [27] and ITS-4 [28] from DNA that was extracted using the Quiagen DNeasy plant mini kit (Quiagen, Germantown, MD, USA). The PCR products were purified using the QIAquick PCR purification kit (Quiagen, Germantown, MD, USA). Putative identifications were based on Basic Local Alignment Search Tool (BLAST) [29] searches of the ITS sequence data in the GenBank database.

2.3. DNA Extraction and Bioinformatic Analysis from Aspen Root Tissue Using the Roche 454 Platform

Aspen root tissue was surface-sterilized as above and a sub-sample from each root system was freeze-dried, then ground with a mortar and pestle, and the DNA was extracted from a 0.25 g quantity using the DNeasy plant DNA extraction kit (Quiagen, Hilden, Germany). The ITS region of ribosomal DNA of any fungi present in or on the roots was amplified using ITS PCRprimer constructs that included the 454-sequencing adaptor A and the DNA capture bead anneal adaptor B for compatibility with subsequent sequencing using the Roche 454 sequencing platform (Roche/454 Life Sciences, Basel, Switzerland). All amplicons were then purified using an Agencourt® AMPure® XP magnetic PCR cleanup system (Beckman Coulter, Inc., Brea, CA, USA) to eliminate primer dimers (fragments < 80 bp) and fragments smaller than 325 bp using a ratio of 0.7:1. The clean PCR amplicons were quantified with the Quant-iT™ PicoGreen® dsDNA assay kit (Invitrogen, Eugene, OR, USA). The DNA concentrations were measured with a Fluoroskan Ascent fluorometer (Thermo Electron Corporation, Vantaa, Finland), with an excitation wavelength of 486 nm and emission wavelength of 585 nm. Pooled DNA samples (75 ng each) were sent for 454 pyrosequencing to the McGill University and Genome Québec Innovation Centre (Montréal, QC, Canada), which performed emulsion PCR with the Lib-L GS FLX Titanium PCR kit (Roche/454 Life Sciences, Basel, Switzerland) according to the manufacturer's instructions and sequenced the samples unidirectionally on one-quarter of a PicoTiterPlate with a GS FLX Titanium sequencer (Roche/454 Life Sciences).

Stringent treatment of 454 DNA pyrosequences (primer mismatch 3; barcode mismatch optimized by service provider), was executed to prevent formation of a disproportional number of spurious operational taxonomic units (OTUs) which were interpreted asproxies for fungal species, and to produce a credible and biologically relevant number of OTUs [30–32]. Analyses were performed with mothur v1.28.0 [33]. Sequences were de-noised (using Pyronoise implementation in mothur), then filtered and trimmed (whereby reads shorter than 120 bp, after removal of barcodes, tags, and primers, were discarded; unambiguous positions and a maximum homopolymer length of 9 bp were tolerated). De-replication on the full length of the set of sequences was performed before construction of clusters [34]. The sequence set was then organized into clusters using USEARCH v6.0.307, with

a sequence similarity threshold of 97% to agglomerate reads and form the OTUs and with the most abundant sequence types serving as cluster seeds. There is no single similarity threshold that will accurately reflect the species level throughout the fungal kingdom, so a cutoff of 3% dissimilarity was selected as a compromise, to avoid overestimating fungal diversity and masking cryptic OTUs [31,35,36]. Representative sequences, which are the most frequent sequences in each OTU, were extracted and then screened against a subsample of the Genbank database (and including UNITE data) containing only fungi, using local BLAST v2.2.28+ [29,34,37]. The 25 top best BLAST hits were sought in databases by BLASTn software, with the minimum identity and query coverage parameters set to 80%.

Trophic functions were assigned to taxa according to Tedersoo et al. [38]. Lifestyle associations were used to sub-categorize biotrophs and symbiotrophs. When available, decay types and growth forms were used to sub-categorize the saprotrophs.

2.4. DNA Extraction and Bioinformatic Analysis from Forest Floor Organic and Mineral Soil Samples Using the Illumina Platform

Samples of the forest floor organic material (forest floor fraction) were air-dried and then mixed and ground into smaller fragments with an industrial blender. Mineral soil samples were sieved (using 6.3 mm, 2 mm, and 0.5 mm mesh sizes), and the resulting three soil fractions included: roots (root fraction, > 6.3 mm), coarse soil containing some fine roots (coarse soil fraction, < 6.3 mm and > 2 mm), and fine soil without roots (fine soil fraction, < 2 mm). Between samples, the sieves were washed, thoroughly spayed with 95% ethanol, and allowed to air dry. Other equipment was also washed, rinsed sequentially with water, bleach (10%), and reverse-osmosis water, and then sprayed with 95% ethanol between samples. Forest floor and soil samples were then freeze-dried and ground to a fine powder, and the DNA was extracted from 0.25 g sub-samples using a PowerSoil® DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). Dried samples and DNA were stored at –20 °C. The ITS ribosomal DNA region ITS1-5.8S was amplified using two-step PCR and Illumina fusion primers containing an index sequence for tagging every sequence in a sample [39]. All amplicons were then purified and quantified as described in Section 2.3. Tagged amplicon samples with differing indexes were then pooled in equimolar amounts of 4 ng DNA per sample. Final quantification of each pooled sample, verification of removal of primer artifact, and amplicon quality check were performed with the Agilent 2100 BioAnalyzer system (Agilent Technologies, Santa Clara, CA, USA). Samples for Illumina sequencing were sent to the Next-Generation Sequencing Platform, Genomics Centre (Centre Hospitalier de l'Université de Québec, Université Laval Research Centre, Quebec City, QC, Canada), which performed paired-end 300 bp sequencing using MiSeq Reagent Kit v3 (600 cycles) (Illumina, San Diego, California, USA) with an Illumina MiSeq system (Illumina, San Diego, California, USA).

Raw Illumina forward and reverse DNA reads were reassembled using the PANDASeq paired-end assembler, v2.11, with default parameters (quality threshold of 0.6) [40]. The resulting sequences were then processed by Illumicut [41], which first removed amplification primers, and then removed sequences that were too short (length < 200 bp) or ambiguous (at least one ambiguity). All other parameters were set to default. Trimmed and filtered sequences were then de-replicated using the mothur unique.seqs command (default parameters; [33]). De-replicated sequences were then screened for long homopolymer chains (> 9) using HomopRemover [42]. Singletons were removed from the final BLAST after OTU compression (number of singletons after clustering: 325859; number of singletons after compression: 3510). Sequences with a long homopolymer chain but size greater than one sequence were conserved. Sequences with 97% similarity or more were then clustered using VSEARCH v2.7.0 with default parameters [43]. The resulting OTUs were then identified with the VSEARCH BLAST algorithm applied to a GenBank fungi-only database (custom extraction; March 2017). Trophic assignment was completed as described for the 454 sequencing results.

2.5. Nutrient Analysis

Nutrient concentrations were assessed on both the forest floor and fine soil fractions. The analytical laboratory of the Northern Forestry Centre (Edmonton, AB, Canada) quantified available nitrate (NO_3^-) and ammonium (NH_4^+) using a standard 2 M potassium chloride (KCl) extraction protocol and quantified extractable phosphorus (P) according to the Bray protocol [44]. Total nitrogen (N) was assessed by dry combustion using a TruSpec CN analyzer (Leco Corporation, St. Joseph, MI, USA), followed by thermal conductivity detection.

2.6. Statistical Analyses

The R platform for statistics was used to perform all analyses [45]. Richness and diversity indexes—specifically, the Chao1 [46], abundance coverage estimator (ACE; [47]), Shannon, Simpson inverse (1/D), and Fisher indexes—were calculated using the 'estimate_richness' function of the 'phyloseq' package [48]. Fungal richness and diversity indexes were compared between soil samples at various distances from the forest edge and samples collected at −10 m from the forest edge (i.e., in the interior of the forest) using linear mixed-effects models, with soil fraction as a random effect. Polynomial or logarithmic models were fitted for the response of total, ectomycorrhizal, saprotrophic, and arbuscular mycorrhizal fungal richness to distance from the forest edge. Best-fit polynomial models were selected by backward and forward stepwise elimination of the least significant factors, according to the Akaike information criterion (AIC). Only terms deemed significant by analysis of variance (ANOVA) and marginal *t*-test were included in the final models.

The abundance of soil taxa was always normalized to per-sample library size by dividing the number of reads for a taxa by the total number of reads in that sample (hereafter, relative abundance). Dissimilarities in the relative abundance of soil fungal communities were first assessed by network analysis of Bray–Curtis distances using the 'make_network' function of the 'phyloseq' package [48]. Non-metric multi-dimensional scaling (NMDS) ordination-based ordering of Bray–Curtis distances [48] was then used to assess community dissimilarities, while limiting taxa to those with variance greater than 0.000,01 among samples. The forest floor, coarse soil, fine soil, and root fractions were analyzed both separately and jointly.

The effects of distance from the forest edge on fungal communities in the soil fractions were assessed using ANOVA-like permutation tests (999 permutations) of (partially) constrained ordination analyses [49]. Presence/absence data and relative abundances of fungal taxa (all taxa and those with variance greater than 0.000,01) were analyzed using constrained correspondence analyses (CCAs), and the relative abundances of fungal trophic functions and the number of taxa belonging to each trophic function were determined with redundancy analyses (RDAs), in accordance with best practices for analyzing various types of multi-variate data [50]. Taxa and trophic functions were only described when correlations with constrained ordinations axes were greater than 0.5 or less than −0.5, unless otherwise specified. Similarity between samples in (partially) constrained ordination analyses was assessed using k-means clustering of chi-squared and Euclidean distances.

When applicable, models accounted for the possible confounding effects of transect, seedling species, number of seedlings, dominant tree species, nutrient concentrations, and spatial distance. Principal coordinates of neighborhood matrices (PCNMs) of the geospatial coordinates of each sampling location were used to assess the effects of spatial distance [51]. The effects of nutrients on fungal communities in the forest floor were assessed using the concentrations of NO_3^-, NH_4^+, total N, and extractable phosphorus (P) in forest floor samples; concentrations of these nutrients in fine soils were used for assessing the effects of nutrients on fungal communities in other soil fractions. Only nutrient concentrations significantly associated with fungal responses when soil fractions were treated separately were used for joint assessment of soil fractions. Significant PCNM components and nutrients were selected independently by stepwise variable selection based on AIC-like statistics [52] followed by stepwise elimination of non-significant factors by ANOVA-like permutation tests, whereby terms are added sequentially and tested for marginal effects. The significance of each potentially confounding

factor was first assessed independently in constrained models before inclusion as a condition in the partially constrained ordinations testing for the effects of distance from edge.

3. Results

3.1. Fungi Cultured from Aspen Roots

Of the 24 fungal taxa cultured from aspen roots (Table 1), 15 were saprotrophs, 3 were saprotrophic biotrophs, and 1 was a plant pathogen. Seven of the cultured fungal taxa were known to have mycorrhizal capabilities, despite being classified only as saprotophs, according to Tedersoo et al. [38]. Only 28% (5/18) and 39% (7/18) of cultured taxa identified to the species level were detected using Roche 454 sequencing of aspen roots and Illumina sequencing of soil fractions, respectively (Table 1). Nonetheless, 72% and 83% of cultured taxa identified to the genus level were detected using Roche 454 sequencing of aspen roots and Illumina sequencing of soil fractions, respectively (Table 1).

Table 1. Fungal taxa cultured from aspen roots and identified with Sanger sequencing. Representative isolates have been deposited in the culture collection of the Northern Forestry Centre (NoF) GenBank accession number of representative isolate, with data on the number of young aspen from which cultures were isolated (maximum 11), the associated trophic function (TF), and whether taxa were detected at the level of species (Y = exact match, N = no match, NA = Cultured taxa not identified to species or genus) or genus (number of taxa) in 454 pyrosequencing of communities in aspen roots (Pyro. aspen roots). Results from Illumina sequencing of communities are also shown for soils sampled in 2016 (Illumina soils).

Fungal Ta xa Cultured	Representative Culture	Representative GenBank Accession	# of Aspen	TF *	Pyro. Aspen Roots		Illumina Soils	
					Species	Genus	Species	Genus
Cylindrocarpon olidum	NoF 3158	MT294406	3	PP	N	0	N	2
Fusarium acuminatum	NoF 3159	MT294407	1	SB	N	2	N	13
Ilyonectria crassa †	NoF 3124	MT294410	1	SB	N	2	N	2
Tolypocladium inflatum‡	NoF 3144	MT294423	1	SB	Y	1	N	1
Lachnum pygmaeum	NoF 3127	MT294411	2	ST	N	2	Y	7
Mycena epipterygia	NoF 3165	MT294413	1	ST	N	5	N	34
Mycena leptocephala	NoF 3163	MT294414	1	ST	N	5	N	34
Nodulisporium sp.	NoF 3148	MT294416	1	ST	NA	0	NA	0
Oidiodendron echinulatum	NoF 3152	MT294417	1	ST	N	1	N	13
Porodaedalea pini	NoF 3172	MT294420	1	ST	N	0	N	0
Scedosporium minutisporum	NoF 3135	MT294422	1	ST	N	0	N	1
Trichocladium opacum	NoF 3133	MT294424	1	ST	Y	1	Y	2
Cadophora finlandica	NoF 3116	MT294403	5	ST ‖	N	1	Y	18
Cladophialophora chaetospira	NoF 3119	MT294404	1	ST ‖	Y	1	Y	7
Cryptosporiopsis ericae	NoF 3122	MT294405	1	ST ‖	N	1	N	0
Leptodontidium orchidicola	NoF 3121	MT294412	3	ST ‖	N	0	N	4
Oidiodendron pilicola	NoF 3120	MT294418	2	ST ‖	N	1	Y	13
Phialocephala fortinii	NoF 3123	MT294419	11	ST ‖	Y	2	Y	14
Rhizoscyphus ericae§	NoF 3128	MT294421	2	ST ‖	Y	1	Y	2
Acremonium sp.	NoF 3147	MT294401	2	UK	NA	1	NA	16
Auriculariales order	NoF 3160	MT294402	1	UK	NA	NA	NA	NA
Helotiales order	NoF 3169	MT294408	1	UK	NA	NA	NA	NA
Hypocreales order	None	MT294409	1	UK	NA	NA	NA	NA
Nectriaceae family	NoF 3145	MT294415	2	UK	NA	NA	NA	NA

Note: * UK, unknown; PP, plant pathogen; ST, saprotroph; SB, saprotrophic biotroph; NA, not applicable; † Previously known as *Cylindrocarpon destructans* var. *crissum*; ‡ Previously known as *Elaphocordyceps subsessilis*; § Previously known as *Pezizella ericae*; ‖ Species with known mycorrhizal capabilities, according to the literature.

3.2. Fungi from Aspen Roots Identified by Roche 454 Analysis

Roche 454 high-throughput sequencing of surface-sterilized aspen roots collected from the undisturbed forest detected 166 unique taxa, of which 92 were detected (exact match) in soil fractions collected along the transects and analysed by the Illumina method (Supplementary Table S1). When the response of these 92 taxa to the distance from the forest edge was assessed across all soil fractions (using relative abundance, according to sample library size), 28 were associated with the interior of the undisturbed forest and 24 with the reclaimed area ($p = 0.001$; Supplementary Table S1). In contrast,

when the response was assessed in relation to the presence or absence of fungal taxa, only 14 taxa were significantly associated with the forest interior and 10 with the reclaimed area ($p = 0.001$; Supplementary Table S1). Of these, more saprotrophs and plant pathogens were associated with the reclaimed area (17 and 8 taxa, respectively) than with the interior of the undisturbed forest (8 and 5 taxa, respectively). Most mycorrhizal taxa (11 ectomycorrhizae and 2 ericoids) were associated with the forest interior, whereas no such taxa were specifically associated with the reclaimed area. Only three taxa from aspen roots showed high variance across soil fractions and were consistently associated with either the forest interior or the reclaimed area, specifically *Piloderma sphaerosporum* Jülich (ectomycorrhizae) in the forest interior and *Paraphoma* sp. L13 and *Cadophora* sp. 9232S2 (saprotroph) in the reclaimed area.

3.3. Soil Fungal Communities Identified by Illumina Analysis

Following data cleaning, a total of 13,653,146 ITS sequences were identified, representing 2796 fungal taxa, using the Illumina platform across all soil fractions. Fewer fungal taxa were detected when soil fractions were treated separately (1950 in coarse soil, 2091 in fine soil, 2373 in forest floor, and 1850 in roots), of which 50 taxa were uniquely found in coarse soil, 71 in fine soil, 286 in forest floor, and 63 in roots (Supplementary Table S2). Of the 189 fungal taxa detected only in mineral soil fractions (fine and coarse soil), 86 were detected in more than one sample (Supplementary Table S3). Some taxa were detected only in the reclaimed area (n = 703), the forest interior (n = 295), or the forest edge (n = 44) (Supplementary Table S4).

Fungal richness was greatest at 20 m into the reclaimed area (580 taxa) but was not significantly different from that at the –10 m sampling site in the forest interior (521 taxa) ($p = 0.376$; Figure 2A and Supplementary Figure S1). Fungal richness was lowest at the –5 m sampling site in the forest interior (289 taxa), followed by that at the forest edge (354 taxa), corresponding to roughly half and two-thirds the richness deeper in the forest interior ($p = 0.001$ and 0.019, respectively). The Fisher, Chao1, and ACE indexes also showed significantly lower diversity at –5 m as compared with the –10 m in the forest ($p < 0.01$; Supplementary Figure S1). The Fisher, Simpson inverse, and Shannon indexes showed significantly greater diversity at 10 m and 20 m into the reclaimed area compared to –10 m in the forest ($p < 0.01$).

Unconstrained analysis of fungal community data consistently separated samples from the forest edge and interior from those in the reclaimed area, but also showed that factors other than distance from the forest edge contributed to similarity/dissimilarity in fungal communities among samples. Network analysis of Bray–Curtis distances consistently clustered forest edge and interior samples separately from those in the reclaimed area; however, some community similarity was independent of distance from the forest edge (Figure 3). Ordering samples on the basis of NMDS ordination-based ordering of Bray–Curtis distances of taxa with relative variance greater than 0.000,01 consistently separated the forest edge and interior from the reclaimed area, whether soil fractions were treated separately or jointly (Supplementary Figure S2). Coarse soils and fine soils from the forest edge and interior of transect A, clustered separately from those obtained from transects B and C (Supplementary Figure S2). Furthermore, samples from the forest edge and interior generally clustered by transect when all soil fractions were analyzed jointly (Supplementary Figure S2). Ordering samples on the basis of NMDS ordination-based ordering of Bray–Curtis distances of trophic functions consistently separated samples from the forest edge and interior from those in the reclaimed area (Supplementary Figure S3).

The distance from the forest edge was significantly associated with the relative abundance and the presence or absence of fungal taxa with high variance, whether soil fractions were considered jointly (accounting for 4.22% and 9.09% of inertia, respectively) or separately (6.61%–11.41% and 8.99%–17.43%, respectively) (Table 2; Figure 4A,B). Although still significant, the distance from the forest edge had generally less explanatory power when accounting for all fungal taxa (4.04%–10.82%; Table 2). Among taxa with high variance, 26 fungal taxa were consistently associated with the forest interior and 14 with the reclaimed area ($p < 0.001$; Table 3). The distance from the forest edge was significantly associated

with the relative abundance of trophic functions across all soil fractions, whether soil fractions were considered separately or jointly (Table 4). Specifically, the relative abundance of ectomycorrhizal fungi was greater in the forest interior, whereas the relative abundances of facultative yeast and fungi with no assigned functions were greater in the reclaimed area ($p < 0.05$; Figure 4C). When soil fractions were analyzed separately, the relative abundances of fungi classified as brown rots and saprotrophs were also greater in the forest floor, whereas more plant pathogens were found in the fine soil ($p < 0.05$). The number of fungal taxa belonging to various trophic functions was significantly associated with distance from the forest edge when all soil fractions were considered jointly (Table 4). The number of arbuscular mycorrhizal taxa was greater in the reclaimed area, and the number of ectomycorrhizal taxa was greater in the forest interior ($p = 0.007$; Figures 2B and 4D).

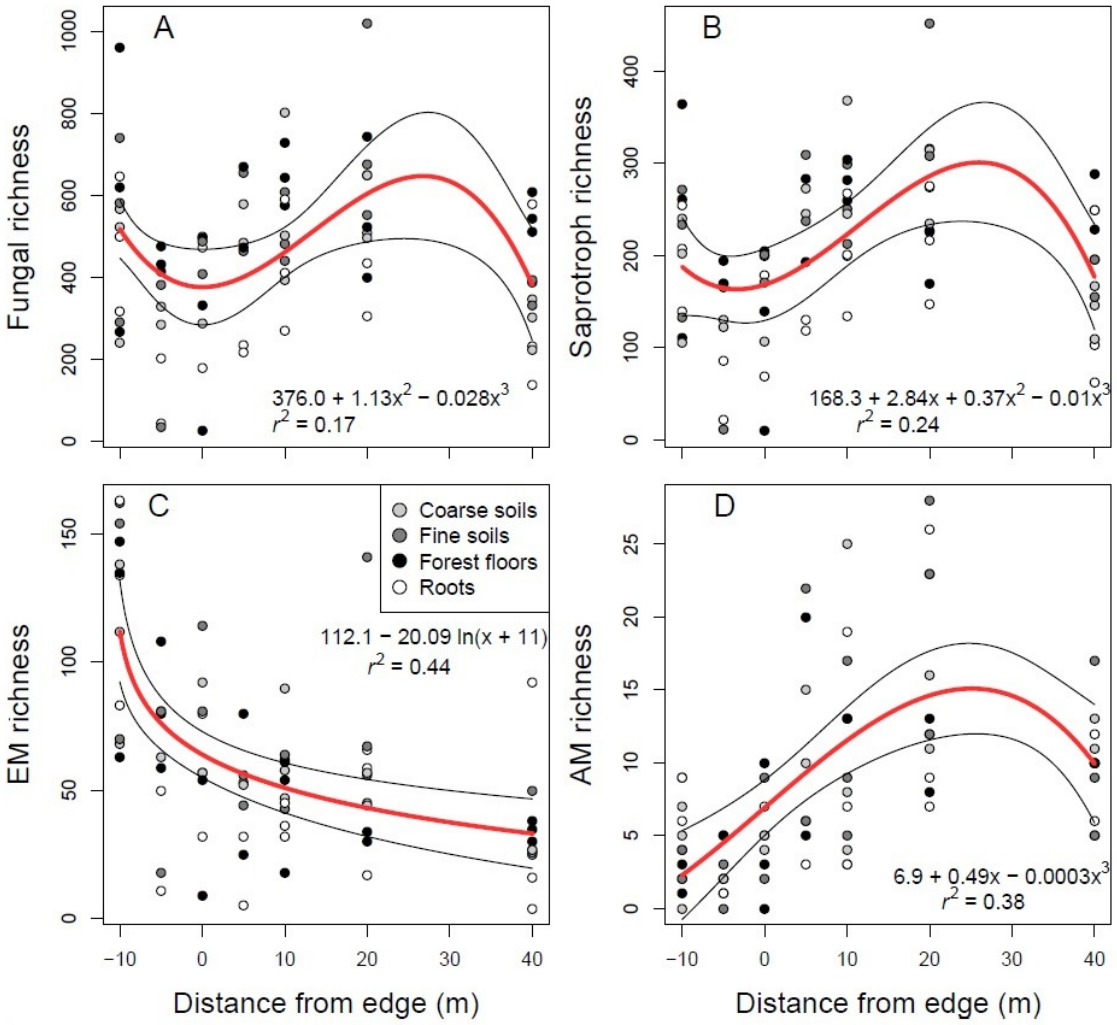

Figure 2. Best-fit modeled responses of fungal richness to distance from the forest edge (red lines), with 99% confidence interval (black lines), where negative distance values represent the forest interior and positive distance values represent the reclaimed area. (**A**) All fungi; (**B**) saprotrophs; (**C**) ectomycorrhizae (EM); and (**D**) arbuscular mycorrhizae (AM).

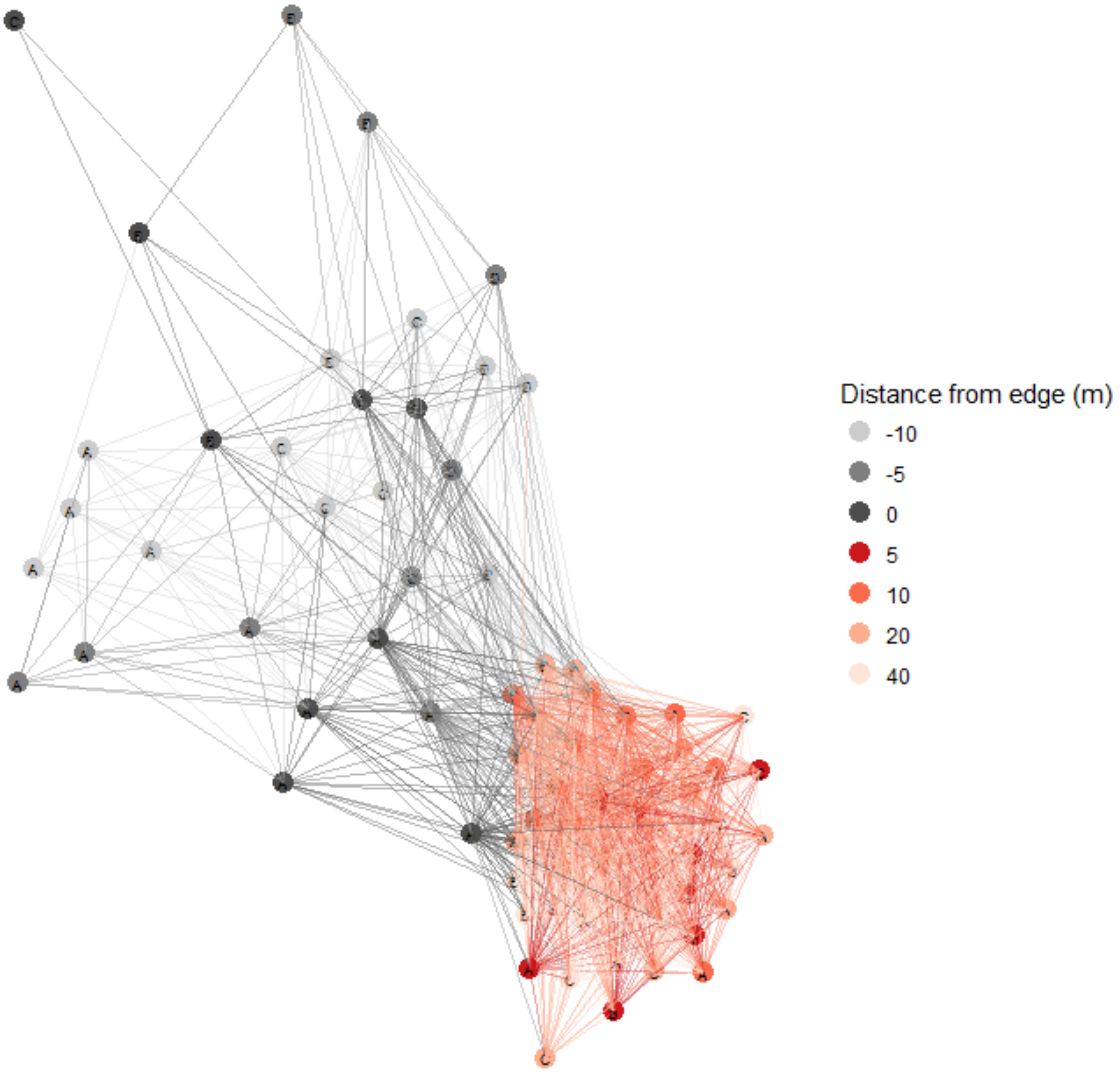

Figure 3. Network analysis of fungal taxa (in terms of relative abundances) in forest floor, coarse soil, fine soil, and root samples, using Bray–Curtis distances less than 0.91 (minimum threshold displaying all 74 samples). Each sample is labeled by transect (A, B, or C).

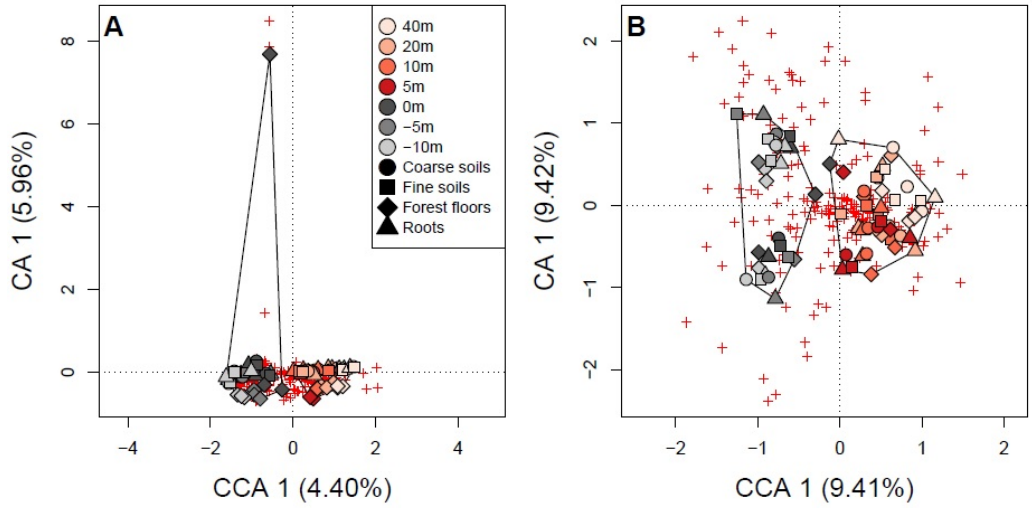

Figure 4. *Cont*.

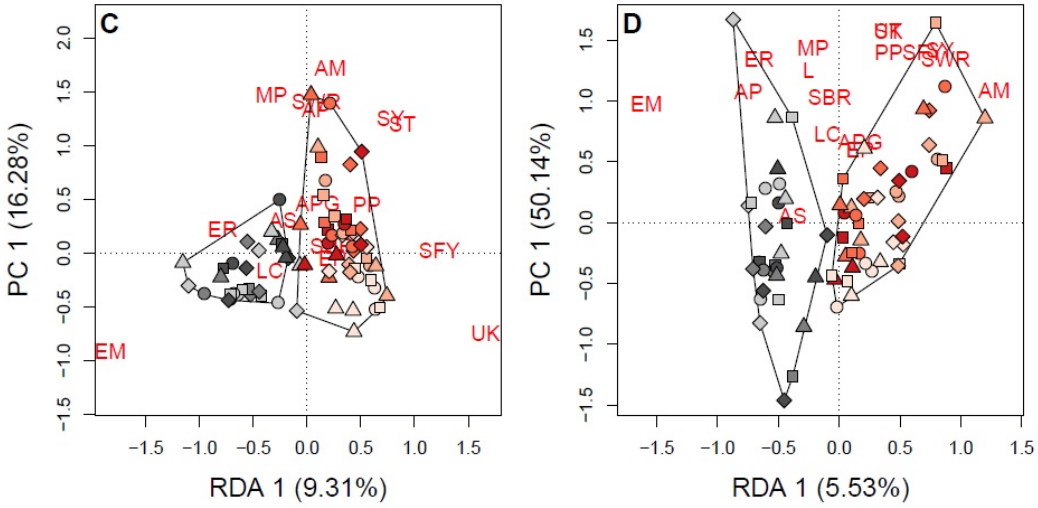

Figure 4. The effects of distance from the forest edge as depicted by partially constrained ordinations of the relative abundances and presence or absence of fungal taxa with variance greater than 0.000,01 across samples (**A**,**B**, respectively), the relative abundance of fungal trophic functions (**C**) and the number of fungal taxa belonging to fungal trophic functions (**D**) in root, forest floor, fine soil, and coarse soil fractions assessed jointly, while removing effects of soil fraction. Both species and sites scores are symmetrically scaled by the square root of eigenvalues for plot clarity. Polygons represent k-means clustering based on axes 1 and 2. High relative abundance of *Tomentella coerulea* and *Hebeloma circinans* are responsible for the divergent forest floor sample at 10 m in panel A. AM, arbuscular mycorrhiza; AP, animal parasite; APG, animal pathogen; AS, animal endosymbiont; EM, ectomycorrhiza; EP, endophyte; ER, ericoid fungus; L, lichenized fungus; LC, lichenicolous fungus; MP, mycoparasite; PP, plant pathogen; SBR, saprotrophic brown rot; SFY, saprotrophic facultative yeast; ST, saprotroph; SWR, saprotrophic white rot; SY, saprotrophic yeast; UK, unknown;.

Table 2. Significance (expressed as *p* values) of distance from the forest edge in (partially) constrained ordinations (by constrained correspondence analyses) of the relative abundance (Rel. abun.) or the presence or absence (Pres./abs.) of fungal taxa in coarse soils, fine soils, the forest floor, roots, or all samples combined. Percent values represent the proportions of inertia explained by the constraints. Instances where confounding factors were not significant as constraints are designated with "NA" (not applicable).

Response Variable		All Taxa	Highly Variable Taxa						
		Conditions	Conditions						
		None	None	Transect	Seedling Species	No. of Seedlings	Dom. Tree Species	Nutrients	PCNMs *
Rel. abun.	All soil fractions †	0.001 4.04%	0.001 4.22%	0.001 4.24%	0.001 3.07%	NA	0.001 4.23%	0.001 3.10%	0.001 2.11%
	Forest floor	0.017 6.72%	0.024 6.61%	NA	NA	NA	NA	0.467 5.33%	NA
	Coarse soils	0.004 9.91%	0.004 10.27%	NA	NA	NA	NA	0.245 6.44%	NA
	Fine soils	0.001 10.82%	0.003 11.41%	NA	NA	NA	NA	0.119 7.26%	0.005 10.70%
	Roots	0.002 8.89%	0.004 8.98%	NA	NA	NA	NA	0.189 6.49%	NA
Pres./abs.	All soil fractions †	0.001 4.60%	0.001 9.09%	0.001 9.19%	0.001 7.90%	0.001 8.74%	0.001 9.08%	0.005 2.34%	0.001 2.64%
	Forest floor	0.001 8.97%	0.001 12.14%	NA	NA	NA	0.001 12.12%	0.390 4.54%	0.140 5.90%
	Coarse soils	0.001 9.52%	0.001 17.43%	NA	NA	NA	0.002 17.29%	0.027 8.10%	0.230 5.86%
	Fine soils	0.002 9.26%	0.002 16.01%	NA	NA	NA	0.001 15.77%	0.067 6.80%	0.028 8.86%
	Roots	0.008 8.13%	0.023 8.99%	NA	NA	NA	0.027 8.91%	0.678 4.52%	0.943 3.44%

Note: * PCNMs, principal coordinates of neighborhood matrices; † Each model was conditioned by soil fraction.

Table 3. Highly variable (defined as variance greater than 0.000,01 across samples) fungal taxa that were consistently detected more often (in terms of both relative abundance and presence/absence) in the interior of the undisturbed forest than in the reclaimed area. Taxa are ordered by total read number (RN) across all soil fractions. The trophic function (TF) associated with each taxon is listed.

RN	Forest Interior	TF *	RN	Reclaimed Area	TF *
107,334	*Amphinema* sp. ML-1	EM	10,056	*Podospora intestinacea*	ST
71,115	*Russula fragilis* var. *fragilis*	EM	9094	*Leptospora rubella*	UK
55,212	*Tricholoma platyphyllum*	EM	9035	*Psathyrella abieticola*	ST
52,951	*Tomentella fusco-cinerea*	EM	8030	*Phlebia* sp. DLL2011-1	SWR
38,287	*Laccaria* sp. AWW564	EM	7092	*Hypholoma capnoides*	ST
30,365	*Cortinarius colymbadinus*	EM	6491	*Paraphoma* sp. L13 †	UK
29,191	*Piloderma sphaerosporum*†	EM	5167	*Plectosphaerella* sp. FPGLXJ06	PP
14,544	*Hygrophorus* sp. EL-2014	EM	4995	*Epulorhiza* sp. SO 035	UK
13,770	*Sebacina* sp. Seb13I	EM	4053	*Cadophora* sp. 9232S2 †	ST
12,855	*Cortinarius paragaudis*	EM	3511	*Trogia venenata*	ST
12,308	*Trechispora stellulata*	ST	2747	*Seimatosporium vitis*	PP
9853	*Sistotrema* sp. PC14	ST	1113	*Trichoderma aureoviride*	ST
5241	*Mycena amicta*	ST	1071	*Rhizoctonia* sp. 70B	SBR
4377	*Inocybe fulvipes*	EM	1009	*Dendrosporium* sp. 1 RB-2011	ST
3870	*Clavariadelphus sachalinensis*	ST			
3707	*Tomentella* sp. 4 RT-2012	EM			
3618	*Sistotrema oblongisporum*	ST			
3481	*Tricholoma saponaceum* var. *saponaceum*	EM			
2593	*Tomentella* sp. YM1903	EM			
2137	*Tomentella subtestacea*	EM			
1981	*Thyronectria coryli*	UK			
1880	*Inocybe calida*	EM			
1400	*Clavariadelphus ligula*	ST			
1389	*Sclerotinia nivalis*	PP			
659	*Hypochnicium albostramineum*	SWR			
118	*Tricholoma magnivelare*	EM			

Note: * UK, unknown; SBR, saprotrophic brown rot; EM, ectomycorrhiza; PP, plant pathogen; ST, saprotroph; SWR, saprotrophic white rot; † Species detected using pyrosequencing of DNA from aspen roots.

Table 4. Significance (expressed as *p* values) of distance from the forest edge in (partially) constrained ordinations (by redundancy analyses) of the relative abundance of fungal trophic groups or the number of taxa belonging to each trophic group in coarse soils, fine soils, the forest floor, roots, or all samples combined. Percent values represent the proportions of inertia explained by the constraints. Instances where confounding factors were not significant as constraints are designated with "NA" (not applicable).

Response Variable		Conditions						
		None	Transect	Seedling Species	No. of Seedlings	Dom. Tree Species	Nutrients	PCNMs *
Rel. abun.	All soil fractions †	0.001 8.89%	0.001 9.06%	0.001 5.87%	0.001 7.12%	NA	0.007 2.52%	0.210 1.50%
	Forest floor	0.001 13.39%	NA	NA	NA	NA	0.203 5.76%	0.247 5.98%
	Coarse soils	0.011 14.84%	NA	0.042 9.19%	NA	NA	0.041 8.82%	NA
	Fine soils	0.003 16.11%	NA	0.012 11.26%	NA	NA	0.065 8.11%	0.146 8.42%
	Roots	0.034 10.84%	NA	0.198 5.72%	NA	NA	0.039 10.13%	0.007 11.97%
No. of taxa	All soil fractions †	0.007 5.01%	0.005 4.85%	0.012 3.98%	NA	0.006 4.96%	0.103 2.45%	0.056 2.48%
	Forest floor	0.114 10.14%	NA	NA	NA	NA	0.288 5.17%	NA
	Coarse soils	0.088 11.17%	NA	NA	NA	NA	0.002 18.00%	0.083 10.45%
	Fine soils	0.546 4.30%	NA	NA	NA	NA	NA	0.228 5.60%
	Roots	0.426 5.16%	0.286 4.34%	NA	NA	NA	0.662 2.87%	0.229 4.66%

Note: * PCNMs, principal coordinates of neighborhood matrice; † Each model was conditioned by soil fraction.

Overall plant richness was not significantly different between the reclaimed clearing and the forest interior (mean ± standard deviation 4.9 ± 0.2 and 5.5 ± 0.3, respectively; $p = 0.106$); however, understory cover (56.3 ± 3.6 and 17.4 ± 5.7, respectively) and richness (3.8 ± 0.2 and 2.2 ± 0.3, respectively) of plants associated with arbuscular mycorrhizae were greater in the reclaimed area ($p < 0.001$ for both). Furthermore, the understory cover of ericoid plants (namely *Vaccinium vitis-idaea* L.) decreased from the forest interior across the edge to the reclaimed area (Supplementary Table S5). Transect, seedling species, dominant tree species, and number of seedlings significantly accounted for 2.47% to 18.27% of variance in fungal taxa (Supplementary Table S6) and 3.23% to 62.59% of variance in trophic functions (Supplementary Table S7), but in most cases did not confound the significance of the distance from the forest edge (Tables 2 and 4). The effects of distance from the edge were confounded by seedling species only when the relative abundance of fungal trophic functions was assessed with root fractions treated separately, despite consistently greater abundance of ectomycorrhizal fungi in the forest interior (Table 4). Transect effects were related to the richness, diameter at breast height, age, height, density, and basal area of overstory trees in the forest interior and was mostly associated with the variance in the number of fungal taxa belonging to various trophic functions in roots (43.85%; Supplementary Table S7). Among the variables corresponding to transect, average tree height of the three tallest trees in the forest interior (19 m, 18.7 m, and 14.7 m for transects A, B, and C, respectively) had the greatest explanatory power for the number of taxa belonging to various trophic functions in roots (42.6%; data not shown), which correlated with greater numbers of animal parasites, arbuscular mycorrhizae, endophytes, ericoid mycorrhizae, facultative yeasts, lichens, mycoparasites, plant pathogens, saprotrophs, white rot fungi, yeasts, and fungal taxa with no associated functions. The identity of dominant tree species (white spruce for transect A and black spruce for transects B and C) was more strongly associated with the presence or absence of fungal taxa in each soil fraction (Supplementary Table S6). However, the identity of seedling species was particularly associated with the relative abundance of trophic functions in coarse soils, fine soils, and roots (58.19%–62.59%; Supplementary Table S7). More specifically, greater abundances of arbuscular mycorrhizae, white rot fungi, and animal parasites, and to a lesser extent, mycoparasites (correlation with RDA axis, $r > 0.43$), were found in samples collected at 10 m and 20 m along transect C, where willow (*Salix* sp.) seedlings were intermixed with white birch (*Betula papyrifera* Marsh.) or white spruce ($p < 0.01$).

The PCNM 3, which was correlated with distance from the edge ($r = –0.775$, $p < 0.001$; Supplementary Figure S5), confounded the effects of distance from the edge when the presence or absence of fungal taxa was assessed in the forest floor or coarse soil fractions and when the relative abundance of trophic functions was assessed in the forest floor or fine soil fractions (Tables 2 and 4). The relative abundance of trophic functions and the number of taxa belonging to these groups were also confounded by PCNM 3 when all soil fractions were analyzed jointly (Table 4). However, the relative abundance of ericoid mycorrhizae, and to a lesser extent, mycoparasites (correlation with RDA axis, $r = 0.49$), was significantly greater in the forest interior than in the reclaimed area when PCNM 1 was accounted for (marginal permutation test for axis, $p < 0.01$). Furthermore, accounting for spatial layout (other than PCNM 3) revealed that significantly more taxa identified as yeasts and facultative yeasts were found in the reclaimed area than in the forest interior (marginal permutation test for axis, $p < 0.01$).

In forest floor, both NH_4^+ and total N concentrations decreased in the reclaimed area with increasing distance from the edge ($p < 0.01$; Supplementary Figure S5). Average total N concentrations in forest floor at 10 m, 20 m, and 40 m into the reclaimed area (0.39%, 0.33%, and 0.22%, respectively) were less than that found at the forest edge and interior (1.17%) ($p < 0.01$). In mineral soils, NO_3^-, NH_4^+, and total N concentrations generally increased in the reclaimed area with distance from the forest edge ($p < 0.05$; Supplementary Figure S5). Total N concentrations in the reclaimed area were on average 2.3 times those at the forest edge interior ($p < 0.001$). Nutrient concentrations did not differ among transects (ANOVA, $p > 0.05$). In most cases, nutrient concentrations in soils (mainly total N) confounded the significant associations of distance from the edge with fungal taxa and

trophic functions (Tables 2 and 4). Yet when soil fractions were analyzed separately, the associations of total N with functional groups were opposite in mineral soil fractions from those in forest floor. For example, greater total N concentration in the forest floor fraction was associated with lower arbuscular mycorrhizae abundance, whereas greater total N concentration in fine soil was associated with greater ectomycorrhizal abundance in coarse soil, fine soil, and root fractions. The same was true for all trophic functions significantly associated with N in both mineral soil fractions, specifically facultative yeasts, plant pathogens, saprotrophs, white rot fungi, yeasts, and fungi not functionally characterized. Overall, N concentration in forest floor was inversely related to that in mineral soil (Supplementary Figure S5 and Supplementary Table S8). The P concentration in fine soils was associated with the relative abundance of various taxa in mineral soils and roots ($p < 0.05$). In fine soils, coarse soils, and roots, the abundance of 23, 26, and 41 taxa, respectively, was positively associated with P concentration, and the abundance of 26, 34, and 45 taxa, respectively, was negatively associated with P concentration (Supplementary Table S9).

4. Discussion

The process of land reclamation following industrial disturbance is essential to the recovery of ecosystem services. During this process, fungi play an important role in facilitating the establishment of seedlings, through beneficial mycorrhizal associations that result in increased nutrient availability and improved seedling growth [23]; however, there can be a dearth of fungal inocula in soils that have been stockpiled for use in land reclamation [21]. Thus, the natural dispersal of fungi into a reclaimed area is critical for optimal plant establishment [26]. In this study, we assessed the communities of fungi within aspen roots in an undisturbed natural boreal mixedwood forest, and soils from the undisturbed forest, at the forest edge, and within an adjacent area that had been reclaimed 1 year before our study. This study thus represents an early baseline assessment of the undisturbed forest and the adjacent reclaimed area. The transect locations used in the current study are known, and it will, therefore, be possible to resample the area in the future to observe changes in the soil fungal community over time.

Before undertaking the current study, we had assessed the community of soil fungi at Gateway Hill, a certified reclaimed area in Northeastern Alberta [26]. In that previous study, we identified a total of 296 unique fungal taxa across all samples (forest floor, coarse soil, fine soil, and root fractions combined) using the Roche 454 sequencing platform and a total of 1369 unique fungal taxa present in a nearby undisturbed spruce stand using the Illumina platform [26]. In the current study, we identified 2796 taxa in the combined reclaimed and adjacent undisturbed forest areas using the Illumina platform, which was 1427 taxa more than were identified in the undisturbed spruce stand using the Illumina platform at the Gateway Hill area. The identification of more species in the current study is likely related to differences between the sites assessed: a single natural site at Gateway Hill [26] and both natural and reclaimed sites in the current study.

The forest edge is very different from the interior of a forest stand because altered abiotic conditions affect biotic composition [53–55]. Greater plant and animal richness at forest edges can result from the overlapping occurrence of some species from neighboring seral stages, in addition to the presence of edge-specific species [54,55]. The observation of lower fungal richness and diversity at or near the forest edge suggests that this ecotone is detrimental to many interior- and clearing-specific taxa. Only a few taxa were unique to the forest edge, where they would be able to take advantage of the ecotone's particular abiotic conditions and/or biotic interactions. The distance over which the edge effect occurred was generally short (≤ 5 m). In essence, differences in environmental conditions that exist across the edge from the undisturbed forest into the reclaimed area appeared to be reducing fungal richness from the forest edge towards the interior, while tolerant fungi from forest refugia were colonizing neighboring soils in the reclaimed area.

Network analysis and NMDS ordination-based ordering of Bray–Curtis distances suggested a distinct shift in composition and organization of the fungal community 5 m into the reclaimed area. During assessment of the variance specifically associated with distance from the forest edge, k-means

clustering of chi-squared and Euclidean distances further supported a distinct transition of fungal community composition and function at 5 m into the reclaimed area. Spatial distance typically increases dissimilarity among soil microbial communities [56–59]; however, at our experimental scale, the spatial components representing the greatest spatial distance (i.e., PCNM 1 and 2) were rarely associated with fungal community dissimilarities. Although spatial layout accounted for an important proportion of the variance in fungal communities, accounting for spatial components other than PCNM 3, which correlated with the distance from edge, did not confound the effects of distance from the edge. Hence, the effects of distance from the edge are unlikely to be solely associated with greater spatial separation. Nonetheless, in some cases, accounting for spatial layout revealed nested underlying effects, such as the significant decrease in the relative abundance of ericoid mycorrhizae with increasing distance from the forest edge.

As suggested by network analysis, fungal communities at the forest edge and in the forest interior were more different in composition and were organized at a finer spatial scale than communities within the reclaimed area. Community distinctness often reflects shifts in habitat structure; for example, Nacke et al. [60] showed that both soil bacterial and fungal communities varied according to soil depth as well as proximity to tree species. The greater environmental homogeneity in the reclaimed area likely resulted from mixing of the soil during processes of stockpiling and reapplication to the site. Some of the heterogeneity in forest stands corresponded to measured environmental parameters such as dominant tree species (exemplified by separation of transect A from transects B and C, reflecting the difference across forest transects in prexisting stand composition); however, measured environmental and spatial parameters often do not account for the full extent of fungal variability [58].

4.1. Mycorrhizal Taxa

The greater abundance and number of ectomycorrhizal taxa in the forest interior are likely attributable to the greater availability of roots within intact forest refugia [2,25] and the concomitant increase in nutrient availability from the host trees [61] and root substrate from which emanating extramatrical hyphae can emerge [61,62]. Similarly, the abundance of understory plants known to form arbuscular associations offered greater host availability to arbuscular mycorrhizal taxa in the reclaimed area. The transition from ectomycorrhizal communities between the forest and 5 m into the reclaimed area likely corresponds to limited root growth into the reclaimed area from the forest edge. Roots emerging from forested refugia are the primary source of ectomycorrhizal inoculation of disturbed (e.g., cleared) land; hence, the decreasing number of ectomycorrhizal taxa detected with increasing distance from the forest edge [2,25] or from isolated trees [63] in harvest cutblocks. Sharp declines in ectomycorrhizal richness at the reclaimed site occurred closer to the forest edge than has been reported for clear-cuts (10 m; [25]) and may reflect the greater extent of root disturbance during soil removal and replacement as compared to forestry practices.

Co-adaptation of tree species and soil microorganisms, especially ectomycorrhizal symbionts, could explain the shifts in fungal community composition with dominant tree species (e.g., white spruce versus black spruce) in the interior of the undisturbed forest [64–66]. The height of the tallest trees in the forest interior correlated with the number of taxa associated with various trophic functions on roots; however, tree height was not correlated with stand age or nutrient concentrations, but may have been confounded with tree species (Supplementary Table S4). Tree height may be representative of soil waterlogging, because this variable mostly separated transect C from transects A and B. Shorter black spruce trees were present in the interior of the undisturbed forest along transect C, which was wetter and covered with a thicker moss layer. It is known that bacterial communities thriving in moss produce antifungal metabolites [67], which may account for the lower number of fungal taxa across most trophic functions on transect C.

The presence of ericoid fungi in root samples increased with the increasing presence of ericoid shrubs (e.g., *Rhododendron groenlandicum* and *V. vitis-idaea*) that occurred with increasing distance from the stand edge. The ectomycorrhizal and ericoid fungi, found in greater relative abundance on roots in

the interior of the undisturbed forest, may have been hosts for mycoparasites, which were also found in greater abundance in root samples from the forest interior. Mycoparasites can take a considerable amount of time to become established (e.g., [68]). Thus, the greater abundance of mycoparasites in the forest interior, despite the abundance of fungi with no associated functions in the reclaimed area, could be due to the longer period since forest establishment. Alternatively, the mycoparasites detected may be specific to ectomycorrhizal and/or ericoid hosts. Most research on mycoparasites has focused on the development of biological control agents (see [69,70]); to our knowledge, no studies have investigated the natural associations of mycoparasites with mycorrhizal fungi.

4.2. Saprotrophs and Soil Nutrients

Saprotrophs made up a considerable proportion of the culturable taxa isolated from aspen roots. Despite using selective MMN media to facilitate the culturing of mycorrhizal taxa, our methods greatly underestimated mycorrhizal richness and the proportion of total fungal richness represented by these fungi. Obligate symbionts are notoriously difficult to culture [2] and are likely depleted in reclaimed soils [21,24]. Despite these limitations, important saprotrophic taxa with known mycorrhizal properties were isolated. Saprotrophic taxa are increasingly known for their intricate associations with mycorrhizal taxa and so-called dark septate endophytes [21,71]. Further investigation into the role of such culturable taxa on seedling growth is warranted, as these would be readily available candidates for inoculation of nursery stocks.

The greater abundance of facultative yeasts and saprotrophs in the reclaimed area, especially in the forest floor, are likely a response to the stockpiling of organic material before soil placement in the reclaimed area. Stockpiling of forest floor material would have created a more homogenous and abundant supply of organic matter for saprotrophs to colonize. Mineralization of organic matter by saprotrophic fungi and other N-utilizing microbes in stockpiled soil would eventually lead to nitrification [72] and potential net N loss to de-nitrification or leaching [73]. Combined with a lack of major inputs of organic matter from falling litter, the result would be low N concentration in the reclaimed area. In the interior of the undisturbed forest, low N concentrations in mineral soils could have resulted from N utilization by the larger trees [74]. In turn, these large trees are perpetually shedding fresh litter onto the forest floor, leading to the accumulation of thicker, N-rich F and H soil layers.

The concentration of P in soils affected the relative abundance of specific fungal taxa, similar to edaphic preferences observed at global scales [38]. Shifts in the community composition of ectomycorrhizal fungi are also affected by N concentrations in forest soils [75]. However, the reverse effects of N concentration in the forest floor and mineral soil fractions at our site suggest that shifts in fungal community function and composition are instead related to distance from the forest edge.

4.3. Plant Pathogens and Seedlings

Plant richness was relatively low and did not differ between the forest interior and the reclaimed area, and thus could not account for the increasing abundance and richness of plant pathogens in mineral soils from the reclaimed area [76–78]. The lack of an association between plant richness and pathogen richness could be indicative of the generalist host preference of these plant pathogens, which may challenge the resilience of regenerating flora [78]. Alternatively, plant pathogenic fungi may be taking advantage of younger or more stressed hosts, they may be specific to herbaceous plants found in the reclaimed area (e.g., arbuscular plant species), or they may have been translocated with reclamation soil material.

Plant pathogens growing on aspen roots did not appear to limit tree establishment within the forest interior. However, many of these root-associated plant pathogens were more abundant in soils from the reclaimed area. Thus, seedlings planted in the reclaimed area would likely be more exposed to plant pathogens, especially if beneficial mycorrhizal symbionts are rarer (e.g., [24]). Sampling and sequencing biases [79] could account for some of the differences in taxa detected between the

high-throughput platforms. For example, collecting soil cores reduces the probability of gathering ectomycorrhizal taxa with short-range hyphal exploration strategies, which would be found within seedling roots. Furthermore, the longer sequence length provided by 454 sequencing technologies might have led to more specific fungal identification that did not match the taxa identified through bioinformatics processing of Illumina sequences.

5. Conclusions

A distinct transition in soil fungal communities from forest-associated to recently reclaimed area-associated assemblages was observed within 5 m of the forest edge. Fungal communities detected within undisturbed forest soils were rich in ectomycorrhizal taxa, whereas those within the reclaimed area contained more arbuscular taxa. Ectomycorrhizal fungi appeared to recolonize reclaimed soils close to the forest edge, which suggests that the natural forest is a source for these fungi. Soils in the reclaimed area were dominated by saprotrophic fungi and taxa of unknown function, yet some of these taxa are known to associate with the roots of small aspen and may be symbiotic in nature. More plant pathogens were detected in the reclaimed area, and these included taxa associated with aspen roots. Risks to the resilience of seedling establishment in reclaimed sites depend on the pace of ectomycorrhizal colonization from nearby intact refugia and the unknown functional properties of fungi in disturbed soils.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/4/427/s1, Figure S1: Boxplots of fungal community richness (Observed) and alpha diversity calculated using Chao1, ACE, Shannon, Simpson inverse (1/D), and Fisher indexes across all soil fractions in response to distance from the forest edge (where negative distance values represent the interior of the undisturbed forest and positive distance values represent the reclaimed area)., Figure S2: Dissimilarity analyses of the relative abundances of fungal taxa with variance greater than 0.000,01 across samples (197 taxa across all soil fractions, 112 taxa from coarse soil samples, 102 taxa from fine soil samples, 171 taxa from forest floor samples, and 136 taxa from root samples) at various distances from the forest edge across three transects (A, B, and C)., Figure S3: Dissimilarity analyses of the relative abundances of fungal trophic functions in soil samples at various distances from the forest edge across three transects (A, B, and C). Figure S4: Principal coordinates of neighborhood matrices (PCNMs) of the spatial coordinates where soil samples were collected. The point size in each plot is proportional to the PCNM value. Figure S5: Boxplot of nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (N), and phosphorus (P) concentrations in forest floor and fine soil fractions in relation to distance from the forest edge (where negative distance values represent the interior of the undisturbed forest and positive distance values represent the reclaimed area). Table S1: Fungal taxa detected using pyrosequencing of DNA from aspen roots and Illumina sequencing of soil DNA collected along transects crossing from the interior of the undisturbed forest into the reclaimed area, ordered by total read number (RN) across all soil fractions. Table S2: Fungal taxa detected in only one of the four soil fractions. Taxa are ordered by total read number (RN) across all soil fractions. Table S3: Fungal taxa only detected in mineral soil fractions (i.e., fine and coarse soil). Taxa are ordered by total read number (RN) across all samples. Table S4: Fungal taxa detected only in samples from the forest interior, forest edge or reclaimed area. Taxa are ordered by total read number (RN) across all samples. For the reclaimed area and the forest interior, only the 89 most abundant taxa are listed Table S5: Vegetation present at the site, by distance from the forest edge (where negative distance values represent the interior of the undisturbed forest and positive distance values represent the reclaimed area) and by transect (A, B, or C). Table S6: Significance (expressed as p values) of potentially confounding factors in constrained ordinations (by constrained correspondence analyses) of the relative abundance (Rel. abun.) or the presence or absence (Pres./abs.) of fungal taxa in coarse soils, fine soils, the forest floor, roots, or all samples combined. Table S7: Significance (expressed as p values) of potentially confounding factors in constrained ordinations (by redundancy analyses) of the relative abundance (Rel. abun.). Table S8: Nutrient concentrations* in the forest floor and fine soil fractions. The label NM designates cases where nutrient concentrations were not measured due to absence of the sample. Table S9: Fungal taxa in coarse soils, fine soils, and roots that were significantly favored or hindered by phosphorus (P) concentration (marginal permutation test for axis, $p < 0.05$).

Author Contributions: T.R. and T.T. conceived the experiment. T.R., C.M., and B.T. performed field sampling, processed samples, and undertook the culturing study. C.M., P.G. and J.B. prepared samples for sequencing and performed bioinformatics. P.-E.S. performed statistical analyses. T.R., P.-E.S., and T.T wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Office of Energy Research and Development, Natural Resources Canada through Energy Innovation Program projects FF-OS-024 "Science Solutions for Protecting and Restoring Ecological Integrity of Fragmented in-situ Oil Sands Landscapes" and CFS-19-113 "Restoration of Working Landscapes".

Acknowledgments: Funding for this research was provided by Natural Resources Canada. NRCan would like to acknowledge Imperial Oil Resources Limited for site access and safety orientation to Cold Lake Operations.

Site safety supervision was provided by James Kirstein. We thank Beverly Wilson of the Alberta Ministry of Agriculture and Forestry for pre-harvest forest cover information and airphotos of the DEB site. Suggestions from two anonymous reviewers improved the text.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jeffries, P.; Gianinazzi, S.; Perotto, S.; Turnau, K.; Barea, J.-M. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fertil. Soils* **2003**, *37*, 1–16. [CrossRef]
2. Berch, S.M.; Monreal, M.A.; Kernaghan, G. Mycorrhizas in Canadian forest and agricultural ecosystems. In *Advances in Mycorrhizal Science and Technology*; Khasa, D., Piché, Y., Coughlan, A.P., Eds.; NRC Research Press: Ottawa, ON, Canada, 2009; pp. 1–13.
3. Visser, S. Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytol.* **1995**, *129*, 389–401. [CrossRef]
4. Walbert, K.; Ramsfield, T.D.; Ridgway, H.J.; Jones, E.E. Ectomycorrhiza of Pinus radiata (D. Don 1836) in New Zealand—An above- and below-ground assessment. *Australas. Mycol.* **2010**, *29*, 7–16.
5. Peay, K.G.; Schubert, M.G.; Nguyen, N.H.; Bruns, T.D. Measuring ectomycorrhizal fungal dispersal: Macroecological patterns driven by microscopic propagules. *Mol. Ecol.* **2012**, *21*, 4122–4136. [CrossRef] [PubMed]
6. Teste, F.P.; Simard, S.W.; Durall, D.M. Role mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. *Fungal Ecol.* **2009**, *2*, 21–30. [CrossRef]
7. Lilleskov, E.A.; Bruns, T.D. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. *Mycologia* **2005**, *97*, 762–769. [CrossRef] [PubMed]
8. Schickmann, S.; Urban, A.; Kräutler, K.; Nopp-Mayr, U.; Hackländer, K. The interrlelationship of mycophagous small mammals and ectomycorrhizal fungi in primeval, disturbed and managed Central European mountainous forests. *Oecologia* **2012**, *170*, 395–409. [CrossRef]
9. Fleming, L.V. Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings grown around mature trees. *New Phyt.* **1984**, *98*, 143–153. [CrossRef]
10. Province of Alberta, Oil Sands Facts and Statistics. Available online: https://www.alberta.ca/oil-sands-facts-and-statistics.aspx (accessed on 25 March 2020).
11. Johnson, E.A.; Miyanishi, K. Creating new landscapes and ecosystems the Alberta oil sands. *Ann. N. Y. Acad. Sci.* **2008**, *1134*, 120–145. [CrossRef]
12. Jiang, Q.; Thornton, B.; Russel-Houston, J.; Spence, S. Review of thermal recovery technologies for the Clearwater and Lower Grand Rapids formations in Cold Lake, Alberta. *J. Can. Petrol. Technol.* **2010**, *49*, 57–68. [CrossRef]
13. Powter, C.B.; Chymko, N.R.; Dinwoodie, G.; Howat, D.; Janz, A.; Puhlmann, R.; Richens, T.; Watson, D.; Sinton, H.; Ball, J.K.; et al. Regulatory history of Alberta's industrial land conservation and reclamation program. *Can. J. Soil Sci.* **2012**, *92*, 39–51. [CrossRef]
14. Pinno, B.D.; Errington, R.C. Maximizing natural trembling aspen seedling establishment on a reclaimed boreal oil sands site. *Ecol. Restor.* **2015**, *33*, 43–50. [CrossRef]
15. Audet, P.; Pinno, B.D.; Thiffault, E. Reclamation of boreal forest after oil sands mining: Anticipating novel challenges in novel environments. *Can. J. For. Res.* **2014**, *45*, 364–371. [CrossRef]
16. Howell, D.M.; MacKenzie, M.D. Using bioavailable nutrients and microbial dynamics to assess soil type and placement depth in reclamation. *Appl. Soil Ecol.* **2017**, *116*, 87–97. [CrossRef]
17. Das Gupta, S.; Kirby, W.; Pinno, B.D. Effects of stockpiling and organic matter addition on nutrient bioavailability in reclamation soils. *Soil Sci. Soc. Am. J.* **2019**, *83*, S27–S41.
18. Dhar, A.; Comeau, P.G.; Vassov, R. Effects of cover soil stockpiling on plant community development following reclamation of oil sands sites in Alberta. *Restor. Ecol.* **2019**, *27*, 352–360. [CrossRef]
19. deBortoli, L.A.; Pinno, B.D.; MacKenzie, M.D.; Li, E.H.Y. Plant community composition and tree seedling establishment in response to seeding and weeding treatments on different reclamation cover soils. *Can. J. For. Res.* **2019**, *49*, 836–843. [CrossRef]

20. Tremblay, P.-Y.; Thiffault, E.; Pinno, B.D. Effects of land reclamation practices on the productivity of young trembling aspen and white spruce on a reclaimed oil sands mining site in northern Alberta. *New For.* **2019**, *50*, 911–942. [CrossRef]
21. Bois, G.; Piché, Y.; Fung, M.Y.P.; Khasa, D.P. Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry. *Mycorrhiza* **2005**, *15*, 149–158. [CrossRef]
22. Bois, G.; Coughlan, A.P. Ectomycorrhizal inoculation for boreal forest ecosystem restoration following oil sand extraction: The need for an initial three-step screening process. In *Advances in Mycorrhizal Science and Technology*; Khasa, D., Piché, Y., Coughlan, A.P., Eds.; NRC Research Press: Ottawa, ON, Canada, 2009; pp. 129–137.
23. Neuenkamp, L.; Prober, S.M.; Price, J.N.; Zobel, M.; Standish, R.J. Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. *Fungal Ecol.* **2019**, *40*, 140–149. [CrossRef]
24. Danielson, R.M.; Visser, S.; Parkinson, D. Microbial activity and mycorrhizal potential of four overburden types used in the reclamation of extracted oil sands. *Can. J. Soil Sci.* **1983**, *63*, 363–375. [CrossRef]
25. Outerbridge, R.A.; Trofymow, J.A. Diversity of ectomycorrhizae on experimentally planted Douglas-fir seedlings in variable retention forestry sites on southern Vancouver Island. *Can. J. Bot.* **2004**, *82*, 1671–1681. [CrossRef]
26. Trofymow, J.A.; Shay, P.-E.; Myrholm, C.L.; Tomm, B.; Bérubé, J.A.; Ramsfield, T.D. Fungi associated with tree species at an Alberta oil sands reclamation area, as determined by sporocarp assessments and high-throughput DNA sequencing. *Appl. Soil Ecol.* **2020**, *147*, 103359. [CrossRef]
27. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes—Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef]
28. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M., Gelfand, D., Shinsky, J., White, T., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.
29. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
30. Reeder, J.; Knight, R. The 'rare biosphere': A reality check. *Nat. Methods* **2009**, *6*, 636. [CrossRef]
31. Huse, S.M.; Welch, D.M.; Morrison, H.G.; Sogin, M.L. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ. Microbiol.* **2010**, *12*, 1889–1898. [CrossRef]
32. Kunin, V.; Engelbrektson, A.; Ochman, H.; Hugenholtz, P. Wrinkles in the rare biosphere: Pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ. Microbiol.* **2010**, *12*, 118–123. [CrossRef]
33. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef]
34. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011**, *27*, 2194–2200. [CrossRef]
35. Nilsson, R.H.; Kristiansson, E.; Ryberg, M.; Hallenberg, N.; Larsson, K.-H. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evol. Bioinform. Online* **2008**, *4*, 193–201. [CrossRef] [PubMed]
36. Schoch, C.L.; Seifert, K.A.; Huhndorf, S.; Robert, V.; Spouge, J.L.; Levesque, C.A.; Chen, W. Fungal Barcoding Consortium, Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6241–6246. [CrossRef]
37. Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Madden, T.L. BLAST+: Architecture and applications. *BMC Bioinform.* **2009**, *10*, 421. [CrossRef] [PubMed]
38. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Villarreal Ruiz, L.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef]
39. Bérubé, J.A.; Gagné, P.N.; Ponchart, J.P.; Tremblay, É.D.; Bilodeau, G.J. Detection of *Diplodia corticola* spores in Ontario and Québec based on High Throughput Sequencing (HTS) methods. *Can. J. Plant. Pathol.* **2018**, *40*, 378–386. [CrossRef]

40. Masella, A.P.; Bartram, A.K.; Truszkowski, J.M.; Brown, D.G.; Neufeld, J.D. PANDAseq: Paired-end assembler for Illumina sequences. *BMC Bioinform.* **2012**, *13*, 31. [CrossRef] [PubMed]
41. Gagné, P.N.; Bérubé, J.A. Illumicut, a C++ Program Specifically Designed to Efficiently Detect and Remove Forward and Revers Sequencing Primers in Paired-End Reconstructed Sequences. 2017. Available online: https://github.com/Patg13/Illumicut (accessed on 14 February 2020).
42. Gagné, P.N.; Bérubé, J.A. HomopRemover, a Program Designed to Efficiently Remove Sequences Containing Very Long Homopolymers. 2017. Available online: https://github.com/Patg13/HomopRemover (accessed on 14 February 2020).
43. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* **2016**, *4*, e2584. [CrossRef]
44. Kalra, Y.P.; Maynard, D.G. *Methods Manual for Forest Soil and Plant Analysis*; Information Report NOR-X-319E; Forestry Canada, Northwest Region, Northern Forestry Centre: Edmonton, AB, Canada, 1991.
45. R Core Team. *R: A Language and Environment for Statistical Computing*; Version 3.5.1; R Foundation for Statistical Computing: Vienna, Austria, 2017.
46. Chiu, C.H.; Wang, Y.T.; Walther, B.A.; Chao, A.N. An improved nonparametric lower bound of species richness via a modified Good-Turing frequency formula. *Biometrics* **2014**, *70*, 671–682. [CrossRef]
47. O'Hara, R.B. Species richness estimators: How many species can dance on the head of a pin? *J. Anim. Ecol.* **2005**, *74*, 375–386. [CrossRef]
48. McMurdie, P.J.; Holmes, S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. Version 1.24.2. *PLoS ONE* **2013**, *8*, 11. [CrossRef]
49. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H.; et al. Vegan: Community Ecology Package, R Package, Version 2.4-5. 2017. Available online: https://CRAN.R-project.org/package=vegan (accessed on 14 February 2020).
50. Legendre, P.; Birks, H.J.B. From classical to canonical ordination. In *Tracking Environmental Change Using Lake Sediments, Volume 5: Data Handling and Numerical Techniques*; Birks, H.J.B., Lotter, A.F., Juggins, S., Smol, J.P., Eds.; Springer: Dordrecht, The Netherlands, 2012; pp. 201–248.
51. Borcard, D.; Legendre, P.; Avois-Jacquet, C.; Tuomisto, H. Dissecting the spatial structure of ecological data at multiple scales. *Ecology* **2004**, *85*, 1826–1832. [CrossRef]
52. Godínez-Domínguez, E.; Freire, J. Information-theoretic approach for selection of spatial and temporal models of community organization. *Mar. Ecol. Prog. Ser.* **2003**, *253*, 17–24. [CrossRef]
53. Fagan, W.F.; Cantrell, R.S.; Cosner, C. How habitat edges change species interactions. *Am. Nat.* **1999**, *153*, 165–182. [CrossRef] [PubMed]
54. Ries, L.; Fletcher, R.J.; Battin, J.; Sisk, T.D. Ecological responses to habitat edges: Mechanisms, models, and variability explained. *Annu. Rev. Ecol. Evol. Syst.* **2004**, *35*, 491–522. [CrossRef]
55. Harper, K.A.; MacDonald, S.E.; Burton, P.J.; Chen, J.; Brosofske, K.D.; Saunders, S.C.; Euskirchen, E.S.; Roberts, D.; Jaiteh, M.S.; Esseen, P.-E. Edge influence on forest structure and composition in fragmented landscapes. *Conserv. Biol.* **2005**, *19*, 768–782. [CrossRef]
56. Green, J.L.; Holmes, A.J.; Westoby, M.; Briscoe, O.I.; Dangerfield, M.; Gillings, M.; Geattie, A.J. Spatial scaling of microbial eukaryote diversity. *Nature* **2004**, *432*, 747–750. [CrossRef]
57. Martiny, J.B.H.; Bohannan, B.J.M.; Brown, J.H.; Colwell, R.K.; Fuhrman, J.A.; Green, J.L.; Horner-Devine, M.C.; Kane, M.; Adams Krumins, J.; Kuske, C.R.; et al. Microbial biogeography: Putting microorganisms on the map. *Nat. Rev. Microbiol.* **2006**, *4*, 102–112. [CrossRef]
58. Ramette, A.; Tiedje, J.M. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2761–2766. [CrossRef]
59. Shay, P.E.; Winder, R.S.; Trofymow, J.A. Nutrient-cycling microbes in coastal Douglas-fir forests: Regional-scale correlation between communities, in situ climate, and other factors. *Front. Microbiol.* **2015**, *6*, 13. [CrossRef]
60. Nacke, H.; Goldmann, K.; Schöning, I.; Pfeiffer, B.; Kaiser, K.; Castillo-Villamizar, G.A.; Schrumph, M.; Buscot, F.; Daniel, R.; Wubet, T. Fine spatial scale variation of soil microbial communities under European Beech and Norway Spruce. *Front. Microbiol.* **2016**, *7*, 2067. [CrossRef]
61. Anderson, I.C.; Cairney, J.W.G. Ectomycorrhizal fungi: Exploring the mycelial frontier. *FEMS Microbiol. Rev.* **2007**, *31*, 388–406. [CrossRef] [PubMed]

62. Agerer, R. Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **2001**, *11*, 107–114. [CrossRef]
63. Outerbridge, R.A.; Trofymow, J.A. Forest management and maintenance of ectomycorrhizae: A case study of green tree retention in south-coastal British Columbia. *BC J. Ecosyst. Manag.* **2009**, *10*, 59–80.
64. Burgess, T.; Dell, B.; Malajczuk, N. Variation in mycorrhizal development and growth stimulation by 20 *Pisolithus* isolates inoculated on to *Eucalyptus grandis* W. Hill ex Maiden. *New Phytol.* **1994**, *127*, 731–739. [CrossRef]
65. Schweitzer, J.A.; Bailey, J.K.; Fischer, D.G.; LeRoy, C.J.; Lonsdorf, E.V.; Whitham, T.G.; Hart, S.C. Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology* **2008**, *89*, 773–781. [CrossRef] [PubMed]
66. Hoeksema, J.D.; Piculell, B.J.; Thompson, J.N. Within-population genetic variability in mycorrhizal interactions. *Commun. Integr. Biol.* **2009**, *2*, 110–112. [CrossRef]
67. Opelt, K.; Chobot, V.; Hadacek, F.; Schonmann, S.; Eberl, L.; Berg, G. Investigations of the structure and function of bacterial communities associated with Sphagnum mosses. *Environ. Microbiol.* **2007**, *9*, 2795–2809. [CrossRef]
68. Moltzan, B.D.; Blenis, P.V.; Hiratsuka, Y. Temporal occurrence and impact of *Scytalidium uredinicola*, a mycoparasite of western gall rust. *Can. J. Plant Pathol.* **2001**, *23*, 384–390. [CrossRef]
69. Brimner, T.A.; Boland, G.J. A review of the non-target effects of fungi used to biologically control plant diseases. *Agric. Ecosyst. Environ.* **2003**, *100*, 3–16. [CrossRef]
70. Kaewchai, S.; Soytong, K.; Hyde, K. Mycofungicides and fungal biofertilizers. *Fungal Divers* **2009**, *38*, 25–50.
71. Jumpponen, A.; Trappe, J.M. Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. *New Phytol.* **1998**, *140*, 295–310. [CrossRef]
72. Booth, M.S.; Stark, J.M.; Rastetter, E. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.* **2005**, *75*, 139–157. [CrossRef]
73. Belser, L.W. Population ecology of nitrifying bacteria. *Annu. Rev. Microbiol.* **1979**, *33*, 309–333. [CrossRef]
74. Schimel, J.; Bennett, J. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* **2004**, *85*, 591–602. [CrossRef]
75. Kranabetter, J.M.; Durall, D.M.; MacKenzie, W.H. Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. *Mycorrhiza* **2009**, *19*, 99–111. [CrossRef] [PubMed]
76. Mills, K.E.; Bever, J.D. Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. *Ecology* **1998**, *79*, 1595–1601. [CrossRef]
77. Packer, A.; Clay, K. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* **2000**, *404*, 278. [CrossRef]
78. Hudson, P.J.; Dobson, A.P.; Lafferty, K.D. Is a healthy ecosystem one that is rich in parasites? *Trends Ecol. Evol.* **2006**, *21*, 381–385. [CrossRef]
79. Schmidt, P.-A.; Bálint, M.; Greshake, B.; Bandow, C.; Römbke, J.; Schmitt, I. Illumina metabarcoding of a soil fungal community. *Soil Biol. Biochem.* **2013**, *65*, 128–132. [CrossRef]