**Partial Retention of Legacy Trees Protect Mycorrhizal Inoculum Potential, Biodiversity, and Soil Resources While Promoting Natural Regeneration of Interior Douglas-Fir**

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Clearcutting reduces proximity to seed sources and mycorrhizal inoculum potential for regenerating seedlings. Partial retention of legacy trees and protection of refuge plants, as well as preservation of the

 Partial retention of legacy trees and protection of refuge plants, as well as preservation of the forest floor, can maintain mycorrhizal networks that colonize germinants and improve nutrient supply. However, little is known of overstory retention levels that best protect mycorrhizal inoculum while also providing sufficient light and soil resources for seedling establishment. To quantify the effect of tree retention on seedling regeneration, refuge plants, and resource availability, we compared five harvesting methods with increasing retention of overstory trees (clearcutting (0% retention), seed tree (10% retention), 30% patch retention, 60% patch retention, and 100% retention in uncut controls) in an interior Douglas-fir-dominated forest in British Columbia. Regeneration increased with proximity to legacy trees in partially cut forests, with increasing densities of interior Douglas-fir, western redcedar, grand fir, and western hemlock seedlings with overstory tree retention. Clearcutting reduced cover of ectomycorrhizal refuge plants (from 80 to 5%) while promoting arbuscular mycorrhizal plants the year after harvest. Richness of shrubs, herbs, and mosses declined with increasing harvesting intensity, but tree richness remained at control levels. The presence of legacy trees in all partially cut treatments mitigated these losses. Light availability declined with increasing overstory cover and proximity to leave trees, but it still exceeded 1,000 W m−2 in the clearcut, seed tree and 30% retention treatments. Increasing harvesting intensity reduced aboveground and belowground C stocks, particularly in live trees and the forest floor, although forest floor losses were also substantial where thinning took place in the 60% retention treatment. The loss of forest floor carbon, along with understory plant richness with intense harvesting was likely associated with a loss of ectomycorrhizal inoculum potential. This study suggests that dispersed retention of overstory trees where seed trees are spaced ~10–20 m apart, and aggregated retention where openings are <60 m (2 tree-lengths) in width, will result in an optimal balance of seed source proximity, inoculum potential, and resource availability where seedling regeneration, plant biodiversity, and carbon stocks are protected.

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**The Mother Tree Project**

**Introduction**

Clearcut harvesting, or removal of all trees from forests, reduces mycorrhizal inoculum ([Perry et al., 1987](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B111); [Jones et al., 2003](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B79); [Cline et al., 2007](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B37)) because the symbiotic fungi are dependent on living trees and plants for photosynthate ([Smith and Read, 2008](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B129)). Without a supply of carbon from their hosts, mycorrhizal fungi cannot grow a mycelial network to explore the soil for nutrients and water, and in turn supply them to their partner trees and plants ([Smith and Read, 2008](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B129)). Low levels of mycorrhizal inoculum can be especially problematic for seedlings regenerating in clearcut forests, where early colonization with mycorrhizal fungi is crucial to survival and growth of germinants ([Barker et al., 2013](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B11)). In temperate forests, where most trees form obligate associations with ectomycorrhizal fungi, species composition of the fungal community also changes with clearcutting, where there is a shift toward a low diversity of “early stage” fungi that have small carbon demands and generalist strategies for nutrient acquisition. This simplification of the fungal community is related to the change in host plant species composition and age, along with losses or redistribution of the forest floor by logging and site preparation, as well as modification of soil physicochemical properties and changes in the soil foodweb ([Jones et al., 2003](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B79)). The loss of fungal biodiversity can lead to reduced nutrient and water uptake and resilience of regenerating forests ([Tomao et al., 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B140)).

Along with reductions in mycorrhizal fungal inoculum, clearcutting also reduces host plant diversity as well as carbon and nutrient stocks ([Halpern and Spies, 1995](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B67); [Johnson and Curtis, 2001](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B78); [Zhou et al., 2013](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B151); [Hume et al., 2017](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B74); [Buotte et al., 2019](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B29); [Ding et al., 2019](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B47); [Curzon et al., 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B43)). Clearcutting effects on C stocks result directly from removal of trees and damage to the forest floor and understory plants ([Simard et al., 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B128)), but also indirectly through loss of mycorrhizal fungi, which are the primary vectors of plant carbon into the soil ([Talbot et al., 2008](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B134)). Extensive mechanized clearcutting is of major concern globally because forests store 80% of terrestrial carbon and are home to more than three-quarters of the world's vertebrate and invertebrate species ([Lindenmayer and Franklin, 2002](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B94); [FAO, 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B52)), and failures to regenerate the clearcuts can create persistent deficits in these ecosystem goods and services. Clearcutting and regeneration of the world's most productive forests are of particular concern because these forests have the greatest biodiversity and C storage ([Thompson et al., 2012](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B138); [Liang et al., 2016](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B92); [Lecina-Diaz et al., 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B88); [Buotte et al., 2019](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B29)).

Partial cutting, or variable retention harvesting, has become more commonplace in western Canada and the Pacific Northwest of the USA in response to the negative ecological outcomes of extensive clearcutting and forest simplification, as well as the intensely negative effects these have had on public perceptions of forest management practices ([Beese et al., 2019](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B14); [Franklin and Donato, 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B56)). Variable retention harvesting aims to mimic natural disturbances and processes that provide the structural heterogeneity and ecosystem functions associated with mature and old growth forests ([Franklin and Donato, 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B56)). Legacies, including trees, plants, snags, downed wood, understory plants or forest floor that survive the disturbance, provide continuity in gene pools and sustain complexity while influencing succession and promoting biodiversity, recovery, and resilience ([Michel and Winter, 2009](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B99); [Lindenmayer et al., 2012](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B93); [Baker et al., 2013](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B8)).

Compared with clearcutting, retention of a portion of legacy trees during harvest can mitigate losses in fungal inoculum, host plant diversity and C pools, and in so doing, promote seedling regeneration and forest recovery. Partial cutting involves removing individual trees or groups of trees from the forest in different intensities and patterns for various objectives. These can include provision of a seed source or shelter for natural regeneration, protection of plants, trees and forest floor that support mycorrhizal fungi and soil foodwebs, particularly where custom hand falling instead of feller-bunchers are used, enhancement of growth of the remaining trees, and improvement in habitat for forest dwellers ([Kneeshaw et al., 2002](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B81); [Lindo and Visser, 2003](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B95); [Hannam et al., 2006](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B68)). Natural regeneration of partially cut forests rather than planting of clearcuts is attractive not only due to lower costs, but because the recovering forests are often more species rich and the seedlings and mycorrhizal symbionts are better adapted to local conditions ([Swanson et al., 2011](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B133); [Kranabetter et al., 2015](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B83)). Seedlings can be rapidly colonized by a diversity of fungi when they germinate in close proximity to the residual trees, whose ectomycorrhizal roots and hyphal networks contact and infect the fledgling germinant roots.

The Mother Tree Project was established in British Columbia to compare recovery of the interior Douglas-fir forests, including seedling regeneration, plant diversity, and carbon pools, following clearcutting and partial cutting practices ([Simard et al., 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B128)). Five harvesting intensity treatments were applied in a highly productive forest that retained Douglas-fir mother trees in different densities and configurations. These included: (1) clearcutting, where none of the trees were retained, (2) seed tree retention, where about 10% were retained in a dispersed pattern to provide seed and mycorrhizal fungal inoculum, (3) 30% patch retention, where small patches of trees were retained to provide seed and fungal inoculum for regeneration in the surrounding open gaps, (4) 60% patch retention with thinning from below, where seedlings could establish under the protection and within the networks of the old trees, and (5) control, where the forests were left to develop in the absence of logging disturbance.

We tested the following hypotheses 3 years following the harvest of this experiment. (1) Total ingress of natural regeneration will increase with overstory tree retention. (2) Douglas-fir regeneration will increase with proximity to legacy trees. (3) Diversity and richness of natural regeneration will be greatest at intermediate retention levels because of the environmental heterogeneity. (4) Retention of legacy trees will protect plant richness and diversity, whereas clearcutting will reduce ectomycorrhizal shrubs and promote dominance of arbuscular mycorrhizal pioneers. (5) Light and soil water availability will decline but forest floor substrate will be better protected with increasing tree retention. (6) Carbon pools, particularly live trees, understory plants, and forest floor, will decline with increasing harvesting intensity. In testing these hypotheses, we sought to determine the tree retention levels at which natural regeneration densities are balanced with resource availability and mycorrhizal fungal inoculum potential (i.e., the potential for the mycorrhizal fungal inoculum of refuge plants to colonize the root tips of regenerating seedlings).

**Materials and Methods**

**Study Area**

The study took place at an interior Douglas-fir-dominated forest situated near the town of Nelson in British Columbia (B.C.), Canada (49.63°N, 117.03°W). The site is located within the West Kootenay Dry Warm Interior Cedar-Hemlock (ICHdw1) biogeoclimatic variant, with mean annual temperature (MAT) of 6.8°C and mean annual precipitation (MAP) of 868 mm. The climate is continental with warm, dry summers and cool, snowy winters.

At the onset of the study, the forest was 116 years old and Douglas-fir made up 61% of the basal area. Other common tree species were western red cedar [*Thuja plicata* (Donn ex D Don], western larch [*Larix occidentalis* (Nutt.)], western hemlock, [*Tsuga heterophylla* (Raf.) Sarg.], grand fir (*Abies grandis* Dougl. ex D. Don Lindl.), western white pine (*Pinus monticola* Dougl. ex D. Don), ponderosa pine (*Pinus ponderosa* Dougl. Ex P. & C. Laws) and paper birch (*Betula papyrifera* Marsh.). Dominant understory shrubs were beaked hazelnut (*Corylus cornuta*), Douglas maple (*Acer glabrum*), and prickly rose (*Rosa acicularis*), and the major herb species were hooker's fairbells (*Disporum hookeri)*, heart-leaved arnica (*Arnica cordifolia*), Queen's cup (*Clintonia uniflora*), and prince's pine (*Chimaphila umbellata*).

The site had a medium soil moisture regime, southeast aspect, 10–30% slope gradient and occupied a mid-slope position. Elevation averaged 850 m. Soils were predominantly Humo-ferric Podzols with a sandy loam texture, and humus forms were Moders ([Green et al., 1993](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B61); [Soil Classification Working Group, 1998](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B130)). The biogeoclimatic site series were ICHdw1-101 and -104.

**Harvesting Treatments**

Treatments were laid out in a completely randomized design with five harvesting intensity treatments replicated 4 times (total 20 treatment units). Harvesting treatments were randomly assigned to 5 ha treatment units and logging was conducted prior to spring green-up in 2017. The goal of the harvesting treatments was to create a gradient of legacy tree densities where large Douglas-fir were targeted for retention, but alternate species were also selected where Douglas-fir was absent. The treatments included: (i) clearcut (no tree retention); (ii) single tree retention (retention of 25 large Douglas-fir stems ha−1 at 25 m spacing, or ~10% dispersed retention); (iii) small patch retention (retention of 30% of the stand area in 30-m wide unconnected patches, with all trees cut in the remaining 70% of the stand); (iv) high retention (retention of 60% of the stand area with all trees cut in the remaining 40% of the stand); and (v) uncut control (100% retention). Harvesting was carried out using feller-bunchers run along random skid trails, but trees too large for the machine were hand-felled. The stands in the high retention treatment were thinned from below by reaching into the uncut patches with the feller-buncher and removing the smaller stems. Trees were limbed and topped before the boles were skidded to roadside landings.

**Measurement and Sampling Methods**

A one-hectare measurement plot was established in the center of each treatment unit, in which all measurements and sampling were conducted. Inside the measurement plot, a nine X nine grid of 81.10 m2 (*r* = 1.78 m) subplots were established in which natural regeneration and environmental conditions were assessed 3 years after treatment. All seedlings that had regenerated since harvesting (i.e., aged 3 years or less) were counted by species, the average height by regenerating species measured, distance to the nearest Douglas-fir seed tree determined, and percent cover of the overstory trees and each regeneration substrate [mineral, organic, highly decayed wood (decay classes 4 and 5; [British Columbia Ministry of Forests Range British Columbia Ministry of Environment, 2010](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B24)), or recently decayed wood (decay classes 1, 2, and 3), hereafter referred to as “fresh wood”] visually estimated. The distance to the nearest Douglas-fir seed tree was not necessarily related to percent cover of the overstory trees, because the overstory contained a mix of species and ages, with Douglas-fir seed trees not always present where the overstory was dense. In the center of 10 randomly selected subplots per treatment unit, photosynthetically active radiation (PAR) and volumetric water content were also evaluated. PAR was measured in the four cardinal directions using a Sunfleck PAR Ceptometer (Model SF-80, Decagon Devices, Pullman, WA) and averaged. Volumetric water content was measured in mineral soil to 15 cm depth using a Dynamax ML2 Theta Probe (Delta-T Devices Ltd.), which measures changes to the dielectric constant of the soil as a proxy for volumetric soil water content (m3/m3) ([Delta-T Devices Ltd, 1999](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B45)). Volumetric water content and PAR were measured over a 2-day period in late August, following 2 weeks of warm, dry weather.

Carbon and plant diversity data were collected in one central permanent circular 0.04 ha (*r* = 11.28 m) National Forest Inventory (NFI) plot per treatment unit in July/August prior to logging, and these plots were re-assessed both one and three summers following harvesting. Data collection followed the NFI ground sampling guidelines ([Canadian Forest Inventory Committee Canadian Forest Service, 2008](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B30)) as follows. All live and dead standing trees >9 cm DBH and >1.3 m height within the plot boundary were tagged at the base and species, diameter at breast height (1.3 m), height, crown length, and condition were recorded. Nested within each 0.04 ha plot was one circular 50 m2 subplot (*r* = 3.99 m) in which assessments were made of density, diameter and height of live and dead trees and shrubs <9 cm DBH and >1.3 m tall, and height, diameter and decay class of stumps >4 cm in diameter and <1.3 tall. Visual assessments were made of percent cover of all live tree and shrub species occurring within a 314 m2 subplot (*r* = 10.0 m) and of all herbaceous, ground-level shrub and bryoid species occurring within a 100 m2 (*r* = 5.64 m) subplot. Each plant species was identified by its known dominant mycorrhizal functional group (ectomycorrhizal, or arbuscular, arbutoid, orchid, and ericoid mycorrhizal). Coarse woody debris (CWD) (>7.5 cm diameter) was measured along two perpendicular 30-m line transects and small woody debris (SWD) (1.1–7.5 cm diameter) pieces were counted by size class along 10 m of each transect. Ground substrate type and depth of organic and decayed wood were recorded every 2 m along each CWD transect (total 30 stations per NFI plot).

Samples were collected for laboratory analysis at one circular 1 m2 (*r* = 0.56 m) microplot at each end of the CWD transects (four microplots per NFI plot). Post-logging microplots were established 1.5 m clockwise on the 0.04 ha boundary from the previous microplot locations. All bryoids, herbs, trees/shrubs <1.3 m in height, and fine woody debris (FWD) (<1 cm diameter) occurring within the microplots were collected separately. A 20 × 20 cm area of forest floor extending from the ground surface to the underlying substrate was measured for thickness and collected. Mineral soil, including rocks <7.5 cm diameter and roots, was collected from all four microplots in 10-cm diameter holes at 0–15 cm depth. The excavated holes were lined with plastic and the volume of water that filled the hole was measured with a graduated cylinder for use in calculating bulk density ([Walter et al., 2016](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B144); [Al-Shammary et al., 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B3)). Cobbles (rocks >7.5 cm across) were weighed in the field and then discarded.

Site data was collected to describe the ecological properties of each NFI plot. A soil pit was dug to >60 cm depth, mineral soil horizons were delineated, and their depth, texture, and coarse fragment content were estimated. Mineral soil and forest floor were classified at least to Order ([Green et al., 1993](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B61); [Soil Classification Working Group, 1998](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B130)). The biogeoclimatic variant of each NFI plot was determined from field maps ([British Columbia Ministry of Forests Lands, 2019](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B23)) and soil moisture regime, nutrient status and site series were estimated using vegetation, soil, and site features ([Braumandl and Curran, 1992](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B20)).

**Laboratory Analysis**

The plant and FWD samples were oven dried and weighed then discarded. The mineral soil samples were oven dried, sieved and separated into roots, particles <2 mm, and gravel (2–7.5 cm) components and each fraction was weighed. The forest floor samples were oven dried, roots, gravel, and charcoal removed and weighed, then sieved into <8 mm and >8 fractions, which were also weighed. All oven drying was done at 70°C for 72 h. Subsamples of each <2 mm mineral and <8 mm forest floor fraction were sent to the Ministry of Environment laboratory in Victoria, B.C. for determination of C and nitrogen (N) concentrations.

**Data Analysis**

Descriptive statistics for C storage in each major pool and the biodiversity components were calculated for each harvesting intensity treatment in each of the climatic regions using the four replicate treatment units per treatment. Carbon storage (Mg ha−1) in aboveground dead and live trees >1.3 m tall, stumps (<1.3 m tall), downed coarse, small, and fine woody debris, understory plants, forest floor, and mineral soil were calculated according to the Canadian Forest Service (CFS) protocol. Root biomass of live trees was calculated as aboveground tree biomass multiplied by 0.29 or 0.20 for coniferous forests with an aboveground biomass of 50-150 Mg ha−1 or >150 Mg ha−1, respectively, as suggested in Table 4.4 ([IPCC, 2006](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B75)). Root biomass of each dead tree was calculated as the root biomass when the tree was alive minus the fine root proportion of total root biomass (P), where *P* = 0.072 + (0.354)\*(e\*\*-0.060RB) where RB = total root biomass ([Li et al., 2003](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B91)). Based on visual characteristics such as lack of needles and sloughing bark, all dead trees were assumed to have died several years prior to their measurement, in which case all fine roots would be dead, based on a fine root turnover rate of 64.1% per year ([Kurz et al., 2009](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B86)). Root biomass of trees cut during harvesting was calculated as root biomass when the tree was alive minus the dead fine roots (0.641\*P). Biodiversity of trees and plants were estimated as: (i) richness (the number of species occurring in a 0.0314 ha plot for trees and shrubs, and in a 0.01 ha plot for herbs and bryoids), and (ii) Shannon's diversity index ([Shannon and Weaver, 1949](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B122)), calculated as *H*′ = 1 –∑ [*p*i ln(*p*i)] where *H*′ is Shannon's index and *p*i is the proportion of total density of species *i*. For the regeneration and resource availability measures, the subplot measurements were averaged for each treatment unit, and these means were used to compare the parameters among the harvesting intensity treatments.

Statistical analyses were conducted in Excel and results were considered statistically significant at *P* < 0.05. We investigated how natural regeneration, plant diversity, mycorrhizal functional groups, water and light availability, and carbon stocks responded to legacy tree retention [0% retention (clearcut), 10% retention (seed tree), 30% retention (small patch), 60% retention (high retention), and 100% retention (control)] using one-way ANOVA or, where responses were measured more than once, ANCOVA (*n* = 4). The general form of the ANCOVA model was:

Yi=μ+τi+βi(Xi−χ)+εiYi=μ+τi+βi(Xi-χ)+εi

where *Yi* is the response variable; μ is the general mean; τ*i* is the fixed effect parameter for the *i*th harvesting intensity treatment; β*i* is the estimated coefficient, *Xi* is the covariate, and ε*i* is the residual ([Steel and Torrie, 1980](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B131)). Means were compared using the Tukey–Kramer *post-hoc* test. Linear relationships between variables were measured using the Pearson product-moment correlation coefficient.

**Results**

Three years after harvesting, total natural regeneration density was lowest in the clearcuts and generally increased with overstory tree retention to where it was greatest in the uncut controls, with the exception of the 30% patch retention treatment where the harvested gaps behaved similarly to the clearcuts ([Figure 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F1), [Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1)). The trends were species-specific, however, where regeneration densities of coniferous species (interior Douglas-fir, western hemlock, western redcedar, and grand fir) tended to be greatest in the 60 and 100% (control) retention treatments, whereas those of broadleaf tree species (paper birch, black cottonwood [*Populus balsamifera* ssp. *trichocarpa* (Torr. Et A. Gray) Brayshaw] and trembling aspen (*Populus tremuloides* Michx.) tended to be greatest in the 0% (clearcut) and 10% (seed tree) retention treatments ([Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1)). Height of interior Douglas-fir seedlings significantly declined with increasing overstory tree retention, whereas western redcedar, western hemlock and grand fir tended to be tallest in the 30 or 60% retention treatments ([Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1)). Although the density of broadleaf tree regeneration was less than that of conifer seedlings, the broadleaves were substantially taller. Species richness of seedling regeneration did not significantly differ among the tree retention treatments ([Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1)).

**FIGURE 1**



**Figure 1**. Natural regeneration density by tree species across the legacy tree retention treatment gradient in the ICHdwl variant at Redfish Creek (*n* = 4). ANOVA test statistics are shown in [Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1).

**TABLE 1**



**Table 1**. Effect of tree retention (mean ± one standard error) on overstory cover, photosynthetically active radiation (PAR), volumetric water content, density, height, and species richness of natural regeneration, and cover of seedbed substrates in the ICHdw1 variant at Redfish Creek (*n* = 4).

Douglas-fir regeneration density declined with distance from the nearest Douglas-fir seed tree, with the greatest densities within 10 m and declining by more than half distally ([Figure 2](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F2)). Regeneration density continued to decline from 20 to 60 m, beyond which germinants were rare. Germinant density of Douglas-fir was unrelated to cover of the substrates except that it was negatively affected by fresh wood (*r* = −0.16), which was most abundant in the low tree retention treatments. Density of grand fir generally increased with organic substrate cover (*r* = 0.15) and decreased with fresh wood (−0.15), while western hemlock tended to regenerate more densely on highly decayed wood (*r* = 0.15). Density of western redcedar germinants was equal among substrates.

**FIGURE 2**



**Figure 2**. Density of interior Douglas-fir natural regeneration with distance to nearest Douglas-fir seed tree, expressed as **(A)** averages of subplots in 5 m distance classes, and **(B)** raw subplot data (*n* = l,210 subplots) in the ICHdwl variant at Redfish Creek. In **(B)**, the large number of subplots with lower densities, bringing down the averages shown in **(A)**, is obscured by overlapping data points.

Species richness of bryoid, herb and shrub functional groups 3 years after harvesting was lowest in clearcuts, and generally increased with overstory tree retention ([Figure 3A](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F3), [Table 2](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T2)). Diversity of bryoids and herbs also tended to be lower in clearcuts, but differences between treatments were not significant ([Figure 3B](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F3), [Table 2](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T2)). Tree species richness and diversity varied little by harvesting treatment after 3 years when all ages of trees, including germinants, were included in the functional group ([Table 2](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T2)).

**FIGURE 3**



**Figure 3**. Plant species **(A)** richness and **(B)** diversity of the tree, shrub, herb, and bryoid functional groups in the tree retention treatments before and 1 year after harvesting in the ICHdwl variant at Redfish Creek (*n* = 4). ANCOVA test statistics are shown in [Table 2](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T2).

**TABLE 2**



**Table 2**. Effect of tree retention (mean ± one standard error) on Shannon's Diversity Index and species richness in the bryoid, herb, shrub, and tree functional groups 1 year after harvesting in the ICHdw1 variant at Redfish Creek.

Prior to harvesting, the cover of plant species that were ectomycorrhizal averaged 80.6%, followed by arbuscular (45.2%), arbutoid (3.7%), orchid (0.9%), and ericoid (0.1%) mycorrhizal ([Figure 4](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F4)). One year post-harvest, cover of plants declined with harvesting in all tree retention treatments, but the most pro-found impact occurred in the clearcuts, where cover of ectomycorrhizal plants declined to 6.1%, or less than half the cover of arbuscular mycorrhizal plants (13.4%). Arbuscular mycorrhizal plants were also more prolific (42.0%) than ectomycorrhizal plants (31.2%) in the 30% patch retention treatment 1 year-post harvest, whereas ectomycorrhizal plants remained dominant in the seed tree, 60% retention and control treatments. By the third year post-treatment, ectomycorrhizal plants had recovered to 24.1% cover in the clearcuts and 28.6–40.7% in the higher retention treatments, and surpassed cover of arbuscular mycorrhizal plants in most cases. Cover of arbutoid, orchid and ericoid plants declined in all of the harvesting treatments, but ericoid plants recovered to pretreatment levels in the 60% retention treatment within 3 years.

**FIGURE 4**



**Figure 4**. Percent cover of plants by mycorrhizal functional group across the tree retention treatments before and 1- and 3-years after the harvesting treatments were applied.

Photosynthetically active radiation significantly increased from 166 W m−2 in the uncut control to over 1,000 W m−2 in the clearcut, seed tree, and 30% patch retention treatments 3 years after harvesting ([Figure 5A](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F5), [Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1)). Light availability increased exponentially with declining overstory cover, with levels exceeding 1,000 W m−2 below 30% cover ([Figure 5B](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F5)). Volumetric water content tended to be greatest in clearcuts, but differences among treatments were not significant and values were low in all treatments ([Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1)).

**FIGURE 5**



**Figure 5**. Photosynthetically active radiation across the **(A)** legacy tree retention treatments and **(B)** tree cover gradient in the ICHdwl variant at Redfish Creek 3 years after treatment (*n* = 4). ANOVA test statistics for **(A)** are shown in [Table 1](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T1).

Total ecosystem C stocks 1 year after harvesting averaged 242.6 ± 43.3 Mg ha−1 in the uncut controls, with 168.5 ± 37.5 Mg ha−1 aboveground and 74.1 ± 5.9 Mg ha−1 belowground (69 and 31%, respectively) ([Figure 6](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F6), [Table 3](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T3)). The average portion in each pool was live trees (49.3%), dead trees (11.2%), coarse woody debris (7.0%), small and fine woody debris (1.7%), stumps (<0.1%), understory plants (<0.1%), tree roots (14.5%), forest floor (8.4%), and mineral soil to 15 cm (7.6%). Prior to treatment there were no significant differences in C stocks among the five treatments, except C in coarse and total woody debris was highest in the 30 and 60% retention treatments (*p* < 0.05; [Table 3](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T3)). Post-harvest, aboveground C stocks were lowest in the most intensive harvesting treatment (clearcutting) and highest for the least intensive treatment (60% retention) and controls. Post-harvest total ecosystem C stocks in the clearcut were one-third the pre-harvest value, while three-quarters of the total ecosystem C stocks was retained following the 60% retention treatment. Forest floor C declined in the clearcut, seed tree and 60% retention treatments, but not in the 30% patch retention treatments where more of the coarse woody debris was mixed into the forest floor by the logging equipment. Mineral soil C to 15 cm depth was unaffected by harvesting ([Figure 6](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F6), [Table 3](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T3)). The total C stocks in coarse woody debris was unaffected by harvesting, but there was significantly more small woody debris in the low retention treatments, and greater fine woody debris in the harvesting treatments than the control, representing slash left from the logging operations. The understory plant community represented a minor C pool in all treatments and had recovered to only 14–20% of control levels after 3 years in the intermediate retention treatments ([Figure 5](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#F5), [Table 3](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T3)).

**FIGURE 6**



**Figure 6**. Distribution of carbon stocks among above and belowground pools in the ICHdwl variant at Redfish Creek 1 year after treatment (*n* = 4). Mean carbon pools are presented for each harvesting treatment across the legacy tree retention gradient. Belowground pools are represented with brown shading and aboveground pools with green shading. ANCOVA test statistics are shown in [Table 3](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#T3).

**TABLE 3**



**Table 3**. Effect of tree retention (mean ± *one* standard error) on carbon stocks in live and dead trees, understory plants, downed wood, stumps, forest floor, mineral soil (0–15 cm), and roots 1 year after harvesting in the ICHdw1 variant at Redfish Creek.

**Discussion**

Our study revealed a large reduction in ectomycorrhizal fungal inoculum potential with clearcut harvesting, and this was associated with considerably lower regeneration of interior Douglas-fir in spite of greater light availability than in treatments where various densities of overstory trees were retained. The reduction in ectomycorrhizal fungal inoculum was the direct result of removal of legacy trees and refuge plants as well as loss of forest floor C stocks. Retention of legacy trees in dispersed or small aggregate patterns mitigated most of these effects and promoted an abundance of conifer regeneration.

**Plant Communities and Refuge Plants**

The reduction in richness of shrub, herb and bryoid species in the clearcut and seed tree treatments compared with the control and high retention treatments supports our fourth hypothesis and reflects the extensive ground disturbance and altered microclimatic conditions associated with the intense harvests ([Bartels et al., 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B12)). Ectomycorrhizal shrub and broadleaf species declined from 81 to 6% cover with clearcutting, including loss of kinnickinick (*Arctostaphylos uva-ursi*), Bebb's willow (*Salix bebbiana*), alder (*Alnus viridis* subsp. *sinuata* (Regel) Ä. Löve & and D. Löve), paper birch (*Betula papyrifera* Marsh.), and trembling aspen (*Populus tremuloides* Michx.), which are known to share ectomycorrhizal fungal species in common with interior Douglas-fir ([Hagerman and Durall, 2004](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B64)). Loss of cover of these shrubs and trees in the clearcuts would have been accompanied by a dramatic decline in ectomycorrhizal fruiting bodies, particularly of late-stage fungi, based on studies in similar forests ([Bradbury et al., 1998](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B19); [Durall et al., 1999](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B51), [2006](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B50)). Arbuscular mycorrhizal plant cover also declined from 42% preharvest to 13% in the clearcuts predominantly fireweed (*Epilobium angustifolium* L.), pinegrass (*Calamagrostis rubscens*), white hawkweed (*Hierarcium albiflorum*) and Hooker's fairybells (*Disporum hookeri*)], but they remained relatively dominant or co-dominant with the ectomycorrhizal shrubs for 4 years. Clearcutting has commonly been found to encourage an influx of arbuscular mycorrhizal pioneer species, including herbs and grasses ([Bradbury, 2004](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B18); [Aikens et al., 2007](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B1); [Craig and MacDonald, 2009](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B40)). These shade-intolerant colonizers have competitive advantages in dispersal and reproduction allowing them to dominate in areas with limited crown cover and high levels of forest floor disturbance ([Beese and Bryant, 1999](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B13); [Newmaster and Bell, 2002](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B104); [Halpern et al., 2005](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B66); [Lencinas et al., 2011](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B89); [Arsenault et al., 2012](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B5); [Baker et al., 2013](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B8); [Haughian, 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B69)).

By contrast, retention of legacy trees in the 30 and 60% patch retention treatments better protected a variety ectomycorrhizal shrubs and trees as well as forest floor that are sources of ectomycorrhizal fungal inoculum for regenerating interior Douglas-fir, in support of our fourth hypothesis. By 3 years post-treatment, shrub species richness had recovered to control levels in these treatments, and cover values were reaching 40%. Ectomycorrhizal shrubs compete well in more intact ecological conditions because their fungal symbionts can access organic nutrients from the forest floor. This is supported by studies finding late-stage ectomycorrhizal fungi fruiting within 7 meters of residual trees in partially cut forests ([Durall et al., 1999](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B51)), and seedlings colonized by a variety of fungal species when they are planted within a few meters of the forest edge ([Hagerman et al., 1999](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B65)). They can also share organic compounds with conifer seedlings linked into their mycorrhizal networks ([Simard et al., 2012](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B126)). Later successional understory arbuscular species like Pacific yew (*Taxus brevifolia*) and Douglas maple (*Acer glabrum* var. *douglasii*) were also protected and their reproductive success was possibly enhanced by the aggregates, and the arbuscular networks of these species likely played a role in the establishment and colonization of the abundant western redcedar germinants.

Richness and abundance of bryoids were maintained in the forest patches of the 30% and 60% retention treatments, where forest floor and coarse woody debris substrates were best protected and the microclimate was wetter, cooler and shadier; by contrast, mosses were almost eliminated from the clearcut and seed tree treatments, as commonly found in other studies ([Arsenault et al., 2012](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B5); [Baker et al., 2013](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B8); [Bartels et al., 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B12); [Curzon et al., 2020](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B43)). Thus, our aggregated retention treatments generated sufficient resource and structural heterogeneity to support rich communities not only of tree, shrub, and herb species, but mosses as well ([Clark and Covey, 2012](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B35); [Haeussler et al., 2017](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B63)). It is also likely that nearby source populations in adjacent intact forests and retained patches accelerated recolonization and re-establishment of these species in the small harvested gaps, as found in other studies ([Halpern et al., 2005](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B66); [Lencinas et al., 2011](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full%22%20%5Cl%20%22B89); [Baker et al., 2016](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B9); [Hu et al., 2018](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B73)).

**Carbon Pools**

Forest floor C stocks (Mg ha−1) declined by 80–85% compared with pre-harvest levels one year after the clearcut, seed tree and 60% retention treatments but did not decline in the 30% retention treatment or controls, generally in support of our sixth hypothesis. Meta-analyses of harvesting effects on temperate forest floors around the world report an average 30% loss of forest floor C, regardless of whether clearcut or partial harvesting is employed ([Zhou et al., 2013](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B151); [James and Harrison, 2016](https://www.frontiersin.org/articles/10.3389/ffgc.2020.620436/full#B77)), indicating feller-buncher and skidding practices at our site were particularly damaging. The loss of forest floor carbon

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