Exploring fungal strategies: a comparative analysis of peroxidase enzyme secretion in ectomycorrhizal and arbuscular mycorrhizal fungi

by

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Abstract

Understanding the role of mycorrhizal associations in soil organic matter (SOM) dynamics is important for managing ecosystem functions and mitigating climate change impacts on forests. In this study, I compared soils under 100% *Pseudotsuga menziesii* D. (Douglas-fir), characterized by ectomycorrhizal (EM) associations, under 100% *Thuja plicata* T. (western red cedar) with arbuscular mycorrhizal (AM) associations, and under a 50:50% balanced mix of both tree species. I used peroxidases, a lignin modifying group of enzymes secreted by mycorrhizae to access nutrients in organic structures, to test between nutrient acquisition strategies of EM and AM. I found 2.75 times as much total forest floor peroxidase concentration in the Douglas-fir plots with EM associations compared to the western red cedar plots with AM associations. This indicates the importance of mycorrhizal symbionts in nutrient acquisition. My results suggest that the ability of EM to produce peroxidases that degrade complex molecules within SOM is a distinct nutrient acquisition strategy employed by this group of mycorrhizae. I also observed lower total peroxidases in mineral soil compared to forest floor, potentially due to the 30-year age of the stand and the early stage of development of the fungal community in mineral soil layers. Covariate analysis revealed that exchangeable potassium, sodium, manganese (Mn), and the carbon-to-nitrogen ratio (C:N) were all positively correlated with total forest floor peroxidase concentration. Exchangeable Mn and the C:N ratio were likely the most influential factors due to the impact of C:N on fungal community compositions. My findings underscore the importance of mycorrhizal symbionts in shaping soil carbon dynamics and highlight the potential for targeted management strategies to optimize soil organic carbon storage and reduce loss of carbon through respiration.

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Introduction

Soil carbon cycling

Understanding soil carbon dynamics is important, as it plays a pivotal role in shaping the future of our planet's climate (Averill *et al.*, 2014; Hawkins *et al.*, 2023). Different anthropogenic terrestrial impacts such as deforestation and soil cultivation can contribute significantly to the release of carbon into the atmosphere, with some cultivated soils losing as much as two thirds of their original carbon content (Lal, 2004, 2013). Within forests, a significant proportion of carbon, ranging from 25% to 63% of gross primary production, is allocated belowground in the form of soil organic carbon (SOC) (Litton *et al.*, 2007). This allocation not only serves as a fundamental component of the forest carbon budget but also influences the physical, chemical, and biological properties of the soil (Ekblad *et al.*, 2013; Kranabetter *et al.*, 2021). The potential to enhance SOC sequestration on a broader scale carries significant implications for mitigating the adverse impacts of climate change, as recognized by various studies (Jandl *et al.*, 2007; Ekblad *et al.*, 2013; Kranabetter, 2019). Forest ecosystems are responsible for storing upwards of 70% of the total terrestrial SOC in the worldand greater than 80% of aboveground carbon (Jandl *et al.*, 2007). Understanding the mechanisms underlying the dynamics of SOC allocation and turnover is essential for devising effective strategies to increase carbon sequestration in forest ecosystems (Lal, 2004, 2013; Kranabetter *et al.*, 2021). However, feedback loops between soil carbon dynamics and climate change introduce complexities that require further investigation (Xu *et al.*, 2011).

Mycorrhizal associations

The presence of mycorrhizal fungi changes how carbon is distributed within the host plant and affects both the quantity and quality of carbon that is stored within SOM (Frey, 2019; Hawkins *et al.*, 2023). Accurately measuring belowground carbon allocation to mycorrhizal fungi is challenging due to the difficulties in visualizing diffuse mycelia in soil and distinguishing mycorrhizal hyphae from saprotrophic fungi (Frey, 2019). Thus, estimating the impact of mycorrhizae on carbon sequestration from host plant allocation is an uncertain parameter in ecosystem carbon budgets and carbon cycling models (Xu *et al.*, 2011; Frey, 2019).

In addition to carbon, the availability of soil nutrients plays a pivotal role in understanding ecosystem function and resilience. Nutrient sources, such as nitrogen (N) and phosphorus (P), are both typically limiting factors of plant growth in forest soils (Martin *et al.*, 2016). To avoid this limitation, many plants establish mycorrhizal associations with fungal symbionts, such as ectomycorrhiza (EM) and arbuscular mycorrhizae (AM), exchanging carbon in the form of sugars for nutrients in their inorganic form (Read and Perez-Moreno, 2003; Martin *et al.*, 2016). It is the distinct strategies in nutrient acquisition employed by these mycorrhizal types that further shape soil carbon dynamics (Craig *et al.*, 2018; Frey, 2019; Hicks Pries *et al.*, 2023).

Due to evolutionary differences between AM and EM, resulting in disparities in morphology and physiology, they play distinct roles in principal aspects of SOC cycling (Soudzilovskaia *et al.*, 2015). Originally derived from a diverse group of saprotrophs including brown-rot and white-rot fungi, EM are paraphyletic and have independently evolved numerous times across multiple fungal orders (Tedersoo *et al.*, 2010; Frey, 2019). While EM evolved to have a reduced set of genes capable of encoding plant cell well degrading enzymes, they are still able to decompose lignocellulose (Mycorrhizal Genomics Initiative Consortium *et al.*, 2015; Frey, 2019). This component of plant cell walls contributes from roughly 15% to 40% of dry plant biomass, and its complex structure aids in its resistance to degradation (Dighton and White, 2005). The EM target this molecule not as a means of carbon gain, but to acquire nutrients within the molecular structure (Dighton and

White, 2005). By releasing oxidative enzymes, which targets polyphenolic compounds within the lignin, EM can convert organic nutrients to their inorganic form (Hofrichter, 2002; Bödeker *et al.*, 2009). These newly accessed nutrients can then be exchanged with the plant symbiont for energy-rich sugars. Typically, EM form associations with woody plants, including *Pseudotsuga menziesii* D. (Douglas-fir) (Martin *et al.*, 2016).

In contrast, AM belong to the Mucoromycota family within the monophyletic phylum Glomeromycotan (Dighton and White, 2005; Vigneron *et al.*, 2018; Frey, 2019). Instead of secreting enzymes to break down organic molecules to acquire nutrients, AM act as an extension of the plant's body and scavenge inorganic nutrients from the soil (Tedersoo *et al.*, 2010; Mycorrhizal Genomics Initiative Consortium *et al.*, 2015; Vigneron *et al.*, 2018; Frey, 2019). AM have obligate associations with over 70% of vascular plants across herbs, grasses, and woody plants, including *Thuja plicata* T. (western red cedar) (Gorzelak *et al.*, 2017). Thus, alterations in the abundance of AM and EM associated plants can induce significant changes in the ecosystem's SOC budget, influencing overall carbon sequestration dynamics (Soudzilovskaia *et al.*, 2015).

Manganese peroxidase

Peroxidases are a class II family of lignin-modifying enzymes that play an important role in SOM decomposition (Hofrichter, 2002; Bödeker *et al.*, 2009; Frey, 2019). Enzyme classes are based on the structural and functional traits of enzymes, and peroxidases are characterized as class II enzymes by their ability to oxidize a variety of substrates with hydrogen peroxide as a co-substrate (Bödeker *et al.*, 2009). Within this family, manganese peroxidase (MnP) is an important lignin-modifying enzyme. It is a regulatory bottleneck of soil carbon turnover due to the limited availability of its cofactor, manganese (Mn) (Hofrichter, 2002; Bödeker *et al.*, 2009; Stendahl *et al.*, 2017; Kranabetter, 2019). Exchangeable Mn has been shown to be one of the strongest predictors of carbon storage in the organic horizon, which represents approximately one third of belowground carbon (Stendahl *et al.*, 2017). The availability of Mn was demonstrated to be a better model of carbon storage in the organic horizon than both litter production and climatic factors (Stendahl *et al.*, 2017). MnP is also the most common lignin-modifying peroxidase and is produced by many saprotrophic and mycorrhizal fungi (Hofrichter, 2002). While MnP and other enzymes within the peroxidase family are secreted by EM to access nutrients from complex organic structures like lignin, it is unclear if AM secrete these enzymes as a form of nutrient acquisition (Hofrichter, 2002; Frey, 2019). To test this hypothesis, I conducted a comparative analysis between soils under Douglas-fir (DF) stands (with EM) and western red cedar (CW) stands (with AM).

Objectives

I aimed to investigate the impact of mycorrhizal association, specifically EM and AM, on peroxidase production and carbon dynamics in forest soils. While most literature is based on results from naturally occurring stands, I conducted a comparative analysis in experimental plots with either host to test the abundance of enzymes secreted in the soil. Soils under trees associated with EM or AM fungi were compared to determine if both mycorrhizal types release enzymes within the peroxidase family to increase inorganic N and P acquisition. Due to the evolutionary heritage of EM, I expect to find greater concentrations of peroxidases within the DF plots, whereas AM associated CW will have lower concentrations. Additionally, I sought to explore the factors contributing to variation in peroxidase levels within the forest floor and mineral soil layers, with a focus on factors such as soil nutrient availability, and soil fertility. Since host trees located in N-limited soils typically display greater N mobilization through their mycorrhizal symbionts, I hypothesize that plots with a higher C:N ratio will have greater peroxidase production. This production will also likely be subject to the availability of enzyme cofactors like Mn, so with increasing Mn availability there will be increased peroxidases. Through these objectives, I aimed to provide insights into the mechanisms driving soil organic matter decomposition and carbon sequestration in forest ecosystems, ultimately contributing to our understanding of how mycorrhizal symbioses shape soil carbon dynamics and ecosystem function.

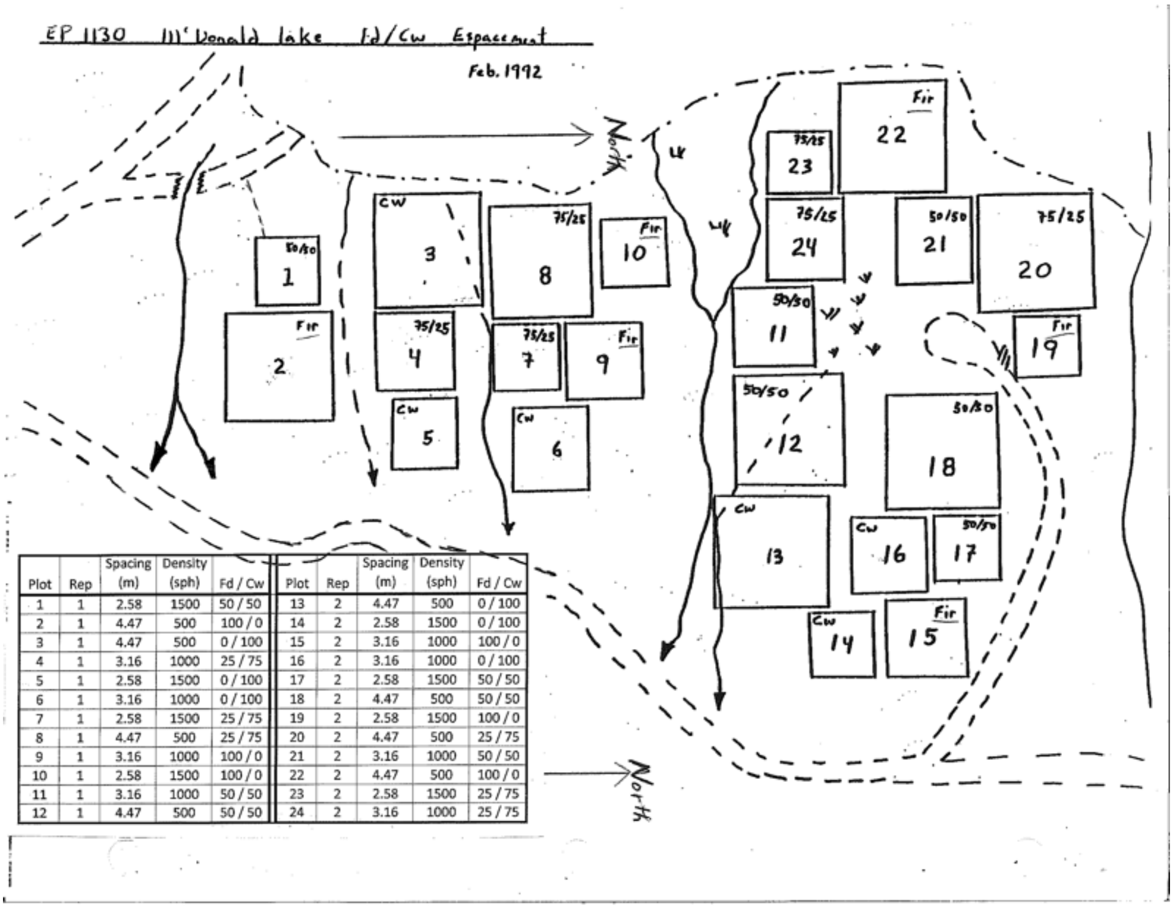
Methodology

Study site and design

The BC Ministry of Forests Experimental Project (EP) site EP1130 was planted in 1992 to test the ecological and economic potential of mixed tree species plantations compared to monocultures (Omari *et al.*, 2021). It is located to the northwest of the City of Victoria, within the Sooke Water Supply Area. The growth response of CW and coastal DF planted in four mixture treatments (1:0, 1:1, 1:3, and 0:1) and three planting densities (500, 1000, and 1500 stems per hectare) was assessed over a period of 22 years (Omari *et al.*, 2021). For my study, this experimental site was used to compare soil enzyme concentrations under DF, which is associated with EM, under CW, which is associated with AM, and under a 50:50 mixture of the two species. Within each of the four mixture treatments, there are six plots that include two replicates of three inter-tree spacings (2.58 m, 3.16 m and 4.47 m). Soil samples were collected from six plots of the 100% CW, 100% DF, and the 50:50 mixture treatments, making a total of 18 plots sampled. I chose the 100% treatment groups as they were ideal to test for the presence or absence of enzymes associated with the mycorrhizal symbiont of each tree species, while the 50:50 mixture treatment provided an opportunity to compare enzyme secretion when both mycorrhizal types are present.

Figure 1. Photos of an array of treatments plots within EP1130 where sampling took place. Photographs were taken on May 31, 2023. Plot 9 (left) is 100% Douglas-fir at low productivity (spacing of 3.16 m). Plot 1 (middle) is a 50/50 plot of Douglas-fir and western red cedar of intermediate productivity (2.58 m spacing). Plot 3 (right) is 100% western red cedar at high productivity (spacing of 4.47 m) Productivity was determined with tree height and size.

Sample collection was completed between the 29th and 31st of May 2023. Within each plot, forest floor samples were taken 1.5 m away from the stems of nine randomly selected trees. A sample of forest floor roughly 10 cm by 10 cm was removed. Fresh litter material such as cones, needles, and branches was extracted from the sample, then the sample was placed in a plastic bag. Another two samples (from two different trees) were then collected and placed in the same bag to make one composite sample. A total of three composite samples were collected in each plot (9 microsites in total per plot). Once all forest floor samples were removed, an auger was used to extract the exposed mineral soil to a depth of approximately 15 cm at each sampling location. The mineral soil was then removed from the auger and bagged using the same sample composite procedure. The augers and knives were cleaned with 95% ethanol to prevent cross contamination of soil among plots.



Plot	Rep	Spacing (m)	Density (sph)	Fd / Cw
1	1	2.58	1500	50 / 50
2	1	4.47	500	100 / 0
3	1	4.47	500	0 / 100
4	1	3.16	1000	25 / 75
5	1	2.58	1500	0 / 100
6	1	3.16	1000	0 / 100
7	1	2.58	1500	25 / 75
8	1	4.47	500	25 / 75
9	1	3.16	1000	100 / 0
10	1	2.58	1500	100 / 0
11	1	3.16	1000	50 / 50
12	1	4.47	500	50 / 50
13	2	4.47	500	0 / 100
14	2	2.58	1500	0 / 100
15	2	3.16	1000	100 / 0
16	2	3.16	1000	0 / 100
17	2	2.58	1500	50 / 50
18	2	4.47	500	50 / 50
19	2	2.58	1500	100 / 0
20	2	4.47	500	25 / 75
21	2	3.16	1000	50 / 50
22	2	4.47	500	100 / 0
23	2	2.58	1500	25 / 75
24	2	3.16	1000	25 / 75

Figure 2. Map of the BC Ministry of Forests Experimental Project (EP) 1130 where sampling was conducted. Treatments of 100% Douglas-fir, 100% western red cedar, and the 50:50 mixture were sampled, whereas the 75:25 treatment was not sampled. Map courtesy of J.M. Kranabetter.

Following collection at the field site, the forest floor and mineral soil samples were brought back to the lab and refrigerated. The next day, each of the composite samples was homogenized through a 4.75 mm sieve. The homogenized samples were then further divided for chemical analyses, including moisture content, percent carbon, nitrogen and sulphur, and exchangeable soil cations. Between 5 and 10 g was removed to determine gravimetric moisture content. Roughly 150 mL in volume was separated for ergosterol and peroxidase analysis, and the remaining sample was used for chemical analysis.

Peroxidase assay analysis

The peroxidase assay approach is based on an oxidative coupling reaction between 3-dimethylaminobenzoic acid (DMAB) and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) with the addition of Mn^{2+} and H_2O_2 (Ngo and Lenhoff 1980). This approach was modified for forest soils by Kyaschenko *et al.* (2017). In the presence of Mn^{2+}, H_2O_2, and MnP, the reaction between DMAB and MBTH creates a blue-purple colour (Castillo *et al.*, 1994). This colour's absorbance peaks at 590 nm and can be used as a proxy to measure enzyme activity through spectrophotometry (Castillo *et al.*, 1994). Two subsamples were taken from each of the three composite samples for a total of six aliquots weighed out from both the forest floor and mineral soil for each plot. Aliquots were composed of 0.5 teaspoon of field-moist soil, roughly equivalent to 0.5 g of dry forest floor and 1.0 g of dry mineral soil. The weight of the aliquots was recorded after they were placed in 50 mL centrifuge tubes, then 5 mL of 50 mM sodium acetate buffer (pH = 5) was added. The forest floor mixture was homogenized with the buffer for 45 seconds using a VirTishear homogenizer (SP Scientific, Stone Ridge NY, USA) at medium speed and the mineral soil for 60 seconds using a vortex. The volume was then adjusted to 50 mL with acetate buffer, and the tubes were inverted to ensure the solutions remained homogenous. Solutions for buffers and reactants were made and stored according to the instructions outlined in the Appendix.

Samples were placed in a centrifuge for 10 minutes at 10,000 RPM, which was followed by 5 minutes at 3,500 RPM. After the centrifugation, two 5 mL portions of supernatant from each sample were transferred to two 15 mL centrifuge tubes, for a total of 10 mL removed from each 50 mL tube. One subsample remained at room temperature, while the second subsample was placed into an aluminium bead bath for 30 minutes at 120 - 130°C. After boiling was complete, the samples were cooled to room temperature in ice.

To plate the samples, solutions A and B were prepared daily based on instructions listed in the Appendix and stored at 4°C. Solution A was composed of 2.5 mL sodium lactate buffer, 2.5 mL sodium succinate buffer, 0.5 mL DMAB solution, 0.5 mL MBTH solution, and 1 mL $MnSO_4$ solution. The $MnSO_4$ solution was added to activate MnP, so the absorbance readings of wells that had solution A contained all active peroxidase enzymes, including MnP. Solution B incorporated 1 mL ethylenediaminetetraacetic acid (EDTA) solution, which is a chelator of MnP that inhibits activity (Ngo and Lenhoff, 1980). Absorbance readings from wells with solution B showed all active peroxidases except for MnP. An H_2O_2 solution was prepared from 70% H_2O_2 by combining 25.5 µL H_2O_2 with sodium acetate buffer in a 50 mL centrifuge tube until a final volume of 50 mL was reached. This H_2O_2 solution was added to ensure activation of peroxidase enzymes. Enzyme activity could then be calculated using the final absorbance values from each of the wells.

Table 1. Set-up of the 96-well plate for peroxidase analysis. S represents 50 µl of the extracted sample while BS is 50 µl of the boiled (control) sample. A indicates the addition of 140 µl of solution A, which contains $MnSO_4$ and activates MnP. B indicates the addition of 140 µl of solution B that contains EDTA, a chelator that binds to MnP and inhibits activity. V shows samples with addition of 10 µl of H_2O_2 occurred. Cells without H_2O_2 are considered a secondary control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1 A V	S1 B V	S1 B	BS1 A V	S3 A V	S3 B V	S3 B	BS3 A V	S5 A V	S5 B V	S5 B	BS5 A V
B	S1 A V	S1 B V	S1 B	BS1 A V	S3 A V	S3 B V	S3 B	BS3 A V	S5 A V	S5 B V	S5 B	BS5 A V
C	S1 A V	S1 B V	S1 B	BS1 A V	S3 A V	S3 B V	S3 B	BS3 A V	S5 A V	S5 B V	S5 B	BS5 A V
D	S1 A V	S1 B V	S1 B	BS1 A V	S3 A V	S3 B V	S3 B	BS3 A V	S5 A V	S5 B V	S5 B	BS5 A V
E	S2 A V	S2 B V	S2 B	BS2 A V	S4 A V	S4 B V	S4 B	BS4 A V	S6 A V	S6 B V	S6 B	BS6 A V
F	S2 A V	S2 B V	S2 B	BS2 A V	S4 A V	S4 B V	S4 B	BS4 A V	S6 A V	S6 B V	S6 B	BS6 A V
G	S2 A V	S2 B V	S2 B	BS2 A V	S4 A V	S4 B V	S4 B	BS4 A V	S6 A V	S6 B V	S6 B	BS6 A V
H	S2 A V	S2 B V	S2 B	BS2 A V	S4 A V	S4 B V	S4 B	BS4 A V	S6 A V	S6 B V	S6 B	BS6 A V

The setup of the 96-well plate is outlined in Table 1 and followed the same structure for each plot. Prior to adding reactants, 50 µL of the room temperature sample was added to the first three rows of wells. I then added 50 µL of the boiled sample to the fourth row of wells as a control. Next, 140 µL of solution A or B were added to the wells, based on the arrangement found in Table 1. The final addition to the plate was 10 µL of H_2O_2 solution in the designated wells, then the plate was promptly inserted into the Gen5 Microplate Reader (BioTek Instruments, Winooski Vermont, USA). Absorbances were recorded at 590 nm at 25°C for 3-minute intervals over the duration of 1 hour using Gen 5 Microplate Reader Software. The absorbance value for the colour produced from the reaction between DMAB and MBTH peaks at 590 nm and reflects the absorbance of enzymes in the peroxidase family. Wells containing the boiled sample (BS), or solution B and no H_2O_2 solution (S B) were designated as the control.

Enzyme activity calculations

The peroxidase and MnP concentration (expressed in µmol h^{-1} g^{-1}) were calculated by adapting equations from DeForest (2009). The absorbance at 590 nm of the blue-purple colour created from the reaction of DMAB and MBTH was determined by subtracting the mean absorbance of wells containing solution A and H_2O_2 from the control samples (containing solution B without the addition of H_2O_2). The difference between average absorbance of wells containing solution A and H_2O_2 and solution B with H_2O_2 could be used to calculate MnP activity. Once the control sample absorbance was subtracted, wells containing solution A reflected the absorbance of all peroxidase enzymes, whereas wells with solution B and H_2O_2 reflect the activity of all peroxidases except MnP. The equations used in the calculations are:

$$\text{Peroxidase Absorbance} = S\,A\,V - S\,B$$

$$\text{MnP Absorbance} = (S\,A\,V - S\,B) - (S\,B\,V - S\,B)$$

$$\text{Activity } (\mu\text{mol h}^{-1}\text{ g}^{-1}) = \frac{\text{Absorbance x 50 mL}}{7.9\ \mu\text{mol}^{-1}\text{ x Time (h) x 0.2 mL x Soil (g)}}$$

where SAV and SB indicate absorbance readings, and S is used to denote the addition of the extracted subsample (50 µl), while A and B represent solutions A and B, respectively. V shows the addition of the H_2O_2 solution. The volume of the homogeneous solution used during analysis is 50 mL, and the micromolar extinction coefficient, calculated by DeForest is 7.9 μmol^{-1} (2009). Each well had 0.2 mL of sample volume added, and the dry weight of the soil was determined by measuring the difference in weight from the 50 mL centrifuge tube before and after the soil was added.

Enzyme activity was determined based on spectrophotometer readings after one hour. To standardize the results, if the readings reached an asymptote prior to the end of the hour, enzyme concentration was calculated before the asymptote was reached. It is likely that when a plateau was reached before the one-hour mark, the enzyme concentration was so high that all reactants were converted, so the linear activity rate best represented the sample (Ngo and Lenhoff, 1980). Typically, peroxidase activity increased linearly with time, so adjustments were rarely necessary (Ngo and Lenhoff, 1980).

Statistical analysis

Statistical analyses were performed using R version 4.2.3 (R Core Team, 2022). First, the mean of the four replicate wells for each subsample was calculated, then using the means of the subsamples, a final mean was calculated for each composite sample. For each plot, we calculated the mean value of MnP, and residual peroxidases for both the forest floor and mineral soil. Forest floor peroxidase data from the three mixture treatments were tested for significant differences among treatments and planting densities using two-way analysis of variance (ANOVA). Mixed linear models with mixture, density, and the interaction between mixture and desnity were used to determine the source of variation between mean total forest floor peroxidase values in each plot. Both treatment groups and their interaction were added as fixed effects to the model, while the plots were added as a random effect. With the models, I aimed to capture unexplained variability within the total forest floor peroxidase concentration by accounting for the correlation within treatment groups and their interaction, and the variation occurring among these groups. I ran an individual mixed linear model with the same parameters for fixed and random effects, but with the addition of a covariate to determine if it would improve the fit of the model. These covariates included exchangeable soil cations, C:N ratio, and included soil moisture. The change in Bayesian information criterion (BIC) was calculated for each covariate that was entered into the model, and results were ranked from greatest fit to least based on the lowest change in BIC.

Results

Forest floor

I calculated a mean total forest floor peroxidase of 33.4 ± 7.1 µmol per g of soil for plots of 100% DF, 12.1 ± 1.1 µmol per g of soil for 100% CW plots, and 23.4 ± 3.3 µmol per g of soil within 50:50% DF:CW plots (Table A1). The results of the two-way ANOVA showed that there were no statistically significant differences among the three species mixtures for total forest floor peroxidases (Table 2). However, the total peroxidases were 2.75 times higher in the 100% DF mixture than in the 100% CW mixture. There were no statistically significant differences among the density treatments, or between mixture and density treatments for total peroxidases (Table 2). There is a clear trend of increasing forest floor peroxidase activity from the 100% CW to the 100% DF treatments, with the 50:50 plot approximately intermediate between the two (Fig. 3). For forest floor MnP, there were no statistically significant differences between any of the means for mixture, density, or their interaction (Table 3). The other forest floor peroxidases did not show significant differences between the mixture treatment means, nor were there significant differences among the density treatments or the interaction in the two-way ANOVA (Table 4). However, the trend of increasing total forest floor peroxidase appears to be driven by other forest floor peroxidases rather than MnP, as MnP levels remained relatively constant between mixture treatment groups.

Table 2. Two-way ANOVA results for significance testing between mixture and planting density treatments for total forest floor peroxidase activity. Mixture treatments were 100% Douglas-fir, 50:50% Douglas-fir and western red cedar, and 100% western red cedar. Planting densities were 500, 1000 or 1500 stems per hectare.

	df	**Sum of Squares**	**Mean Squares**	**F-value**	**p-value**
Mixture	2	1354.37	677.19	3.47	0.08
Density	2	612.22	306.11	1.57	0.26
Mixture x Density	4	1559.79	389.95	2.00	0.18
Residuals	9	1758.42	195.38		

Table 3. Two-way ANOVA results for significance testing between mixture and planting density treatments for total forest floor MnP. Mixture treatments were 100% Douglas-fir, 50:50% Douglas-fir and western red cedar, and 100% western red cedar. Planting densities were 500, 1000 or 1500 stems per hectare.

	df	**Sum of Squares**	**Mean Squares**	**F-value**	**p-value**
Mixture	2	62.82	31.41	1.62	0.25
Density	2	45.25	22.63	1.17	0.35
Mixture x Density	4	194.38	49.10	2.53	0.11
Residuals	9	174.69	19.41		

Table 4. Two-way ANOVA results for significance testing between mixture and planting density treatments for other forest floor peroxidase activity. Mixture treatments were 100% Douglas-fir, 50:50% Douglas-fir and western red cedar, and 100% western red cedar. Planting densities were 500, 1000 or 1500 stems per hectare.

	df	**Sum of Squares**	**Mean Squares**	**F-value**	**p-value**
Mixture	2	835.67	417.83	3.39	0.08
Density	2	339.95	169.98	1.38	0.30
Mixture x Density	4	724.09	181.02	1.47	0.29
Residuals	9	1109.49	123.28		

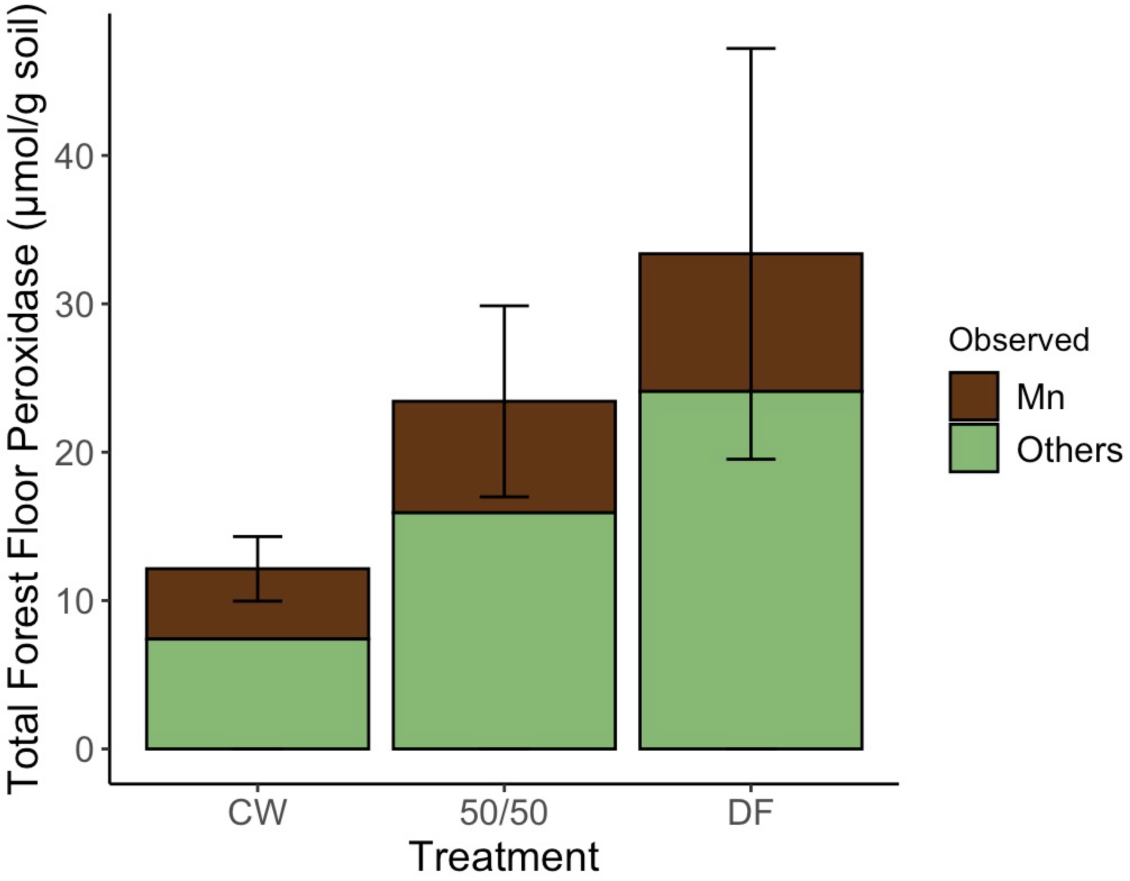


Figure 3. Total forest floor peroxidases (µmol per g of soil) as a function of mixture treatments. MnP is shown in brown and all other peroxidases in green. DF = 100% Douglas-fir, 50/50 = 50:50% DF:CW, and CW = 100% coastal western red cedar. Error bars represent 95% confidence intervals of the total peroxidase amounts.

Mineral soil

I calculated a mean total mineral soil peroxidase of 0.46 ± 0.08 µmol per g of soil for plots of 100% DF, 0.50 ± 0.10 µmol per g of soil for 50:50% DF:CW plots, and 0.37 ± 0.03 µmol per g of soil within 100% CW plots (Table A2). The mineral soil samples showed no significant differences among the mixture or density treatments of the total peroxidases, and there were also no significant differences between any of the treatments for MnP (Table 5; Table 6). Other peroxidases within the mineral soil layer showed no statistical significance between treatment groups (Table 7.) The total concentration of peroxidases found within the mineral soil was also much lower than the peroxidase concentration within the forest floor samples (Fig. 3; Fig. 4).

Table 5. Two-way ANOVA results for significance testing between mixture and planting density treatments for total mineral soil peroxidase activity. Mixture treatments were 100% Douglas-fir, 50:50% Douglas-fir and western red cedar, and 100% western red cedar. Planting densities were 500, 1000 or 1500 stems per hectare.

	df	Sum of Squares	Mean Squares	F-value	p-value
Mixture	2	0.051	0.026	0.35	0.72
Density	2	0.049	0.025	0.33	0.73
Mixture x Density	4	0.356	0.089	1.20	0.38
Residuals	9	0.668	0.074		

Table 6. Two-way ANOVA results for significance testing between mixture and planting density treatments for total mineral soil MnP. Mixture treatments were 100% Douglas-fir, 50:50% Douglas-fir and western red cedar, and 100% western red cedar. Planting densities were 500, 1000 or 1500 stems per hectare.

	df	Sum of Squares	Mean Squares	F-value	p-value
Mixture	2	0.021	0.011	1.08	0.38
Density	2	0.006	0.003	0.30	0.75
Mixture x Density	4	0.121	0.030	3.09	0.07
Residuals	9	0.088	0.010		

Table 7. Two-way ANOVA results for significance testing between mixture and planting density treatments for other mineral soil peroxidase activity. Mixture treatments were 100% Douglas-fir, 50:50% Douglas-fir and western red cedar, and 100% western red cedar. Planting densities were 500, 1000 or 1500 stems per hectare.

	df	Sum of Squares	Mean Squares	F-value	p-value
Mixture	2	0.039	0.019	0.40	0.68
Density	2	0.025	0.012	0.26	0.78
Mixture x Density	4	0.071	0.018	0.37	0.82
Residuals	9	0.433	0.048		

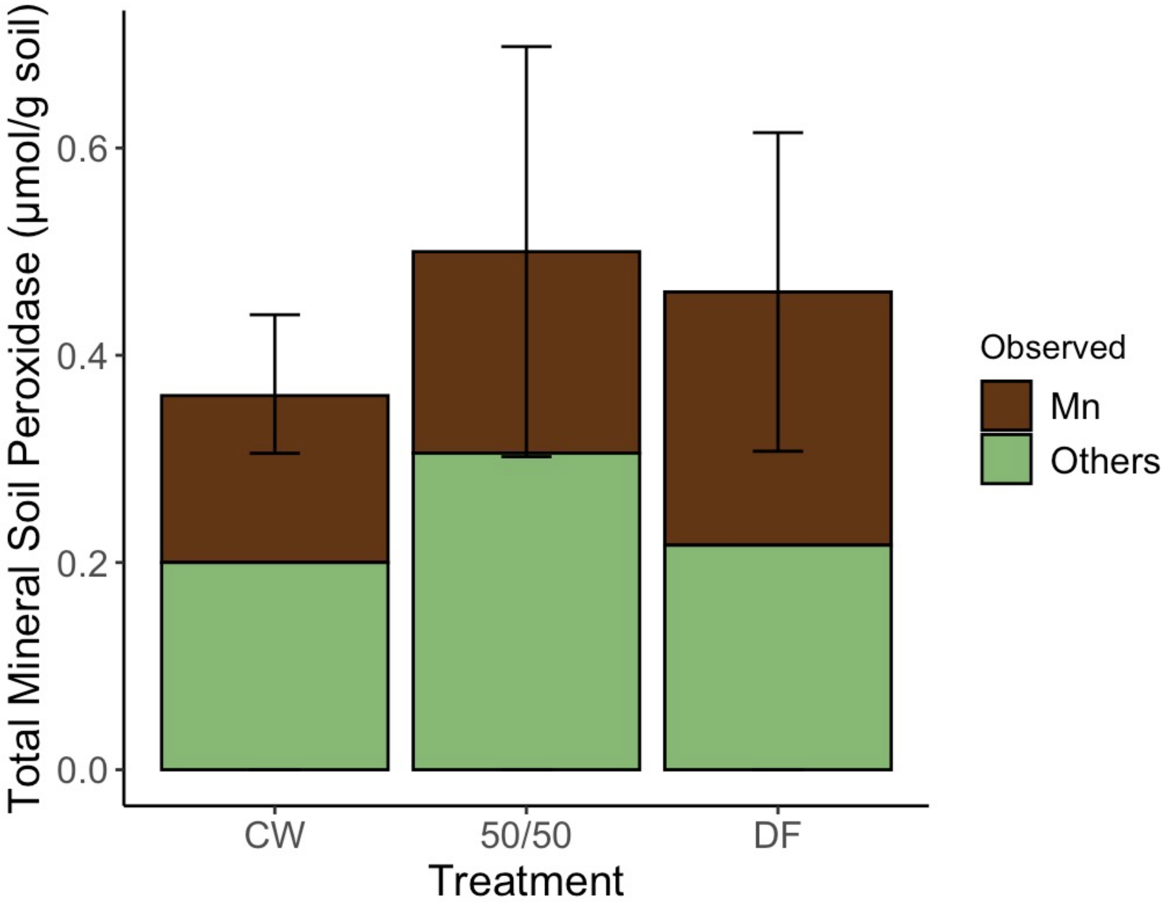


Figure 4. Total mineral peroxidases (µmol per g of soil) as a function of mixture treatments. MnP is shown in brown and all other peroxidases in green. DF = 100% Douglas-fir, 50/50 = 50:50% DF:CW, and CW = 100% coastal western red cedar. Error bars represent 95% confidence intervals of the total peroxidase amounts.

Peroxidase variation within forest floor plots

Forest floors under DF and in the 50:50 plots show an increase of total forest floor peroxidases as the amount of exchangeable Mn increases (Fig. 5). Total peroxidases in the forest floor under CW remain consistently low, regardless of the exchangeable Mn availability (Fig. 5). The C:N ratio had a similar relationship as exchangeable Mn on the total forest floor peroxidase, with the greatest increase seen under DF and no change among CW plots (Fig. 6). In the DF treatment, large variation is observed among the average total peroxidase concentration in each plot, hinting at other factors influencing total peroxidase concentration, in addition to the forest floor exchangeable Mn and the C:N ratio (Fig. 5; Fig.

6).

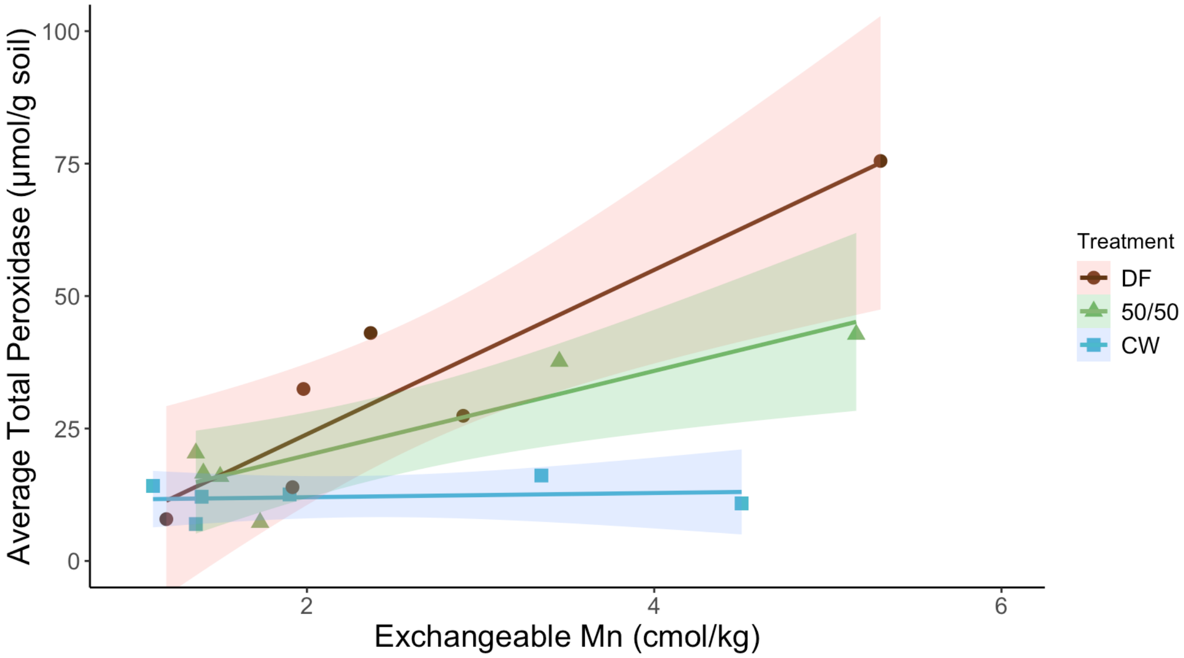


Figure 5. Total forest floor peroxidases (µmol g per soil) as a function of exchangeable Mn (cmol/kg) of the sample. Each plot is represented by a single point with DF = 100% Douglas-fir (circle), 50/50 = 50:50% DF:CW (triangle), and CW = 100% western red cedar (square). Lines of best-fit were calculated using a linear model and the shaded area represents the SE of the line of best-fit.

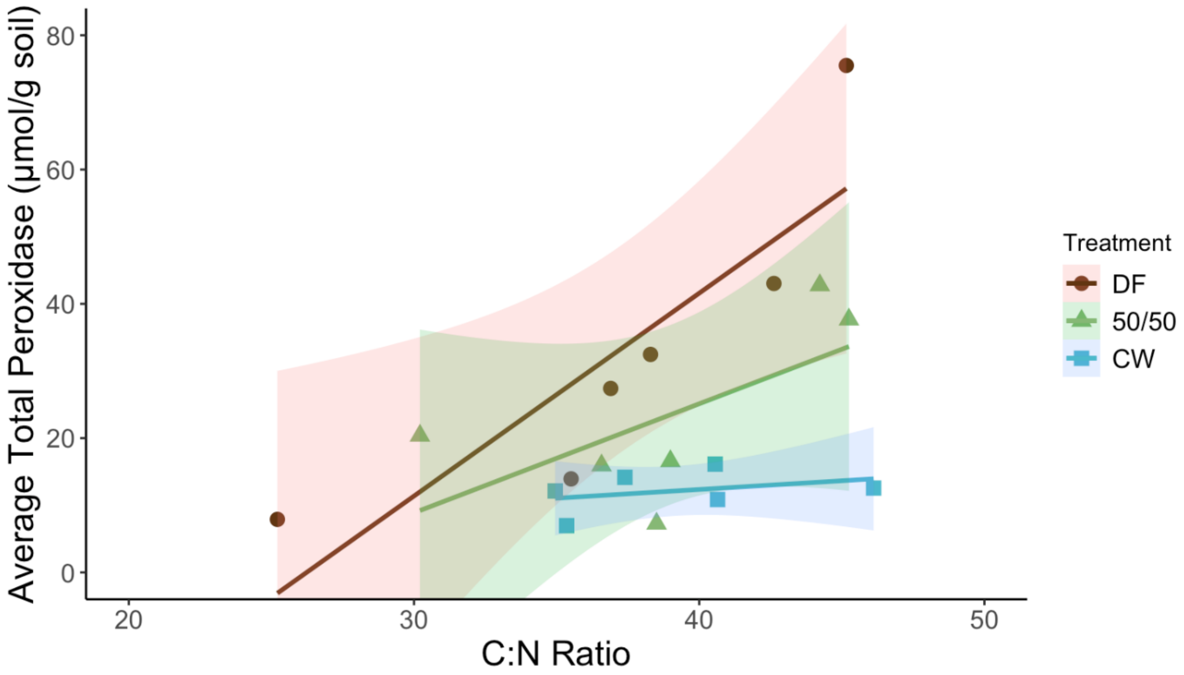


Figure 6. Total forest floor peroxidases (µmol g per soil) as a function of C:N ratio of the sample. Each plot is represented by a single point with DF = 100% Douglas-fir (circle), 50/50 = 50:50% DF:CW (triangle), and CW = 100% western red cedar (square). Lines of best-fit were calculated using a linear model and the shaded area represents the SE of the line of best-fit.

Results of the individual mixed linear models that incorporated the fixed effects of mixture, density, and their interaction, as well as the random effect of plot number showed that the addition of exchangeable potassium (K) ion had the greatest explanatory power of total peroxidase concentration (Table 6). The model including exchangeable sodium (Na) ion as a covariate had the second best fit according to change in BIC, and exchangeable Mn was third (Table 6).

Table 8. Ranking of covariates in mixed linear models with mixture, density, and the interaction of the two. Each model accounted for the treatments as fixed effects and the plots as a random effect, but a new model was generated to determine if the added covariate improved the model overall fit. The fit of covariates was determined using the change in BIC, with the best fit obtained through the lowest delta BIC value.

Covariate	BIC	Δ BIC
Exch. K (cmol kg $^{-1}$)	458.07	0.00
Exch. Na (cmol kg $^{-1}$)	475.20	17.13
Exch. Mn (cmol kg $^{-1}$)	476.02	17.95
Exch. Fe (cmol kg $^{-1}$)	485.95	27.88
Sulphur (%)	487.15	29.08
Carbon (%)	491.65	33.58
Nitrogen (%)	491.65	33.58
Exch. Al (cmol kg $^{-1}$)	491.77	33.70
Exch. Mg (cmol kg $^{-1}$)	495.47	37.40
No Covariate	494.54	36.47
Carbon:Nitrogen	498.39	40.32
Effective CEC (cmol kg $^{-1}$)	498.84	40.76
Exch. Ca (cmol kg $^{-1}$)	499.07	41.00

Since the exchangeable K and Na produced the best model fit but are not the most biologically relevant covariates, I plotted them against the C:N ratio and exchangeable Mn to determine if there was any correlation (Fig. 7). Both covariates were positively correlated with the C:N ratio and exchangeable Mn; however, exchangeable K exhibited a much greater range in values compared to exchangeable Na (Fig. 7). All raw data values used in the models can be found in the appendix (Table A3; Table A4, Table A5).

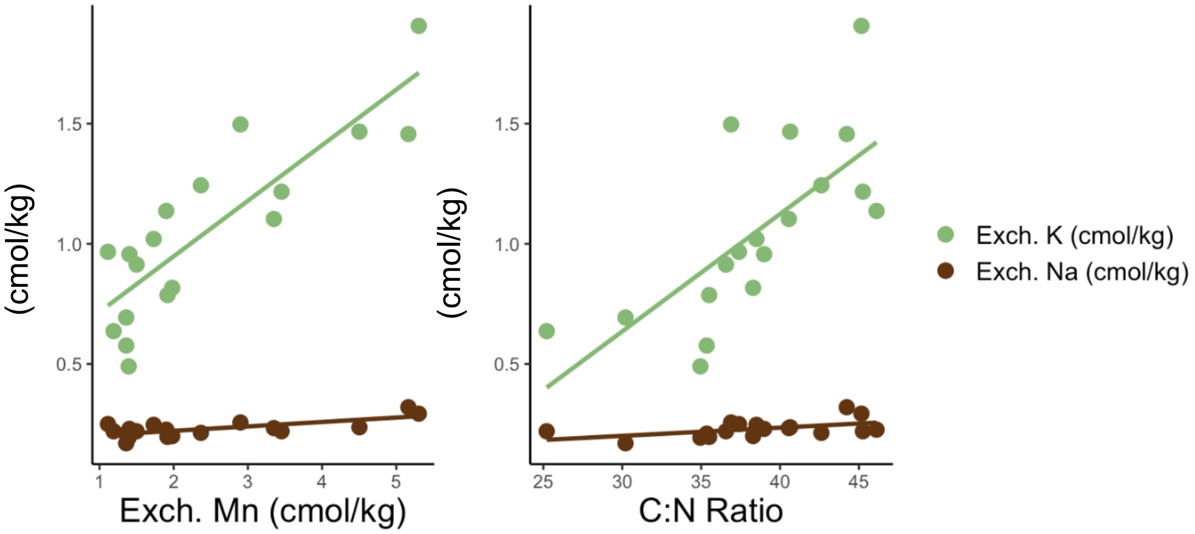


Figure 7. Relationships for exchangeable manganese (Exch. Mn) (cmol/kg) or carbon-to-nitrogen ratio (C:N Ratio) with exchangeable sodium (Exch. Na) (cmol/kg) or exchangeable potassium (Exch. K) (cmol/kg), in forest floor samples. Scatter plots depict the correlations, with linear regression lines fitted to the data. Colours represent Exch. K (green) and Exch. Na (brown).

Discussion

Differences between mycorrhizal symbionts of Douglas-fir and western red cedar

Through the comparison of soils planted with trees forming EM associations (DF), with those planted with trees forming AM associations (CW), or a balanced 50:50 mix of trees with both associations (DF:CW), I demonstrated 2.75 times more mean total peroxidase production within the 100% DF plots compared to 100% CW plots (Fig. 3). The increased total forest floor peroxidase was also linked to factors such as the C:N ratio and availability of exchangeable Mn within the plots, both of which played a role in the consistently higher peroxidase levels observed in 100% DF in comparison to 100% CW plots (Fig. 3; Fig. 4; Table 6).

The observed differences among mixture treatments can be attributed to the ability of select EM to degrade the soil organic matter (Dighton and White, 2005; Tedersoo *et al.*, 2010; Mycorrhizal Genomics Initiative Consortium *et al.*, 2015; Frey, 2019). AM fungi typically rely on free-living soil microbial decomposers for nutrient access because they lack the ability to convert organic to inorganic forms of N and P (Talbot *et al.*, 2008; Frey, 2019). However, AM can produce other types of enzymes such as phosphatases, which act to mineralize inorganic nutrients (Joner and Johansen, 2000). In contrast, EM fungi originated from a diverse group of saprotrophic ancestors and maintain the capacity to break down complex organic molecules like lignocellulose within the soil, granting them greater access to organic N and P sources compared to AM (Tedersoo et al., 2010; Averill et al., 2014; Frey, 2019). This capacity is based upon the potential for enzymatic activity within groups of EM fungi, including the production of peroxidases, which play a crucial role in SOM decomposition (Dighton and White, 2005; Bödeker *et al.*, 2009; Frey, 2019).

The genetic limitation of AM in producing enzymes including those from the peroxidase family, explains the overall lower amounts within 100% CW plots. The reduced presence of peroxidases does not indicate the success of the mycorrhizal community in those plots, but hints at the presence of different acquisition strategies. Minor fluctuations seen among the 100% CW plots could be attributed to the activity of saprotrophs, or plants with associations other than AM found within the plots, for example, salal (Xiao and Berch, 1996).

The observed differences between mixture treatments can be attributed to the distinct methods employed by EM and AM to gain nutrients (Fig. 3). The genetic limitations of AM in production of some enzymes, coupled with their reliance on an alternative nutrient acquisition strategy, align with consistently lower peroxidase values in the respective experimental plots. These findings highlight the distinct ecological roles and strategies employed by EM and AM in shaping soil organic matter dynamics.

Differences between mycorrhizal types within the forest floor and mineral soil

In this investigation of the mycorrhizal associations between plots of 100% CW, 100% DF, and 50:50% CW and DF, I showed lower total peroxidases in the mineral soil compared to the forest floor (Fig. 3; Fig. 4). There were also no significant differences of MnP or other peroxidases among the mixture and planting density treatment groups within the mineral soil (Table 5). These results contrasted increased total peroxidases in 100% DF plots found in the forest floor (Fig. 3). The contrast between the forest floor and mineral soil horizons may be linked to the young age of the secondary growth stand; however, there is also a lower amount carbon stored in mineral soils (Piirainen *et al.*, 2015). While less carbon is sequestered into the mineral soil, a similar trend between the mixture treatments was expected (Piirainen *et al.*, 2015). Given that EP1130 is only 30 years old, I propose that the fungal community has not yet fully established within the mineral soil due to the limited amount of OM within the soil layer (Rosling *et al.*, 2003; Tedersoo *et al.*, 2003; Colin *et al.*, 2021).

Both AM and EM have been shown to colonize the mineral soil to acquire nutrients (Koele *et al.*, 2014; Colin *et al.*, 2021). The EM groups most commonly found colonising the mineral soil include ascomycetes and euagarics; however, the success of mineral-associated fungal communities is primarily reliant on the forest plant cover (Tedersoo *et al.*, 2003; Colin *et al.*, 2021). At the plot scale, fungal communities are vertically structured and strongly linked to the land cover at the surface (Rosling *et al.*, 2003; Colin *et al.*, 2021). As the trees in the stand age and increase the total plant land cover, the fungal colonization of lower soil profiles, like the mineral soil, should also increase (Colin *et al.*, 2021). EM can cause weathering through acidification of the rhizosphere, which releases mineral nutrients to the mycorrhizae (Koele *et al.*, 2014). However, mineral soil within a younger plot can have a limited amounts of OM due to leaching and erosion that may result from timber harvest (Kalbitz and Kaiser, 2008). This is a possible explanation for the drastically reduced presence of EM peroxidases within the mineral soil of EP1130. After the clear cut, OM in the mineral soil possibly declined and has not yet recovered within the 30 years since replanting. Thus, the reduced OM potentially resulted in a significantly less developed fungal community in the mineral soil layer.

Covariates contributing to total forest floor peroxidase concentration

I tested the relationships of a series of soil attributes measured by soil chemical analysis with total peroxidase concentration to explore possible factors contributing to the variation in total peroxidase values in the forest floor (Fig. 5). Models demonstrating the lowest change in BIC were selected as having the best fit to the data, although it is acknowledged that this may not always reflect the most ecologically accurate representation (Schielzeth and Nakagawa, 2013). Exchangeable potassium (K) and sodium (Na) resulted in the lowest change in BIC, followed by exchangeable Mn (Table 6). While existing research supports a correlation of greater EM enzyme secretion with lower exchangeable K levels in soils, similar evidence in relation to exchangeable Na is lacking (Potila *et al.*, 2009). I predict that both K and Na may co-correlate with other soil factors, such as other nutrients or soil pH, This results in both exchangeable K and Na being disproportionately represented in the model, in contrast to more biologically significant variables like exchangeable Mn and C:N.

Both K and Na are mobile cations prone to leaching and migrating deeper into the soil profile (Grasso *et al.*, 1992; Mouhamad, 2015). Soils with higher productivity levels tend to exhibit a more active moisture regime (significant moisture fluctuations throughout the year), resulting in lower cation concentrations (Nigh, 2006). Thus, plots with a lower C:N within EP1130 have lower exchangeable soil cations, like K and Na. When examining the relationship between C:N or exchangeable Mn with K and Na levels, there is evidence of collinearity with exchangeable K and Na (Fig. 6) (Stendahl *et al.*, 2017). As exchangeable Mn increases in these results, so too does exchangeable K and Na, though Na is on a different scale (Fig. 6). Such patterns are common in soil profiles, since it is unusual for only one parameter to vary along an edaphic gradient (Nigh, 2006; Kranabetter *et al.*, 2021). The collinearity between exchangeable Mn and K has also been previously documented in organic soil horizons like the forest floor (Stendahl *et al.*, 2017). In lower productivity plots (high C:N), host trees rely more on N mobilizing EM symbionts, and higher amounts of soil exchangeable K and Na cations are present due to the reduced activity in the moisture regime (Nigh, 2006; Mouhamad, 2015; Frey, 2019). The amount of total peroxidases within each plot is then limited by the availability of exchangeable Mn, the required cofactor in oxidative decomposition by MnP (Hofrichter, 2002; Kranabetter *et al.*, 2021).

The C:N ratio exerts a significant influence not only on total peroxidase concentration but also on the fungal community structure within the forest floor (Sterkenburg *et al.*, 2015; Guo *et al.*, 2020). Numerous studies have demonstrated that variations in soil fertility, as reflected by the C:N ratio, shape the composition of the EM fungi guild (Sterkenburg *et al.*, 2015; Guo *et al.*, 2020). As the C:N ratio affects nutrient availability and OM decomposition rates, it indirectly dictates the types of EM fungi that thrive in a particular environment (Sterkenburg *et al.*, 2015; Frey, 2019). For example, high C:N ratios typically signify nutrient-poor conditions, favouring EM with efficient nutrient mobilization capabilities, whereas low C:N ratios (nutrient-rich) lead to the dominance of EM specialized in exploiting readily available resources (Sterkenburg *et al.*, 2015; Frey, 2019; Guo *et al.*, 2020). Since not all EM produce enzymes or peroxidases, shifts in the fungal community composition driven by variations in soil fertility can directly impact the total peroxidase production potential of the soil ecosystem (Tedersoo *et al.*, 2010; Guo *et al.*, 2020). Due to the complexity of soil dynamics, it is difficult to reach definitive conclusions, and while both C:N and exchangeable Mn may be driving peroxidase activity, further experimentation is necessary to disentangle these co-limiting factors.

Future directions and conclusions

I determined that the quantity of total peroxidases in the forest floor was three-fold higher in plots characterized with EM associations (DF) compared to those with AM associations (CW), but was being influenced by other soil factors such as C:N and exchangeable Mn. Differing evolutionary backgrounds of EM and AM and distinct nutrient acquisition strategies are the predominant reason for these differences. DF exclusively associates with EM, which are derived from a diverse group of saprotrophic fungi, thus some taxa are capable of producing enzymes to convert nutrients from their organic to inorganic forms (Dighton and White, 2005; Mycorrhizal Genomics Initiative Consortium *et al.*, 2015; Frey, 2019). In contrast, CW associates with AM, which functions as a nutrient scavenger, mineralizing and collecting nutrients in their inorganic form (Vigneron *et al.*, 2018; Frey, 2019).

The type of mycorrhizal symbiont has been shown to play an independent and larger role in soil carbon cycling than the impacts of temperature, precipitation and net primary production (Averill *et al.*, 2014). My study underscores how the effect of mycorrhizal guild is context dependent via soil fertility, rather than a generalized attribute across all forest landscapes. In future forestry practices, mycorrhizal choice driven by tree species choice could prove to be a critical link in managing SOC sequestration and respiration, which act as important predictors and potential mitigators of climate change (Lal, 2004; Averill *et al.*, 2014; Averill and Hawkes, 2016). Patterns in SOC sequestration over the long term from experimental studies are needed to better understand the contribution of mycorrhizal guild.

The targeted immobilization of exchangeable Mn accessed by EM could result in an increase of SOC sequestration (Kranabetter *et al.*, 2021). Mn has been previously shown to play an important role in carbon turnover as a co-factor of MnP, an enzyme secreted by EM that can break down polyphenolic compounds (Hofrichter, 2002; Dighton and White, 2005; Kranabetter, 2019). Reducing the efficacy of this co-factor in the environment would result in lower rates of carbon turnover but greater sequestration over time; however, the amount, duration, and potential unintended consequences of this treatment are still unknown, so further small-scale research is recommended (Kranabetter *et al.*, 2021).

Soils that host EM fungal communities have been associated with a significant 67% decrease in soil carbon respiration compared to soils lacking EM hyphae, as indicated by Averill and Hawkes (2016). This reduction is likely the result of competition between EM hyphae for nutrients such as N within the soil, which was found to independently influence the balance of SOC in the ecosystem (Averill and Hawkes, 2016). Notably, this decrease in soil carbon respiration implies a mitigating effect equivalent to a change in soil warming greater than 2°C (Averill and Hawkes, 2016). This underscores the importance of understanding soil community interactions in predicting ecosystem responses to global climate change and soil warming (Averill and Hawkes, 2016). Deeper exploration of the ecological implications of mycorrhizal communities is essential for gaining insights into their impacts and potential for mitigating climate-induced effects on SOC cycling, as highlighted by and in other studies (Orwin *et al.*, 2011; Kranabetter *et al.*, 2021).

The 3-fold difference observed in peroxidase concentration between EM and AM associations underscore the pivotal role of mycorrhizal symbionts in shaping soil organic matter dynamics, with DF associated EM exhibiting notably higher peroxidase concentration levels. This highlights the potential of mycorrhizal fungi to modulate soil nutrient availability and decomposition processes, thereby influencing the overall health and functioning of forest ecosystems.

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Appendix

Buffers and solutions for the peroxidase enzyme assay

Sodium buffers

50 mM sodium acetate buffer: dissolve 4.1015 g sodium acetate in 800 mL DI H_2O, bring the volume up to 1 L, and adjust the pH to 5 using glacial acetic acid

100 mM sodium lactate buffer: dissolve 1.1206 g sodium lactate in 80 mL DI H_2O, bring the volume up to 100 mL, and adjust the pH to 4.5 using glacial acetic acid

100 mM sodium succinate buffer: dissolve 1.6205 g sodium succinate in 80 mL DI H_2O, bring the volume up to 100 mL, and adjust the pH to 4.5 using glacial acetic acid

Store sodium buffers at 4°C for up to 1 week.

Solutions

2 mM EDTA solution: dissolve 7.4 mg Na_2-EDTA·$2H_2O$ in 10 mL acetate buffer

1 mM $MnSO_4$ solution: dissolve 8.45 mg $MnSO_4 \cdot 4H_2O$ in 50 mL acetate buffer

50 mM DMAB solution: dissolve 82.6 mg 3-dimethylaminobenzoic acid in 10 mL acetate buffer

1 mM MBTH solution: dissolve 2.3 mg 3-methyl-2-benzothiazolinone hydrazone hydrochloride in 10 mL acetate buffer

Store solutions at 4°C for 1 month (DMAB) or several months (EDTA, $MnSO_4$, MBTH).

Table A1. Mean values and standard errors (SE) of MnP and residual peroxidases found in forest floor samples (µmol per gram of soil). Means are calculated from the three composite samples collected within each plot.

Treatment	**Plot**	**MnP (μmol g^{-1})**	**SE of MnP**	**Residual Peroxidase (μmol g^{-1})**	**SE of Residual Peroxidase**
DF	2	3.4	0.9	24.0	4.5
	9	17.6	6.4	25.4	10.6
	10	12	2.0	20.4	3.1
	15	16.3	4.2	59.3	22.9
	19	3.4	0.5	4.5	1.0
	22	2.9	0.3	11.0	2.9
50:50	1	2.2	0.5	5.1	1.2
	11	4.7	1.0	11.9	2.4
	12	4.1	0.8	11.8	4.6
	17	11.7	1.3	26.0	3.2
	18	17.3	1.3	25.4	1.2
	21	5.0	0.4	15.3	2.7
CW	3	3.0	0.7	9.1	1.6
	5	2.9	0.6	4.1	2.0
	6	6.9	1.3	7.3	2.1
	13	5.2	1.0	7.4	1.4
	14	4.5	0.5	6.4	1.4
	16	5.9	1.2	10.2	1.7

Table A2. Mean (± SE) MnP and residual peroxidases content found in mineral soil (µmol per gram of soil). Means are calculated from the three composite samples collected in each plot.

Treatment	Plot	MnP (µmol g^{-1})	SE of MnP	Residual Peroxidase (µmol g^{-1})	SE of Residual Peroxidase
DF	2	0.3	0.1	0.6	0.2
	9	0.2	0.0	0.3	0.0
	10	0.1	0.0	0.2	0.1
	15	0.2	0.0	0.1	0.1
	19	0.1	0.0	0.1	0.0
	22	0.4	0.0	0.0	0.0
50:50	1	0.5	0.2	0.7	0.2
	11	0.1	0.0	0.2	0.1
	12	0.1	0.0	0.5	0.2
	17	0.1	0.0	0.1	0.0
	18	0.1	0.0	0.2	0.1
	21	0.2	0.0	0.2	0.1
CW	3	0.1	0.0	0.2	0.0
	5	0.1	0.0	0.3	0.0
	6	0.2	0.0	0.3	0.1
	13	0.1	0.0	0.1	0.1
	14	0.2	0.0	0.2	0.1
	16	0.2	0.1	0.1	0.0

Table A3. Mean peroxidase values (μmol g^{-1}) of forest floor (FF) and mineral soil (MIN) subsamples within plots of 100% DF, 100% CW, and 50:50% DF:CW. Both MnP and residual peroxidases were added to determine the total peroxidase values of the forest floor and mineral soil.

Plot	**Treatment**	**Replicant**	**Mean FF MnP (μmol g^{-1})**	**Mean FF Others (μmol g^{-1})**	**Mean Total FF Peroxidases (μmol g^{-1})**	**Mean MIN MnP (μmol g^{-1})**	**Mean MIN Others (μmol g^{-1})**	**Mean Total MIN Peroxidases (μmol g^{-1})**
1	50/50	1	1.2	3	4.2	0.3	0.6	0.9
1	50/50	2	3	7.1	10.1	0.3	0.4	0.7
1	50/50	3	2.4	5.1	7.5	0.9	1.0	1.9
2	DF	1	5.2	32.6	37.8	0.6	0.8	1.3
2	DF	2	2.2	17.3	19.5	0.3	0.9	1.3
2	DF	3	2.8	22.1	24.9	0.1	0.1	0.3
3	CW	1	2.3	7.4	9.7	0.2	0.1	0.3
3	CW	2	4.4	12.2	16.6	0.1	0.2	0.4
3	CW	3	2.4	7.7	10.1	0.1	0.1	0.2
5	CW	1	2.7	2	4.7	0.1	0.3	0.4
5	CW	2	2.1	2.2	4.3	0.1	0.2	0.3
5	CW	3	4	8	11.9	0.1	0.3	0.4
6	CW	1	8.5	11.4	19.8	0.2	0.4	0.6
6	CW	2	4.3	4.5	8.8	0.2	0.2	0.5
6	CW	3	7.9	6.1	13.9	0.3	0.4	0.6
9	DF	1	30.4	46.6	77	0.3	0.2	0.5
9	DF	2	11.4	16.2	27.6	0.2	0.3	0.5
9	DF	3	11.1	13.4	24.5	0.2	0.3	0.5
10	DF	1	13.4	25.1	38.5	0.2	0.3	0.5
10	DF	2	8.1	14.5	22.7	0.1	0.1	0.2
10	DF	3	14.6	21.6	36.2	0.1	0.2	0.3
11	50/50	1	6.7	15.6	22.3	0.1	0.1	0.2
11	50/50	2	3.3	7.5	10.7	0.1	0.3	0.4
11	50/50	3	4.2	12.6	16.7	0.1	0.1	0.3
12	50/50	1	4.5	14.3	18.8	0.2	0.6	0.8
12	50/50	2	2.6	3	5.6	0.1	0.2	0.3
12	50/50	3	5.2	18.2	23.4	0.1	0.8	0.9
13	CW	1	7.1	10.1	17.1	0.1	0.1	0.2
13	CW	2	4.5	6.8	11.3	0.1	0.1	0.2
13	CW	3	3.9	5.3	9.3	0.2	0.2	0.4
14	CW	1	5.5	9.1	14.6	0.2	0.3	0.4
14	CW	2	3.7	4.3	8	0.2	0.3	0.6
14	CW	3	4.3	5.7	10	0.2	0.1	0.3
15	DF	1	16.2	70.7	86.9	0.2	0.2	0.4
15	DF	2	9.2	15.2	24.3	0.1	0.0	0.1
15	DF	3	23.5	91.9	115.3	0.2	0.1	0.3
16	CW	1	5.4	10.1	15.5	0.3	0.2	0.5
16	CW	2	4.2	7.3	11.6	0.1	0.0	0.2
16	CW	3	8.2	13.1	21.3	0.1	0.1	0.2
17	50/50	1	13.6	26.3	39.9	0.2	0.1	0.2
17	50/50	2	9.1	20.4	29.4	0.1	0.1	0.3
17	50/50	3	12.4	31.3	43.7	0.1	0.2	0.3
18	50/50	1	16.6	25.5	42.1	0.1	0.1	0.2
18	50/50	2	15.6	23.4	39.1	0.1	0.1	0.2
18	50/50	3	19.8	27.3	47.1	0.2	0.3	0.5
19	DF	1	4.1	6	10.1	0.1	0.2	0.3
19	DF	2	3.8	4.9	8.6	0.2	0.1	0.3
19	DF	3	2.4	2.5	5	0.1	0.1	0.1
21	50/50	1	5.2	20	25.2	0.1	0.1	0.2
21	50/50	2	5.6	15.2	20.9	0.2	0.3	0.5
21	50/50	3	4.3	10.7	15	0.2	0.1	0.2
22	DF	1	3.5	15.5	19	0.5	0.0	0.5
22	DF	2	2.9	12.1	15	0.4	0.0	0.4
22	DF	3	2.3	5.5	7.8	0.5	0.0	0.5

Table A4. Exchangeable soil cations and effective cation exchange capacity (CEC) (cmol kg^{-1}) of forest floor subsamples within plots of 100% DF, 100% CW, and 50:50%.

Plot	Treatment	Replicant	Exch Al (cmol kg^{-1})	Exch Ca (cmol kg^{-1})	Exch Fe (cmol kg^{-1})	Exch K (cmol kg^{-1})	Exch Mg (cmol kg^{-1})	Exch Mn (cmol kg^{-1})	Exch Na (cmol kg^{-1})	Effective CEC (cmol kg^{-1})
1	50/50	1	0.47	30.26	0.04	0.78	5.60	1.35	0.22	38.72
1	50/50	2	0.57	42.03	0.05	1.26	6.88	2.26	0.28	53.34
1	50/50	3	0.90	31.43	0.08	1.02	7.03	1.58	0.24	42.27
2	DF	1	0.15	55.49	0.03	1.46	7.31	2.89	0.21	67.53
2	DF	2	0.17	53.23	0.02	1.56	8.25	3.17	0.27	66.66
2	DF	3	0.16	56.79	0.02	1.47	9.03	2.64	0.29	70.40
3	CW	1	0.13	54.59	0.02	0.64	6.14	1.75	0.20	63.48
3	CW	2	0.24	31.59	0.03	0.35	4.54	0.71	0.16	37.62
3	CW	3	0.46	46.59	0.04	0.48	7.93	1.72	0.22	57.45
5	CW	1	0.55	33.89	0.07	0.57	4.40	1.02	0.20	40.69
5	CW	2	0.43	29.80	0.04	0.45	5.12	1.24	0.18	37.26
5	CW	3	0.39	40.88	0.07	0.71	7.94	1.82	0.25	52.06
6	CW	1	0.50	41.15	0.04	1.52	8.66	1.49	0.31	53.68
6	CW	2	0.75	22.10	0.06	0.53	4.97	0.80	0.19	29.40
6	CW	3	0.28	45.42	0.05	0.85	8.61	1.05	0.25	56.50
9	DF	1	0.54	31.73	0.07	1.84	4.37	2.53	0.26	41.34
9	DF	2	0.45	30.21	0.04	0.92	4.62	2.26	0.20	38.71
9	DF	3	0.89	28.74	0.09	0.97	3.90	2.31	0.18	37.07
10	DF	1	0.82	36.36	0.06	0.86	6.06	1.55	0.23	45.95
10	DF	2	0.72	19.19	0.03	0.51	2.33	2.56	0.16	25.50
10	DF	3	1.10	27.27	0.10	1.08	4.10	1.83	0.21	35.68
11	50/50	1	0.25	49.59	0.03	1.03	7.34	1.49	0.23	59.95
11	50/50	2	0.51	33.47	0.05	0.81	6.02	1.33	0.24	42.44
11	50/50	3	0.27	35.79	0.04	1.03	5.90	1.39	0.22	44.64
12	50/50	1	0.17	48.77	0.03	0.90	7.54	1.60	0.23	59.24
12	50/50	2	0.17	45.52	0.03	0.83	7.08	1.44	0.22	55.29
12	50/50	3	0.15	47.44	0.03	1.01	6.74	1.46	0.21	57.05
13	CW	1	0.46	33.58	0.05	1.43	7.00	1.71	0.25	44.47
13	CW	2	0.53	31.83	0.05	0.97	6.48	2.12	0.23	42.19
13	CW	3	0.75	29.49	0.08	1.01	6.19	1.87	0.20	39.59
14	CW	1	0.43	39.27	0.04	1.79	6.64	6.43	0.23	54.83
14	CW	2	0.61	31.47	0.06	1.29	6.70	2.70	0.22	43.06
14	CW	3	0.55	32.04	0.04	1.32	5.58	4.38	0.26	44.16
15	DF	1	0.39	34.32	0.04	1.74	5.70	6.19	0.30	48.68
15	DF	2	0.70	26.60	0.05	1.42	4.01	3.59	0.25	36.63
15	DF	3	0.69	30.23	0.07	2.56	4.62	6.13	0.33	44.62
16	CW	1	0.66	26.51	0.06	0.93	5.38	3.28	0.24	37.06
16	CW	2	0.51	24.61	0.07	1.11	6.07	2.88	0.22	35.46
16	CW	3	0.96	23.77	0.08	1.27	4.86	3.89	0.24	35.07
17	50/50	1	0.38	36.08	0.04	1.36	3.63	4.11	0.21	45.82
17	50/50	2	0.80	24.92	0.07	0.95	3.08	2.20	0.22	32.24
17	50/50	3	0.45	35.32	0.04	1.34	4.16	4.05	0.23	45.58
18	50/50	1	0.45	30.86	0.05	1.23	5.91	6.21	0.32	45.02
18	50/50	2	0.95	32.24	0.06	1.49	4.60	4.31	0.33	43.97
18	50/50	3	0.22	50.24	0.02	1.65	7.58	4.97	0.31	64.98
19	DF	1	0.21	49.55	0.03	0.61	6.43	1.42	0.23	58.49
19	DF	2	0.25	46.79	0.03	0.59	7.63	1.33	0.21	56.83
19	DF	3	0.40	43.91	0.07	0.71	7.49	0.82	0.22	53.62
21	50/50	1	0.19	39.27	0.03	0.58	4.67	1.15	0.14	46.03
21	50/50	2	0.12	51.27	0.02	1.00	6.03	1.60	0.19	60.25
21	50/50	3	0.20	51.00	0.03	0.50	7.87	1.33	0.18	61.11
22	DF	1	0.19	42.31	0.03	0.91	7.21	2.07	0.22	52.93
22	DF	2	0.15	45.42	0.03	0.64	5.65	1.74	0.18	53.80
22	DF	3	0.10	58.08	0.02	0.81	7.11	1.94	0.19	68.26

Table A5. Nitrogen (%), carbon (%), C:N ratio, sulphur (%), salal cover estimate, and gravimetric moisture for forest floor (FF) subsamples within plots of 100% DF, 100% CW, and 50:50%. Gravimetric moisture was also calculated for mineral soil (MIN) subsamples.

Plot	Treatment	Replicant	N FF (%)	C FF (%)	C:N FF	S FF (%)	Salal Estimate (%)	Moisture FF	Moisture MIN
1	50/50	1	0.850	29.293	34.462	0.098	2	0.681	0.784
1	50/50	2	1.133	41.458	36.591	0.122	2	0.645	0.814
1	50/50	3	1.011	44.949	44.460	0.118	2	0.647	0.764
2	DF	1	1.006	36.618	36.400	0.110	10	0.618	0.675
2	DF	2	1.258	47.898	38.075	0.137	10	0.567	0.818
2	DF	3	1.296	46.932	36.213	0.147	10	0.469	0.823
3	CW	1	1.266	46.999	37.124	0.139	25	0.363	0.800
3	CW	2	0.561	18.937	33.756	0.061	25	0.464	0.710
3	CW	3	1.279	43.447	33.970	0.147	25	0.389	0.779
5	CW	1	0.854	33.132	38.796	0.095	50	0.478	0.799
5	CW	2	0.681	23.244	34.132	0.078	50	0.646	0.825
5	CW	3	1.264	41.872	33.127	0.146	50	0.404	0.786
6	CW	1	1.292	50.465	39.060	0.153	95	0.323	0.843
6	CW	2	0.732	30.213	41.275	0.088	95	0.583	0.730
6	CW	3	1.359	43.279	31.846	0.175	95	0.396	0.755
9	DF	1	1.018	41.568	40.833	0.118	80	0.574	0.873
9	DF	2	0.677	31.163	46.031	0.078	80	0.586	0.832
9	DF	3	0.740	30.333	40.991	0.087	80	0.617	0.858
10	DF	1	1.205	38.854	32.244	0.139	5	0.605	0.839
10	DF	2	0.660	28.961	43.880	0.074	5	0.615	0.873
10	DF	3	1.190	46.118	38.755	0.139	5	0.457	0.863
11	50/50	1	1.437	43.868	30.527	0.163	20	0.523	0.861
11	50/50	2	0.975	48.037	49.269	0.119	20	0.531	0.829
11	50/50	3	1.088	40.443	37.172	0.129	20	0.532	0.858
12	50/50	1	0.943	42.682	45.262	0.111	15	0.465	0.773
12	50/50	2	0.792	24.081	30.405	0.086	15	0.562	0.840
12	50/50	3	0.824	28.062	34.056	0.097	15	0.491	0.799
13	CW	1	1.142	48.239	42.241	0.148	80	0.490	0.835
13	CW	2	0.868	43.725	50.374	0.111	80	0.463	0.850
13	CW	3	0.933	42.665	45.729	0.120	80	0.535	0.800
14	CW	1	1.190	46.819	39.344	0.147	75	0.660	0.883
14	CW	2	1.023	39.672	38.780	0.117	75	0.615	0.808
14	CW	3	1.104	48.359	43.803	0.128	75	0.583	0.842
15	DF	1	0.883	40.774	46.177	0.105	85	0.588	0.882
15	DF	2	0.812	38.935	47.950	0.102	85	0.691	0.900
15	DF	3	1.028	42.503	41.345	0.119	85	0.576	0.891
16	CW	1	0.818	41.980	51.320	0.099	50	0.674	0.885
16	CW	2	1.168	36.368	31.137	0.108	50	0.687	0.894
16	CW	3	1.067	41.845	39.217	0.124	50	0.700	0.901
17	50/50	1	0.795	35.458	44.601	0.094	30	0.744	0.874
17	50/50	2	0.701	32.699	46.646	0.081	30	0.596	0.897
17	50/50	3	0.979	43.566	44.501	0.106	30	0.532	0.868
18	50/50	1	0.837	34.525	41.249	0.098	90	0.572	0.898
18	50/50	2	1.091	49.704	45.558	0.126	90	0.565	0.879
18	50/50	3	1.099	50.420	45.878	0.141	90	0.482	0.866
19	DF	1	1.713	42.467	24.791	0.175	0	0.492	0.870
19	DF	2	1.676	46.076	27.492	0.174	0	0.488	0.840
19	DF	3	2.035	47.508	23.345	0.212	0	0.414	0.873
21	50/50	1	0.886	24.524	27.679	0.095	0	0.623	0.831
21	50/50	2	0.967	31.931	33.021	0.111	0	0.639	0.759
21	50/50	3	1.124	33.628	29.918	0.131	0	0.456	0.798
22	DF	1	0.999	41.602	41.644	0.114	10	0.437	0.777
22	DF	2	0.972	30.062	30.928	0.110	10	0.587	0.843
22	DF	3	1.070	36.323	33.947	0.125	10	0.528	0.842