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Impacts of organic matter removal and vegetation control on nutrition and growth of Douglas-fir at three Pacific Northwestern Long-Term Soil Productivity sites



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ABSTRACT

As intensive management of forest plantations and interest in harvesting biomass for energy continue to increase, there is a need to investigate the longer-term effects of harvest-related disturbances and intensive treatments on soil and site productivity. This research focused on three Pacific Northwestern Douglas-fir soil productivity studies around 15 years since harvest that spanned a range in soil nutrients: high soil N and low base cations (Fall River), low soil N and base cations (Matlock), and high soil N and base cations (Molalla). The studies, which had similar organic matter and vegetation control treatments, were compared for differences in belowground and aboveground nutrients as well as differences in periodic stand volume growth. Five years of annual vegetation control (AVC) resulted in the greatest losses of belowground N and base cations compared to one year of vegetation control (IVC) at planting, but also resulted in significantly greater stand volume growth at Fall River and Matlock. Whole tree removal (WT) resulted in lower soil NO₃⁻ at Fall River but greater soil NO₃⁻ at Matlock due to greater colonization by N-fixing Scotch broom. There was also a decrease in soil exchangeable K due to WT and WT plus coarse woody debris removal (WT +) at Fall River, which had the lowest initial soil exchangeable K. There was either no effect (Matlock and Molalla) (0-15 years) or a decrease (Fall River) (0-5 years) in stand volume growth due to WT removal. At Fall River, WT, WT+, and AVC treatments had no detectable effect on volume growth from 10 to 15 years. Overall, longer-term effects of organic matter removals and vegetation control on soil and site productivity were variable at each site due to pre-treatment soil nutrition and competition from understory vegetation.

1. Introduction

The Long-Term Soil Productivity (LTSP) study in North America is a research network designed to determine the effects of organic matter removal and soil compaction on forest productivity (Powers 2006). The focus on organic matter removal and soil compaction was due to the importance of soil porosity and organic matter in determining forest productivity and the sensitivity of these parameters to management (Powers et al., 1990). Some LTSP sites included additional vegetation control treatments to determine the effects of intensive vegetation management after harvest (Ares et al., 2007) as well as to remove potential confounding in competing vegetation abundance attributable to the organic matter treatments (Morris and Lowery, 1988).

Summary results from the LTSP studies have been highly site specific with some dependency on time since harvest (Fleming et al., 2006; Thiffault et al., 2011; Ponder et al., 2012). The greatest effects of organic matter removal and soil compaction in young stands (< 15 years old) appear to be attributable to changes in microclimate, whereas treatment effects in older stands (i.e., at the canopy closure stage) appear to be associated with changes in soil nutrient concentrations and soil water availability (Thiffault et al., 2011). For example, seedling survival tended to increase due to improved microclimate and planting efficiency after whole tree harvests, but height growth in planted trees was reduced 10-25 years post-harvest compared to bole only harvests

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(Thiffault et al., 2011). Competing vegetation control has improved planted seedling nutrition and growth up to 10 years post-harvest due to less competition from understory species for water, nutrients, and light (Roberts et al., 2005; Fleming et al., 2006; Ponder et al., 2012).

Potential nutrient losses from organic matter removals and increased leaching due to vegetation control could have considerable effects on site nutrition (Powers, 2006). For example, annual vegetation control has been shown to increase NO_3^- leaching for two to four years after harvest (Slesak et al., 2009). Conversely, traditional organic matter removals (bole-only harvest) tend to result in more NO_3^- leached after harvest than more intensive organic matter removals (whole-tree harvest) (Strahm et al., 2005; Slesak et al., 2009), likely because aboveground pools of organic matter (e.g. slash and O-horizon) are the dominant sources of NO_3^- (Ares et al., 2007; Slesak et al., 2016a). In a *meta*-analysis (Thiffault et al., 2011), whole tree treatments mostly resulted in lower soil macronutrient contents compared to bole only treatments 1–20 years after harvest due to higher and permanent

nutrient removal from the system. LTSP treatment effects on site nutrition will depend on pre-treatment soil nutrient availability because the limiting essential nutrients are preferentially incorporated into aboveground biomass during the most productive time in stand development (i.e., around canopy closure) compared to non-limiting soil nutrients (Vitousek and Reiners, 2006). Therefore, impacts of permanent losses of limiting nutrients through organic matter removals and leaching may be exacerbated.

Although traditional soil extractions and incubations are commonly used for response assessment in LTSP studies, ion exchange resins have been used on LTSP sites to examine changes in soil nutrient availability (Duarte, 2002; Lewandowski et al., 2016; Lewandowski et al. 2019). Ion exchange resins provide measures of nutrient availability in the soil solution by adsorbing available cations or anions over time based on the charge of the resin. Organic matter removal increased $\rm NH_4^+$ adsorption by exchange resins relative to unharvested trembling aspen (*Populus tremeloides* Michx.) stands in the first year after harvest and decreased



Fig. 1. Three affiliate sites of the North American Long-term Soil Productivity study in Washington and Oregon, USA. Map is sourced from ArcGIS (ArcMap version 10.6, Esri, Redlands, CA).

 NO_3^- adsorption in the second year (Lewandowski et al. 2016). Vegetation control has also been found to increase availability of NH_4^+ and NO_3^- adsorption by Plant Root SimulatorTM (PRS) probes compared to control plots in white spruce (*Picea glauca* (Moench) Voss) and jack pine (*Pinus banksiana* Lamb.) stands (Hangs et al., 2004). Therefore, ion exchange resins show a potential to increase the understanding of treatment effects due to organic matter removal and vegetation control treatments in LTSP studies.

Coast Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco var. menziesii) is the primary plantation species utilized in Pacific Northwest forests. While extensive throughout the coastal Pacific Northwest, Douglas-fir productivity is highly variable and dependent on climate. soil development, and available soil water and N (Steinbrenner, 1979; Littke et al., 2016). Here we report on three Douglas-fir LTSP affiliate studies with distinct climates and contrasting soil textures that were established 15-20 years ago to study the effects of organic matter removals and competing vegetation control on soil productivity. Our objectives were to examine treatment effects on aboveground and belowground nutrient properties and Douglas-fir stand volume growth and how they vary with pre-treatment soil nutrient availability. We tested the following hypotheses: 1) whole tree removals and annual vegetation control will have significant negative effects on nutrient concentrations in the forest floor, mineral soil, and planted-tree biomass, with greater effects from higher levels of organic matter removals, 2) stand volume growth will respond according to observed treatment effects on aboveground and belowground nutrient concentrations, and 3) PRS probe ion adsorption will reflect significant treatment effects on aboveground and belowground nutrient concentrations.

2. Methods

2.1. Site descriptions

The three sites in this study are referred to as (1) Molalla, (2) Matlock, and (3) Fall River (Fig. 1). At Molalla (near Dodge, OR), the parent material is basic igneous agglomerate and the soil series is classified as a Kinney cobbly medial loam (Table 1; Soil Survey Staff, 2019). The youngest soil parent material is found at Matlock (near Matlock, WA) where glacial outwash was deposited during the

Pleistocene (Kruckeberg, 1991) and the soil series is classified as a Grove very gravelly loamy sand (Soil Survey Staff, 2019). All sites were analyzed for total and available soil nutrients prior to treatment. Details on pre-treatment soil sampling and analyses can be found in Ares et al. (2007) and Slesak et al. (2011). According to regional soil data, Fall River contained a greater than average soil total N content and the highest LTSP soil total N content, yet had the lowest soil exchangeable Ca content among sites and low soil exchangeable Ca, K, and Mg contents compared to regional data (Table 1). The Matlock site held less than average regional soil total N and exchangeable cation contents, yet exchangeable Ca and Mg contents were higher than those at Fall River. The Molalla site had greater than average regional total soil N and exchangeable cation contents. All sites contained less than average soil extractable P contents.

At Matlock and Molalla, treatments were applied in 2003 in a randomized complete block design with four replications of each treatment (Fig. 2). Initial vegetation control at Matlock included triclopyr ester to control Scotch broom and this treatment was repeated across the site in 2004 and 2007 because of a severe Scotch broom infestation and associated high mortality of planted Douglas-fir (Harrington and Schoenholtz, 2010). Five years of annual vegetation control (AVC) at Matlock aimed for operational treatments including sulfometuron (2003) and glyphosate and clopyralid (2005–2008). All plots at Molalla received glyphosate in 2003, while the AVC treatments included operational treatments of glyphosate (2004 and 2006), sulfometuron (2003–2004), atrazine (2006–2007), and triclopyr ester (2008) (Harrington and Schoenholtz, 2010). Both Matlock and Molalla were planted in spring 2004 with 1,111 trees per ha in 0.09-ha measurement plots with treated buffers (Table 1).

Fall River (near Brooklyn, WA) is situated on the Pomona basalt flow with a component of volcanic ash and the soil series is classified as a Boistfort silt loam (Ares et al., 2007; Soil Survey Staff, 2019) (Table 1). Fall River was harvested in 1999 (Table 1; Fig. 2). There were four treatment blocks. Annual vegetation control treatments aimed for 95% vegetation control and included sulfometuron (2000 and 2002), glyphosate (2000–2003), atrazine (2001–2002), clopyralid (2002–2003), and hexazinone (2003–2004) (Ares et al., 2007). Fall River was planted with Douglas-fir at 1,600 trees per ha in March 2000 within 0.1-ha measurement plots with treated buffers (Table 1; Fig. 2).

Table 1

Descriptive site variables and pre-treatment soil data to 1-m depth compared to regional Douglas-fir site and soil averages (n = 47-156) with minimums and maximums in parentheses (Holub, 2011; Littke et al., 2014; James et al., 2016).

Variable	Unit	Fall River	Matlock	Molalla	Regional Averages (Range)
Plot Size	ha	0.1	0.09	0.09	N/A
Latitude	degrees	46.72	47.21	45.2	46.3 (42.6–50.3)
Longitude	degrees	-123.42	-123.44	-122.29	-123.0 (-126.8-121.6)
Elevation	m	334	35	549	353 (46–1,341)
Mean Annual Temperature ^a	С	9.6	10.7	9.8	10 (6.7–12)
Mean Annual Precipitation ^a	mm	2,300	2,000	1,800	1,900 (700–3,900)
Soil Parent Material	geology	Basalt	Glacial Outwash	Basic Agglomerate	Glacial, Igneous, Sedimentary
Geologic Age	period	Miocene	Pleistocene	Miocene	(Pleistocene-Eocene)
Available Water Supply ^b	cm	18.3	6.5	19.3	15.8 (3.8–28)
Soil Total N Content	kg N ha ⁻¹	13,010 ^c	4,498 ^d	9,844 ^d	8,232 (280-24,639)
Soil Exchangeable Ca Content	kg Ca ha $^{-1}$	803 ^c	744 ^d	9,930 ^d	5,640 (136-63,201)
Soil Exchangeable Mg Content	kg Mg ha ⁻¹	349 [°]	358 ^d	4,024 ^d	1,485 (12–12,691)
Soil Exchangeable K Content	kg K ha ⁻¹	511 [°]	188 ^d	2,496 ^d	737 (8–7,342)
Soil Extractable P Content	kg P ha ⁻¹	38 ^c	59 ^d	26 ^d	73 (5–456)
Previous Stand King's Site Index [Current Stand]	m at 50 years	42 [46 ^e]	36 [26 ^e]	36 [34 ^e]	42 (28–55) ^e
Stand Density at Planting	trees hectare ⁻¹	1,600	1,111	1,111	N/A

^a Wang et al., 2012.

^b Soil Survey Staff, 2019.

^c Ares et al., 2007.

^d Soil samples from the Matlock and Molalla sites were converted from 0.6 m (Slesak et al., 2016a) to 1 m using equations relating 0.6-m depth soil nutrients to 1-m depth soil nutrients (K. Littke, unpublished data; J. James, personal communication).

Current King's site index (King, 1966) is based on 15-year (this study) and juvenile (12-31 years) tree height measurements.



Fig. 2. Timeline of harvest, planting, vegetation control, measurements, and foliar and soil sampling at the three sites.



Fig. 3. Mean residual coarse woody debris with standard errors after bole only (BO), whole tree (WT), and whole tree plus coarse woody debris removals (WT +) at Fall River, Matlock, and Molalla. Sampling methods are described in Ares et al., 2007; Harrington and Schoenholtz, 2010.

All sites received the main organic matter removal (OM) treatments applied in the LTSP study (bole-only harvest (BO) and whole tree harvest (WT)) to test the effect of OM removals on soil and site productivity. A whole tree harvest with coarse woody debris removal (WT+: WT plus large legacy wood, surface red rot, and live and dead coarse woody debris greater than 0.6 cm) treatment was only applied at Fall River (Ares et al., 2007). The BO treatment removed just the merchantable logs from the plot. The WT treatment removed most aboveground tree parts including needles and branches, but stumps and roots were left on site. None of these treatments match what would be done operationally but were instead targeted to cover a range of possible biomass and nutrient removals. Average residual biomass for each site and treatment are shown in Fig. 3.

Fall River is the most productive site according to estimates of King's site index prior to the previous harvest (42 m at 50 years) (King, 1966) (Table 1). Matlock and Molalla were estimated to have the same site index (36 m at 50 years) based on the previously harvested stands, but the Molalla site is much more productive overall than Matlock (Table 1; Slesak et al., 2016a). Douglas-fir diameter at breast height (1.3 m) (DBH) and height were previously measured on the LTSP sites at 0, 5, 10, and 15 years (Devine et al., 2011; Holub et al., 2013; Slesak et al., 2016a) (Fig. 2). Stem volume was calculated based on tree size. For trees shorter than breast height, volume was calculated as the sum of the volume of a cylinder and the volume of a neiloid using the radius at 15-cm height. The equations of Omule et al. (1987) (DBH < 2.5) and Bruce and DeMars (1974) (DBH \geq 2.5-cm; small tree equation for height < 5.5-m and larger tree equation for height ≥ 5.5 -m) were used for trees with measurable DBH. Total stand volume at each plot was summed from all trees for each measurement. Periodic stand volume growth per plot was calculated as the difference between the stand volume between two adjacent measurements divided by the associated time interval (Fig. 2).

2.2. Soil sampling and analyses

During April 2017, four sampling areas were randomly selected in each plot. One forest floor sample of known area (182 cm²) was removed from the sample area, and an 8-cm wide by 15-cm deep core was hammered into the mineral soil to measure bulk density, pH, and nutrient concentrations. Forest floor and mineral soil samples were brought back to the lab in a cooler and stored at 4 °C.

Forest floor and mineral soil samples were air-dried for at least 48 h or until dry to the touch. Forest floor and mineral soil samples were composited for each plot by sample type. Mineral soil samples were sieved to 4.75 mm to separate the fine-fraction. Forest floor samples were ground to 2 mm in a Wiley mill and then finely-ground in a ball mill. A subsample of forest floor and mineral soil was oven-dried at 105 °C for 48 h to estimate total dry weight.

Forest floor and mineral soil pH was analyzed using a 1:1 slurry of DI water after sitting for ten minutes. Total C and N of forest floor and mineral soil samples were analyzed by dry combustion with a CHN 2400 analyzer (PerkinElmer Inc., Akron OH). NH_4^+ and NO_3^- were extracted using 3 g of mineral soil and 30 ml of 2 M KCl, shaken for 1 h, filtered, and analyzed with an auto-analyzer (O-I-Analytical Co). Exchangeable cations were extracted by shaking 30 ml of 1 N NH₄Cl with 3 g of forest floor and mineral soil samples for 1 h. Mineral soil P was analyzed using the Bray-1 method (Bray and Kurtz, 1945). Total forest floor nutrients were analyzed using method EPA 3050b (U.S. EPA, 1996). Exchangeable cations, Bray P, and total nutrients were analyzed on an ICP-AES (ThermoFisher Scientific Co., Waltham MA).

Plant Root SimulatorTM (PRS) probes (Western Ag Innovations, Inc., Saskatoon, Saskatchewan, Canada) are commonly used to measure available anions and cations *in situ* in agriculture and forestry research with minimal associated soil disturbance. At each sampling pit, one of four anion and cation PRS probes per plot was placed at 5-cm depth in a vertical position to avoid water pooling on the probe. PRS probes were removed 12 weeks after placement and washed free of soil particles with deionized (DI) water, composited by plot, and sent to the manufacturer for analysis of NO₃⁻, NH₄⁺, Ca, K, Mg, H₂PO₄, SO₄, and micronutrients. NO₃⁻ and NH₄⁺ are analyzed colorimetrically with an automated flow injection analysis system while the other nutrients are analyzed using inductively-coupled plasma spectrometry. PRS probe data is presented as the amount of ions accumulated over the area of the resin membrane (10 cm²) over the burial period (84 days).

2.3. Vegetation sampling

Prior to the growing season in 2016, current-year foliage of the planted Douglas-fir were sampled from all LTSP sites (Root, 2017). Five trees per plot were sampled at Fall River and ten trees per plot were

sampled at Matlock and Molalla. At least two branches were sampled from the third whorl on the south side of each tree. The central and lateral current-year shoots from each branch were used for analysis. Foliar samples were stored at 4 $^{\circ}$ C until oven drying at 70 $^{\circ}$ C. The weight of 100 oven-dried needles was measured for each plot.

In August of 2018 (Matlock and Molalla) and 2019 (Fall River), competing vegetation was sampled on 10 subplots from each plot at Matlock and Molalla and 20 subplots per plot were sampled at Fall River. Subplots were 0.2 m^2 and were randomly located near buffer trees. At Fall River, the small amount of competing vegetation within subplots required a bulk sampling of competing vegetation throughout the buffer plot to obtain a sample large enough for nutrient analyses. Competing vegetation biomass from all subplots was composited by plot. Competing vegetation was separated into overstory (trees and large shrubs) and understory (herbaceous and small shrubs) in the lab. Samples from each component and plot were dried at 70° C.

Total foliar and competing vegetation C and N were measured by dry combustion using a CHN analyzer (CHN 2400, PerkinElmer Inc., Akron OH). Total metals were measured using the methods of EPA 3050b (U.S. EPA 1996) on an ICP-AES (ThermoFisher Scientific Co., Waltham MA).

2.4. Statistical analysis

Data from each site were analyzed separately because of the differences in treatments, plot size, and experimental design. An alpha level of 0.1 was used to determine significance for all analyses. Treatment effects on forest floor, soil, PRS adsorption, competing vegetation, and foliar nutrient variables were examined using a Type II sums of squares ANOVA from a linear regression model with a blocking factor using the "Anova" function in the "car" package (Fox and Weisberg, 2018) in R (R Statistical Software version 3.4.2, The R Foundation for Statistical Computing). Non-significant treatment effects (p > 0.10) were removed from each model unless the treatment effects involved in an interaction. No factor interactions were included in analysis of Fall River data due to a fractional factorial design. Significant treatment contrasts were determined using a Holm-Bonferroni method (Holm 1979) (p < 0.10).

The fixed effects of the organic matter removal and vegetation control treatments, measurement period, and their interactions on stand volume growth were modeled using repeated measures, mixed model analysis of variance (ANOVA) in SAS (SAS Institute Inc, 2013) for the 0–5, 5–10, and 10–15 year measurement periods. The model included random effects of blocking. No factor interactions were included in analysis of Fall River data due to a fractional factorial design. When treatment differences were detected for a given site, multiple comparisons of least-squares means were conducted with Bonferroni probabilities to control the Type I error rate (Quinn and Keough, 2002).

3. Results

3.1. Fall River

At Fall River, the whole-tree harvest with coarse woody debris removal (WT +) treatment resulted in significantly lower forest floor mass, soil NO₃⁻, soil exchangeable K, PRS Ca and K, and foliar Ca, but higher PRS P adsorption, competing vegetation Ca concentration, and foliar Al than the BO treatment (Table 2). The whole-tree harvest (WT) treatment had lower soil NO₃⁻, soil exchangeable K, and PRS K, but higher foliar Al than the BO treatment. WT+ resulted in significantly lower forest floor mass and foliar Ca and Mg, but greater forest floor exchangeable Al and PRS P adsorption than the WT treatment. Lower PRS K adsorption in the WT and WT+ treatments matched lower extraction of exchangeable K from the top 15-cm of soil in these treatments.

The annual vegetation control (AVC) treatment resulted in many

decreases in aboveground and belowground nutrient concentrations. There was significantly lower soil exchangeable Ca and Mg, forest floor total N, forest floor exchangeable Ca, K, and Mg, PRS NO₃⁻ and Mg, and foliar Ca in AVC versus initial vegetation control (IVC) (Table 2 and Fig. 4A). Greater forest floor exchangeable Al and competing vegetation N concentration were also associated with AVC relative to IVC. PRS NO₃⁻ and Ca adsorption matched vegetation treatment effects on forest floor total N and foliar Ca, respectively.

3.2. Matlock

Even though pre-treatment soil nutrient pools were often the lowest at Matlock, this site contained the fewest statistically significant responses to the experimental treatments (Table 3). The whole-tree harvest (WT) treatment resulted in greater soil exchangeable Al, PRS NO_3^- , and foliar N than the bole-only harvest (BO) treatment (Fig. 4B). The BO treatment contained significantly lower soil pH and soil exchangeable K than the WT treatment.

The IVC treatment contained greater forest floor total P, PRS NO₃⁻ and Mg, and foliar N than the AVC treatment (Table 3). The AVC treatment resulted in greater understory competing vegetation Al and foliar Mg and Al concentrations. The interaction between organic matter removal and vegetation control treatments resulted in significantly lower forest floor C:N ratios and forest floor exchangeable Al and higher forest floor total N and soil NH₄⁺ in the whole-tree harvest with initial vegetation control (WTIVC) treatment versus the whole-tree harvest with annual vegetation control (WTAVC) treatment. The WTAVC treatment also had significantly higher forest floor C:N ratios and forest floor exchangeable Al than the bole-only harvest with annual vegetation control (BOAVC) treatment.

3.3. Molalla

Organic matter removal treatments at Molalla resulted in few significant changes in nutrient concentrations (Table 4). Forest floor mass and PRS NH_4^+ were significantly greater in the WT treatment than in the BO treatment. The BO treatment contained significantly greater overstory competing vegetation N and P than in the WT treatment, while overstory competing vegetation Al was significantly lower in the BO treatment.

At Molalla, the greatest effects on nutrient variables were associated with the AVC treatment, yet there were no significant effects of treatments on foliar nutrition (Table 4). Soil exchangeable K and Mg, forest floor pH, understory competing vegetation Al, and PRS Ca and Mg were significantly lower in AVC than in IVC. Forest floor total P, soil Bray P, and understory competing vegetation Ca and Mg concentrations were significantly greater in AVC than in IVC. Interactions between organic matter removal and vegetation control treatments resulted in significantly higher forest floor exchangeable Mg in the WTIVC treatment than in the WTAVC treatment. The WTIVC treatment also resulted in significantly higher PRS K adsorption than in the BOIVC treatment.

3.4. Stand volume growth

Only one site (Fall River) showed a significant reduction in stand volume growth due to organic matter removals; the WT treatment had significantly lower stand volume growth than the BO and WT + treatments in the first measurement period (0–5 years) (Table 5; Fig. 5). The WT treatments at Matlock and Molalla did not have a significant effect on stand volume growth relative to the BO treatments.

At Fall River, there was significantly greater stand volume growth in the AVC treatment than in the IVC treatment in the first two measurement periods (0–10 years), but no significant difference in the last measurement period (10–15 years) (Table 5; Fig. 5). The AVC treatment significantly increased stand volume growth compared to the IVC treatment at each measurement period at Matlock. No significant

Table 2

Results of the analysis of variance for below- and aboveground responses to organic matter removal (OM) and vegetation control (V) at Fall River. Models with significant treatment effects are shown by variable (all variable means are listed in Supplementary material). Only treatments with significant effects (p-value < 0.10 in bold) are included in the models. Means and contrasts are shown for each significant treatment. Means for a given variable that are significantly different (p < 0.10 with Holm-Bonferroni correction) within each treatment type have different lowercase letters. There were no statistically significant interactions tested for OM and V due to a fractional factorial design.

Nutrient Variable	Unit	ANOVA N	Iodel	OM Treatmen	ts		V Treatments	
		ОМ	V	BO $(n = 8)$	WT(n = 4)	WT+ (n = 4)	IVC $(n = 4)$	AVC (n = 12)
		p-value	p-value	mean	mean	mean	mean	mean
Soil NO3	µg/g	0.02		1.87b	0.85 a	1.20 a		
Soil Exchangeable (Ex.) Ca	µg/g		< 0.01				457b	227 a
Soil Ex. K	µg/g	0.03		158b	104 a	107 a		
Soil Ex. Mg	µg/g		< 0.01				100b	53 a
Forest floor (FF) Mass	g/cm ²	0.07		0.29b	0.29b	0.21 a		
FF Total N	%		0.02				1.20b	0.98 a
FF Ex. Ca	µg/g		0.01				3163b	2322 a
FF Ex. K	µg/g		0.02				546b	430 a
FF Ex. Mg	µg/g		< 0.01				728b	540 a
FF Ex. Al	µg/g	< 0.01	0.09	77 ab	71 a	138b	51 a	104b
Plant Root Simulator (PRS) NO3-	µg/10 cm ² /12 weeks		0.02				20.5b	8.3 a
PRS Ca	µg/10 cm ² /12 weeks	0.09		243b	187 ab	110 a		
PRS K	µg/10 cm ² /12 weeks	< 0.01		153b	95 a	61 a		
PRS Mg	µg/10 cm ² /12 weeks		0.02				132b	83 a
PRS P	µg/10 cm ² /12 weeks	0.04		0.66 a	0.63 a	1.58b		
Competing Vegetation (CV) N	%		0.07				2.03 a	2.45b
CV Ca	%	0.08		0.52 a	0.58 ab	0.73b		
CV K	%	0.09		2.74b	2.42 ab	2.13 a		
Foliar Ca	%	0.02	0.03	0.14b	0.15b	0.11 a	0.15	0.13
Foliar Mg	%	0.02	0.02	0.077 ab	0.082b	0.072 a	0.082	0.075
Foliar Al	%	< 0.01		0.018 a	0.022b	0.021b		

differences in stand volume growth were found among treatments during all growth periods at Molalla.

4. Discussion

4.1. Treatment effects on soil nutrients and volume growth

The results from the three LTSP sites support the first hypothesis that organic matter removal and vegetation control have a significant effect on forest floor, soil, competing vegetation, and foliar nutrition concentrations. Most of these effects were detrimental (e.g., reductions in soil nutrient concentrations), but contrary to hypothesis 2 there were some positive effects of intensive treatments on stand volume growth. While the effect of vegetation control on stand volume growth has been greater than the effect of organic matter removals in past measurements, data from the most productive site (Fall River) suggests that trends could be changing such that the effect of AVC is lessening and the consequences of nutrient removals through leaching and whole tree removal are increasing (Fig. 5). Because these stands are at or reaching canopy closure, they are approaching peak demand for soil nutrients compared to earlier in their development when improvements in microclimate were more important (Thiffault et al., 2011; Harrington et al., 2013).

At all sites, AVC resulted in many changes in nutrient variables presumably due to the large effects of AVC on microclimate and uptake of nutrients. For example, in the second and third growing seasons at the Fall River site, there was a significant increase in soil moisture due to AVC, and seedling volume growth was positively correlated with summer soil moisture (Roberts et al., 2005). Up to 5 years after vegetation control treatments, there was significantly greater soil moisture and seedling growth and lower bulk soil respiration (only measured at Matlock and Molalla) due to AVC, but there was little effect on soil temperature (Roberts et al., 2005; Devine and Harrington, 2007; Slesak et al., 2010; Devine et al., 2011).

There was significantly less PRS NO_3^- adsorption in AVC treatments (at Fall River and Matlock), which could be due to significantly

greater NO₃⁻ leaching shortly after harvest in AVC treatments compared to IVC treatments and/or greater uptake by Douglas-fir in the AVC treatment (Slesak et al., 2009). Slesak et al. (2009) found significantly greater NO₃⁻ leaching after harvest due to AVC than IVC at Matlock and Molalla, yet there was no significant effect of AVC on PRS NO₃⁻ at Molalla. Furthermore, at Matlock additional N fixation due to Scotch broom (Cytisus scoparius (L.) Link) colonization resulted in significantly higher PRS NO₃⁻ adsorption in the IVC treatment. NO₃⁻ leaching was not measured after harvest in the IVC treatment at Fall River, but it would be expected to be lower than in the AVC treatment due to greater N uptake by the larger competing vegetation biomass (2,940 kg/ha (IVC) versus 53 kg/ha (AVC) at five years) (Roberts et al., 2005; Devine et al., 2011). Similar to five-year data, the competing vegetation at Fall River contained significantly greater N concentrations in the AVC treatment at 20 years than in the IVC treatment with greater competing vegetation biomass in the IVC treatment (1.2 kg/ha versus 4.9 kg/ha; data not shown). However, there was no significant effect of the vegetation control treatments on Douglas-fir foliar N concentration.

Although there were decreases in soil N and base cation concentrations in the AVC treatments, stand volume growth was increased over IVC treatments from 0 to 10 years at Fall River and during all measurement periods at Matlock due to less competition with understory species for light, water, and nutrients. Increased growth in stem volume and biomass of planted trees due to AVC has also been found in many studies (Fleming et al., 2006; Ponder et al., 2012; Wagner et al., 2006; Zhang et al., 2017). In a study of vegetation control on threeyear-old white spruce and jack pine, greater stem volume growth was associated with greater PRS NO₃⁻ and NH₄⁺ adsorption due to vegetation control compared to the control treatment (Hangs et al., 2004). The declining effect of AVC on periodic stand volume growth during the 10–15 measurement period at Fall River (Fig. 5B) was also observed on ponderosa pine stands from ages 15–25 (Zhang et al., 2013).

In comparison, post-harvest effects of whole tree removals resulted in increased soil temperatures and N mineralization (only measured at Fall River) with slightly negative (Fall River) or no effects on seedling growth (Matlock and Molalla) (Licata 2004; Roberts et al. 2005;



Fig. 4. Box and whisker plots of PRS NO_3^- adsorption at 5-cm depth over 12 weeks as affected by the organic matter removal and vegetation control treatments at Fall River (A), Matlock (B), and Molalla (C). Median (solid) and mean (dashed) lines and outliers (dots) are shown for each treatment. Individual treatments with different lowercase letters are significantly different (p < 0.10) within each treatment factor.

Table 3

Results of the analysis of variance for below- and aboveground responses to organic matter removal (OM) and vegetation control (V) at Matlock. Models with significant treatment effects are shown by variable (all variable means are listed in Supplementary material). Only treatments with significant effects (p-value < 0.10 in bold) are included in the models. Means and contrasts are shown for each significant treatment. Means for a given variable that are significantly different (p < 0.10 with Holm-Bonferroni correction) within each treatment type have different lowercase letters.

Nutrient Variables	Unit	ANOVA	Model		OM Treatme	nts	V Treatments		OM*V Trea	atments		
		ОМ	V	OM*V	BO (n = 8)	WT (n = 8)	IVC $(n = 8)$	AVC (n = 8)	$\begin{array}{l}\text{BOIVC}\\(n = 4)\end{array}$	$\begin{array}{l} \text{BOAVC} \\ (n = 4) \end{array}$	WTIVC $(n = 4)$	WTAVC $(n = 4)$
		p-value	p-value	p-value	mean	mean	mean	mean	mean	mean	mean	mean
Soil pH	no unit	0.02			5.20b	5.03 a						
Soil NH4 ⁺	µg∕g	0.63	0.07	< 0.01					3.98 ab	5.44b	6.66b	1.98 a
Soil Exchangeable (Ex.) K	µg∕g	0.06			109b	85 a						
Soil Ex. Al	µg∕g	0.04			60 a	85b						
Forest floor (FF) Total N	%	0.89	0.19	0.03					1.00 ab	1.11 ab	1.24b	0.84 a
FF C:N Ratio	no unit	0.76	< 0.01	< 0.01					36b	35b	27 a	46c
FF Ex. Al	µg∕g	0.27	0.04	< 0.01					41 ab	37 a	31 a	56b
FF Total P	µg∕g		0.09				1045b	943 a				
Plant Root Simulator (PRS) NO ₃ ⁻	μg/10 cm ² / 12 weeks	0.01	0.09		0.59 a	4.91b	4.11b	1.39 a				
PRS Mg	μg/10 cm ² / 12 weeks		< 0.01				86b	61 a				
Foliar N	%	< 0.01	0.02		1.53 a	1.73b	1.70b	1.56 a				
Foliar Mg	%		0.05				0.087 a	0.093b				
Foliar Al	%		0.01				0.017 a	0.020b				

Table 4												
Results of the analysis of variance for below	w- and aboveground re	sponses to	organic 1	natter rem	oval (OM) an	d vegetation co	ntrol (V) at M	olalla. Models w	ith significant tr	reatment effects :	are shown by v	ariable (all variable
means are listed in Supplementary materia variable that are significantly different (p	al). Only treatments wit < 0.10 with Holm-B	h signific onferroni	ant effect correctic	s (p-value n) within e	< 0.10 in bo	ld) are included nt type have di	l in the model fferent lowerd	ls. Means and co case letters.	ntrasts are show	n for each signif	icant treatmen	t. Means for a giver
NutrientVariable	Unit	ANOVA I	Model		OM Treatme	ints	V Treatmen	ts	OM*V Treatm	ents		
		MO	Λ	OM*V	BO (n = 8)	WT (n = 8)	IVC $(n = 8)$) AVC($n = 8$)	BOIVC(n = 4)) $BOAVC(n = 4)$	WTIVC(n = 4) WTAVC(n = 4)
		p-value	p-value	p-value	mean	mean	mean	mean	mean	mean	mean	mean
Soil Exchangeable (Ex) K	μ8/8		0.03				490b	378 a				
Soil Ex Mg	ug/g		0.02				282b	210 a				
Soil Bray P	ng/g		0.09				7.8 a	10.8b				
Forest floor (FF) Mass	g/cm ²	< 0.01			0.18 a	0.23b						
FF pH	no unit		0.03				5.68b	5.47 a				
FF Ex Mg	hg/g	0.69	0.05	0.03					994 ab	1012 ab	1167b	886 a
FF Total P	ng/g		0.04				971 a	1054b				
Plant Root Simulator (PRS) NH4 ⁺	μg/10 cm ² /12 weeks	0.03			0.19 a	0.96b						
PRS Ca	$\mu g/10 \text{ cm}^2/12 \text{ weeks}$		0.06				963b	668 a				
PRS K	$\mu g/10 \text{ cm}^2/12 \text{ weeks}$	< 0.01	0.77	0.01					228 a	295 ab	380b	299 ab
PRS Mg	$\mu g/10 \text{ cm}^2/12 \text{ weeks}$		0.04				246b	163 a				
Competing Vegetation (CV) Overstory (OS) N	% N	0.10			0.87b	0.63 a						
CV Understory (US) Ca	%		0.04				0.72 a	0.88b				
CV US Mg	%		0.06				0.23 a	0.27b				
CV US AI	%		< 0.01				0.040b	0.012 a				
CV OS AI	%	< 0.01			0.0041 a	0.0045b						
CV OS P	%	0.10			0.122b	0.098 a						

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Harrington and Schoenholtz, 2010; Slesak et al., 2010). There was a significant negative effect of whole tree removal on stand volume growth from 0 to 5 years at the Fall River site (Fig. 5). In a meta-analysis of organic matter removal studies, Thiffault et al. (2011) concluded that the effects of whole tree removals on early stand productivity are more dependent on the changes to microclimate, while the effects on older stand productivity are more dependent on changes in nutrient availability.

4.2. Additional organic matter removals

In support of the first hypothesis, there were distinct effects of the WT + versus WT and BO treatments at Fall River. Compared to the BO treatment, the WT+ treatment resulted in a greater amount of significant differences than in the WT treatment such as lower forest floor mass and PRS Ca but greater PRS P and competing vegetation Ca concentration. Both the WT and WT+ treatments resulted in a decrease in soil NO₃⁻ and exchangeable K, PRS K adsorption, but greater foliar Al concentration. In the spring and summer the year after study establishment, the WT+ treatment at Fall River also experienced warmer soil temperatures which resulted in greater N mineralization than in the BO (with AVC) treatment (Licata, 2004; Devine and Harrington, 2007). However, there was significantly less leaching of NO_3^{-2} -3 years after planting in this treatment than in the BO (with AVC) treatment (Strahm et al., 2005); the WT treatment was not tested in the Strahm et al. (2005) study. The decrease in soil K due to both WT and WT+ treatments is concerning because soil exchangeable K was low at Fall River in pre-treatment soil contents compared to regional soils (Table 1). Because plant biomass contains high concentrations of K it is probable that the WT and WT+ treatments resulted in a large permanent removal from the site instead of greater uptake by trees.

4.3. Influence of Pre-treatment nutrition on treatment response

Pools of limiting essential nutrients are thought to be concentrated in aboveground biomass relative to soil pools, limiting losses from the system in the absence of disturbance (Vitousek and Reiners, 2006). These unique sites contained a range of pre-treatment soil nutrient availabilities and site productivity that affect nutrient retention in forests. The main limiting nutrients in pre-treatment soils at the LTSP sites compared to regional averages appear to be exchangeable Ca, Mg, and K (Fall River and Matlock), N (Matlock), and P (Molalla) (Table 1).

Fall River contained the highest total soil N of the three LTSP sites yet contained the lowest soil exchangeable Ca and Mg that was reflected in moderately-deficient foliar Ca and Mg (Ballard and Carter, 1986). The first hypothesis was confirmed at this site because the loss of Ca, Mg, and K through annual vegetation control and organic matter removals resulted in lower amounts of these essential limiting nutrients that would otherwise be sequestered from loss out of the system through preferential uptake by Douglas-fir (Vitousek and Reiners, 2006). Also, the AVC and organic matter removal treatments at Fall River resulted in greater forest floor exchangeable Al and foliar Al, respectively, further reducing availability of base cations in these treatments (Fig. 6). Similarly, a meta-analysis of LTSP sites with different levels of organic matter removals found a decrease in soil exchangeable base cations associated with an increase in soil exchangeable Al (Achat et al., 2015). Organic matter removal and AVC treatments have resulted in a decrease in Ca from the forest floor, soil, and foliage compared to BO and IVC treatments, respectively, from this base cation-deficient soil (Fig. 6).

Hynicka et al. (2016) found that basalt-derived soils with high N and low Ca (like Fall River) were reliant on atmospheric Ca due to a depletion in Ca weathered from parent material. Due to the high N soil at Fall River, it is probable that consistent N leaching has been stripping base cations from this soil over time (Perakis et al., 2006). Leaching studies at the Fall River site showed much higher N leaching after

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Table 5

Results of the analysis of variance for stand volume growth (m³ ha⁻¹ yr⁻¹) responses to organic matter removal (OM) and vegetation control (V) at Fall River, Matlock, and Molalla. Least squares means for a given treatment type and measurement period followed by different lowercase letters differ significantly (p < 0.10). There were no statistically significant interactions between OM and V at Matlock and Molalla and no interactions were tested at Fall River due to a fractional factorial design.

Site	Model			Measurement Period	OM Treat	ments		V Treatmen	V Treatments	
	OM*Year	V*Year	OM*V*Year		во	WT	WT+	IVC	AVC	
	p-value	p-value	p-value		mean	mean	mean	mean	mean	
Fall River	0.06	< 0.01	NA	0–5 years	0.95b	0.81 a	0.96b	0.74 a	1.10b	
				5–10 years	12.13	10.95	12.05	10.44 a	13.10b	
				10–15 years	27.31	25.91	26.57	26.41	26.77	
Matlock	0.62	0.02	0.66	0-5 years	0.14	0.10	NA	0.07 a	0.19b	
				5–10 years	1.53	1.04		0.73 a	2.17b	
				10-15 years	6.48	4.60		3.44 a	8.65b	
Molalla	0.36	0.31	0.32	0-5 years	0.24	0.21	NA	0.19	0.26	
				5–10 years	5.21	4.36		4.17	5.45	
				10-15 years	19.92	15.99		16.14	19.73	



Fig. 5. Average difference in five-year volume growth increment compared to the bole only treatment (A) and the initial vegetation control treatment (IVC) (B) at Fall River (FR), Matlock (MAT), and Molalla (MOL) sites. WT = whole tree; AVC = annual vegetation control. Lines with asterisks were significantly different during that measurement period (p < 0.1; Table 5).

harvesting than at Matlock (Devine et al., 2012), and that the highest leaching was found in the BOAVC treatment over the WT + AVC treatment (Strahm et al., 2005). Significantly lower forest floor N concentration, forest floor, soil, foliar cation concentrations, and PRS NO_3^- and cations were found in the AVC treatment compared to the IVC treatment (Tables 2–4; Figs. 4 and 6), which would support greater leaching of base cations along with N after harvest than in the IVC treatment. Leaching was not measured in the IVC treatment at the Fall River site, but it was likely lower than AVC because of increased uptake

by competing vegetation as in Matlock and Molalla (Slesak et al., 2009). In addition, the WT+ treatment contained the lowest PRS Ca and K adsorption and foliar Ca supporting the further loss of limiting available cations through removal of whole trees and smaller coarse woody debris (Fig. 6).

Even though Matlock contained low pre-treatment soil base cation contents, hypothesis 1 was not supported at this site because there were few significant differences in cation nutrient variables found at 15 years post-harvest (Fig. 6). There was an increase in foliar Mg concentration in the AVC treatment, which is likely due to less competition for the nutrient from understory vegetation. Another study at the Matlock site found higher soil K in the absence versus presence of Scotch broom (Slesak et al., 2016b). In this study, we did not observe soil K differences due to vegetation control treatments, but there was significantly higher soil K in the BO treatment than in the WT treatment, which could be associated with a loss of K from organic removals and differences in Scotch broom cover between the two organic matter removal treatments (Harrington and Schoenholtz, 2010).

At Matlock, limiting pre-treatment N was associated with significant treatment effects on aboveground and belowground N. However, contrary to the first hypothesis, there was a large increase in site N with IVC and WT treatments due to significantly greater colonization by Scotch broom in those treatments (Harrington and Schoenholtz, 2010), which fixes N through Rhizobium bacteria (Fig. 6). However, the increase in available N in the IVC treatment at the Matlock site has not resulted in more growth because Scotch broom (around 25% ground cover) reduced survival and growth of planted seedlings by 20% and 59%, respectively, at five years (Harrington and Schoenholtz, 2010; Slesak et al., 2016a). Slesak et al. (2016b) examined the effects of presence or absence of Scotch broom on soil nutrients and found no change in total soil N at the Matlock site, which they concluded to be due to less N fixation by Scotch broom on low productivity sites. In this study, we also did not find a change in total soil N concentration, but were able to measure an increase in available soil N due to Scotch broom colonization through a decrease in forest floor C:N ratio and an increase in forest floor N concentration and PRS NO₃⁻ adsorption (Fig. 6). Other factors, such as competition from understory species for available soil water, are also important variables influencing tree growth at Matlock.

The first hypothesis was supported at Molalla, where the soil contained high levels of exchangeable base cations prior to treatment and showed a large negative treatment effect on forest floor and soil cations (Table 1 and Fig. 6). In previous studies at Molalla, Slesak et al. (2011 and 2016a) noticed lower increases in exchangeable K and Mg due to AVC compared to IVC. Soil extractions and adsorption by PRS probes further shows that available soil Ca and Mg are lower due to AVC.



Fig. 6. Effects of soil nitrogen and cations on changes in aboveground and belowground variables due to whole tree removal (A) and annual vegetation control (B) treatments. The " < " and " > " symbols designate significant treatment effects.

While there was a negative effect of AVC on soil nutrients at Molalla, the second hypothesis was not supported because there was no negative effect of the treatments on stand volume growth due to adequate pre-treatment soil N and base cations (Fig. 6).

Molalla contained the lowest pre-treatment soil P contents, yet the first hypothesis was not supported according to P availability after treatments. Higher forest floor and soil P was measured in the AVC treatment, which is likely due to less uptake by understory species. Similarly, Slesak et al. (2016a) previously reported lower decreases in P due to the AVC treatment compared to the IVC treatment at the Molalla site ten years after harvest. Thiffault et al. (2011) found that the effects of whole tree removals on soil P were greatest on older soils, but the response ratio of WT:BO was below 1 in 84% of the studies reviewed. While Molalla had low pre-treatment available soil P, foliar P concentrations were only slightly deficient (0.15%) (Ballard and Carter, 1986) and not much lower than at Fall River and Matlock (0.17 and 0.16, respectively) that have moderate pre-treatment soil P contents, which suggests that Molalla is exhibiting a limiting nutrient relationship by accumulating more P in the aboveground biomass to limit loss of P (Vitousek and Reiners, 2006).

4.4. PRS probe adsorption

The Fall River, Matlock, and Molalla sites have been extensively measured for changes in soil nutrients over time (Licata, 2004; Slesak et al., 2011; Slesak et al., 2016a; Knight, 2013; Dietzen et al., 2017). In support of the third hypothesis, the PRS probes provided a unique view into nutrient availability that is supported by forest floor and soil nutrient data (Tables 2–4), while providing new findings that were not previously observed. Some effects of organic matter removal, vegetation control, and compaction treatments were captured by PRS probe adsorption that were not represented by forest floor and soil extractions.

At the Fall River site, there was significantly lower PRS NO_3^- adsorption in the AVC treatment that was not captured in soil concentrations (Table 2). Furthermore, lower PRS Ca and higher PRS P adsorption in the WT + treatment than in the BO treatment was not measured in soil extractions. Lower PRS Ca adsorption in the WT + treatment and lower PRS Mg adsorption in the AVC treatment supported the finding of lower foliar Ca and Mg than in the BO and IVC treatments, respectively.

Due to Scotch broom colonization, the Matlock site had significantly

higher PRS NO_3^- in the WT and IVC treatments that was not identified by soil concentrations yet was captured in the IVC treatment in a previous study at Matlock (Slesak et al., 2016a). Greater PRS NO_3^- in these treatments was also supported by higher foliar N concentrations in the WT and IVC treatments compared to the BO and AVC treatments, respectively, even though stand volume growth was much lower. The addition of N into the system due to Scotch broom at Matlock is supported by undetectable NO_3^- adsorption by PRS probes in the BOAVC treatment combination (Supplementary material); this treatment combination also had the greatest survival and cover of Douglas-fir and lowest cover of Scotch broom (Harrington and Schoenholtz, 2010).

The PRS probes recorded contrasting NH_4^+ and NO_3^- in the WT and BO treatments at the Molalla site. While PRS NH₄⁺ adsorption was five times higher in the WT treatment, PRS NO₃⁻ adsorption was two times higher, although not significantly different, in the BO treatment compared to the WT treatment (Table 5). The change in the type of available N that was adsorbed by PRS probes was not reflected in N leaching after harvest and mineral soil total N extractions from this study and changes in soil N ten years after harvest (Slesak et al. 2009; Slesak et al. 2016a). During the same measurement period, a set of 38 Douglas-fir stands were found to have a positive logarithmic relationship between PRS NO_3^- and King's site index ($R^2 = 0.27$) (King 1966), while there was no correlation between PRS NH4⁺ and site index $(R^2 = 0.06)$ (K. Littke, unpublished data). These findings suggest that decreases in PRS NO3⁻ adsorption due to individual treatment effects at each site (Fall River: AVC < IVC, Matlock: BO < WT, and Molalla: WT < BO) will result in lower soil productivity in those treatments.

5. Conclusions

The effects of organic matter removals and annual vegetation control varied by site due to pre-treatment differences in total and available soil nutrients, treatment intensity, and understory competition. Five years of annual vegetation control resulted in similar losses of forest floor and soil N and base cations from the Fall River, Matlock, and Molalla sites. The effects of whole tree removals were variable such that there was a loss of N and K (Fall River), addition of N (Matlock), and a shift in the type of available N (Molalla) and a reduction in early growth (Fall River). Additional removal of organic matter through coarse woody debris resulted in a loss of Ca from the system at Fall River. While five years of annual vegetation control resulted in losses of forest floor and mineral soil nutrients, there was a positive effect on stand volume growth. However, the improvement in stand volume growth due to the annual vegetation control treatment has decreased in the last measurement period at the most productive site (Fall River), which is likely due to the higher demands for soil nutrients during the canopy closure stage. While Molalla and Matlock are younger, less productive stands, we expect a similar reduction in the annual vegetation control effect as these stands reach the canopy closure stage. The results from this study suggest that excessive vegetation control treatments could potentially be detrimental to the site in the long-term if the improvement in stand growth cannot be supported over time due to a loss of soil nutrients.

CRediT authorship contribution statement

K.M. Littke: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. T.B. Harrington: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. R.A. Slesak: Formal analysis, Writing - original draft, Writing - review & editing. S.M. Holub: Formal analysis, Writing - original draft, Writing - review & editing. J.A. Hatten: Formal analysis, Writing - original draft, Writing review & editing. A.C. Gallo: Formal analysis, Writing - original draft, Writing - review & editing. W.R. Littke: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. R.B. Harrison: Project administration, Funding acquisition, Writing - original draft, Writing - review & editing. E.C. Turnblom: Project administration, Funding acquisition, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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