*Article*

# Investigating Relationships between Nutrient Concentrations, Stem Sinuosity, and Tree Improvement in Douglas-Fir Stands in Western Washington

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**Abstract:** Stem sinuosity is a deformation that occurs in Douglas-fir and other tree species. Nutritional deficiencies, such as those of copper, zinc, boron, and calcium have been positively associated with increased stem form deformities. The objective of this study was to analyze available soil and total foliar concentrations of sinuous Douglas-fir stands and determine if these data provided insights into the role of nutrients on sinuosity across different sites. Relationships among genetic gain levels across different sites were also assessed to evaluate whether trees selected for growth and form were better at mobilizing and sequestering nutrients in soils or needles. As data collected were multivariate, Mantel test and permutational multivariate analysis of variance (PERMANOVA) were used to study effects of site, genetic gain levels on soil, and foliar nutrients, with ordinations used to visualize trends. The stands were found to be deficient in boron and calcium, and moderately deficient with respect to zinc and nitrogen. Combining foliar data with soil nutrients revealed that there was differential uptake of these nutrients as compared to other more abundant micronutrients. Results of univariate and multivariate analyses showed that site was the most important predictor, which explained variation among soil nutrient concentrations. Genetic gain level was a significant predictor for variation among soil nutrients; however, not significantly for foliar concentrations according to multivariate analyses. Trends in the data indicate that proper genetic selection could reduce sinuosity while also increasing growth. The results of this study support the contention that no one single factor can explain stem sinuosity. It is likely that sinuous growth in trees is caused by a combination of unbalanced nutrient concentrations and metal deficiencies, as were seen in this study, in addition to genetic, environmental, and physiological factors.

**Keywords:** stem-form deformity; nutrient deficiency; soil chemistry; genetic gain; forest soils

## 1. Introduction

Mathematically, sinuosity is thought of as a dimensionless ratio denoting actual path length divided by shortest path length of a curve. In trees, stem sinuosity is defined as "any stem crookedness occurring in the segment within two 'whorls'" [1]. In practical terms, it is the measure of stem displacement from its original direction over the total length of the displacement. Sinuous growth in gymnosperms/angiosperms causes tension/compression wood to develop on the convex side of the curved portion. This compression wood is undesirable not only for pulp but also for solid wood due to its unfavorable wood properties, such as 15% to 40% higher specific gravity and higher lignin content.

The high lignin content creates issues with milling and processing of lumber. It has also been shown that wood from severely sinuous trees warps during drying, which is a major structural deficiency and would render the wood unsuitable for many uses [2]. Middleton et al. compared net value of lumber grades for different sinuosity scores and found a 10% decrease in dollar value [3]. Even though this was not statistically significant, it indicated a propensity to avoid buying wood sinuous trees.

Occurrences of sinuosity have been associated to land management, genetics, and tree physiology. A genetic tendency for sinuous growth was shown by Pederick et al. but the exact heritability was not determined [4]. Biomechanical reasons such as long, slender internodes (parts of the stem between the nodes) being more susceptible to bending and contortion could also explain this deformity [5]. Stem-form defects have been linked with intensive land management, particularly fertilizer application which could induce sinuosity in some genotypes of loblolly pine [6,7]. Bent tap roots, caused by impermeable soil pans, in loblolly pine (*Pinus taeda* L.) were also associated with stem-form defects [8]. In their primary growth phase, trees have been shown to be more susceptible to sinuosity [9]. Howe and Jayawickrama showed that at age 11, there was a high correlation between stem-form defects and height (r = 0.95), and diameter at breast height (r = 0.97) [10]. However, it is unclear whether it is representative of coastal Douglas-fir region.

Nutritional deficiencies of Cu, B, Zn, and Ca have also been associated with distorted growth [7,11]. The aforementioned nutrients are also essential for wood formation (i.e., xylogenesis). This becomes a concern as soils in western Washington are known to be moderately weathered and experience copious precipitation, which leads to high leaching rates [12]. Owing to well established and long-term disturbance cycles in the Pacific Northwest, early succession species such as red alder (*Alnus rubra*) play a crucial role in nitrogen fixation, which contributes to the soils being relatively nitrogen rich [13]. Higher soil nitrogen promotes more nitrification and leaching; consequently lowering soil pH of these soils and depleting base cations. Cation movement in soils is influenced by anion concentrations and organic acids associated with soil organic matter (SOM). In acidic forest soils, like the ones found along coastal Washington, Al mobilization can occupy and deplete sites meant for exchangeable cations such as Ca, Mg, and Zn. This has been posited as a mechanism responsible for depletion of forest Ca [14]. Calcium is an essential soil nutrient which plays a major role in lignification, secondary cell wall formation, ion balance, cell wall stabilization, etc. Poplars (*Populus sp.*) grown hydroponically in a low calcium solution were shown to have reduced wood increment, vessel size and fiber length [15].

High levels of leaching can also result in the depletion of boron, an essential micronutrient. Boron is available for plant uptake within a narrow range of soil pHs (5.5–7). Boron exists in soil solution as un-dissociated acid ($H_3BO_3$) which is a neutral compound and therefore does not bind to charged soil particles or organic matter. Owing to this property, it can be easily leached out of soils. Boron is known to be a key component in crucial cellular processes, such as maintaining the integrity of the cell wall [16]. Boron deficiency is known to cause a decrease in apical dominance in trees, which consequently results in reduced timber quality and yield and an increase in stem malformation [17]. Studies conducted in south-western British Columbia on Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) as well as western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) stands found boron to be a limiting nutrient, presumably caused by high leaching rates leading to its depletion [18].

Genetic gain was another important aspect of this study. Investments in tree improvement through selection for desired traits, has been integral for Douglas-fir in the Pacific Northwest since the 1960s [19]. Very little attention has been focused on evaluating the impact of genetic variation on nutrient cycling. Studies which investigate the effect of tree species on nutrient cycling, and ecosystem function in general, focus only on differences between species. Examples of these can be seen in comparisons drawn between hardwood and conifer species, which exist due to differences in nutrient management strategies between species such as root exudates, litter fall, etc. Schweitzer et al. showed that differences in genotypes in cottonwood (*Populus deltoids* Bartr.) with respect to condensed tannin production had a significant impact on nitrogen cycling. [20]. Improved trees might also be able to access more nutrients through improved rooting or through root exudates among other processes.

If micronutrient deficiencies are found to be strongly associated with stem-form sinuosity in Douglas-fir, tree improvement programs may have the potential to improve this stem defect. The selection of best traits in trees tends to prioritize form and growth; therefore, if there was a genetic propensity for sinuous growth, tree improvement programs could be a useful practice in this regard.

Extensive research has been done on sinuosity in the American Southeast with loblolly pine, as well as in New Zealand with radiata pine (*Pinus radiata* D. Don) and Douglas-fir and in northern Europe with Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L.). Literature indicates a gap in knowledge in the Pacific Northwest with respect to studies investigating the relationships between soil chemistry and stem defects as compared to some other regions in the world with extensive commercial forestry. Therefore, the first aspect of the project aimed at pinpointing nutritional deficiencies in sinuous Douglas-fir stands through soil and foliar sampling. We also investigated the effects of site and genetic gain on differences in soil and foliar nutrients and posit a link between productivity and sinuosity. This would provide insights to whether tree improvement had any effect on nutrient cycling and whether those differences could explain the differences in sinuosity between trees from different genetic gain levels.

## 2. Materials and Methods

In collaboration with the USDA Forest Service's Pacific NW Research Station Genetics Team and Oregon State University's Northwest Tree Improvement Cooperative, Stand Management Cooperative planted six installations by 2007 to examine the effects of genetics, vegetation control, and spacing on growth, yield, and wood quality. Planting spacings were $7 \times 7$, $10 \times 10$, and $15 \times 15$ feet. Three genetic levels (*G*) of seedlots were tested: (1) *Unimproved* population (random sample of 50 wild trees distributed throughout the Grays Harbor breeding zone). (2) *Intermediate* population or Medium (as shown here) (a mix of pair crosses among 20 parent trees chosen to represent an intermediate level of genetic gain). This seedlot was only tested in the genetic gain trial portion (i.e., at 10′ spacing with standard vegetation control). (3) *Elite* population or High (as shown in this paper) (a mix of crosses among clones of the 20 best parent trees in each breeding unit designed to represent a high level of genetic gain). All parents in the *Elite* and *Intermediate* populations originated from the Grays Harbor vicinity and were selected based on their growth performance in the Grays Harbor progeny tests. We chose plots with $10 \times 10$ spacing and standard vegetation control for this study as examining the effects of various levels of genetic gain (high, intermediate, and unimproved) was the focus of this study. Plots were 0.23 acres (931 m$^2$) in size and separated by 100 feet (30.5 meters). For this study, 3 sites were selected:

1. Donkey Creek (47°19′08.5″ N, 123°47′59.2″ W);
2. Crane Creek (47°23′41.2″ N, 124°03′38.9″ W);
3. Donaldson Creek (46°37′59.6″ N, 123°45′59.8″ W).

Soils and other pertinent characteristics for the three sites were obtained from the Web Soils Survey (NRCS, 2018) and are described in Table 1.

**Table 1.** Soil, elevation, and climatic characteristics of the selected sites [21].

| Site | Crane Creek | Donkey Creek | Donaldson Creek |
|---|---|---|---|
| Soil series | Matheny Creek medial silt loam found on till plains | Mopang medial silt loam (Andisol) found on outwash terraces on till plains | Vesta medial silt loam (Andisol) found on mountainous uplands |
| Parent material | Silty alluvium over gravelly glacial outwash | Old alluvium over glacial drift | Residuum weathered from basalt |
| Drainage class | Somewhat poorly drained | Well drained | Well drained |
| Elevation | 58 meters above sea level | 207 meters above sea level | 110 meters above sea level |
| Temperature and precipitation | 230 to 300 centimeters of precipitation per year and 8–10 °C | 230 centimeters of precipitation and 9 °C | 230 centimeters of precipitation and 9 °C |

A total of fifteen plots were sampled on each site, classified by unimproved, medium, and high genetic gain. Four soil cores were taken per plot to a depth of 15 cm. The locations of these soil cores were randomly selected to avoid both sampling biases and spatial autocorrelation. Soil samples were then composited by weight to denote a representative sample per plot. Four foliar samples were taken from four different trees using a pruning pole to sample the top third of the crown from the same flush. Foliar samples were also composited by weight to denote a representative sample per plot. The sampling was carried out in July 2017.

Individual trees were graded for sinuosity across all three sites in 2017 using a combination of visual scoring and assessing the displacement of sections of the stem from the initial direction of growth. Due to the wide range in values, these values were averaged and relativized to represent a sinuosity rating for a plot.

## 2.1. Chemical Analyses

Soils were analyzed for pH, mineral nitrogen, and several micronutrients, where foliar samples were analyzed for a suite of macro- and micronutrients. The pH of composited soil samples was determined using a 1:1 mixture of soil and distilled water, which was then stirred to create a suspension as per Soil Survey Laboratory Methods Manual (USDA, 2004). For determination for available nitrogen ($NH_4$-N and $NO_3$-N), extraction with 1 M potassium chloride was employed as per Maynard and Kalra [22]. Mehlich 3 extraction was utilized for measuring available fractions of boron, manganese, copper, and zinc [23]. Available calcium was determined by extraction with 1 M ammonium chloride (NH4Cl) [24]. For foliar samples, a total metals digest was performed to determine concentrations of a full suite of nutrients [25]. A CHN analyzer was used to determine %N in the needles. All concentrations were measured using 4 g of samples extracted with 40 mL of extractant and shaken for 2 h. The solutions were filtered using Whatman Filter paper Type 1. ICP-MS (Inductively coupled plasma mass spectrometry) analyses were performed by the Analytical Service Centre at the School of Environmental and Forest Sciences at the University of Washington. For each element, a 5 point calibration curve was plotted with elemental standards diluted to appropriate concentrations.

## 2.2. Statistical Analysis

For the soil and foliar data, the key predictor/explanatory variables in this analysis were site and genetic gain level, owing to the design of the experiment. Site and genetic gain comparisons were conducted using an analysis of variance (ANOVA) test with both variables as fixed effects, and R. Tukey's honest significance difference test was used to determine which treatments and sites, if found significant ($\alpha = 0.05$), were different. Pearson's correlation coefficients were calculated between soil, foliar nutrients, and sinuosity ratings, and visualized using the 'corrplot' function [26]. Heights of the trees were also compared across sites and genetic gains by using an ANOVA. Sinuosity values were averaged and relativized by range by individual site, using the 'decostand' function which is a part of the 'vegan' package [27] in R. This relativization ensures that no one value can disproportionately influence the statistical analysis.

Multivariate analyses were performed to evaluate differences in soil and foliar nutrient concentrations. As soil and foliar concentrations vary across nutrients by orders of magnitude, it was desirable to relativize them by column totals through the 'decostand' function, which is important as most multivariate analyses involve the computation of a distance or a dissimilarity matrix. The analyses in this project used Euclidean distances to compute the distance matrices, which are an extension of the Pythagoras' theorem. A Mantel test was then used to test for correlations between the two distance matrices, which were the soil and foliar nutrient concentrations.

The next step in this analysis involved using a non-parametric test, permutational multivariate analysis of variance (PERMANOVA), to test for the effects of site, genetic gain level, and pH on soil and foliar nutrient concentrations. PERMANOVA uses multiple permutations of the dataset to compare the actual $F$ statistic to multiple permuted $F$-values, whereas parametric tests depend on

known distributions of data. PERMANOVA does not require the data to meet any assumptions of normality or heteroscedasticity but rather requires observations to be interchangeable under the null hypotheses [28]. The 'adonis' function, which is a part of the 'vegan' package, was used to perform this test. As the experimental design had only one treatment factor, those permutations would no longer be interchangeable and therefore restrictions were imposed on the scope of calculations. Restricting the permutations would allow for the study of both site and plot level effects on soil and foliar nutrients, and preserve the experimental ordering of the data. This was achieved by using the 'permute' package in R [29].

An equally important aspect of multivariate analyses is visualization. Ordinations are commonly used in soil microbiology but have also been used for analyzing exchangeable cations across climatic, spatial, and within profile gradients [30]. For this purpose, PCA (Principal Components Analysis) ordinations were used. PCA was performed in R using the 'prcomp' function which is a part of the 'stats' package [31].

## 3. Results

### 3.1. Univariate Analyses

Donaldson Creek was found to have the tallest and most sinuous trees (Figures 1 and 2). Trees from the unimproved genetic gain level were the shortest and the most sinuous.
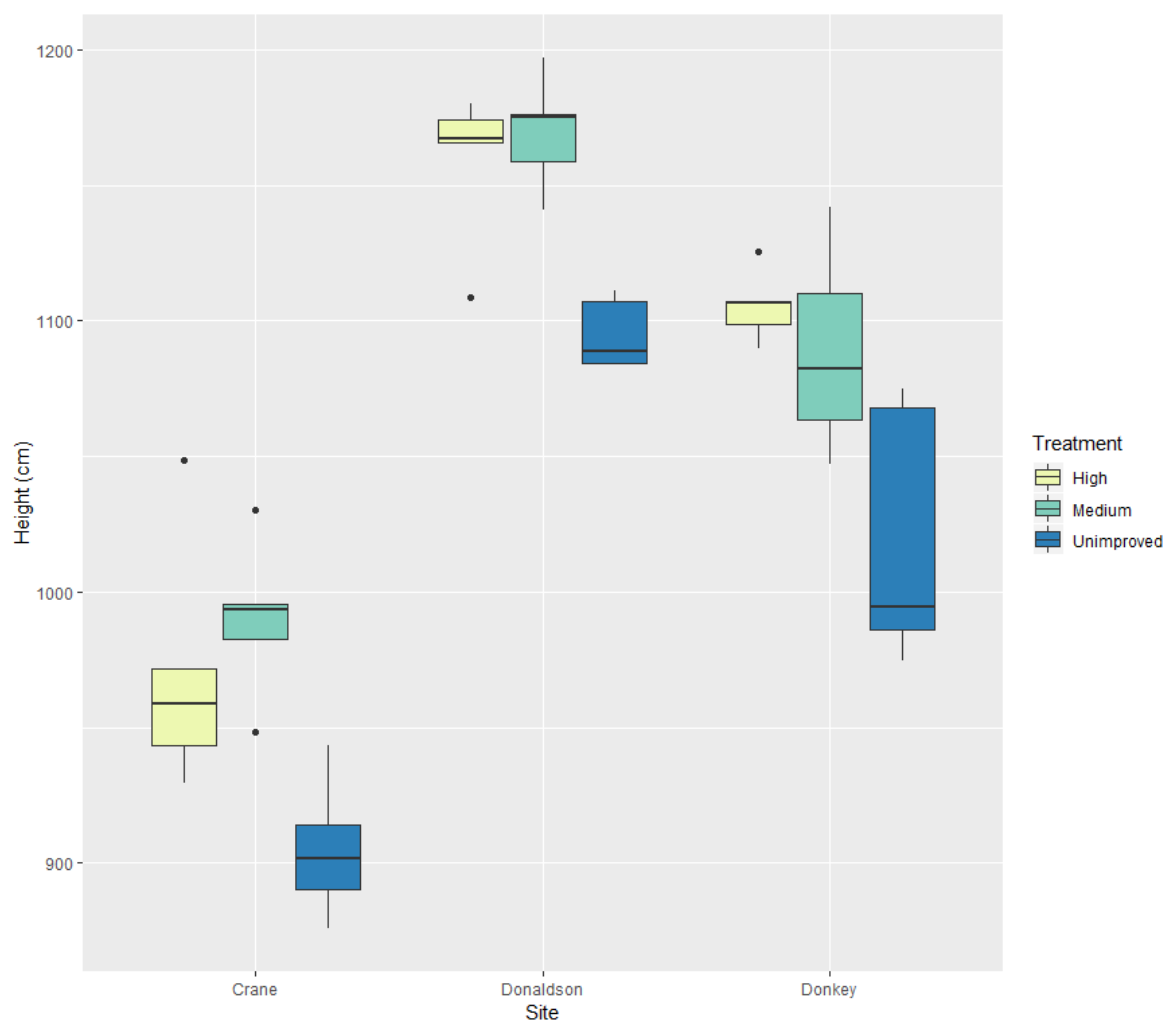


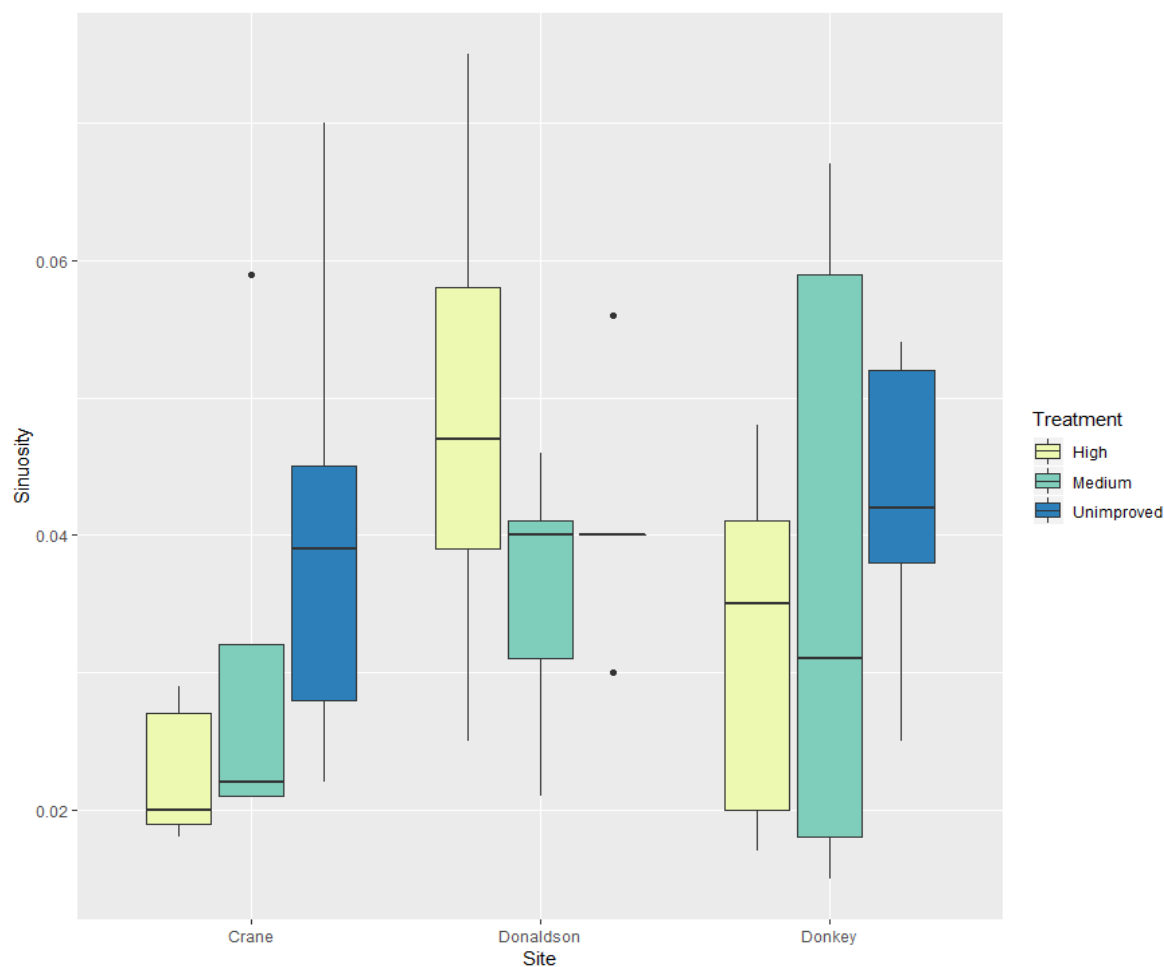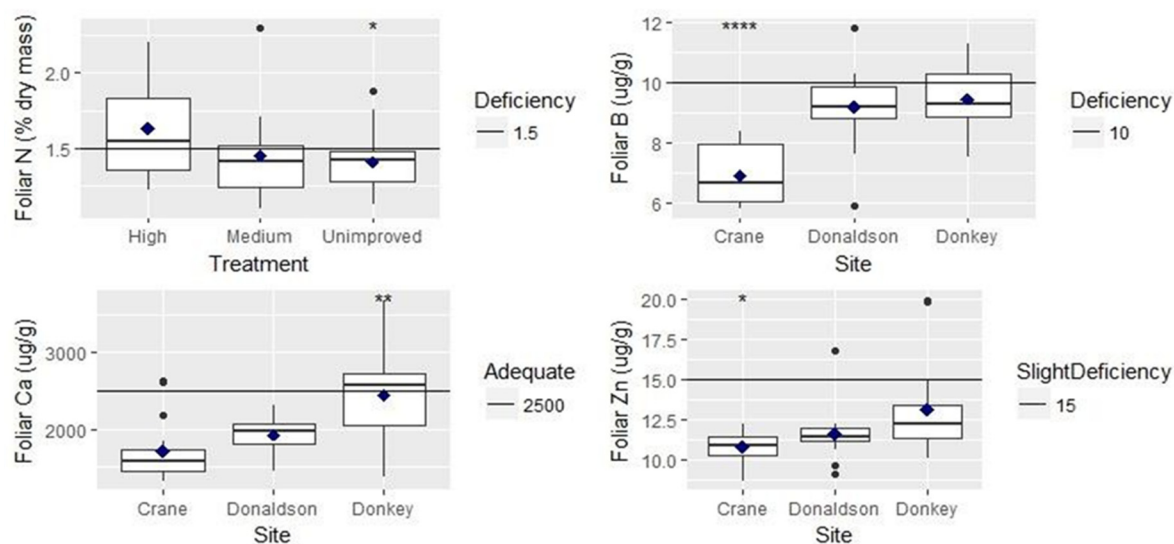**Figure 1.** Tree heights, measured in 2017, across different sites and genetic gain levels.

**Figure 2.** Relativized sinuosity ratings across different sites and genetic gain levels.

Foliar B was found to be <10 ppm across all three sites (Figure 3), indicating a possible deficiency [32]. Deficiencies of nitrogen, calcium, and zinc were found as well across sites and treatments, but to varying degrees, according to concentrations indicated by Ballard and Carter [33]. Trees at Crane Creek had significantly lower levels of B, Ca, and Zn in their foliage (Table 2), and this was also true for other micronutrients as well. Crane Creek was also the site with significantly lower pH (Table 2). None of the trends for Donkey Creek were statistically significant, but trends suggest that they had higher concentrations of B, Ca, Zn, and other micronutrients than the other two sites (Figure 4).

**Table 2.** ANOVA table for all measured variables.

| Variable | Site | Genetic Gain | Description of Significance, If Any |
|----------|:----:|:------------:|------------------------------------|
| Sinuosity | *** | *** | Crane Creek had lowest sinuosity, unimproved trees were the most sinuous |
| Tree height | *** | *** | Crane Creek had the shortest trees, unimproved trees were the shortest |
| pH | *** | ns | Crane Creek had lowest pH |
| Bulk density | | ns | |
| Moisture content | ** | ns | Crane Creek had lowest soil moisture |
| 100 needle dry weight | * | ns | Crane Creek had highest needle weight |
| Organic C | ns | ns | |
| Foliar nitrogen | ns | | Highest genetic gain had highest foliar N |
| Available soil ammonium | ** | ns | Donkey Creek had highest soil $NH_4$-N |
| Available soil nitrate | ** | ns | Donkey Creek had lowest soil $NO_3$-N |
| Foliar boron | *** | ns | Crane Creek had lowest foliar B |
| Available soil boron | | ns | |
| Foliar calcium | *** | | Crane Creek had lowest foliar Ca |
| Available soil calcium | ns | ns | |
| Foliar copper | ** | ns | Crane Creek had lowest foliar Cu |
| Available soil copper | ** | ns | Crane Creek had lowest soil Cu |
| Foliar magnesium | *** | ns | Crane Creek had lowest foliar Mg |
| Available soil magnesium | ns | ns | |
| Foliar manganese | *** | ns | Crane Creek had lowest foliar Mn |
| Available soil manganese | *** | ns | Crane Creek had lowest soil Mn |
| Foliar zinc | * | ns | Crane Creek had lowest foliar Zn |
| Available soil zinc | ns | | |
| Foliar sulphur | * | ns | Donkey Creek had highest foliar S |

Blank space represents significance at $p = 0.1$; * represents significance at $p = 0.05$; ** represents significance at $p = 0.01$; *** represents significance at $p = 0.001$; ns represents non-significant effect.



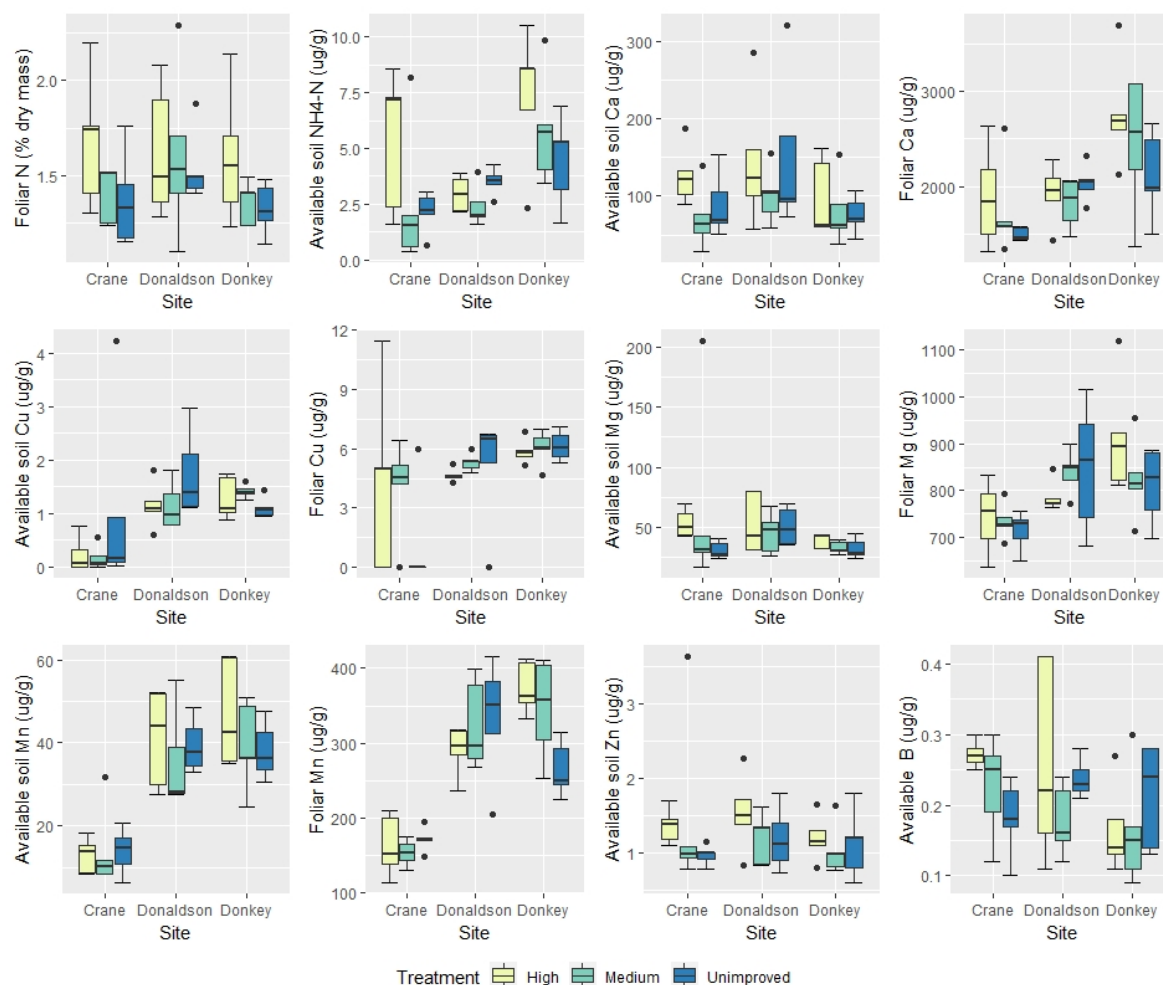**Figure 3.** Nutrient deficiency box plots, values as per Edmonds et al. [32].

**Figure 4.** Soil and foliar nutrients across different sites and genetic gain levels. Soil and foliar nutrients for the same element are shown in pairs.

Results for soil and foliar nutrient concentrations across different genetic gain levels showed that trees from the highest genetic gain level had significantly higher foliar N concentrations than medium gain and unimproved trees (Table 2). They also had higher foliar Ca, Mg, and Zn concentrations, although none of these trends were statistically significant (Figure 4).

Correlation analyses show that foliar B was strongly correlated with foliar Ca and Zn, along with other micronutrients (Figure 5). Foliar B was also found to be negatively correlated with available soil B, and a similar, although weaker, correlation was seen for foliar Ca as well. Sinuosity was found to be negatively correlated with soil B and positively correlated with soil Ca. Tree height was shown to be positively correlated with soil Mn, Cu, and foliar B, Ca, Cu, Mg, Mn, and Zn.

*3.2. Multivariate Analyses*

Soil and foliar nutrients were found to be positively correlated (Mantel statistic (r) = 0.1985), although the correlation was not strong. The PCA ordination acts as further support for this result (Figure 6). It was observed that there was not a high degree of correlation between vectors representing soil and foliar nutrients. PC1, which explained 34% of the variation in the data, was heavily weighted towards foliar nutrients, whereas PC2 had more weight towards soil nutrients. There was a clear separation in the ordination for all the nutrients by site, supporting the univariate analyses.
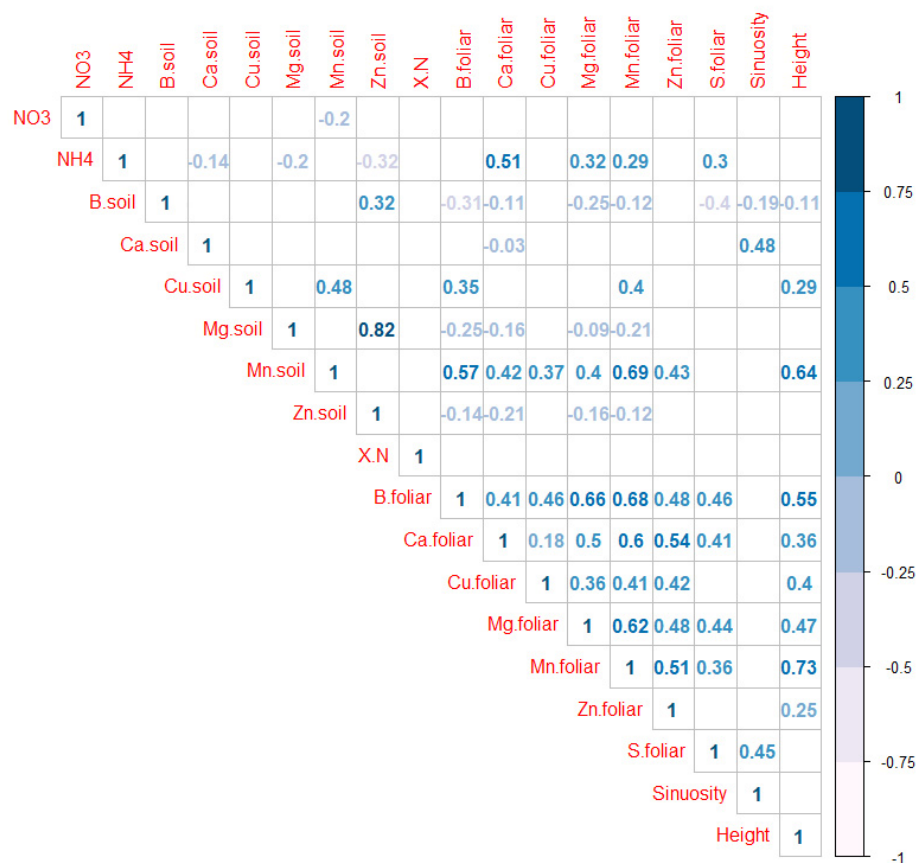
**Figure 5.** Correlation matrix of all nutrients and moisture content. All reported correlation coefficients are significant at $p = 0.05$. X.N refers to % nitrogen in the foliar samples.
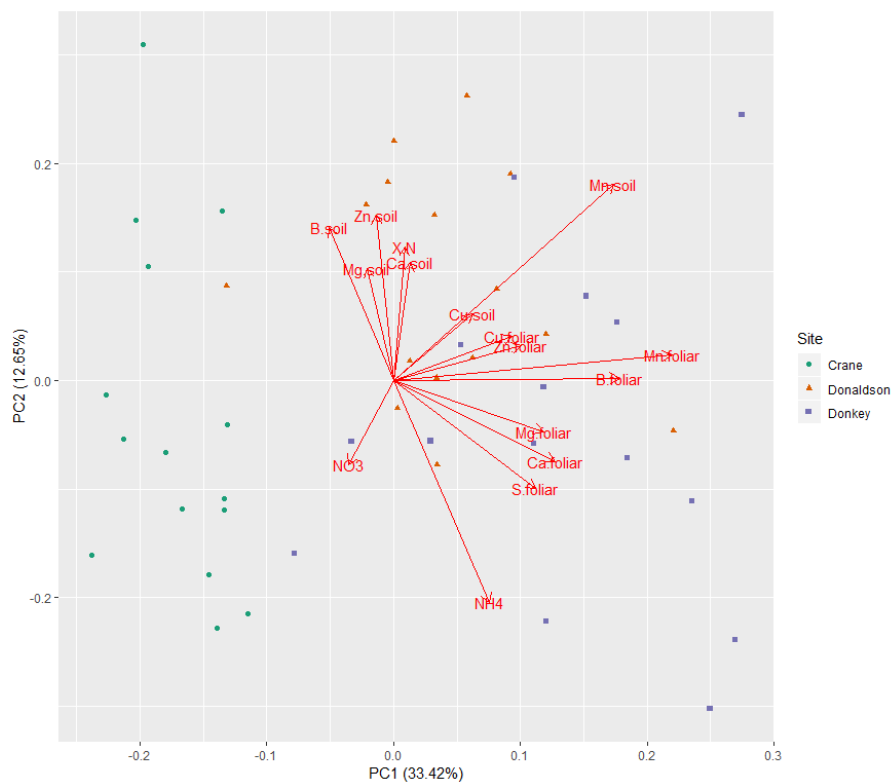


**Figure 6.** Principle components analysis for all nutrients across sites. For ease of interpretation, the first two axes are shown in this ordination.

PERMANOVA results for soil nutrients indicate that genetic gain was significant ($p = 0.09$). Genetic gain was not found to be significant for explaining variation in the foliar nutrient matrix, although it did account for 5% of the variation in the data. Site differences accounted for a significant amount of variation for both soil and foliar nutrient data, but the *p*-values are not calculated due to the low number of permutations (3! = 6).

## 4. Discussion

The majority of sampling was conducted during peak growth for Douglas-fir (July 2017). These concentrations, therefore, would hint at chronic deficiencies of these nutrients, with the likelihood of severe deficiency events being high in periods of low growth. Lower soil nutrient concentrations for Donkey Creek, with respect to B, Ca, and Zn, were found to be linked to trees on that site having higher levels of the aforementioned nutrients in their foliage (Figure 4). Trends for soil Cu, Mn were different (Figure 4); however, foliar concentrations for these nutrients seemed to be sufficient for Douglas-fir. Therefore, the same nutrient cycling regimes would not apply to these micronutrients.

There was a high degree of correlation observed between different foliar nutrient concentrations across these plots. Foliar B and S were found to be correlated (Figure 5), supporting Carter et al. findings [18]. Negative correlations between foliar and soil concentrations of B and Ca (Figure 5), however, would indicate that cycling for these nutrients was different as compared to other micronutrients. The lack of correlation with foliar N (Figure 5) could be indicative that nitrogen uptake, or what is most commonly associated with growth, is not correlated to uptake of other nutrients. This is important to understand as sufficiency of a primary growth nutrient could lead to higher demand of other nutrients, possibly exacerbating these deficiencies. Donaldson Creek was the site with the highest sinuosity (Figure 1) and although it had the tallest trees [34] (Figure 1) (Table S1), the foliar concentrations were less than adequate (Table S2), therefore supporting the hypothesis that more productive and faster growing trees could be prone to higher sinuosity if there are deficiencies of key nutrients.

These results suggest that incidences and severity of sinuosity are related to higher productivity. More productivity would increase demand for other essential micronutrients such as B, Ca, and Zn, which are present in insufficient concentrations in trees. It is also known that trees that grow faster possess leaders that have slower cambium growth leading to slower synthesis of structural secondary xylem, thereby rendering them susceptible to bending [9]. This could be hypothesized as a reason for trees at Donaldson Creek having the highest sinuosity. Previous experiments evaluating the effect of nutrient inputs on stem-form sinuosity support this idea. Higher calcium availability, in a controlled experiment, led to an increase in stem sinuosity in a calcium limited environment [35]. Espinoza found that trees with nitrogen fertilization had higher sinuosity, and calcium additions did not decrease stem sinuosity [7]. However, calcium additions, in conjunction with nitrogen fertilization, were able to decrease stem sinuosity. Other reasons for Donaldson Creek possessing the highest sinuosity could be related to its geographical location closer to the coast as compared to other sites, which could lead to different microclimatic conditions, which have been shown to be associated with the severity of sinuosity [2].

Trees from the highest genetic gain level had significantly higher foliar N than medium gain level and unimproved trees (Figure 3). This trend persisted within individual sites, however it was not significant (Figure 4) ($p = 0.15$). They also had higher foliar Ca, Mg, and Zn concentrations (Figure 4). Both findings could be explained by better nutrient uptake capacity of trees selected for better growth and form, which would be one potential mechanism. The plots with the highest genetic gain trees had higher available soil N, Zn, and B concentrations (Figure 4) (Table S3), which could be explained by increasing the efficiency of nutrient cycling through litterfall. Further research would be required to confirm this proposed mechanism. Pregitzer et al. found differences in plant leaf litter for cottonwood phenotypes across an elevational gradient which could influence below-ground soil C and N dynamics [36]. Madritch et al. also found differences in aspen litter decomposition by

genetic identity [37]. These findings which are seen in univariate and multivariate analyses, combined with previous literature, highlight the importance of genetic variation for ecosystem function within a single species. This has implications for the deployment of improved families by provenance for this species. Trees from a higher genetic gain level had lower sinuosity scores as compared to medium and unimproved trees (Figure 2). This highlights the potential of genetic selection to improve upon stem-form defects, especially considering that sinuosity is heritable to a moderate degree, from values of 0.59 as calculated by Adams and Howe, to 0.41 and 0.36 at ages 12 and 24 as calculated by Temel and Adams [38,39]. More efficient and tighter nutrient cycling could potentially be a mechanism through which they maintain growth and stem form, which was evident by higher concentrations of critical nutrients such as N, B, and Ca.

## 5. Conclusions

This study found that there were mild to severe deficiencies of nitrogen, calcium, zinc, and boron in Douglas-fir across all three sites. There were differences between sites with respect to sinuosity as well as multiple soil and foliar nutrient concentrations. Donaldson Creek, which is the most productive site with respect to height, was found to possess the most sinuosity among the three sites sampled. Trees from a higher genetic gain level had fewer occurrences of stem sinuosity and results support the hypothesis that these trees cycle nutrients efficiently and optimize nutrient uptake while at the same time maintain better growth. Results of the multivariate analyses also indicate significant differences in nutrient cycling between site and genetic gain levels. The results support the deployment of improved genetic provenances to mitigate against sinuosity. Pushing for higher productivity through silvicultural inputs could also have negative consequences for wood quality. Proximity to coast can also be a factor which should be considered for plantation forestry as it appears to have a negative impact with respect to stem-form sinuosity.

The results of this study support the contention that no single factor can explain stem sinuosity. It is likely that sinuous growth in trees is caused by a combination of unbalanced nutrient concentrations and metal deficiencies, as were seen in this study, in addition to genetic, environmental, and physiological factors. Building upon the results of this study, there is definitely potential for controlled, fertilization, and genetics-based studies looking at boron, nitrogen, calcium, and zinc combinations, and their effects on sinuosity in Douglas-fir.

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