

Nutrient cycling and wood chemistry of sinuous Douglas-fir stands across different genetic gain levels in western Washington

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Abstract

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Stem sinuosity is a deformation which occurs in Douglas-fir and other tree species, which can be thought of as the displacement of the stem from its original direction over the total length of displacement. 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 analyse available soil and total foliar concentrations of sinuous Douglas-fir stands, which were planted as a part of a genetic gains trial, which would give information about trends in nutrient cycling across sites and different levels of genetic gain. Increment wood cores, taken at breast height, were also analysed through FT-IR spectrophotometry and compared against sinuosity scores of the trees in order to get a more rigorous measurement of sinuosity. As the data collected was multivariate, Mantel test and PERMANOVA were used to study effects of site, genetic gain levels and pH on soil and foliar nutrients, with PCA and NMDS ordinations being used to visualize those trends. Relationships among genetic gain levels across different sites

were also assessed to evaluate whether trees from a provenance suited to higher growth were better at mobilizing and sequestering nutrients in soils or needles. The stands were found to be deficient in boron and calcium, and moderately deficient with respect to zinc and nitrogen concentrations in the foliage. Trees from higher genetic gain level were able to accumulate nutrients more in their foliage, and there also seemed to be some differences with respect to available soil nutrients in the soils around them. Sinuosity was found to be negatively correlated with available soil boron, nitrogen, calcium, nitrate and foliar nitrogen while it was positively correlated to foliar sulphur, calcium, copper, zinc and available soil ammonium. Results of multivariate analyses show that site was the most important predictor which explained variation among soil nutrient concentrations. Genetic gain level explained some differences in soil/foliar nutrient concentrations, although not significantly so. Trends in that dataset and supporting data indicate the potential of proper genetic selection to reduce sinuosity while increasing growth. Absorbances obtained from wood cores taken at breast height were not found to be significant predictors of stem-form sinuosity and did not hint towards high lignin to cellulose ratios in the wood, although the principle components analysis did pinpoint a wavenumber range associated with glucosidic bonds as explaining the variation in the spectral dataset. This is encouraging and its potential for exploring differences in wood chemistry from sinuous sections of the stem would be worth investigating. 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

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DEDICATION

This is dedicated to my family, without whom none of this would've been possible.

Chapter 1. NUTRIENT CYCLING IN SINUOUS DOUGLAS-FIR STANDS

1.1 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” (Campbell, 1965). 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 that curved portion. This compression wood is undesirable not only for pulp but also for solid wood due to its unfavourable wood properties, such as 15% to 40% higher specific gravity and higher lignin content. 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 not suitable for many uses (Rune and Warensjo, 2002). Middleton et.al (1989) compared net value of lumber grades for different sinuosity scores and found a 10% decrease in dollar value, which, although was not statistically significant, indicated a propensity to avoid buying wood from those trees.

Sinuosity has been associated to a variety of causes, ranging from land management, genetics to tree physiology. A genetic tendency for sinuous growth was shown by Pederick (Pederick et.al, 1984) but the degree to which it was passed down is questionable. 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 (Downes et.al, 1994). Stem form defects have been linked with intensive land management, particularly fertilizer application, in some genotypes of loblolly pine (McKeand et al., 2006; Espinoza, 2009). Bent tap roots, caused

due to impermeable soil pans, in loblolly-pine were associated with stem-form defects as well (Gatch et.al, 1999).

Nutritional deficiencies of copper, boron, zinc and calcium have been linked to distorted growth (Hopmans, 1990) as most of the aforementioned nutrients are essential for xylogenesis. In their primary growth phase, trees have been shown by studies to be more susceptible to sinuosity (Grob and Carlson, 1994). Howe and Jayawickrama (2002) showed that at age 11, there was a high correlation between stem form defects and height ($r=0.95$) and diameter at breast height ($r=0.97$) although it is unclear whether it is representative of coastal Douglas-fir region.

Sinuosity is colloquially referred to by foresters as *speed-wobble*, so such results would support that observation.

Soils in western Washington span a variety of topography and parent material, owing to the glacial and volcanic activity in the past. They are known to be moderately weathered and experience copious precipitation which leads to high leaching rates (Ugolini, 1968). Owing to well established and long-term disturbance cycles in the Pacific Northwest, early succession species such as red alder play a crucial role in nitrogen fixation, which contributes to the soils being relatively nitrogen rich (Perakis et.al 2011). Higher soil nitrogen promotes more nitrification and leaching and as a consequence, pH of these soils decreases, thereby 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^{+3} mobilization can occupy and deplete sites meant for exchangeable

cations such as Ca, Mg and Zn and this has been posited as a mechanism responsible for depletion of forest Ca (Lawrence et.al, 1995). Calcium is an essential soil nutrient which plays a major role in lignification, secondary cell wall formation, ion balance, cell wall stabilization etc. Poplars grown hydroponically in a low calcium solution were shown to have reduced wood increment, vessel size and fibre length (Lautner et.al, 2007).

High levels of leaching can also result in the depletion of an essential micronutrient, boron. 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 and deposition of material for the same (Hu, 1994). Boron deficiency is known to cause a decrease in apical dominance in trees, which consequently results in reduced timber quality and yield (Lehto, 2010) and an increase in stem malformation. Studies conducted in south-western British Columbia in Douglas-fir as well as hemlock stands have shown boron to be a limiting nutrient (Carter et.al, 1986), which would be concurrent with high leaching rates found in this region. This study aimed to focus primarily on these two nutrients, although relationships with available nitrogen, zinc and copper, which have been shown to be associated with stem-form sinuosity, were also investigated.

Genetic gain was another important aspect of this study. Tree improvement, through selection for desired traits, is a practice which has become integral to forestry, and Douglas-fir is no exception to that. Douglas-fir improvement programs date back to the 1960s in the Pacific Northwest (Adams et.al, 1990) and a considerable amount of resources have been put into such schemes.

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. Very little attention has been focused on evaluating the impact of genetic variation on nutrient cycling within a species. Schweitzer et.al (2004) showed that plant genes in cottonwood had a significant impact on nitrogen cycling through condensed tannin inputs in foliage. 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 silvicultural defect. The selection of best traits in trees do tend to concentrate on form and growth, therefore if there was a genetic propensity for sinuous growth, tree improvement programs could be a useful practice in this regard.

Based upon supporting literature, the first aspect of the project aimed at pinpointing nutritional deficiencies in sinuous Douglas-fir stands through soil and foliar sampling through univariate analyses. The second aspect aimed to investigate the effects of site and genetic gain level on differences in soil and foliar nutrients and posit a link between sites with higher productivity and those which had more sinuous trees. This would also give indicate whether tree improvement had any affect on nutrient cycling in these stands and whether those differences could explain the differences in sinuosity between trees from different genetic gain levels.

1.2 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, SMC planted six new type IV installations by 2007 to examine the effects of genetics, vegetation control and spacing on growth, yield and quality of wood. Genetic gain (high, intermediate and low) would be the primary focus of this study. 3 sites were selected for this study:

1. Donkey Creek by Humptulips on Highway 101 north of Aberdeen
2. Crane Creek in the Quinault reservation north of Aberdeen,
3. Donaldson Creek between Raymond and South Bend by Highway 101.

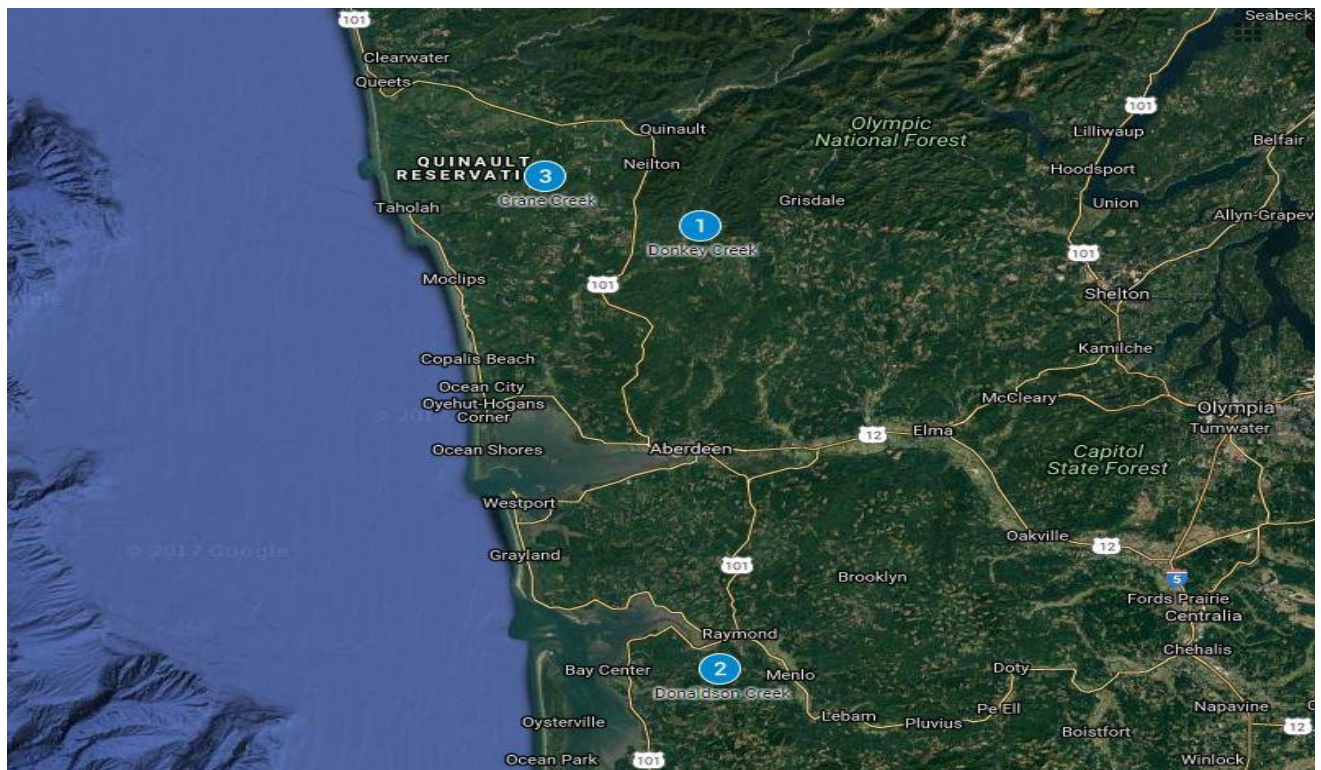


Figure 1: Selected type IV installations sampled for this study

Soils and other pertinent characteristics for the three sites were obtained from the Web Soils Survey (NRCS, 2018)

Table 1: Soil, elevation and climatic characteristics of the selected sites (NRCS, 2018).

Site	Crane Creek	Donkey Creek	Donaldson Creek
Soil order	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 metres above sea level	207 metres above sea level	110 metres above sea level
Temperature and precipitation	90 to 120 inches of precipitation per year and 47-50 degrees F	90 inches of precipitation and 48 degrees F	90 inches of precipitation and 48 degrees F

Fifteen plots were sampled on each site, classified by unimproved, medium and high genetic gain. Four soil cores were taken per plot down to a depth of 15 centimetres. The locations of these soil cores were randomly picked to avoid both sampling biases and spatial autocorrelation. These were then composited to denote a representative sample per plot. Four foliar samples were taken from four different trees, randomly picked per plot and also adjusted to avoid spatial autocorrelation, using a pruning pole to get up to the top third of the tree, which were then composited to denote a representative sample per plot. Care was taken to ensure that the needles which were collected were from the same flush.

Individual trees had been graded for sinuosity across all three sites in 2010. Due to the wide range in values due to observer error, these values were normalized to a 0-6 scale, where 0 would denote no stem defects and 6 would indicate severe stem deformities. These values were then

averaged to represent a sinuosity rating for a plot. This step was performed so as to enable the usage of sinuosity in analyses linking it to soil and foliar nutrient concentrations.

Chemical analyses

The pH of the 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 ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$), extraction with potassium chloride was employed as per Maynard and Kalra (1993). Mehlich 3 extraction was utilized for measuring available fractions of boron, manganese, copper and zinc (Mehlich, 1984). Available calcium was determined by extraction with ammonium chloride (NH_4Cl) (Truby and Aldinger, 1989). For foliar samples, a total metals digest was performed to determine concentrations of a full suite of nutrients (EPA 200.7). A CHN analyser was used to determine % nitrogen in the needles.

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. The null hypotheses which this study aimed to test were the following:

- There is no difference in soil nutrient concentrations between sites/treatments
- There is no difference in foliar nutrient concentrations between sites/treatments
- There is no relationship between soil and foliar nutrient concentrations.
- There is no relationship between soil and foliar concentrations and stem-form sinuosity.

For site and genetic gain comparisons, an ANOVA with both as fixed effects was performed for the soil and foliar nutrient concentrations. This was then followed by Tukey's honest significance difference test to determine which treatments and sites, if found significant, were different from one another. This was then visualized as multiple boxplots using the 'ggplot' package (Wickham, 2009).

This was then followed up by a multivariate analysis in which soil and foliar nutrient concentrations were analyzed together as compared to evaluating them individually, which is the case with ANOVA. The first step in this analysis was separating and relativizing the response matrices. Separating response variables from explanatory variables facilitates more streamlined statistical analyses and was performed by using the 'filter' function which is a part of the 'dplyr' package (Wickham and Francois, 2015). As the magnitude of soils and foliar nutrient concentrations vary from individual nutrient to other, it was desirable to relativize them by

column totals through the 'decostand' function which is a part of the 'vegan' package (Oksanen et.al, 2017). This relativization ensures that no one nutrient disproportionately influences the statistical analyses more than the others, 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. For instance, this study measured 7 different soil nutrients across 45 plots. On applying the Euclidean distance measure, a 45x45 dissimilarity matrix would be calculated regardless of the number of nutrients measured. A Mantel test was then used to test for correlations between the two distance matrices, which for this project were the soil and foliar nutrient concentration distance matrices.

The next step in this analysis involved making use of a non-parametric test, PERMANOVA (Permutational multivariate analysis of variance), to test for the effects of site, genetic gain level and pH on soil and foliar nutrient concentrations. PERMANOVA makes use of multiple permutations of the dataset to compare the actual F statistic to multiple permuted F-values, as opposed to parametric tests which depend on known distributions of data. PERMANOVA does not require the data to meet any assumptions of normality or heteroscedasticity (Anderson and Ter Braak, 2003). It just requires observations to be interchangeable under the null hypotheses, which makes it a more versatile tool to analyse multivariate response data. The 'adonis' function, which is a part of the 'vegan' package developed by Oksanen et.al (2017), was used to perform this test. As the experimental design had only one treatment factor in addition to sites, those permutations would no longer be interchangeable and therefore restrictions were imposed on the scope of those calculations. Restricting the permutations 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 ‘permutest’ package in R (Simpson, 2012).

An equally important aspect of multivariate analyses is visualization. Ordinations are commonly used in soil microbiology but have also been used for analysing exchangeable cations climatic, spatial and within profile gradients (James et.al, 2016). For this purpose, PCA (Principal Components Analysis) and NMDS (non-metric multidimensional scaling) ordinations were used. Principle Components Analysis makes use of eigen vectors, derived from matrix algebra, to decompose and reduce the high dimensionality of a matrix into linearly orthogonal variables which are known as principle components. These can then be represented as an ordination with the variables comprising the data matrix overlaid on top of it. This was particularly useful for this analysis as it would help visualize trends in soil and foliar nutrients and potentially reveal correlations between the same, or lack thereof. PCA was performed in R using the ‘prcomp’ function which is a part of the ‘stats’ package (R Core Team, 2016) and visualized using the ‘autoplot’ function which is a part of the ‘ggplot2’ package (Wickham, 2009).

Non-metric multidimensional scaling (NMDS) was used to visualize the effects of significant explanatory variables on the distribution of soil and foliar nutrient concentrations across all experimental units. The NMDS ordinations were restricted to two dimensions, for ease of interpretation, using Euclidean distances. Care was taken to ensure that the solution had a stress value lower than 0.2 as recommended by Clarke (1993, p.126) above which the ordination can be misleading and not representative of the true distance matrix, which is the mathematical basis of this ordination. This was achieved by using the ‘metaMDS’ function which is a part of the ‘vegan’ package (Oksanen et.al, 2017) and visualizing the same using the ‘ggplot2’ package (Wickham, 2009).

1.3 Results and discussions

Figure 2 shows the foliar concentrations of nutrients previously linked to stem-form sinuosity. Foliar boron was found to be lower than 10 ppm, as indicated by Edmonds et.al (1989). Deficiencies of nitrogen, calcium and zinc were found as well, but to varying degrees, according to concentrations indicated by Ballard and Carter (1986). Most of the sampling for this project was done in July, which is considered to be a period of intensive growth for Douglas-fir. 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. Boron uptake is tied with soil moisture as it happens through the mass flow of water to the roots (Mengerl and Kirkby, 1982), and this could explain higher levels of boron in the foliage for the site with the highest soil moisture, Donkey Creek (figure 15). Trees at Donkey Creek also had higher levels of calcium and zinc in their foliage, and this was true for manganese, magnesium and copper (figure 15). This could be explained by the differences in pH between the sites, leading to higher availability of soil nutrients which makes it easier for trees to take up.

Figure 15 also shows differences in available soil nutrients between the three sites, and the trends are interesting. Lower soil nutrient concentrations for Donkey Creek, with respect to magnesium, calcium and zinc, would be linked to the fact that trees on Donkey Creek have higher levels of the aforementioned nutrients in their foliage. This was also the case for boron as well (figure 17), indicating tighter cycling of nutrients of which the trees might be deficient. Trends for soil copper, manganese are different but on observing the foliar concentrations of these nutrients, they seemed to be above values which are considered deficient for Douglas-fir. Therefore, the same nutrient cycling regimes wouldn't necessarily be applicable to these micronutrients.

There were intriguing results for soil and foliar nutrient concentrations across different genetic gain levels also. Figure 2 shows that trees from the highest genetic gain level had significantly higher foliar nitrogen concentrations than medium and unimproved trees. They also had higher foliar calcium, magnesium and zinc concentrations. Both findings could be explained by better nutrient uptake capacity of trees selected for better growth and form. The plots with the highest genetic gain trees had higher available soil zinc, magnesium, manganese concentrations and significantly higher soil boron, which could be explained by better nutrient cycling through litterfall. Pregitzer et.al (2013) found differences in plant leaf litter for cottonwood phenotypes across an elevational gradient which could influence below-ground soil carbon and nitrogen dynamics. Madritch et.al (2006) also found differences in aspen litter decomposition by genetic identity. These findings, combined with previous literature, do highlight the importance of genetic variation for ecosystem function within a single species.

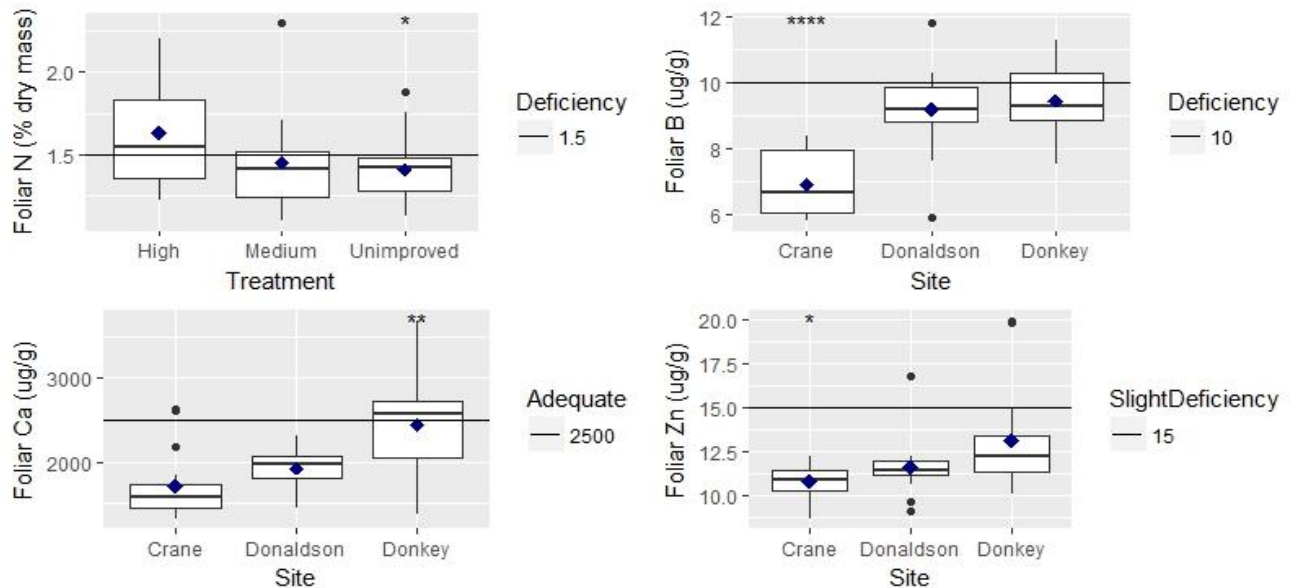


Figure 2: Nutrients which were found to be deficient in the foliage of Douglas-fir trees. Note that for foliar N, site was not found to be significant and therefore that plot wasn't shown.

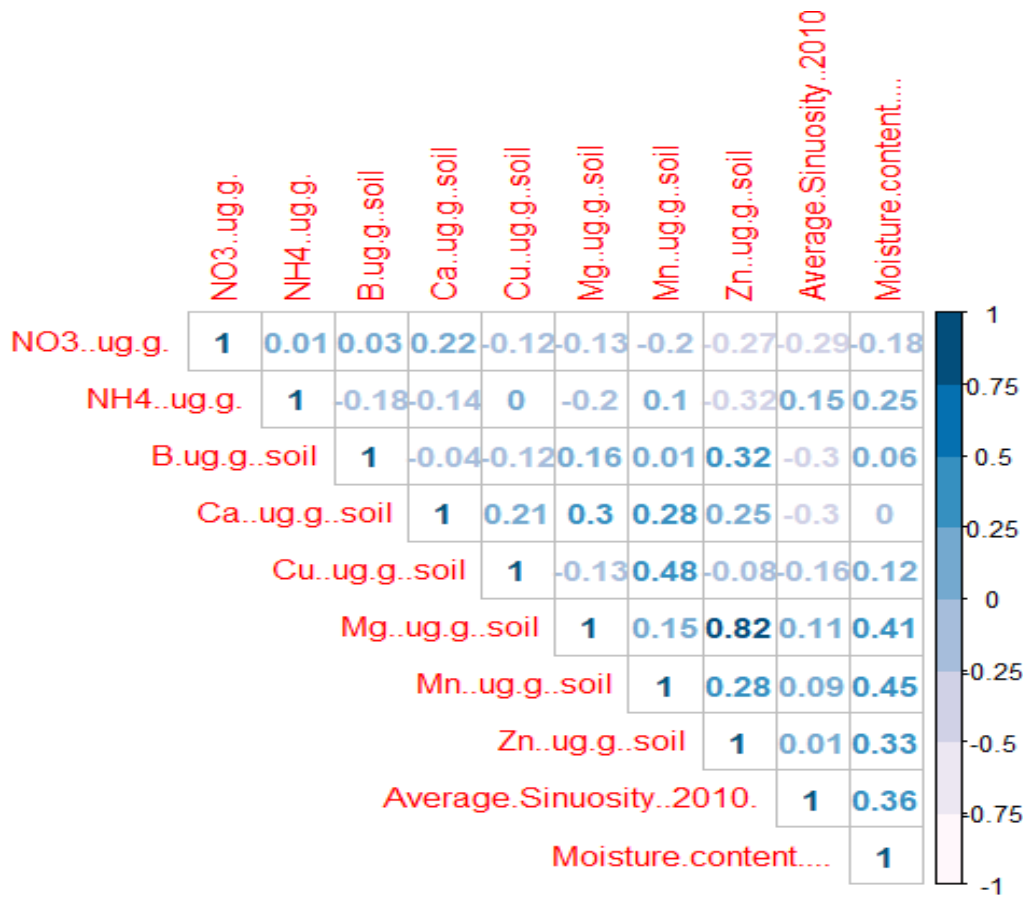


Figure 3: Correlation matrix of all nutrients, sinuosity and moisture content. All reported correlation coefficients are significant at $p=0.05$.

Figure 3 shows a correlation matrix of all available soil nutrients, average sinuosity calculated for all individual plots in 2010, and moisture content of the soils on the plots. Sinuosity was seen to be negatively correlated with available soil boron and calcium, as well as soil nitrate concentrations. Figure 2 shows Douglas-fir to be deficient in all three of these nutrients, to varying degrees. Soil calcium was found to be correlated with other nutrients such as zinc, manganese, copper and magnesium. This would be explained by the fact that they are all available for uptake at higher pHs, so therefore a low pH soil lowers the availability of all these nutrients, potentially exacerbating the nutrient deficiencies for these trees.

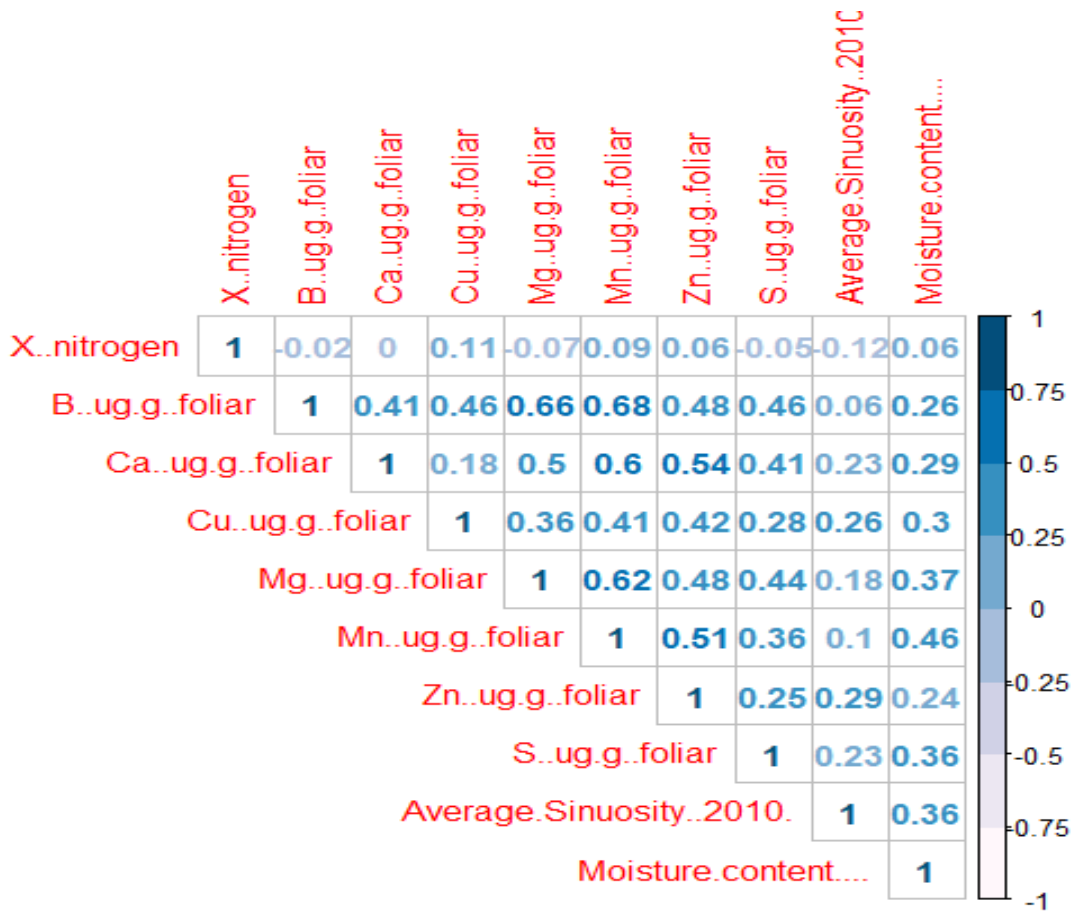


Figure 4: Correlation matrix of all nutrients, sinuosity and moisture content. All reported correlation coefficients are significant at $p=0.05$

This correlation matrix visualizes the result of multiple correlations among foliar nutrients, sinuosity and moisture content of the soil. Sinuosity was found to be weakly positively correlated with foliar boron and manganese, and positively correlated with foliar calcium, copper, magnesium, zinc and sulphur. This is explained by the fact that most foliar nutrients are positively correlated to each other apart from foliar nitrogen. This could be indicative that nitrogen uptake, or what is most commonly associated with growth, is not correlated with other nutrient uptake. This would be important to understand as sufficiency of a primary growth nutrient could lead to

higher demand of other nutrients, possibly exacerbating these deficiencies. Foliar boron and sulphur were found to be correlated, supporting Carter et.al (1986)'s findings.

Donkey Creek was the site with the lowest soil boron and the highest sinuosity (figure 17 and figure 18) although it had significantly higher foliar concentrations. These concentrations were still found to be less than adequate, which necessitates more research into investigating linkages between nutrient deficiencies and stem-form deformities. Results from Table 8, especially those pertaining to soil and foliar nutrients, indicate that Crane Creek was the least productive site and also had the lowest sinuosity among all three sites. Data collection for current year is still ongoing, but observations in the field did support this assertion. Trees at Crane Creek had the least height out of all three sites, as per SMC's field research scientist, and Donkey Creek had the tallest trees. This combined with the results from the table suggest that incidences and severity of sinuosity were related to higher productivity and more available soil nitrogen. This additional nitrogen would cause the trees to grow faster and bigger, but would also increase their demand for other micronutrients, which are already depleted in the soil. With faster growth, the leaders have been known to have slower cambium growth, which might lead to slower synthesis of structural secondary xylem, thereby rendering the tree more susceptible to bending (Grob and Carlson, 1994). This could be hypothesized as one of the reasons why trees at Donkey Creek had the highest sinuosity. Higher calcium availability, in a controlled experiment, led to an increase in stem sinuosity (Littke and Zabowski, 2007) and gypsum fertilization increased soil sulphate, which was found to be positively correlated with sinuosity. Foliar sulphur was positively correlated with sinuosity in this study, which could be interesting to investigate as a part of a controlled experiment in addition to boron and zinc. Espinoza (2009) found that trees with nitrogen fertilization had

higher sinuosity, and calcium additions did not decrease stem sinuosity. However, calcium additions, in conjunction with nitrogen fertilization, were able to decrease stem-sinuosity.

Other reasons for Donkey Creek possessing the highest sinuosity could be related to its geographical location at the top of a hill (highest elevation among sites at 207 metres above sea level), which could lead to different microclimatic conditions, which have been shown to be associated with the severity of sinuosity (Rune and Warensjo, 2002).

Figure 18 shows that trees from a higher genetic gain level had generally lower sinuosity scores as compared to medium and unimproved trees. 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 (1985) to 0.41 and 0.36 at ages 12 and 24 as calculated by Temel and Adams (2000). More efficient and tighter nutrient cycling could be a mechanism through which they maintain growth and stem form, which is evident in figure 2 and table 8.

Multivariate analyses

Table 2 shows the results of the Mantel test between available soil and total foliar nutrient concentrations. Available soil nutrient and total foliar nutrient concentrations were found to be significantly positively correlated, although the correlation was not found to be strong. This points towards other factors which might be influencing nutrient uptake, such as deficiency of a particular micronutrient and its selective uptake, which might necessitate the accumulation of the same in the foliage over other nutrients. Both above and below ground pools for these nutrients were found to be sufficient which might influence the significance of the correlation.

Table 2: Mantel test examining correlation between soil and foliar nutrient concentrations

Variables	Mantel statistic r	Significance
Available soil nutrient concentrations, Total foliar nutrient concentrations	0.1985	0.0143**

** indicates significance (p<0.05)

Figure 5 shows the PCA ordination for the complete data matrix. It was observed that there was not a high degree of correlation between vectors representing soil and foliar nutrients. There was a clear separation in the ordination for all the nutrients by site, supporting the univariate analyses.

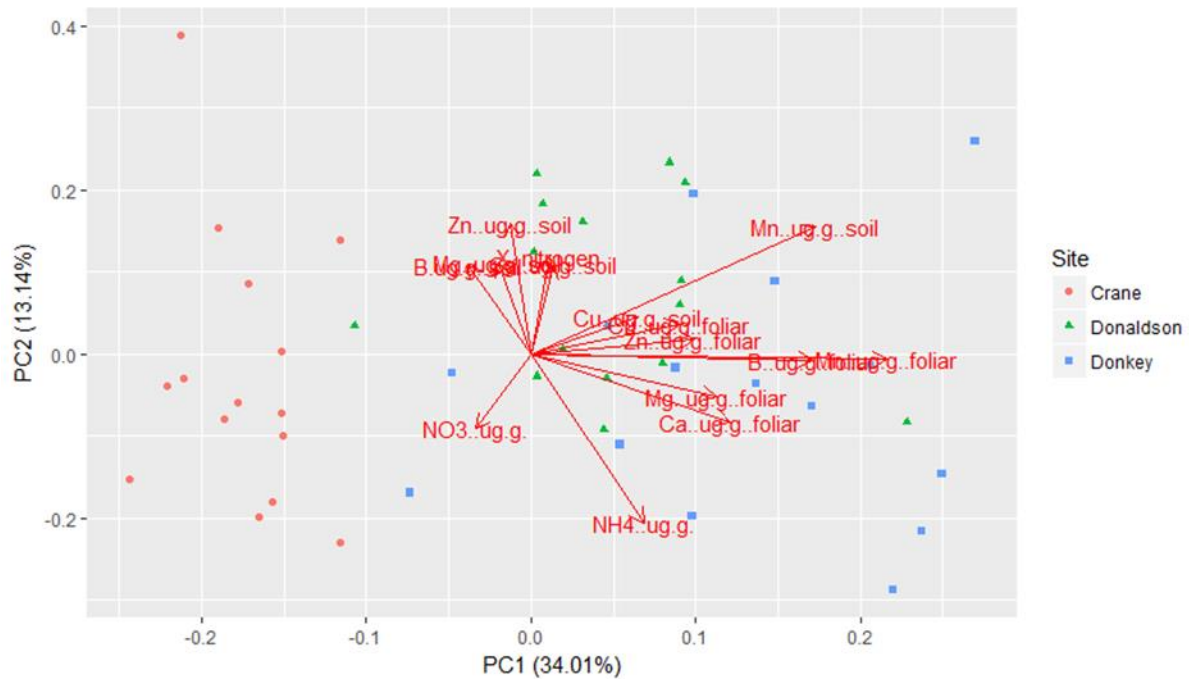


Figure 5: Principle components analysis for all nutrients across sites. For ease of interpretation, the first two axes were showed in this ordination

Table 3 shows the effects of site, genetic gain levels (treatment) on soil nutrient concentrations. Site differences accounted for a significant amount of variation in soil nutrients, although the p-value for the same was not reported down to the fact that there were only 3 sites, so the number of permutations testing for site differences were low ($3!=6$). pH was found to be a significant predictor of soil nutrients, which is not surprising considering the role of pH in the availability of soil nutrients. Elements such as calcium, boron, zinc and magnesium are more available for uptake when the pH is greater than 7. Since the soils in the Pacific Northwest have a low pH, this might indicate deficiencies or tighter nutrient cycling of such nutrients. Genetic gain level was significant at $p<0.1$, pointing towards the ability of trees which come from families which experience higher growth to mobilize more nutrients.

Table 3: PERMANOVA results for the effects on soil nutrients.

Explanatory variables	Df	Sum of squares	Pseudo F	R²	Pr(>F)
Site	2	4.869	8.1353	0.27401	-
pH	1	1.379	4.0641	0.06349	0.001***
Treatment	2	0.930	1.5542	0.05235	0.0697·
Residual	38	10.842			

*** indicates significance at $p=0.001$

· indicates significance at $p=0.1$

Table 4 shows the effect on selected explanatory variables on total foliar nutrient concentrations. Site differences accounted for a significant amount of variation, but the p-value for the same was not reported due to the low number of permutations ($3!=6$) which would go into calculating one. Genetic gain level was also found to account for 5% of the variation in the data, presumably due to the reason that trees from different genetic provenances would have different nutrient management strategies. pH was not found to be a significant predictor for differences in foliar nutrient concentrations.

Table 4: PERMANOVA results for the effects on foliar nutrients.

Explanatory variables	Df	Sum of squares	Pseudo F	R²	Pr(>F)
Site	2	6.648	12.773	0.37331	-
pH	1	0.336	1.057	0.01257	0.229
Treatment	2	0.751	1.442	0.04214	0.145
Residual	38	10.186			

Figures 6 and 8 visualize the effect of site on differences in soil nutrient concentrations. There is visible separation of groups, validating the variance attributed to it in the PERMANOVA results. There was also some overlap, as evident from figure 8, between these ellipses. This is a reminder of the variability of soils within the same site leading to a wider spread of values. In figure 6, plots on Donkey Creek appeared to have the highest pH, as the pH vector seems to be increasing in the region associated with that site, validating the results of the univariate analysis between pH and site.

Figures 7 and 9 visualize the effect of site on differences in foliar nutrient concentrations. There was visible separation by site, which was also seen in the PCA ordination for the same dataset.

There was also some overlap in the 95% confidence ellipses, hinting to why it might not be that strongly significant.

NMDS ordination of soil nutrient concentrations from three sites

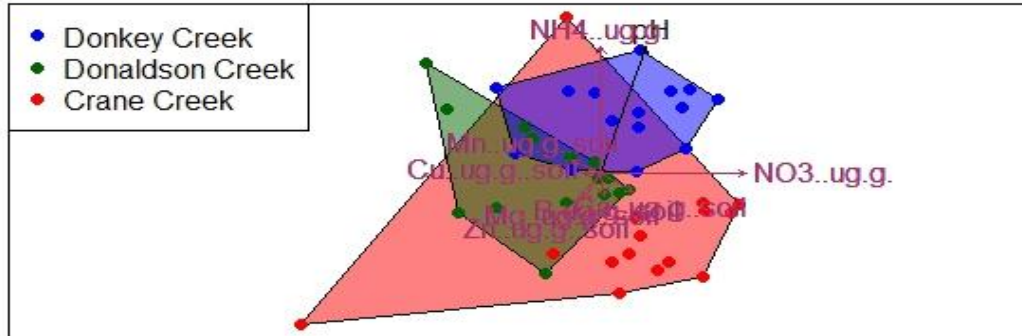


Figure 6: NMDS ordination for soil nutrient concentrations across sites. Final stress= 0.2

NMDS ordination of foliar nutrient concentrations from three sites

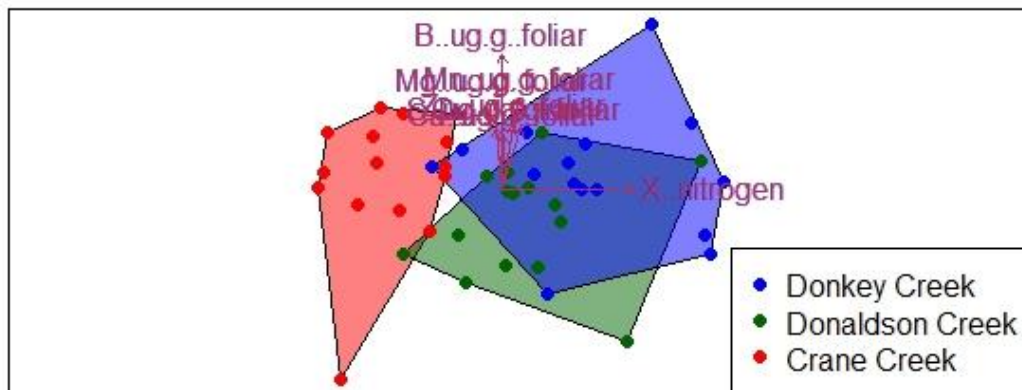


Figure 7: NMDS ordination for foliar nutrient concentrations across sites. Final stress = 0.16

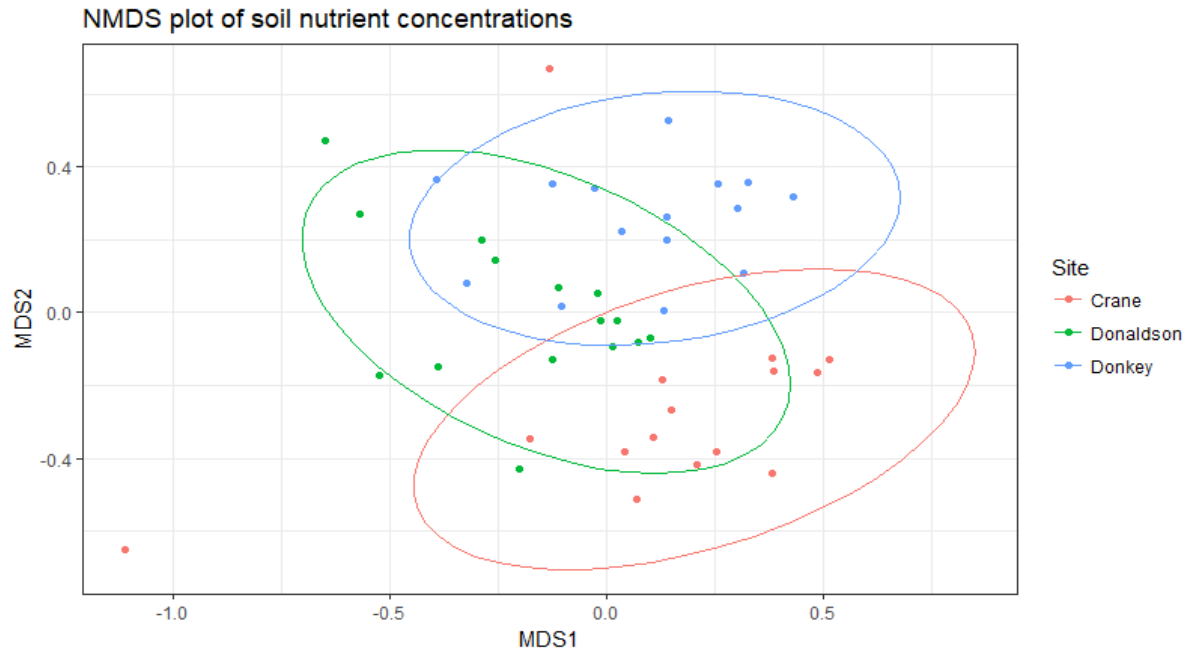


Figure 8: NMDS ordination for soil nutrients across sites. Final stress= 0.209. The ellipses represent 95% confidence intervals around their centroids.

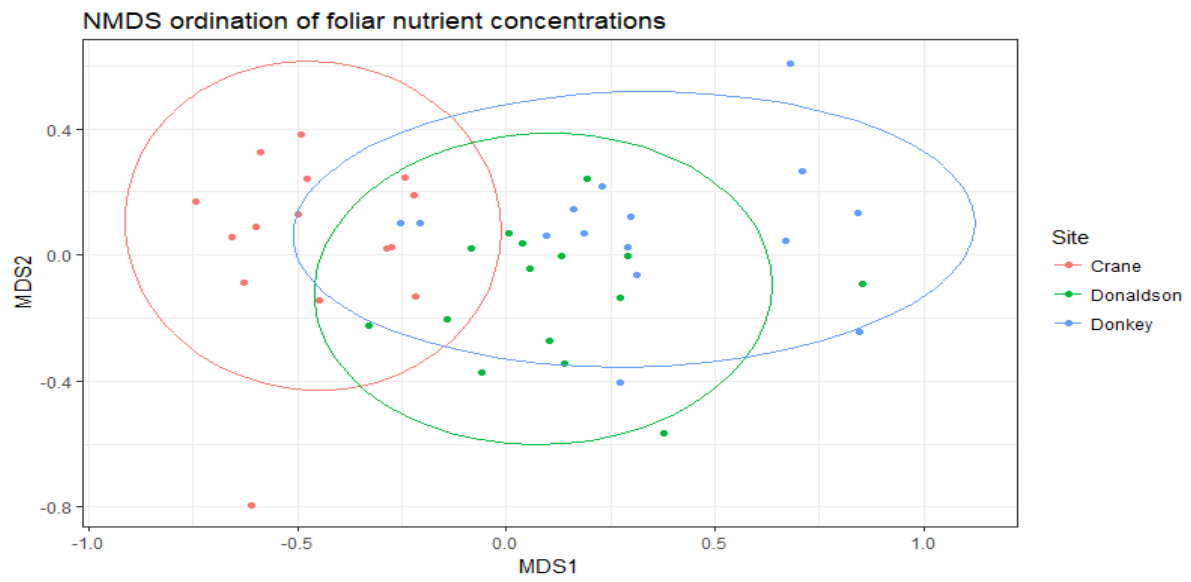


Figure 9: NMDS ordination for foliar nutrients across sites. Final stress= 0.158. The ellipses represent 95% confidence intervals around their centroids.

Figure 10 shows that trees from higher genetic gain levels might be marginally different when it comes to storing nutrients in their foliage, which seems to agree with the variation explained in the PERMANOVA model.

NMDS ordination of foliar nutrients across three genetic gain levels

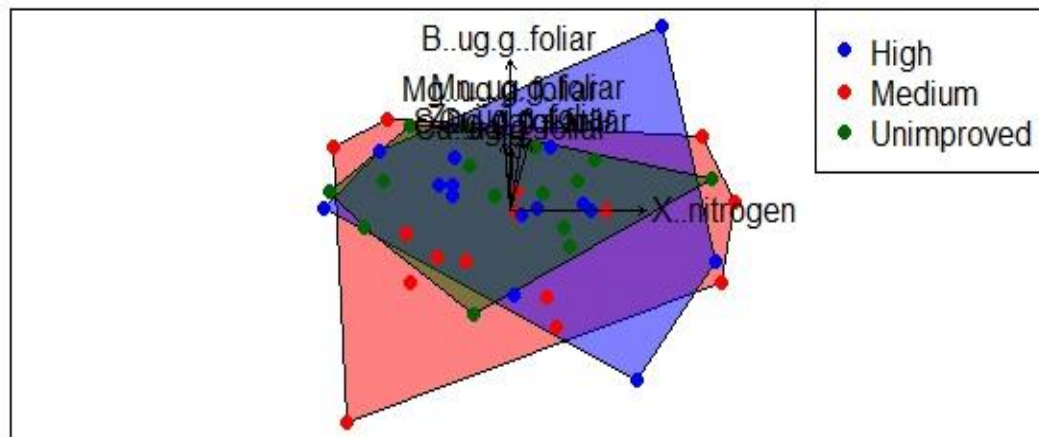


Figure 10: NMDS ordination for foliar nutrients across different genetic gain levels. Final stress = 0.158

1.4 Conclusions

This study found that there were mild to severe deficiencies of nitrogen, calcium, zinc and boron in Douglas-fir across all three sites which were sampled. Sinuosity was also found to be negatively correlated with available soil boron, nitrogen, calcium, nitrate and foliar nitrogen while it was positively correlated to foliar sulphur, calcium, copper, zinc and available soil ammonium. There were differences between sites with respect to sinuosity as well as multiple soil and foliar nutrient concentrations. Donkey Creek, which is the most productive site with respect to multiple foliar and nutrient concentrations as well as height of the trees, 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 are able to cycle nutrients more efficiently and optimize various nutrient ratios while at the same time maintaining better growth. Results of the multivariate analyses also indicate significant differences in nutrient cycling between site and genetic gain levels.

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. Extensive research has been done on sinuosity in the American south-east with loblolly and radiata pine, as well as in New Zealand with radiata pine and Douglas-fir and in northern Europe with Scots pine and Norway spruce. Literature review does indicate that there does seem to be 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. Building upon the results of this study, there is definitely potential for controlled, fertilization-based studies looking at boron, nitrogen, calcium, zinc and their combinations and the effects they have on sinuosity in Douglas-fir.

Lastly, trees from higher genetic gain levels were found to be less sinuous than medium and unimproved ones. Advanced genetic engineering techniques, such as the CRISPR-Cas9 genome editing technology, could also show promise in this regard, considering that previous literature has found sinuosity to be a heritable trait, to varying degrees. These techniques could be explored if genes associated with sinuous growth can be identified.

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Chapter 2. INVESTIGATING THE DIFFERENCES IN WOOD CHEMISTRY BETWEEN SINUOUS DOUGLAS-FIR STANDS

2.1 Introduction

Wood is a hard, fibrous tissue found in the stems and roots of trees and shrubs. It is primarily constituted of cellulose, hemicellulose, lignin and other polymeric organic compounds such as lipids, fatty acids, phenols, waxes etc. These three main wood polymers are the most abundant polymers in the carbon cycle. Wood consists of cellulose fibres embedded in a lignin and hemicellulose matrix. This unique structure gives it mechanical strength (Salmen et.al, 2009), and resistance against pests and microbes (Daniel, 2003).

Compression wood is formed by gymnosperm trees such as Douglas-fir, as a response to environmental or external stresses such as wind, snow which act as impediments to normal growth (Timell, 1986). Stem defects or deformations are known to be associated with compression wood formation as well, which is done to compensate for growth in the opposite direction, as is the case with stem sinuosity. Even though this process is an important defense mechanism for the tree, it does lead to issues for the timber industry. Compression wood is known to have higher specific gravity, higher lignin content and shrinks/warps during drying or upon application of thermal stress (Beard et.al, 1993, Timell, 1986). The longitudinal shrinkage also causes distortions such as bow and crooks to appear in the sawn timber (Ohman, 2002), in addition to issues related with sawing, drilling owing to the higher specific gravity and hardness. Lastly, compression wood increases the amount of lignin compared to cellulose, which reduces pulp yield, which has implications for biofuel and paper industries.

An important practice which is central to the study of stem deformations is the quantification of stem sinuosity. Currently, there does not appear to be a standard way of doing so. A variety of methods, ranging from a “Crook Index”, which multiplies the number of crooks with the deviation of the largest crook (Goddard and Strickland, 1964), to photographic measurements of angle of deviation (Cremer, 1998) are used, but they are prone to observer bias. This bias can be further worsened by unfavourable working conditions in the field such as precipitation events, high temperatures etc., according to field research scientists. Other measures of sinuosity, such as pith sinuosity, require splitting logs and measuring deviation of the pith from a reference line (Spicer et al. 2000), which is a destructive, time intensive method. Leduc et al (2000) recommended the use of subjective ratings over measurement-derived ratings as they would be faster and seemed to be more accurate with respect to their data. Subjective ratings would lack repeatability between different observers and would also be found lacking on detecting slight differences between sinuous stems.

Taking into consideration this variability, it would make sense to explore newer techniques to quantify this phenomenon. In this project, we explored the potential of Fourier-transform infrared spectroscopy to detect differences in wood chemistry between trees with varying levels of sinuosity. The characteristic of sinuous trees having higher compression wood formation in their stems, which would result in higher lignin concentrations as compared to cellulose, was used as the justification for the use of this technology. FT-IR spectroscopy, in addition to being non-invasive and relatively cheap, has been employed for detecting differences between wood species (Liu et al. 2008), or changes in wood chemistry following decay by fungi (Pandey and Pitman, 2003).

2.2 Materials and methods

4 tree cores were taken per plot from the same trees which were sampled for foliage, using an increment borer at breast height. These cores were not composited. The trees in these sites were visually scored for sinuosity in 2010, which were then normalized to a 1-6 scale, 1 indicating least sinuous and 6 denoting high sinuosity, for this analysis. 159 cores were used for final analysis, after discarding the ones not suitable for spectrophotometry due to coarseness. The tree cores were reduced to fine, homogenous wood powder after subjecting them to 5 minutes in a ball mill followed by passing them through a Wiley mill. These were then analysed under an FT-IR spectrophotometer with an ATR (attenuated total reflectance) attachment. The structure of the dataset consists of rows of absorbances recorded at different wavenumbers associated to a tree core. These wavenumbers lay in the middle infrared region and ranged from 4000-450 cm^{-1} . The spectra were then pre-processed prior to importing, using baseline and ATR correction functions which are a part of the Perkin Elmer Spectrum software. The data was subset to 800-1800 cm^{-1} , which represents the fingerprint region.

The null hypothesis which this study aimed to test was the following:

- There is no difference in spectral absorbances between sinuosity ratings.

For the univariate analyses, ratios of absorbances associated with lignin and cellulose were regressed against sinuosity ratings in a multinomial regression analysis. This method was chosen as the response variable, sinuosity ratings, was ordinal. Lignin to cellulose ratios were obtained by dividing the spectral absorbances of wavenumbers associated to cellulosic and lignin compounds. 1505 and 1594 cm^{-1} are wavenumbers which have been associated with aromatic skeletal vibration with a C=O bond, indicative of lignin compounds. Wavenumbers 1030 and

1155 cm⁻¹ both correspond to cellulosic compounds as the latter wavenumber corresponds to C-O-C bond vibrations (glucosidic linkages) and former to aromatic C-H in plane deformation vibrations (cellulose contains these bonds). (Pandey and Pitman, 2003). 800-1800 cm⁻¹ is considered to be the fingerprint region for wood chemistry analyses.

Table 5: Wavenumber characterization, as per Pandey and Pitman (2003). Point refers to points on the plot succeeding the table.

Wavenumber	Wavenumber characterization	Point
1738	C=O stretch in unconjugated ketones, carbonyls and esters	1
1649	Absorbed O-H and conjugated C-O	2
1594	Aromatic skeletal vibration and C=O stretch	3
1505	Aromatic skeletal vibration and C=O stretch	4
1460	C-H deformation in lignin and carbohydrates	5
1424	Aromatic skeletal vibration combined with C-H in plane deformation	6
1372	C-H deformation in lignin and hemicellulose	7
1235	Syringyl ring and C= stretch in lignin and xylan	8
1155	C-O-C vibration in cellulose and hemicellulose	9
1030	Aromatic C-H in plane deformation, C-O deformation in cellulose and hemicellulose.	10

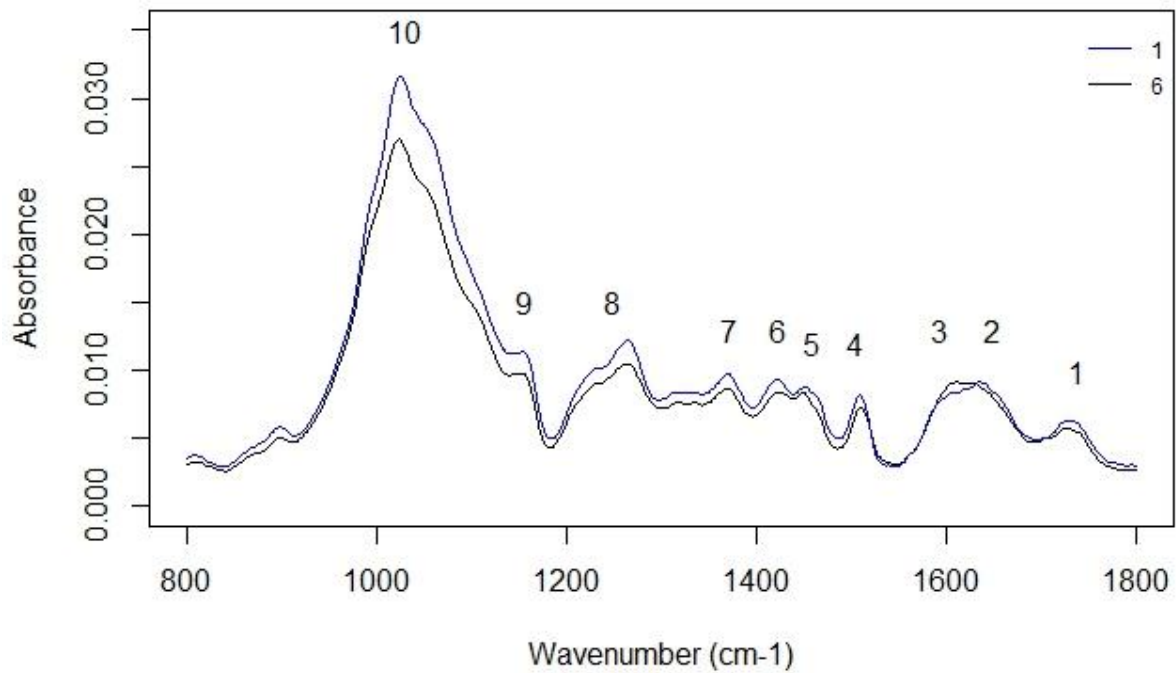


Figure 11: FT-IR spectra of wood cores taken from two trees with different sinuosity scores. Band assignments refer to Table 5.

PERMANOVA was utilized to test for the differences in spectral absorbances by sinuosity scores associated to the tree the wood cores were extracted from.

For spectral absorbances, which can have considerable number of observations owing to the wavelengths at which absorbances are recorded, Principle Components Analysis, or PCA, helps in decomposing them into components, which can be subsequently used for a PERMANOVA or an ANOVA. This technique was utilized for the spectral reflectance data matrix obtained from FT-IR analysis of wood cores.

2.3 Results and discussion

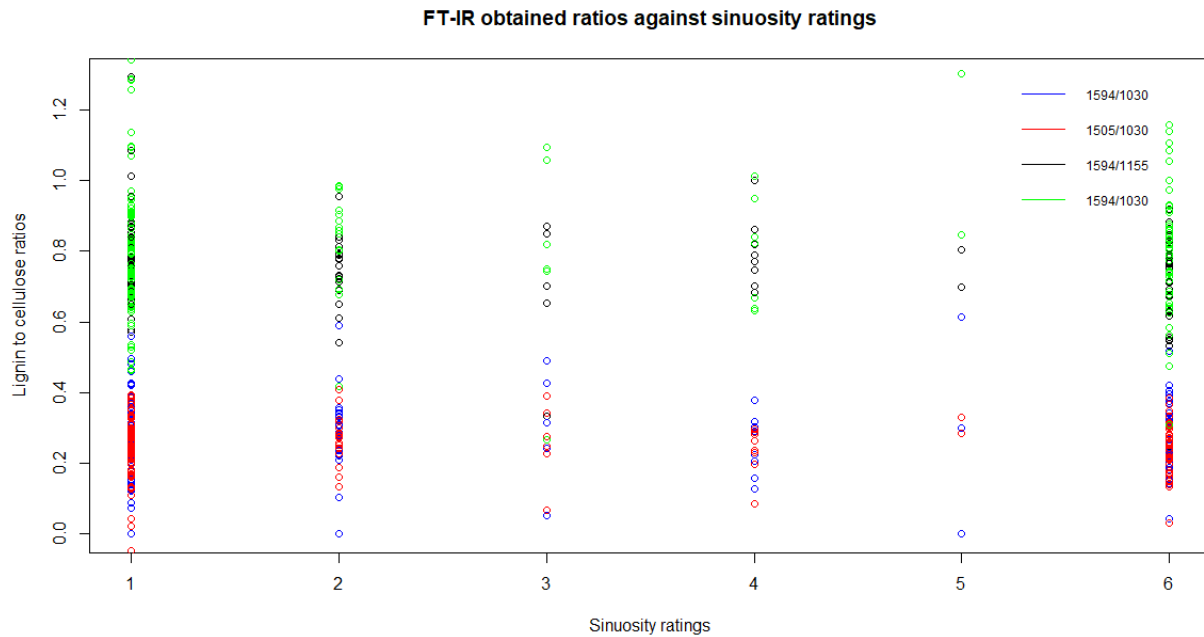


Figure 12: Distribution of 4 FT-IR derived lignin to cellulose ratios at different wavenumbers across sinuosity scores

Figure 12 shows the distribution of FT-IR derived ratio against sinuosity ratings. There seemed to be a wide range of ratios at the lowest sinuosity rating, with no clear pattern emerging from this plot. Multinomial regression analysis showed that lignin to cellulose ratios could not describe enough variation among sinuosity ratings, and were therefore not significant predictors ($p=0.47$)

Table 6 shows that recorded sinuosity scores were not a good predictor for differences in spectral absorbances recorded from FT-IR analysis of wood cores. This lack of predictive power was further evident in Figure 13 which shows a PCA ordination of the spectral data. There did not appear to be any separation by sinuosity scores along PC1. Due to the high number of response

variables, loadings were not reported, but there was a distinct lack of separation in the data by different sinuosity scores. The first principle component accounted for 94.3% of the variation in the data and was found to be significantly associated with the absorbances in the 1010-1040 cm^{-1} range, which is associated with cellulosic functional groups (Table 5). As the first principle component seemed to explain 94.3% of the variation in the response, it was analysed in an ANOVA model against sinuosity scores as shown in Table 7. Sinuosity scores are not able to explain the variation among spectral absorbances. The PCA ordination points towards the oversampling of trees with lower sinuosity scores, which was one of the issues with the selection of trees for this analysis.

Table 6: PERMANOVA results for the effects on spectral absorbances obtained through FT-IR analysis of wood cores

Explanatory variables	Df	Sum of squares	Pseudo F	Pr(>F)
Sinuosity Scores	5	0.0081	0.45957	0.864
Residual	153	3.0602		

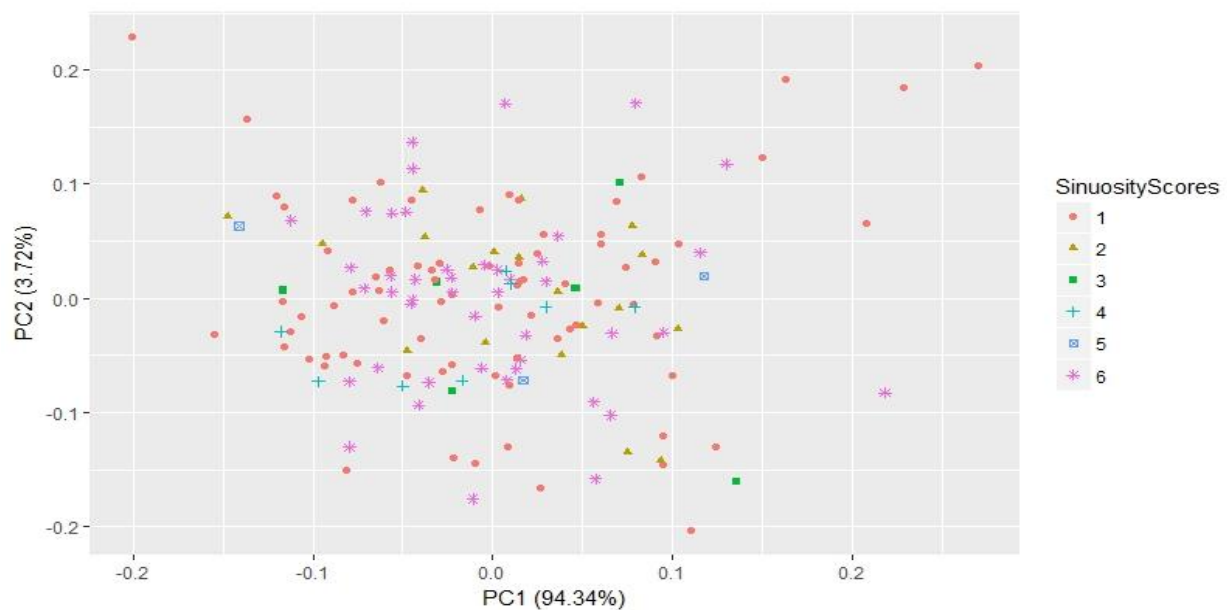


Figure 13: Principle components analysis of spectral absorbances across sinuosity scores.

Table 7: ANOVA table for differences between PC1 loadings across sinuosity scores.

Explanatory variables	Df	Sum of squares	F value	Pr(>F)
Sinuosity Scores	5	0.0369	0.4115	0.8402
Residual	153	2.7492		

Both the univariate and the multivariate analyses were unable to prove any associations between FT-IR derived wood component ratios/absorbances and sinuosity scores. There were some issues with the study which could explain the lack of any relationships. Firstly, the sinuosity scores which were used for this analysis were from when the trees were 6 years old, in 2010, because the sites were still being measured at the time of this study and that is still an ongoing process. Second, the sinuosity grading was done by multiple field crew/contractors, which would bring with it the potential of observer bias in the whole process. This process could also be performed using drone technology as well, combined with an image analysis software which could quantify the degree of sinuosity. This would be a non-invasive procedure and would reduce manpower as well.

There also seemed to be issues with the manner in which the grading was done, as some trees which were marked higher for sinuosity in 2010 and 2017 (for two plots) did not appear to be as sinuous. This observation was supported by members of our field crew, which included a technician, a research scientist and graduate students. On comparing the data which we had from 2010 and 2017 (not used in this thesis as current year data is incomplete), there appeared to be a weak, positive correlation between sinuosity scores for the two years. This was contrary to our expectations of a stronger correlation but could be explained by the fact that trees seem to correct

themselves as they grow older, thereby reducing visible sinuosity or even potentially exacerbating existing sinuosity, both of which would reduce the correlation.

The other important issue with this study was the restriction that cores could only be taken from breast height and not the actual sinuous sections of the stem. As these trees were 13 years old, and averaged 10 metres in height, it was hard to get to those sections even with a ladder.

Compression wood formation is a physiological reaction to any kind of stimuli which tries to force growth in a different direction (Timmell, 1986), so even trees which could be exposed to snags or higher wind-throw could develop compression wood. Therefore, sinuosity would not be the only phenomenon which prompts formation of compression wood, which explains why some trees with little sinuosity had higher lignin to cellulose ratios in figure 12.

2.4 Conclusions

Lignin to cellulose ratios obtained through FT-IR spectrophotometry of wood cores were not found to be associated with sinuosity scores of the trees the cores came from. Multivariate analysis does indicate that wavenumbers associated with cellulosic compounds might explain 90% of the variation in the absorbance dataset in the first principle component, but they were not found to explain sufficient variation among sinuosity scores when regressed against them. It would be interesting to explore whether this technique could be applied to wood from sinuous sections of the tree and whether that could be used as an alternative way of quantifying sinuosity in Douglas-fir. There is also a potential for drone technology to help with the grading of trees in the future as well, reducing manpower and material costs.

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Tables

Table 8: ANOVA table for all measured variables.

Variable	Site	Genetic gain	Description of significance, if any
pH	***		Crane Creek had lowest pH .
Bulk density			
Moisture content	**		Crane Creek had lowest soil moisture
100 needle dry weight	*		Crane Creek had highest needle weight
Foliar nitrogen		.	Highest genetic gain had higher foliar N
Available soil ammonium	**		Donkey Creek had highest soil NH ₄ -N
Available soil nitrate	**		Donkey Creek had lowest soil NO ₃ -N
Foliar boron	***		Crane Creek had lowest foliar B
Available soil boron			
Foliar calcium	***		Crane Creek had lowest foliar Ca
Available soil calcium			
Foliar copper	**		Crane Creek had lowest foliar Cu
Available soil copper	**		Crane Creek had lowest soil Cu
Foliar magnesium	***		Crane Creek had lowest foliar Mg
Available soil magnesium			
Foliar manganese	***		Crane Creek had lowest foliar Mn
Available soil manganese	***		Crane Creek had lowest soil Mn
Foliar zinc	*		Crane Creek had lowest foliar Zn
Available soil zinc			
Foliar sulphur	*		Donkey Creek had highest foliar S

· represents significance at p=0.1

* represents significance at p=0.05

** represents significance at p=0.01

*** represents significance at p=0.001

Table 9: Loadings for the total nutrient PCA

Variables	PC1	PC2
Nitrate (NO ₃ ..ug.g)	-0.08214028	-0.22460094
Ammonium (NH ₄ ..ug.g)	0.16945593	-0.50832121
Boron (B..ug.g..soil)	-0.09451839	0.26830617
Calcium (Ca..ug.g..soil)	0.03507512	0.26916814
Copper (Cu..ug.g..soil)	0.16065766	0.11250638
Magnesium (Mg..ug.g..soil)	-0.04963869	0.27472870
Manganese (Mn..ug.g..soil)	0.42879953	0.38633525
Zinc (Zn..ug.g..soil)	-0.03177086	0.39258224
% nitrogen (X.nitrogen)	0.02752511	0.29278824
Boron (B..ug.g..foliar)	0.42277106	-0.01789193
Calcium (Ca..ug.g..foliar)	0.29979260	-0.20463870
Copper (Cu..ug.g..foliar)	0.22252796	0.09040265
Magnesium (Mg..ug.g..foliar)	0.27763221	-0.12939
Manganese (Mn..ug.g..foliar)	0.53385582	-0.0169502
Zinc (Zn..ug.g..foliar)	0.254456	0.0459

Table 10: Loadings for FT-IR spectra PCA. Only the highest loadings for PC1 were reported due to the high number of wavenumbers in the dataset.

Wavenumbers (cm ⁻¹)	PC1	PC2
1026	0.09772751	0.1284863
1024	0.09767287	0.1283270
1028	0.09738783	0.1275708
1022	0.09727229	0.1270319
1030	0.09669532	0.1255999
1020	0.09635863	0.1249616
1032	0.09578852	0.1227808
1018	0.09512914	0.1214337
1034	0.09451291	0.1194372
1016	0.09351583	0.1167574

Figures

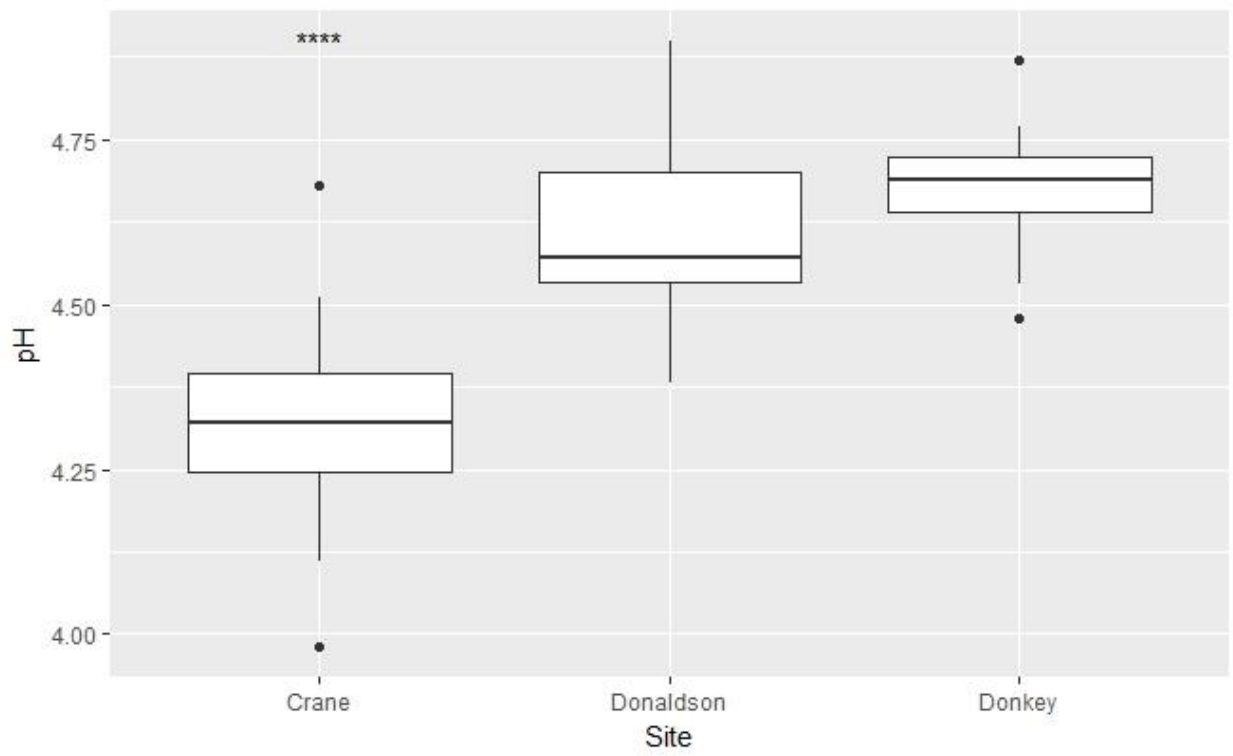


Figure 14: pH values across different sites.

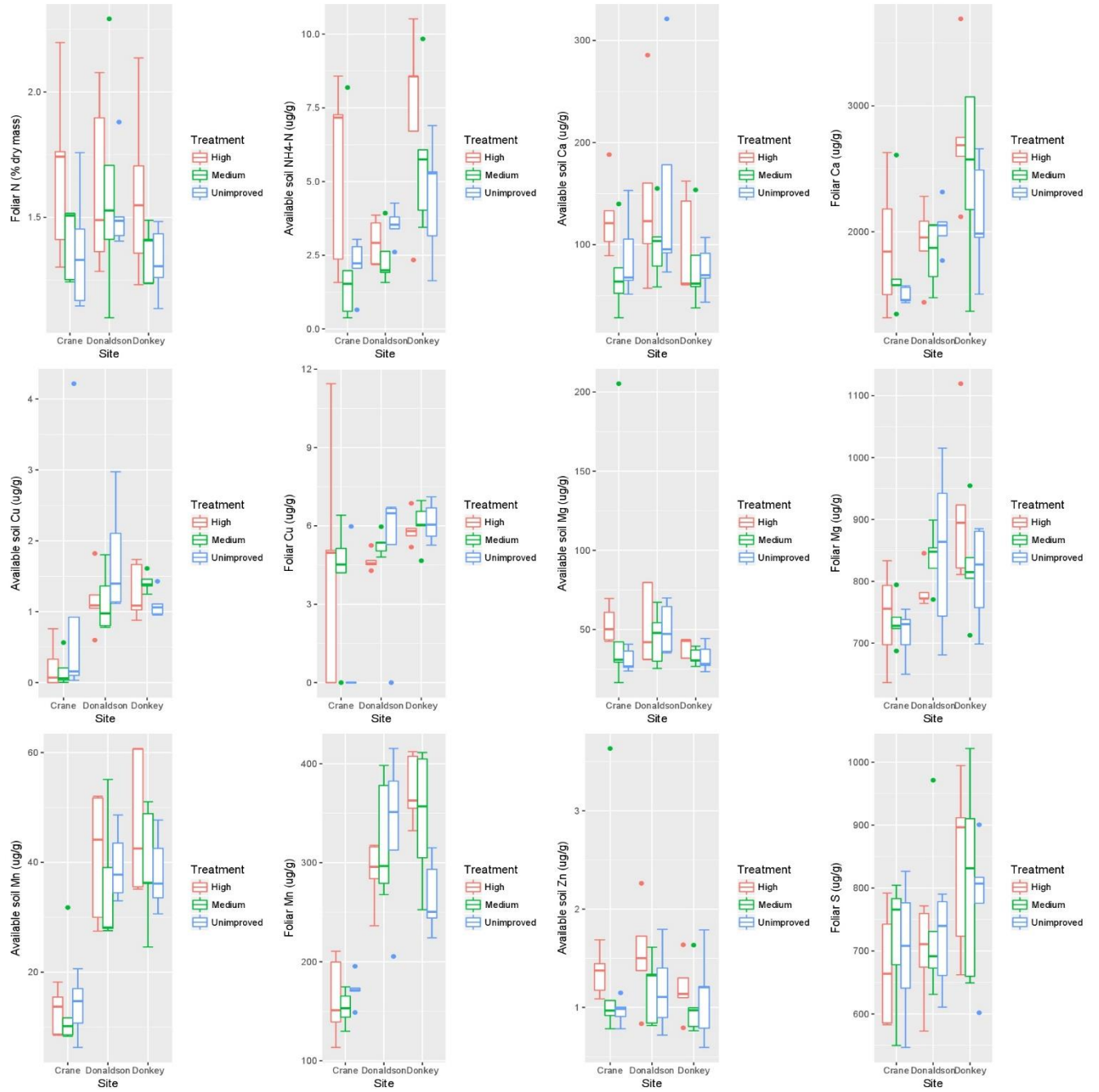


Figure 15: Soil and foliar nutrients across different sites and genetic gain levels.

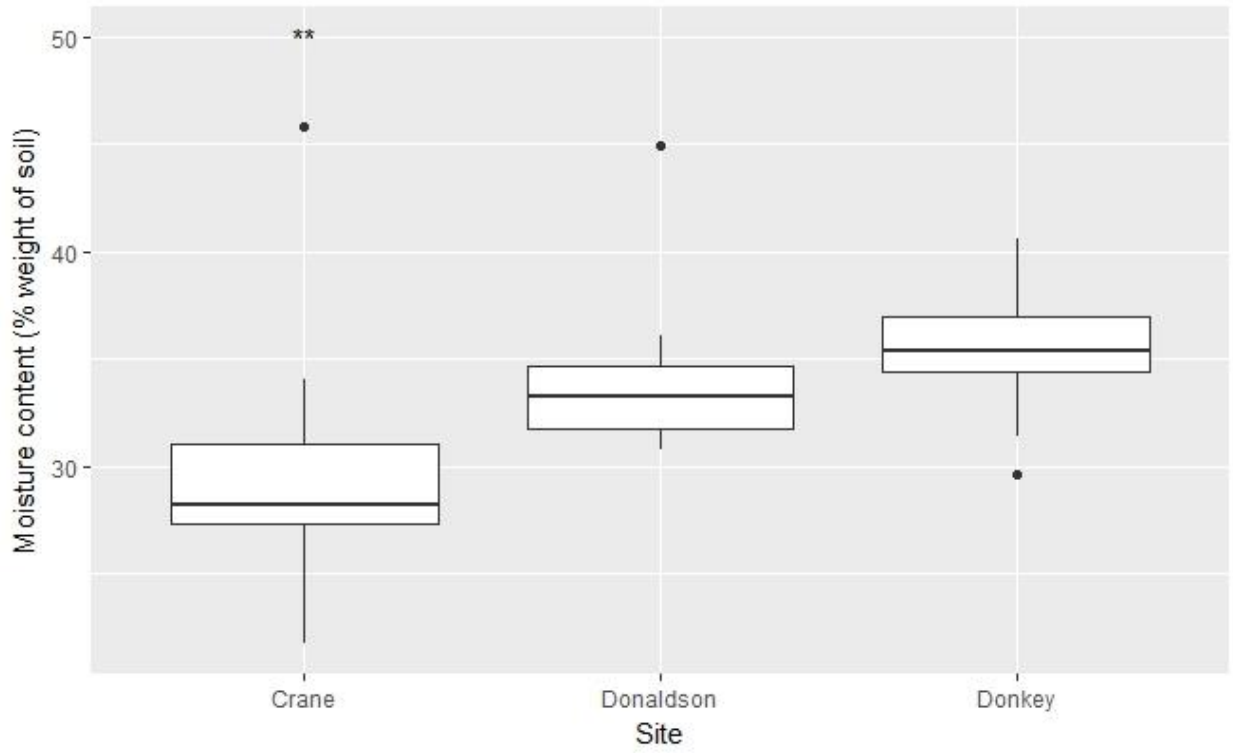


Figure 16: Moisture content across different sites.

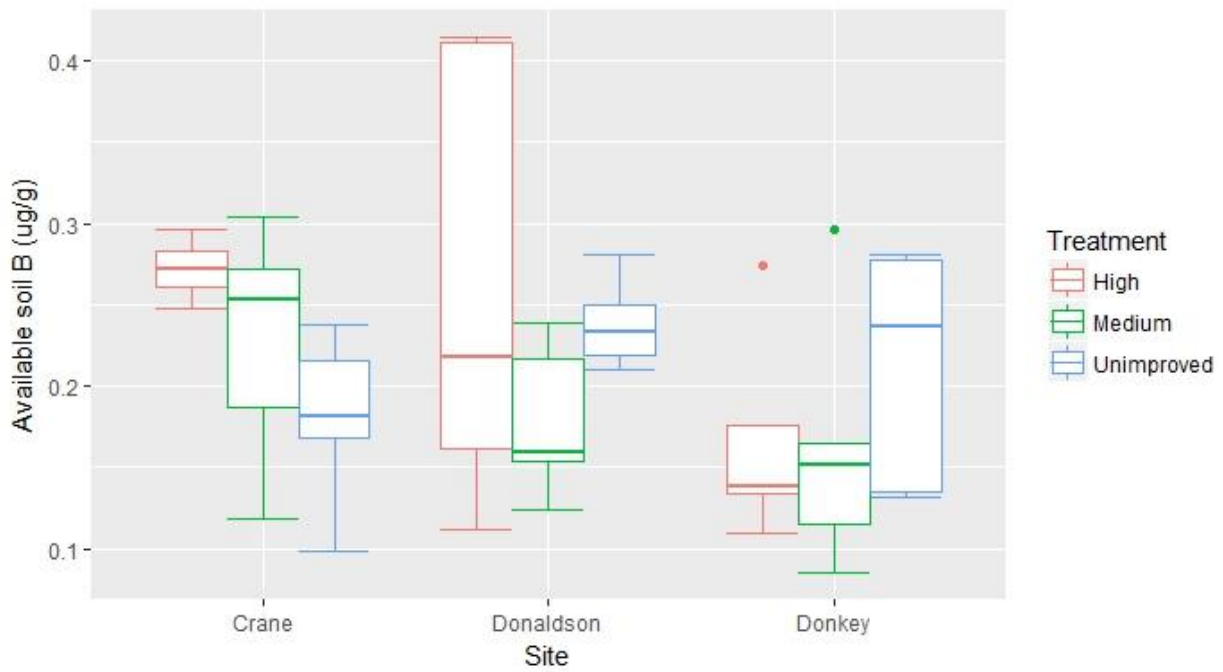


Figure 17: Soil boron across different sites and genetic gain levels.

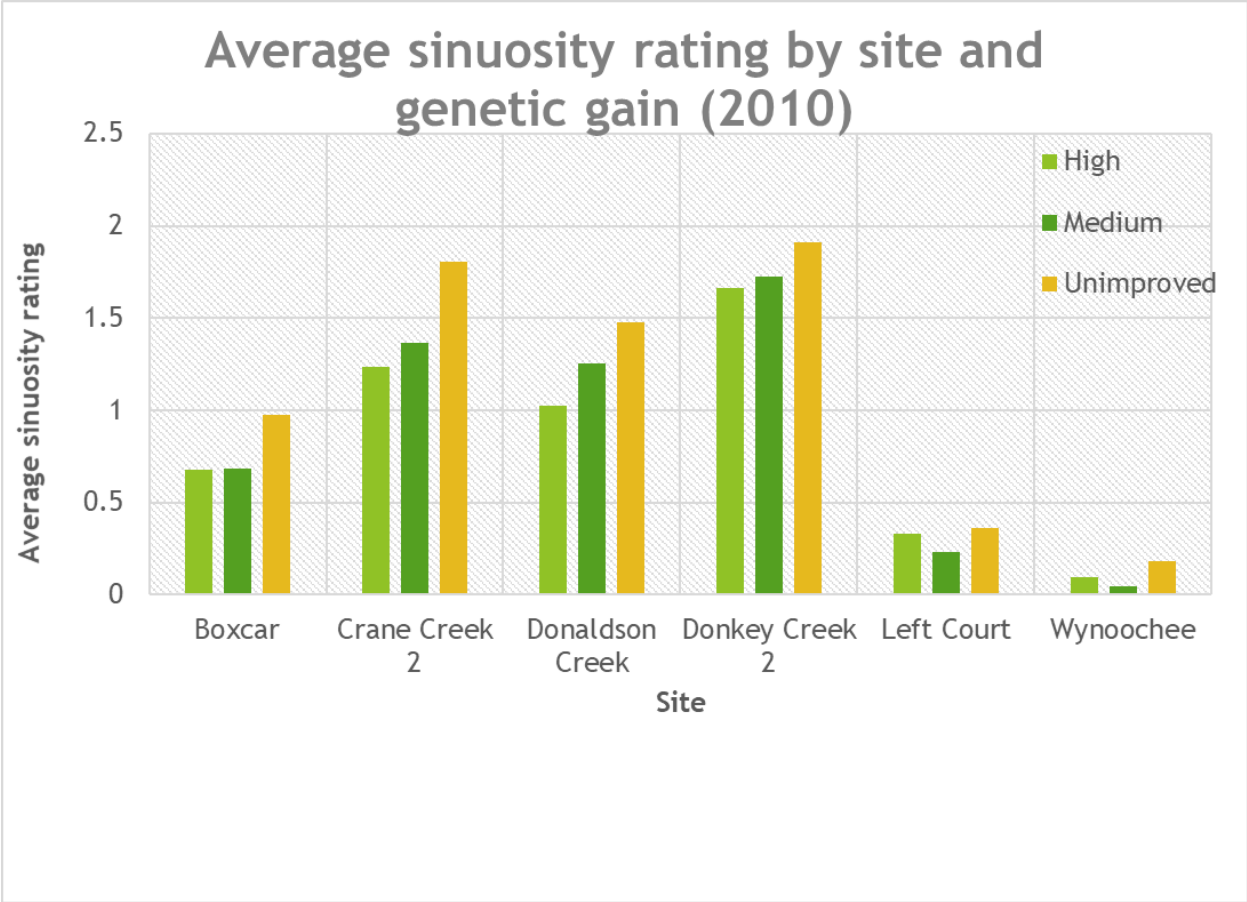


Figure 18: Sinuosity scores, recorded in 2010, across different sites and genetic gain levels.

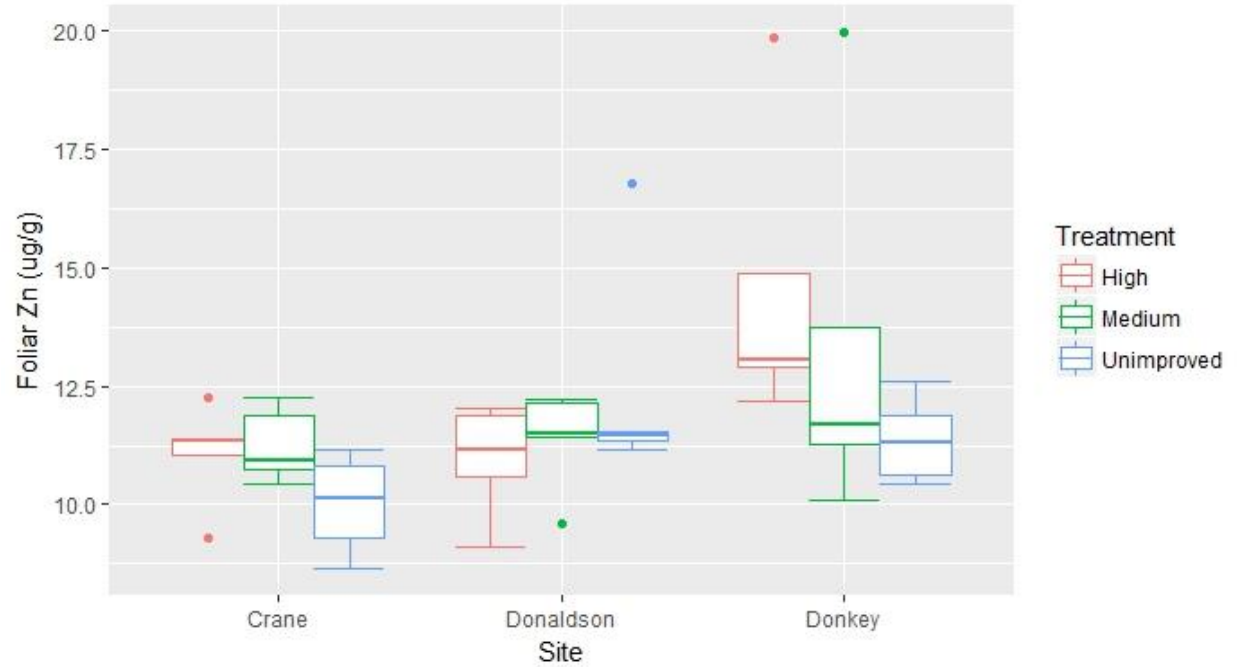


Figure 19: Foliar zinc across different sites and genetic gain levels.