

Nitrate in Forest Soils Following Biosolids Applications in a Working Plantation

Emma L. Leonard

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Committee:

Sally L. Brown

David Butman

Ben Axt

Program Authorized to Offer Degree

School of Environmental & Forest Sciences

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Emma L. Leonard

University of Washington

**Abstract**

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Emma L. Leonard

Chair of the Supervisory Committee:

Sally L. Brown

School of Environmental and Forest Sciences

Biosolids applications to commercial tree plantations is standard practice in the Pacific Northwest. It has shown to increase stand growth, improve the quality of forest soils, soil and above ground carbon sequestration capacity, and plantation economic returns. However, biosolids applications are currently limited to minimize the potential for nitrate leaching to groundwater. Plantation managers reduce the risk of nitrate leaching by determining site-specific application rates that aim to match the nitrogen demand of the forest with that of available mineral nitrogen after additions and losses (e.g. mineralization, volatilization, and denitrification). This study examines a dataset collected through a 30 year-long biosolids monitoring program in a Douglas-fir plantation located in King County, Washington, to identify

risks to groundwater that successive applications and variable rates of biosolids may pose. Soil nitrate, soil total carbon and total nitrogen were examined relative to biosolids application rate, application number, as well as a range of environmental variables including soil series, precipitation and temperature. The results of this study show little evidence that biosolids applications pose a risk to groundwater quality at the rates and frequencies used during the study period. While nitrate concentration in the A horizon was found to increase as the number of applications increased, nitrate in the A horizon had no linear relationship to the application rate. Additionally, there was no evidence of downward movement of nitrate; nitrate in the B horizon did not have any linear correlation with application number or rate. Additionally, the results of this study demonstrate that the nitrate concentration in the A and B horizons showed little change in concentration incrementally ( $\delta$ , i.e. between applications), or over the course of the application period studied ( $\Delta$ , i.e.  $t_0$  to  $t_n$ , where  $n$  is the total number of applications a given unit received during the study period for a given unit). Collectively, these results suggest that the nitrate being added to plantation soils in biosolids applications is largely being taken up by the forest system, rather than being lost to leaching.

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## DEDICATION

To Sally – About a month after finishing my undergraduate degree in Connecticut, I was sitting in your office in Seattle having poured over her work, convinced you were graduate advisor for me. You told me to come back in a few years once I had “lived” a little more. I should have known then that having you an academic advisor would mean having an invaluable mentor and champion in life as well. Thank you.

To Nate – You have been my partner in all things, and this has been no exception. It’s been a weird a windy road at times but I wouldn’t want it any other way. I can’t wait to see where we get to next.

# TABLE OF CONTENTS

<b>ABSTRACT</b> .....	<b>3</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>5</b>
<b>INDEX OF TABLES</b> .....	<b>10</b>
<b>INDEX OF FIGURES</b> .....	<b>13</b>
<b>INDEX OF MAPS</b> .....	<b>17</b>
<b>I. LITERATURE REVIEW</b> .....	<b>18</b>
A. INTRODUCTION .....	18
B. BIOSOLIDS: PRODUCTION, REGULATION, & USE.....	19
C. BENEFITS AND CONCERNS OF LAND-APPLIED BIOSOLIDS .....	23
Existing and Emerging Issues Concerning Land-Applied Biosolids.....	24
D. BIOSOLIDS IN FORESTRY .....	26
Carbon Storage .....	27
Soil Nitrogen Enrichment & Other Benefits of Applied Biosolids in Forestry.....	29
E. MANAGING NITROGEN IN FORESTRY .....	32
Biosolids Nitrogen-Dynamics .....	33
Factors Affecting Nitrogen Dynamics in Biosolids-Amended Forests .....	34
F. DETERMINATION OF BIOSOLIDS APPLICATION RATES .....	36
Environmental Variables Affecting Biosolids in Forestry .....	38
G. STUDY OBJECTIVES & HYPOTHESIS STATEMENT .....	40
<b>II. METHODS</b> .....	<b>43</b>
A. THE KING COUNTY BIOSOLIDS PROGRAM.....	43
Study Site Description & History .....	47
Unit Delineation.....	47
Plantation Tree Species & Understory .....	49

B.	BIOSOLIDS PROGRAM & PLANTATION MANAGEMENT .....	50
	Application Timing.....	50
	Determination of Biosolids Application Rates .....	50
	Unit Site Assessments .....	54
	Soil Sampling & Analysis .....	54
	Dataset Consolidation.....	55
C.	DATA MANAGEMENT & ANALYSIS.....	58
	Statistical Analysis.....	60
	Description and Nomenclature of Variables.....	61
<b>III.</b>	<b>RESULTS.....</b>	<b>65</b>
A.	A HORIZON.....	68
	Nitrate.....	68
	Total Carbon .....	85
	Total Nitrogen.....	89
	C/N.....	94
B.	B HORIZON .....	97
	Nitrate.....	97
	Total Carbon .....	103
	Total Nitrogen.....	106
<b>IV.</b>	<b>CONCLUSIONS .....</b>	<b>110</b>
	<b>REFERENCES .....</b>	<b>113</b>
<b>VI.</b>	<b>APPENDIX .....</b>	<b>127</b>
A.	FIGURES .....	127
	i. Bar Charts.....	127
	ii. Scatter Plots.....	131
B.	TABLES .....	137
	i. Biosolids Composition.....	137
	ii. Soil Series Descriptions .....	138

iii.	ANOVA Analysis Results.....	139
iv.	Summary Table of Means.....	142

## INDEX OF TABLES

**Table 1:** Summary of chemical characteristics of King County WTD biosolids from all three WWTPs that supply biosolids for the forest applications examined in this study. All three plants supply Class B biosolids that have been applied on the plantation examined in this study. Concentrations are reported on a dry weight basis.

**Table 2:** Summary of records included in the study and classified by the number of applications received on a given unit.

**Table 3:** Nitrate table of means comparing treated and untreated soils in the A and B horizon. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was also significant ( $p < 0.05$ ).

**Table 4:** Table of means reporting nitrate\_A and nitrate\_B concentrations following successive biosolids applications. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was not significant.

**Table 5:** Table of means reporting the total change in nitrate ( $\Delta$  nitrate\_A and  $\Delta$  nitrate\_B) concentration at with the total number of applications for a given unit at the completion of the study period. ANOVA of  $\Delta$  nitrate\_A and  $\Delta$  nitrate\_B were not significant.

**Table 6:** Table of means reporting nitrate (nitrate\_A and nitrate\_B) concentration following a biosolids application, at a range of application rates. ANOVA of nitrate\_A and nitrate\_B were both significant ( $p < 0.001$  and  $p < 0.05$ , respectively).

**Table 7:** Table of means reporting nitrate\_A and nitrate\_B concentrations for each soil series observed in the study. ANOVA of nitrate\_A and nitrate\_B were not significant.

**Table 8:** Table of means reporting nitrate\_A and nitrate\_B concentrations for each calendar month soil samples were collected. ANOVA of nitrate\_A and nitrate\_B were not significant.

**Table 9:** Total carbon table of means comparing treated and untreated soils in the A and B horizon. ANOVA of Total Carbon\_A and Total Carbon\_B were not significant.

**Table 10:** Table of means reporting Total C\_A and Total C\_B concentration following successive biosolids applications. ANOVA of Total C\_A and Total C\_B were not significant.

**Table 11:** Table of means reporting the total change in Total C ( $\Delta$  Total C\_A and  $\Delta$  Total C\_B) concentration at the completion of the study period. The total change was calculated as the final carbon concentration minus the initial carbon concentration prior to biosolids application. ANOVA of  $\Delta$  Total C\_A and  $\Delta$  Total C\_B were not significant.

**Table 12:** Total nitrogen table of means comparing treated and untreated soils in the A and B horizon. ANOVA of Total N\_A was trending towards significance ( $p < 0.053$ ). ANOVA of Total N\_B was not significant.

**Table 13:** Table of means reporting Total N\_A and Total N\_B concentration following successive biosolids applications. ANOVA of Total N\_A and Total N\_B were not significant.

**Table 14:** Table of means reporting the total change in Total N ( $\Delta$  Total N\_A and  $\Delta$  Total N\_B) concentration at the completion of the study period. ANOVA of  $\Delta$  Total N\_A and  $\Delta$  Total N\_B were not significant.

**Table 15:** C/N table of means comparing treated and untreated soils in the A and B horizon. ANOVA of C/N\_A and C/N\_B were not significant.

**Table 16:** Table of means reporting C/N\_A and C/N\_B concentration following successive biosolids applications. ANOVA of C/N\_A C/N\_B was not significant.

## INDEX OF FIGURES

**Figure 1:** Biosolids disposal and end uses (Beecher et al., 2022).

**Figure 2:** King County Wastewater Treatment Division summary of the number of dry tons of land-applied biosolids diverted annually to forestry end-uses.

**Figure 3:** Sample worksheet developed by University of Washington that is used to determine the recommended application rate for a given unit. In this case, the unit number is 24-08-06. Soil samples are taken and results for each horizon are reported in the upper left corner of the worksheet. Notes from the ecological assessment are reported in the bottom left corner of the worksheet. The right side of the worksheet requires a number of numeric inputs in order to calculate a recommended application rate. These include an estimate for the nitrogen requirements of the crop and how much will be lost after application as determined by the project manager/project scientist, the pool of nitrogen already available in the soil based on the soil sampling results, and the amount of nitrogen supplied by the biosolids themselves based on the quality reports from the WWTP.

**Figure 4:** Nitrate, Total C and Total N concentrations in treated and untreated soils. Error bars show standard error. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was also significant ( $p < 0.05$ ). ANOVA of Total Carbon\_A and Total Carbon\_B were not significant. ANOVA of Total N\_A was trending towards significance ( $p < 0.053$ ). ANOVA of Total N\_B was not significant.

**Figure 5:** Soil nitrate concentrations for “(A)” and “(B)” soil horizons following successive biosolids applications. Error bars show standard error. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was not significant.

**Figure 6:** Scatter plots of nitrate\_A (a),  $\delta$  nitrate\_A (b) and  $\Delta$  nitrate\_A (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of nitrate\_A and  $\delta$  nitrate\_A were significant ( $p < 0.001$  and  $0.01$ , respectively). ANOVA of  $\Delta$  nitrate\_A was not significant.

**Figure 7:** Bar graph reporting nitrate\_A and nitrate\_B concentrations  $\pm$  SE for each soil series observed in the study. ANOVA of nitrate\_A and nitrate\_B were not significant.

**Figure 8:** Scatter plot of the mean nitrate\_A concentration of each observed soil series against the soil organic carbon (SOC) stock (kg/m<sup>2</sup>) (a) and soil organic matter (SOM)(%) (b) concentrations corresponding to the NRCS Soil Survey series metrics with linear regression in blue and R<sup>2</sup> correlation coefficient.

**Figure 9:** Bar graph of mean nitrate\_A and nitrate\_B concentration  $\pm$  SE for each calendar month soil samples were collected. ANOVA of nitrate\_A and nitrate\_B were not significant.

**Figure 10:** Scatter plot of the nitrate\_A concentration relative to the 20-year average temperature (a) and precipitation (b) of the month in which the soil sample was collected, with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of temperature and precipitation in the A horizon was significant ( $p > 0.01$  and  $p > 0.05$ , respectively).

**Figure 11:** Scatter plot of nitrate\_A concentration (a) and  $\delta$  nitrate\_A concentration (b) relative to the depth at which each soil sample was taken, with linear regression in blue and R2 correlation coefficient. ANOVA of both nitrate\_A and  $\delta$  nitrate\_A were not significant.

**Figure 12:** Scatter plots of total C\_A (a),  $\delta$  total C\_A (b) and  $\Delta$  total C\_A (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R2 correlation coefficient. ANOVA of total C\_A and  $\delta$  total C\_A and  $\Delta$  total C\_A were not significant.

**Figure 13:** Scatter plots of total nitrogen\_A (a),  $\delta$  total nitrogen\_A (b) and  $\Delta$  total nitrogen\_A (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R2 correlation coefficient. ANOVA of total N\_A and  $\delta$  total N\_A and  $\Delta$  total N\_A were not significant.

**Figure 14:** C/N for “(A)” and “(B)” soil horizons following successive biosolids applications. Error bars show standard error.

**Figure 15:** Scatter plots of C/N\_A (a) and C/N\_b (b) relative to application rate with linear regression in blue and R2 correlation coefficient. ANOVA of total C/N\_A was not significant. ANOVA of C/N\_B was significant ( $p < 0.05$ ).

**Figure 16:** Scatter plots of nitrate\_B (a),  $\delta$  nitrate\_B (b) and  $\Delta$  nitrate\_B (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R2 correlation coefficient. ANOVA of nitrate\_B and  $\delta$  nitrate\_B were found to be significant ( $p < 0.05$  and  $p < 0.01$ , respectively). ANOVA of  $\Delta$  nitrate\_B was not significant.

**Figure 17:** Scatter plot of the mean nitrate<sub>B</sub> concentration of each observed soil series against the soil organic carbon (SOC) stock (kg/m<sup>2</sup>) (a) and soil organic matter (SOM)(%) (b) concentrations corresponding to the NRCS Soil Survey series metrics with linear regression in blue and R<sup>2</sup> correlation coefficient.

**Figure 18:** Scatter plot of the nitrate<sub>B</sub> concentration relative to the 20-year average temperature (a) and precipitation (b) of the month in which the soil sample was collected, with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of temperature was trending towards significance ( $p < 0.061$ ). ANOVA of precipitation was not significant.

**Figure 19:** Scatter plots of total carbon<sub>B</sub> (a),  $\delta$  total carbon<sub>B</sub> (b) and  $\Delta$  total carbon<sub>B</sub> (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of total carbon<sub>B</sub>,  $\delta$  total carbon<sub>B</sub>, and  $\Delta$  total carbon<sub>B</sub> was not significant.

**Figure 20:** Scatter plots of total nitrogen<sub>B</sub> (a),  $\delta$  total nitrogen<sub>B</sub> (b) and  $\Delta$  total nitrogen<sub>B</sub> (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of total nitrogen<sub>B</sub>,  $\delta$  total nitrogen<sub>B</sub>, and  $\Delta$  total nitrogen<sub>B</sub> was not significant.

# INDEX OF MAPS

**Map 1:** Location map of the study site and plantation units.

**Map 2:** Satellite imagery of study site and subsection of underlying soil series established by the U.S. Soil Survey.

**Map 3:** Annual record of biosolids application within the plantation. Dataset includes units prior to the join by spatial extent.

**Map 4:** Final selection of units following GIS analysis and record of the grand total number of biosolids applications each unit received at the conclusion of the study.

# I. LITERATURE REVIEW

## A. INTRODUCTION

The land application of biosolids provides an alternative to disposal that utilizes the organic matter and plant nutrients in wastewater residuals. Nitrogen is essential for plant growth and typically deficient in most soils. Use of biosolids as fertilizer requires careful consideration, not only for the nutrient needs of the target crop, but also given the environmental risks excess nitrogen poses to surface water and groundwater. In 1993, the State of Washington published some of the earliest guidelines for the management of biosolids worldwide, which established protocols for the appropriate determination of application rates for land-applied biosolids (Sullivan et al., 1993). Since the publication of these foundational guidelines, researchers' and practitioners' understanding of nitrogen dynamics in biosolids-amended soils continues to improve. Working landscapes where biosolids have been applied successively over a long period of time provide the best opportunity to better understand the benefits and risks posed by the application of biosolids. However, as the use of land-applied biosolids is a relatively new practice, these long-term application sites are rare. Sites with continuous soil monitoring data, such as the site examined in this study, are even rarer. This study analyzes data collected through a long-term biosolids monitoring program in a Douglas-fir plantation located in King County, Washington, to identify risks to groundwater that successive applications and variable rates of biosolids may pose. In doing so, it contributes to our understanding of the environmental variables that influence biosolids-derived nitrogen dynamics in the plant-soil system.

## B. BIOSOLIDS: PRODUCTION, REGULATION, & USE

Biosolids are a stabilized sewage sludge, one of several residual materials produced by wastewater treatment plants. Once processed (e.g. digested; chemically conditioned with lime, polymers, and other products; or thermally conditioned), biosolids have many subsequent uses as a solid, dewatered, or liquid product (U.S. EPA, 1994).

Wastewater treatment plants (WWTPs) use a combination of physical, biological, and chemical processes to separate the liquid from the solid fraction. The remaining dissolved organic matter in the liquid fraction is converted into bacterial biomass and removed, thus reducing the biological oxygen demand of the effluent. Finally, a tertiary step may be employed to remove nutrients, such as nitrogen and phosphorous, from the effluent to mitigate eutrophication following the discharge of the effluent into the environment (e.g. via streams, rivers, wetlands, irrigation, groundwater injection).

Biosolids are the organic product that remains following the treatment of the solid fraction of wastewater. This treatment reduces volatile solids and targets the removal of pathogens in order to satisfy regulatory requirements for the land application of biosolids. The standards to which these solids are treated vary from country to country. In the United States, these standards are enumerated within several regulatory frameworks:

- (1) The U.S. Clean Water Act provides the legal foundation for the use, management, and disposal of biosolids nationwide.

- (2) The U.S. Environmental Protection Agency's Title 40 Code of Federal Regulation (40 CFR) Part 503 Biosolids Rule (also known as the "503 Rule") establishes the treatment standards of biosolids. The 503 Rule sets biosolids quality standards for land application based on (1) pathogen and vector reduction and (2) metal loading and concentration limits. It also provides guidance for application rates based on the nitrogen needs of the receiving crop.
- (3) Biosolids production standards and end uses can also be regulated to a higher degree through municipal, state, and local policies regulating WWTP operations, as well as environmental health and safety.

The U.S. EPA's 503 Rule also specifies a hierarchy of treatment classes for biosolids used in land application. These classifications set limits to the density of pathogens that can remain in the final biosolids product after treatment. The class of biosolids also determines the types of land applications for which the product is suitable. "Class A" biosolids require that pathogens (e.g. *Salmonella* sp., bacteria, enteric viruses, and helminth ova) are below detectable limits. These materials are typically thermally treated, composted, or aerobically digested to achieve adequate pathogen reductions. "Class B" biosolids only require pathogen levels to be monitored and reduced to levels that do not pose a risk to human health, and that site access be limited immediately after application (including crop harvesting, animal grazing, and public access) (U.S. EPA, 1994).

Approximately seven million tons of biosolids are produced in the U.S. every year, and  $1 \times 10^8$  tons worldwide (Thangarajan et al., 2013). The successful management of this large volume of material has led to a growing pressure on WWTPs to develop a range of end uses for biosolids. By conducting inventories of the resources consumed and emissions produced by wastewater

treatments plants, Life Cycle Assessments (LCA's) have proposed a 'fit-for-purpose' water strategy (Nair et al., 2014; Short, 2015). This proposed framework aims to better match water treatment methods and intensity with the effluent water quality requirements of a given region, while simultaneously developing more opportunities for the recovery and reuse of wastewater residuals (Alvarez-Gaitan et al., 2016; Peccia & Westerhoff, 2015). End uses included in these Life Cycle Assessments typically include combustion or anaerobic digestion for energy production, cement production following incineration, and land application as a substitute for synthetic fertilizers (Brown et al., 2011).

Once treated to either Class A or Class B standards, biosolids have many beneficial end uses in soil conditioning and remediation (Brown et al., 2003; Gikas, 2016; Hasan et al., 2021; Mata-Álvarez et al., 2000; Smith, 1996). As such, governments have increasingly favored these end uses, and begun to phase out the landfilling and incineration of wastewater residuals. According to the most recent national biosolids survey, approximately 55% of biosolids produced in the U.S. were applied to soils for agronomic, silvicultural, and/or land restoration purposes, and the remaining fraction disposed of in municipal solid waste landfills, surface disposal units, and/or incineration facilities (Beecher et al., 2022) (Figure 1). As land-applied biosolids are more widely used, our understanding of their properties as a soil amendment grows. Through a growing body of literature, researchers have determined a range of benefits and several mitigated risks associated with the land use application of biosolids. These risks and benefits are discussed in the following section.

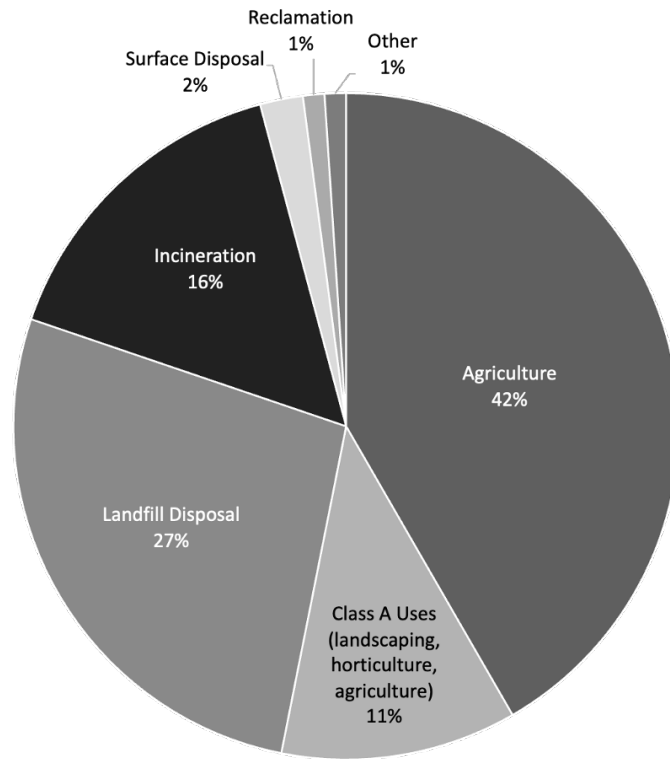


Figure 1: Biosolids disposal and end uses (Beecher et al., 2022).

## C. BENEFITS AND CONCERNS OF LAND-APPLIED BIOSOLIDS

The land application of biosolids provides many of the micro- and macro-nutrients required for plant and crop growth. For this reason, biosolids have been used as a soil conditioner to improve the biological, physical and chemical properties of soils.

**Soil Structure:** In a 4-year trial investigating soil structure following biosolids amendments in a sandy loamy silt soil, aggregate size and stability increased with increasing biosolids amendments (Krause, 1985). Porosity, moisture retention, and aggregate size all increased in the upper 0-15 cm horizon of soils with a decrease in bulk density following biosolids applications, with the effects persisting for at least 4 years. Additionally, results indicated that 80% of the observed increase in water-holding capacity could be attributable to the observed changes in soil texture and increased soil organic C. Reductions in erosion and sedimentation have also been observed following biosolids amendments (Gardner et al., 2010), as well as improved soil porosity, increased aggregate stability, and higher water-holding capacity (Jones et al., 2010, 2012)

**Soil Chemistry:** The clay particles, minerals and organic colloids in applied biosolids have been shown to increase the CEC of degraded soils (Bendfeldt et al., 2001; Gardner et al., 2010), as well as organic matter (OM), and thus soil fertility (Castillego & Castello, 2010; Larney & Angers, 2012). In a 10-year study conducted in sandy agricultural soils where OM was typically less than 1%, the addition of biosolids increased OM content by 200%, and CEC was 2.5 times that of non-amended soils following biosolids applications ranging between 38 and 47 Mg/ha

(Ozores-Hampton et al., 2011). Land-applied biosolids also provide a source of many plant nutrients including N, P, S, Mg, Ca, Cu, Zn, and B (Chambers et al., 2003; Christie et al., 2001) and have widely established use as an effective fertilizer and soil conditioner.

**Soil Ecology:** A forest-based study of the ecological effects of biosolids used soil enzyme activity (primarily soil sulphatase and phosphatase) to evaluate functional diversity and ecosystem health. While the long-term use of inorganic fertilizers has been shown to inhibit the activity of many enzymes (Davies et al., 2022), the soil enzyme health of long-term biosolids-amended soils were unaffected and statistically similar to non-amended forest soils (Xue et al., 2015). Biosolids have also been shown to benefit soil microbial species quantity and diversity. More specifically, research has demonstrated increases in beneficial groups such as actinomycetes, pseudomonads and heterotrophic aerobes (Ozores-Hampton et al., 2011).

## EXISTING AND EMERGING ISSUES CONCERNING LAND-APPLIED BIOSOLIDS

While the benefits of applying biosolids to land clearly has numerous benefits, it is important to note there are several limiting factors to its widespread use. A great deal of research has been conducted with the aim of better understanding the risks associated with biosolids land application (Smith, 1996). Limiting pathogens, nitrogen and phosphorus are of primary concern in the land application of biosolids, as well as in the regulation of class A and class B biosolids.

**Nutrients:** The risk of nutrient leaching and runoff (especially nitrogen) from biosolids-applied soils into surface and groundwater has been well documented and is central to this study (Brockway & Urie, 1983; Edmonds & Cole, 1976, 1977, 1980; Henry, C.L. & Cole, 1983;

Riekerk & Zasoski, 1979). It has been established that the risk of nutrient movement is sufficiently minimized when biosolids application rates do not exceed the assimilative capacity of the soil and target crop (Henry, 2000). However, this assimilative capacity is often difficult to ascertain without extensive site-specific data, which is often lacking.

Pathogens, Metals, VOCs, and PPCPs: In addition to nutrient leaching, the pathogens in biosolids can pose a risk to humans. However, while treatment and monitoring remain critical in managing pathogen densities, there is generally little evidence of the pathogen densities in treated biosolids posing a risk to groundwater or humans (Gottschall et al., 2009; Rusin et al., 2003). There is some risk of buildup of trace and heavy metals in soil with higher land application rates of biosolids, but metals in biosolids-amended soils have been shown to have limited phytoavailability (Kukier et al., 2010). Odor emissions are another potential risk of biosolids land applications, but are fairly negligible in plantation settings. Biosolids release a range of volatile organic compounds (VOCs) and ammonia, which in high concentrations can be toxic to humans (Wijesekara et al., 2016). However, limiting site accessibility immediately following applications significantly limits this risk, as does using nitrogen-based application rates. Finally, pharmaceutical and personal care products (PPCPs) can be found at measurable concentrations in biosolids, but generally degrade over time (Clarke & Smith, 2011; Fu et al., 2016).

## D. BIOSOLIDS IN FORESTRY

When compared to agriculture or many other potential biosolids end-uses, forests and forest soils are extremely well suited to biosolids application. Forests exhibit rapid soil drainage and a high input of organic matter, allowing soluble nutrients to effectively reach the tree root zones.

Forests also have the added benefit of growing perennially, allowing biosolids to be applied year-round. Forests on steep slopes with shallow, rocky, or highly acidic soils can pose a difficulty for biosolids application. However, these difficulties can be ameliorated with careful site selection and monitoring.

The use of biosolids in forestry is typically prioritized over that of agriculture due to its proven effectiveness in increasing plantation economic returns (Briggs et al., 2000; Kimberley et al., 2004; Wang et al., 2013) and productivity of both hardwoods (Cogliastro et al., 2001) and conifers (Henry et al., 1994; Prescott & Blevins, 2005; Wang et al., 2006) while minimizing risk or perception of risk to the human food supply (Magesan & Wang, 2003). Many of the studies that evaluate the impacts of biosolids on forests have focused on short-term (1–5 year) application periods, and several have investigated the effects of applications spanning more than 10 years (Henry et al., 1994; Leonard et al., 2021; Prescott & Blevins, 2005; Xue et al., 2015). These studies are crucial for providing insight into how biosolids influence forests on the time scale of complete crop rotations.

## CARBON STORAGE

The Intergovernmental Panel on Climate Change has proposed that soil carbon sequestration is one of a handful of climate change mitigation strategies that could be implemented at large scales and at low cost (Ciais, P. et al., 2013). Forests are currently the largest terrestrial carbon sink on the planet. At the same time, they present a significant opportunity for increasing carbon sequestration and forest stocks (Pan et al., 2011). The potential for carbon fixation by forests is made all the more significant when taking into account the sequestration of carbon in forest soils. Through the use of tools like the BEAM model (CCME, 2009), land application of biosolids by municipal wastewater treatment plants has been determined to be a viable and carbon-negative alternative to disposal (Brown et al., 2010; Villeneuve, 2011). While many studies have shown increased carbon storage in agriculture and land reclamation sites utilizing biosolids (Bolan et al., 2013; Brown et al., 2011; Chambers et al., 2003; Torri et al., 2014; Wijesekara et al., 2017), the use of biosolids in forestry offers an even greater potential for carbon storage.

In a 25-year-long forestry study conducted by Xue et al. (2022), a C-Change model was used to estimate carbon storage in the above- and below-ground biomass of a *Pinus radiata* forest. While the model did not account for soil carbon storage (only the carbon stored in the stands' biomass and litter layer), following biosolids applications of 18 to 36 Mg/ha every 3 years, researchers observed an 18% increase in C storage (an additional 40 t C/ha) relative to untreated control stands (Xue et al., 2022). A study conducted in a Washington Douglas-fir (*Pseudotsuga menziesii*) plantation took a different approach to determining the effects of biosolids on forest carbon storage. Soil carbon storage was found to be greater in biosolids -amended soils relative to untreated soils ( $172 \pm 39$  Mg C/ha and  $112 \pm 44$  Mg C/ha, respectively) following four

applications over a 12 year period, totaling 42 Mg/ha of applied biosolids. This study conducted a carbon balance in which the carbon associated with biosolids treatment and transportation and other emissions were compared to the carbon credits from soil and biomass carbon storage. For one of the soil series studied, researchers found a credit of 5.15 Mg CO<sub>2</sub> per Mg of biosolids applied. The other soil series, higher in organic matter, did not show any change in soil carbon storage following biosolids applications (Leonard et al., 2021).

Added soil C storage often corresponds to an increase in biomass and enhanced tree growth. A number of studies have shown significant increases in stand volume, as well as economic value, following biosolids applications. Harrison et al. (2002) measured increases in DBH (14.7%), height (2.7%), per-stem basal area (27%), per-stem average volume (33%), and per-stem dry weight (38%) in a 6-year-old Douglas-fir (*Pseudotsuga menziesii*) plantation after only a single application of biosolids, relative to controls. In a study spanning 16 to 19 years, Ouimet et al. (2015) found that radial tree growth increased 18% for red pine (*Pinus resinosa*), 62% in white spruce (*Picea glauca*), and up to 700% in oak (*Quercus* sp.) after a single biosolids application at a range of rates (Ouimet et al., 2015). A study conducted in a radiata pine (*Pinus radiata*) plantation New Zealand found that after two applications of biosolids to a 6-year-old stand, significant growth responses by DBH and height (and corresponding basal area and volume) were observed. Researchers found that stands gained 0.74 years of volume growth compared to control stands at the standard application rate of 300 kg N/ha (Kimberley et al., 2004; Wang et al., 2013). In the same study, the stand receiving the high application rate of 600 kg N/ha gained 1.10 years of growth over control trees. Despite increases in the diameter of large branches (a factor that would potentially decrease the value of the lumber produced by the stand), the study concluded that the increase in volume growth would correspond to an estimated 14% increase in

revenue at the standard biosolids application rate (300 kg N/ha) and a 24% revenue increase in stands grown at the high application rate (600 kg N/ha) (Kimberley et al., 2004; Wang et al., 2013). Collectively these results indicate that biosolids present a useful tool in increasing soil carbon sequestration in forest soils, and simultaneously presents an added economic benefit of increased timber value.

## SOIL NITROGEN ENRICHMENT & OTHER BENEFITS OF APPLIED BIOSOLIDS IN FORESTRY

The global agricultural demand for synthetic nitrogen fertilizers has seen a dramatic rise from 11.6 Tg in 1961 to 104 Tg in 2006 (FAO, 2009). After 40 to 50 years of synthetic N fertilization in Marrow Plots, America's oldest field experiment, fertilization was recently found to substantially exceed grain N removal. Synthetic fertilizer N depletes soil organic matter by promoting microbial C utilization and N mineralization. This finding contradicts the principle that synthetic nitrogen fertilization builds soil OM—a principle that has been the basis for agricultural practices globally (Mulvaney et al., 2009). It is therefore necessary that the use of synthetic N fertilizers be called into question, and researchers instead consider the benefits of alternative practices that can sustain agriculture globally.

While typically less nutrient-dense than inorganic fertilizers, biosolids offer a rich source of nitrogen—typically 2% to 9%—depending on the wastewater source and treatments utilized by the WWTP. Because forest soils are typically N-deficient, plantations are fertilized in order to increase growth (Heilman, 1981). A minimum of 80% of the nitrogen in biosolids is organic,

requiring decomposition and mineralization before it can be assimilated by the forest stand. However, much of the remaining nitrogen is soluble  $\text{NH}_4$ . As such, biosolids provide both a short-term and readily available form of inorganic nitrogen, as well as a slow-release form of nitrogen (as organic nitrogen forms are broken down in the long-term). As a microbially mediated process, the assimilation of nitrogen into the forest-soil system is difficult to trace, and the direct effects of biosolids-derived nitrogen on plantations are difficult to quantify. Microbial biomass N (MBN) has been used successfully in agricultural studies of biosolids applications as an estimate of mineralized-N pools. These studies have shown that MBN increased with increasing rates of biosolids applications (Villa & Ryals, 2021).

Soil total N and foliar N has also been used as a metric for biosolids-N uptake. In a 13-year plantation study conducted in New Zealand, biosolids were applied in a 16-year-old stand every 3 years at standard and high application rates (15 Mg/ha and 30 Mg/ha, respectively) (Xue et al., 2015). Foliar N concentrations in control treatments averaged 1.2% N, remaining consistently N-deficient and below the ideal level of 1.5% for healthy tree growth. However, stands where biosolids were applied averaged 1.4% N and 1.5% N by the conclusion of the study at the standard and high application rates, demonstrating the benefits of biosolids fertilization.

Additionally, N concentrations in soils at the 0–25 cm depth had increased relative to control soils at both the high and standard application rate (0.3, 0.5 and 0.6 g/kg total N, respectively). Soils at the 25–50 cm depth increased relative to control soils at both the standard and high application rate (0.2, 0.3 and 0.4 mg/kg, respectively). Statistical analysis revealed that the 0–25 cm soils treated at the standard and high application rates were both statistically higher in total N than the control soils. However, at the 25–50 cm depth, the total N concentration of standard rate soils were similar to the control, while total N was statistically higher in soils that received the

high application rate. Crucially, these results indicate that movement of N into the lower soil horizons can be effectively mitigated through the appropriate determination of application rates, while still increasing total N availability in the upper soil horizons and benefiting tree growth (Xue et al., 2015).

Finally, Wang et al. (2004) used  $^{15}\text{N}$  isotopes to trace the fate of biosolids-N in a *Pinus radiata* plantation, allowing the researchers to estimate the storage and loss of N in biomass, soils and groundwater. This study found that 50% (451 t N) of the total biosolids-N was stored in the forest: soil (263 t N), tree foliage (28 t N), wood (91 t N), roots (50 t N), understory vegetation (14 t N), and groundwater (4.8 t N) (Wang et al., 2004, 2005). These results make it clear that forests have a high capacity for assimilating biosolids-derived nitrogen. However, the rates of application are constrained by the high mobility of N and the risk of ground- and surface-water contamination.

## E. MANAGING NITROGEN IN FORESTRY

Despite the many benefits of biosolids applications in forests, careful consideration should be given to avoid exceeding a forest's ability to assimilate nitrogen from the added biosolids. It has been thoroughly established that failure to do so can result in nitrification and nitrate leaching (Brockway & Urie, 1983; Riekerk & Zasoski, 1979; Sidle & Kardos, 1979). If nitrate leaching is severe enough, groundwater and nearby water bodies may be put at risk of eutrophication. In an early study conducted in University of Washington's Pack Forest, researchers demonstrated that application rates greater than 47 Mg/ha resulted in significant nitrate leaching, and that younger stands are less susceptible to leaching than recently cleared sites and mature stands. Most of the nitrate leaching occurred during the first year, decreased significantly in the second year, and returned to below U.S. EPA drinking water standards (< 10 mg/L) in the third year (Edmonds & Cole, 1976, 1977, 1980; Henry, C.L. & Cole, 1983). Excessive nitrate has other implications in forest management. Harrison et al. (1994) found that following excessive biosolids applications at a Christmas tree farm, nitrate leaching was associated with the simultaneous leaching of Mg and other base cations, resulting in decreased soil pH and available plant nutrients (Harrison et al., 1994). These foundational studies demonstrated the value of biosolids in forestry as well as the importance of appropriate nitrate management, and in doing so were the basis for the development of the best management practices used in the PNW today (U.S. EPA, 1995; Washington Department of Ecology, 1982).

## BIOSOLIDS NITROGEN-DYNAMICS

It is clear from the results of these plantation studies that sustainable application rates for forests must be determined by first establishing the fate of biosolids following their application, then identifying which elements are retained in the forest system, and where; how and why losses occur; and whether the magnitude of this loss is environmentally acceptable as a source of pollution.

Nitrogen in biosolids is present in both inorganic ( $\text{NH}_4$ ) and organic pools. While the  $\text{NH}_4$  is immediately available to plants, organic-N must be mineralized before plants can utilize it. While not all of the organic-N pool is readily mineralized, the mineralizable fraction is transformed through the microbially mediated breakdown of organic matter and, when the N-requirements of the microbial biomass are exceeded, subsequent release of  $\text{NH}_4$ .  $\text{NH}_4$  may be assimilated by plants directly or oxidized by nitrifying bacteria to produce plant-available  $\text{NO}_3^-$ . The availability of plant-available N (PAN) in biosolids for the target crop is determined by the availability and concentration of these nutrient pools.

$$\text{PAN} = \text{NH}_4\text{-N} + (\text{NO}_3\text{-N} + \text{NO}_2\text{-N}) + \text{N}_m$$

Where  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$  are ammonium-nitrogen, nitrate-nitrogen, and nitrite-nitrogen, respectively, and  $\text{N}_m$  is the mineralizable fraction of organic-N. The N-composition of the biosolids themselves varies widely, changing relative concentrations of organic-N and  $\text{NH}_4\text{-N}$ , as well as the fraction of mineralizable-N, depending on the source material and treatment technologies at the WWTP (Rigby et al., 2016). Additionally, as a microbially mediated process, the rate at which the organic fraction will mineralize is dependent on a number of factors

including application rate, soil type, temperature, moisture content, and the pH of the soil (Rigby et al., 2016).

## FACTORS AFFECTING NITROGEN DYNAMICS IN BIOSOLIDS-AMENDED FORESTS

While nitrogen dynamics in plant-soil systems are highly complex and difficult to predict, there are several basic parameters that guide the safe use of biosolids in forestry.

**Biosolids Composition:** A small fraction of the nitrogen in biosolids is soluble and readily available to plants as ammonium ( $\text{NH}_4$ ). Typically, a minimum of 80% of the nitrogen in biosolids is in the form of organic-N. These organic forms of nitrogen must first be decomposed by soil microbes, releasing mineral-N as ammonium (e.g. mineralization). For example, aerobically treated biosolids decompose more rapidly than anaerobically treated products, and typically contain more  $\text{NH}_4$ -N, which also mineralizes more readily (Garau et al., 1986; Wang et al., 2003). Biosolids treatment processes such as composting and digestion can reduce the amount of readily mineralizable organic-N by as much as 15.6% through ammonia volatilization in order to produce a more stable, slow-release product (Tubail et al., 2008). As with any fertilizer, it is also important to avoid over-fertilization, which could result in potentially harmful losses of N to the environment through ammonia volatilization and denitrification, as well as leaching and runoff.

**Rate of N-mineralization:** The rate of organic-N mineralization is highly dependent on the C/N ratio of the biosolids themselves—the higher the ratio, the slower the rate. Approximately 25% of the organic-N fraction of the anaerobically digested biosolids is mineralized within one year

of its application, with progressively slower rates in the years that follow (Rigby et al., 2016). As much as half of the organic N in some biosolids may remain stable for decades. However, this fraction is highly dependent on treatment methods: it is roughly 10% for composted biosolids and 30% for aerobically digested biosolids (U.S. EPA, 1995). It is for this reason that the rate of mineralization is largely considered the rate-limiting step in the assimilation of biosolids-N by forest plantations.

**Likelihood of N-immobilization:** Another factor often considered is the C/N ratio of the soil. Nutrient-poor and high C/N soils, like the forest soils observed in the Pacific NW, have a higher likelihood of immobilizing this added nitrogen in the biomass of the soil microbial community, rather than the microbial community producing free  $\text{NH}_4$  to be taken up by plants or nitrified (Henry, 2000). In N-limited soils, the N-requirements of the soil's microbial biomass are not exceeded. Excess  $\text{NH}_4$  is therefore not released into the soil or made available to the plantation. In soils with a C/N greater than 30:1, immobilization of added nitrogen is likely, while soils with a C/N below 20:1 are more likely to mineralize added N.

**Likelihood of N-losses by Volatilization and Denitrification:** The fraction of  $\text{NH}_4$ -N in biosolids and the ammonium generated following the mineralization of organic-N that is lost through volatilization depends on a number of conditions, including soil pH, temperature, and wind speed. Volatilization is expected to decrease as the stand matures (i.e. as the growing canopy reduces the exposure of the forest floor to solar radiation and wind). In a 14-week laboratory study conducted by Robinson and Polgase (2000), most losses of N from biosolids took place in the first week following biosolids application, and after 3 weeks 81% of the  $\text{NH}_4$ -N applied had been lost through volatilization. Loss of N by the denitrification of nitrate to  $\text{N}_2$  were minimal, as denitrification largely occurs in warmer, oxygen deficient, water-logged soils. While these

conditions are unlikely to be found in temperate forest soils, biosolids application still requires careful site selection (Robinson & Polglase, 2000). Additionally, higher temperatures tend to increase the rate of volatilization, as does poorly incorporated and large particles of biosolids (Donovan & Logan, 1983).

Plant Requirement of N: The agronomic N-requirements of the plantation are typically estimated based on the existing nitrate concentration of the soil, the size and age of the stand, and the nitrogen demands of the understory. Mature forests and recently cleared land are at high risk of leaching, while young plantations have a high N-requirement and are at low risk of leaching (Henry et al., 1994). Additionally, younger stands must compete with the N-demands of the understory. As the stand matures and canopy closure reduces understory density, more of the biosolids-N applied on a plantation becomes available to the stand. As such, when calculating the application rate for a plantation and determining the amount of plant-available nitrogen required for healthy tree growth, the N-demands of the understory must be taken into account alongside the demands of the stand (Henry et al., 2000).

## F. DETERMINATION OF BIOSOLIDS APPLICATION RATES

While the use of biosolids in forestry has the potential to greatly benefit forest productivity, biosolids managers determine application rates with the aim of balancing the likelihood of the soil leaching any added nitrate with the nitrogen requirements of the plantation. With the nitrogen dynamics of the plant-soil system in mind, there are two methodologies by which biosolids application rates for forestry are determined.

The 'N balance approach' determines application rates by matching the N demand of the forest with that of available mineral nitrogen after additions and losses (e.g. mineralization, volatilization, and denitrification). First, the plant-soil N-demand is determined by combining plant N-demand (by both the target tree species and its understory) and adding or subtracting the N supplied or immobilized by the soil, respectively. Second, the pool of phytoavailable N supplied by the biosolids is determined, taking into account net losses and transformations.

The 'C/N and OM approach' instead aims to amend soils in a way that maintains a balanced C/N and strives to increase soil OM. As stated in above, when soil amendments have a C/N below 20:1 (as is the case with biosolids), N mineralization is probable. However, immobilization becomes likely when amendments have a C/N greater than 30:1. As such, the application rate of N-rich biosolids is buffered by the simultaneous addition of a C-rich amendment (such as sawdust) so as to immobilize excess biosolids-N and minimize N-losses (Cowley, 2000).

The Washington State Department of Ecology (DOE) requires that application rates be made under the guiding principle of having no discernable increase in groundwater nitrate concentration. All applications of biosolids must be pre-approved, recorded, and accord with the site-specific land application plan required by the DOE. The determination of rates must be at 'agronomic rates' and account for outside sources of nitrogen, such as from the forest understory and stored soil nitrogen (Washington State Legislature, 2007). However, accounting for these factors requires that biosolids managers consider a number of site-specific environmental factors that can significantly influence the response of a plantation to biosolids, especially as those environmental factors shape the fate and transport of nitrogen in a forest system.

## ENVIRONMENTAL VARIABLES AFFECTING BIOSOLIDS IN FORESTRY

The fate and transport of biosolids in a forest ecosystem are influenced by any number of factors, including tree species, climate, soil typology, and topography. Meeting these basic principles requires that application rates be determined on a forest-by-forest basis, if not on an even smaller scale, and that nitrogen dynamics above- and below-ground should be continually monitored and assessed in order to better inform the calculus that goes into developing safe and effective biosolids application rates.

**Harvest & Thinning:** Nitrification rates are known to increase in soils immediately following timber harvest. The accumulation of  $\text{NO}_3$  can lead to a temporary but substantial increase in nitrate leaching. However this sudden loss of N can be minimized by maintaining soil OM and restocking the site as quickly as possible (Borman et al., 1968; Gross & Harrison, 2018).

Additionally, the tannins from bark and leaves has been found to inhibit nitrification in soils, indicating that harvest residues should be left on site (Bollen & Lu, 1969).

**Stand Age:** The N-requirements of a stand vary with age. In a Douglas-fir (*Pseudotsuga menziesii*) plantation, N uptake was found to be as high as 110 lb N/ac/yr in young stands, but as low as 25 lb N/ac/yr in mature stands (Dycke et al., 1984). In a study conducted by Henry et al. (2000), nitrate leaching was monitored using plate lysimeters following biosolid applications in Douglas-fir stands of variable ages. It was determined that stand age greatly affected the assimilatory capacity of the forest: the 15-year-old stand had the lowest losses (average 73 kg-N  $\text{ha}^{-1} \text{yr}^{-1}$ ), followed by the 1-year-old stand (average 115 kg-N  $\text{ha}^{-1} \text{yr}^{-1}$ ), and finally the 55-year-old stand leaching the most (average 479 kg-N  $\text{ha}^{-1} \text{yr}^{-1}$ ). It was posited that due to the closed

canopy of the 55-year-old stand, understory, solar radiation, and wind velocities would have been minimized, so that understory N-demand and N-loss to ammonia volatilization and denitrification were reduced. Additionally, the N-demand of the 55-year-old stand may have been highest due to the fact that the canopy size would remain relatively constant, whereas the canopies of the 1-year-old and 15-year-old stands would have been in a state of rapid growth (Henry et al., 2000).

Seasonality: Mineralization and the production of nitrate occur through the microbial oxidation of ammonia. Microbial activity increases in warmer and dryer months. As temperature increases, the activation energy required for oxidation, and the incidence of saturated, poorly oxygenated soil environments is reduced (Barbarika Jr. et al., 1985; Campbell et al., 1994). As such, soil nitrate concentrations determined from soil samples collected in winter will not be inherently representative of year-round nitrate availability. Nitrate leaching is also seasonally dependent. In a U.K. study examining the leaching tendencies of liquid sludges, 50-70% of the nitrogen applied was available in the first year following application, when the sludge was applied in the spring. Only 20-30% of applied N was available when the sludge was applied in winter due to increased winter rainfall (Hall, 1983). Runoff of applied biosolids-N is also more likely during periods when precipitation exceeds the rate of soil infiltration (Pierzynski et al., 2005).

Soil Characteristics: The rate of biosolids mineralization has been observed to be higher in sandy soil textures than that of soils with higher clay content due to the higher soil aeration. However, the opposite trend has also been observed. This is likely due to the fact that soils with higher clay content typically have higher OM and microbial activity (Rigby et al., 2009). In a plantation study of biosolids-N dynamics, faster N-mineralization was observed in brown/more-basic soils relative to volcanic/acidic soils (Wang et al., 2003). Nitrifying bacteria's optimal soil pH is

typically between 7 and 9, and inhibited below a soil pH of 4.5 (Nieder & Benbi, 2008). As such, acidic soils, like those in forest systems, leach less nitrate due to the restriction of nitrifying bacteria and conversion of ammonia-N to nitrate-N (Sierra et al., 2001). Soils with low anion exchange capacity (AEC) (i.e. low clay content) are also more susceptible to nitrate leaching due to their low electrochemical affinity for nitrate anions. Additionally, soil C/N strongly dictates the likelihood of immobilization of applied N. As forest soils are typically N-limited, application of biosolids-N promotes the growth of soil microbial populations. At the same time, it can reduce soil organic carbon and plant-available nitrogen due to increased microbial demand (Henry, 2000). Given the range of soil characteristics that affect N-dynamics, these dynamics are typically very difficult to predict. In order to account for the soil properties of biosolids-applied plantations, repeated soil sampling of the site is generally required.

## G. STUDY OBJECTIVES & HYPOTHESIS STATEMENT

This research seeks to provide insight into plantation management protocols for the safe and effective use of biosolids as a plantation fertilizer. The study will analyze data collected through a large-scale biosolids monitoring program in a Douglas-fir plantation located in King County, Washington. It was in 1993, in Washington State, that some of the earliest guidelines for the management of biosolids worldwide were developed. It is in working landscapes such as this, where biosolids have been applied successively over a long period of time with a continuous monitoring record, that provide the best opportunity to better understand the benefits and risks posed by the application of biosolids, and grow our understanding of their use.

This research will evaluate changes in soil nitrate concentrations following the long-term application of biosolids in a working plantation as a proxy for the potential of leaching of soil nitrate to groundwater. This analysis will also consider a number of factors that may be associated with the modification of soil-nitrogen-dynamics.

Forestry is an ideal setting for the land-application of biosolids. Yet while biosolids provide many benefits to stand and soil productivity, excessive nitrate loading poses risks to groundwater quality. Previous studies of forests in this region have established that biosolids application rates exceeding 47 Mg/ha result in nitrate leaching above U.S. EPA limits (Henry et al., 1994). Application rates used in this study were well below this maximum limit. However, there is no active program to monitor groundwater NO<sub>3</sub> concentrations following forest application of biosolids in this region. As a result, monitoring the N-dynamics in the soil profile is required in order to better identify any potential for downward movement of N through the soil profile and potential risk of leaching, as well as the potential for excessive nitrogen accumulation following successive applications. It is also important to determine forest-specific dependencies on environmental factors (seasonality, soil type, etc.), and how these factors change according to difference in application rates.

This study will examine soil nitrate concentrations through the A and B horizons of the soil profile, measured over the course of 30 years of biosolids applications at a range of application rates and environmental conditions. The scale of this study presents an ideal opportunity to evaluate the effects of long-term forest application of biosolids, as well as potential variables that influence those effects. Identifying risk of N-leaching is of primary concern. N-leaching would

be evidenced by the movement of nitrate from the A horizon into the B horizon, and would be indicative of biosolids application rates and/or frequency exceeding the assimilative capacity of the plantation. Additionally, enrichment of nitrate in the B soil horizon would be expected with increased biosolids loading rates.

Soil total carbon and total nitrogen may also serve as tools to estimate the potential for nitrate leaching. Biosolids applications (as an organic amendment) may increase total soil carbon if applied frequently enough and at high enough rates. However, over-application of biosolids may be evidenced by a decline in total carbon and nitrogen, as seen in the soils of ‘America’s oldest field experiment’ after 50 years of synthetic-N fertilization (Mulvaney et al., 2009). However, biosolids provide an added benefit that synthetic-N fertilizers do not—they also supply a significant amount of organic-N and prevent the depletion of soil carbon. Additionally, the C/N ratio of the soil can affect N-dynamics, as carbon is required for N-mineralization. Soils with higher C/N would be expected to correspond with higher rates of N-mineralization, and therefore minimal nitrate accumulation and downward movement in the soil profile over time.

Finally, the assimilation of biosolids-N is influenced by a number of environmental factors, some of which will also be examined in this study in order to better identify contexts that favor N-assimilation and minimize leaching. Variables such as soil series and climate may cause soils to respond differently to successive biosolids applications, and correspond to different nitrate concentrations in the soil profile.

## II. METHODS

### A. THE KING COUNTY BIOSOLIDS PROGRAM

The King County Wastewater Treatment Division (WTD) Biosolids Program consists of three WWTPs which produce biosolids that meet EPA Class B standards for beneficial use. The West Point plant began the production of biosolids in 1978, and the South Plant in 1988. Together, these two plants produce 25,000 dry tons of biosolids annually. Both plants meet standards for Class B volatile solids and pathogen reduction requirements through anaerobic digestion. The third plant, Brightwater, uses a membrane bioreactor and has been producing Class B biosolids since 2012. Together, these three plants produce 28,000 dry tons of biosolids annually. The amount of biosolids distributed to each end-use varies from year to year. A small percentage of biosolids had been sent to a private composter who ceased operations several years ago. The vast majority of the material is used as a soil amendment for agricultural crops in Central Washington and commercial forests in eastern King County (74% and 25% of total biosolids produced in 2011, respectively). Figure 1 reports the number of dry tons the King County biosolids program distributed to forestry end-uses annually, from the start of the program in 1978 until now. The chemical characteristics of the biosolids used in this study are summarized in Table 1.

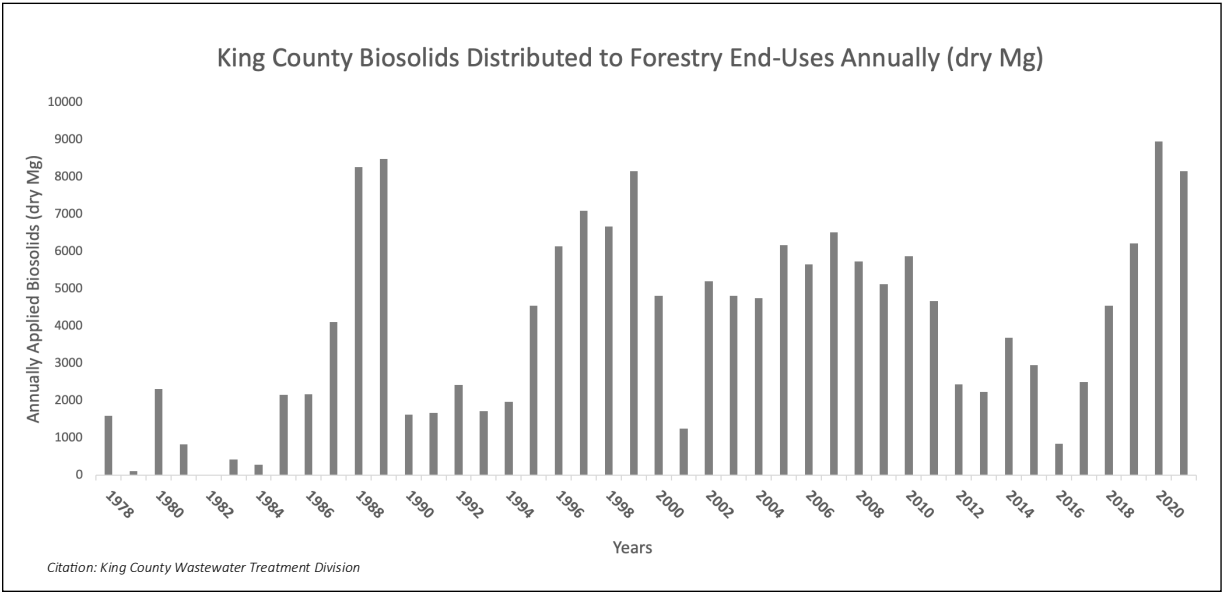


Figure 2: King County Wastewater Treatment Division summary of the number of dry tons of land-applied biosolids diverted annually to forestry end-uses.

**Summary of Biosolids Composition**

Biosolids Analyte	Unit	Brightwater Plant		South Plant		West Point Plant		U.S. EPA Limit
		Mean	SD	Mean	SD	Mean	SD	
Total Solids	%	20.62	0.01	23.34	0.01	27.39	0.01	
Total Volatile Solids	%	58.93	2.92	62.48	1.93	68.84	4	
pH		9.1	0.32	9	0.15	8.9	0.42	
Total Kjeldahl-N	g/kg	77	4.79	72.2	5.97	64.9	6.30	
Ammonia-N	g/kg	9.73	1.03	11.2	1.24	8.48	0.73	
Organic-N	g/kg	67.2	4.99	61.1	5.98	56.0	6.09	
Total P	g/kg	16.1	3.52	24.0	3.87	15.2	2.13	
Total K	g/kg	1.14	0.13	1.73	0.25	1.14	0.20	
Total Sulfate	g/kg	0.38	0.24	0.179	0.10	0.19	0.10	
As	mg/kg	4	0.35	6	0.56	6	1.13	41
Ba	mg/kg	155	14	170	12	187	56	
Be	mg/kg	0.19	0.02	0.11	0.01	0.14	0.03	
B	mg/kg	60	9	17	4	11	1.38	
Cd	mg/kg	1	0.07	2	0.2	2	0.22	39
Ca	mg/kg	22080	1615	25855	2447	17577	2470	
Cr	mg/kg	17	1.64	23	2	28	7	
Cu	mg/kg	272	24	341	27	372	43	1500
Fe	mg/kg	9440	744.35	18702	3861	15698	3909	
Pb	mg/kg	12	1.33	22	4	63	14	300
Mg	mg/kg	6952	3052	7850	1901	5186	1097	
Mn	mg/kg	887	99	408	73	741	377	
Hg	mg/kg	0.46	0.16	0.56	0.26	0.7	0.33	17
Mb	mg/kg	7	0.59	7	1.02	11.1	2	
Ni	mg/kg	16	1.38	17	2	22	5	420
Se	mg/kg	6	0.74	6	0.62	5	0.84	100
Ag	mg/kg	3	0.35	4	2	3	0.61	
Zn	mg/kg	779	90	967	112	887	111	2800
Fecal Coliform	org/g dry	1292135		90.368		205737		
Salmonella	org/4g dry	89.3		21.1		54.1		

*Citation: King County Wastewater Treatment Division. 2021 Loop Quality Data Summary. <https://kingcounty.gov/services/environment/wastewater/resource-recovery/loop-biosolids/leading-with-science.aspx>. accessed 1/13/23.*

Table 1: Summary of chemical characteristics of King County WTD biosolids from all three WWTPs that supply biosolids for the forest applications examined in this study. All three plants supply Class B biosolids that have been applied on the plantation examined in this study. Concentrations are reported on a dry weight basis.

In 1995, the Washington Department of Natural Resources (Washington DNR) partnered with King County WTD to grow the capacity of the Mountains to Sound Greenway biosolids forestry program. There are two forest plantations where biosolids are applied in the Greenway. One plantation is owned and operated by the WA Department of Natural Resources, and the other by private owners. The latter has changed ownership several times through the course of the

biosolids program. While the amount of King County biosolids used for forest fertilization varies over the years, 2,500 to 8,900 Mg of King County biosolids were applied annually between the years 2017 and 2021.

In accordance with Washington Department of Ecology guidelines, King County WTD's biosolids application rates are determined at a site-specific level, and limited to ensure that leaching of nitrate below the vadose zone is below detection limits (Washington State Legislature, 2007). These guidelines were established to minimize any measurable impacts that biosolids applications may have on the nitrate concentration in groundwater and surface water.

Biosolids application rates aim to balance the likelihood of the soil leaching any added nitrate, with the nitrogen requirements of the plantation. Nitrogen demands for the trees and understory, as well as the potential for soil N storage in the soil, are all accounted for in estimating the N demand of a particular unit. While nitrogen dynamics in plant-soil systems are complex and difficult to predict, there are several basic parameters biosolids program managers use to guide the development of application rates: biosolids composition, rate of N-mineralization, likelihood of N-immobilization, likelihood of N-volatilization, and the N-requirements of the plantation and understory. Nitrogen dynamics and biosolids application rates are discussed in greater detail in sections E and F of this chapter.

In this study, biosolids application rates were determined for each application unit (i.e. unit by unit). The methodology and data used by King County WTD to determine biosolids application rates are discussed at greater length in the sections that follow, but generally ranged from 4.5 to 22 Mg/ha ( $\bar{x} = 11.5 \pm 3.27$  Mg/ha) throughout the plantation.

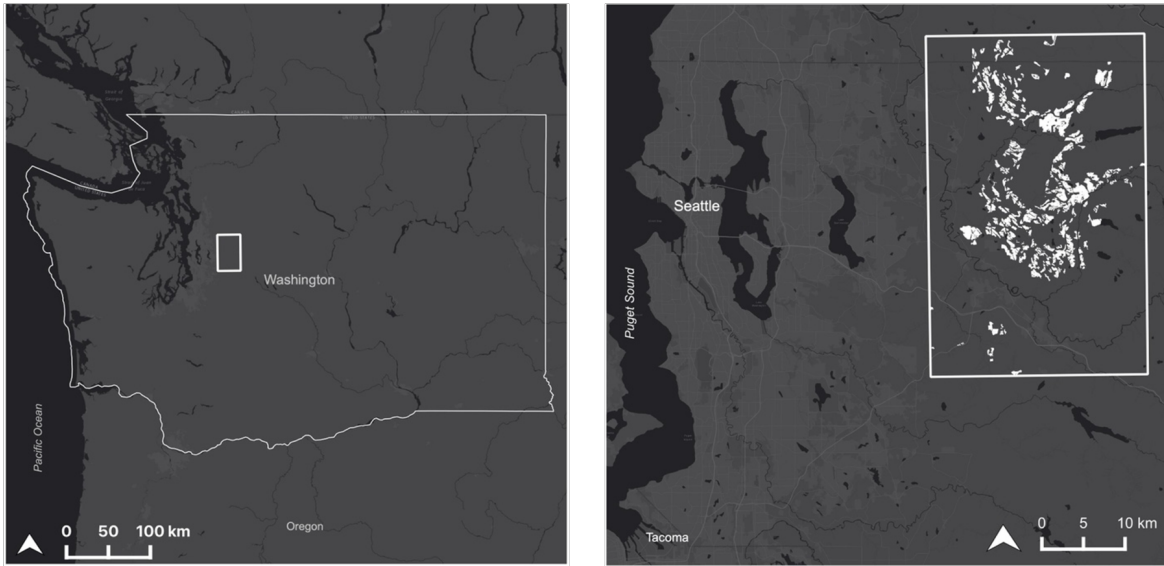
## STUDY SITE DESCRIPTION & HISTORY

The plantation investigated in this study is composed of two parcels. The Marckworth parcel is owned by Washington DNR. The second parcel is currently managed by the resource management company Campbell Global. This property has been previously referred to in previous studies as the “Weyerhouser Tree Farm” as it was historically owned by the timber production company Weyerhaeuser. Both parcels were existing tree plantations before biosolids applications began. These parcels are located in King County, Washington: the Marckworth DNR parcel at 47.743°N, -121.838°W, and the Weyerhaeuser parcel at N 47.646°N, -121.764°W. The parcels share a boundary and King County WTD manages the biosolids applications as a single plantation. Map 1 show the location of the plantation.

## UNIT DELINEATION

The plantation spans approximately 65,000 hectares of forested land. Of this, 7,300 hectares were identified as having the appropriate site conditions for biosolids applications in accordance with U.S. EPA and Washington State regulations. These biosolids-compatible regions have been subdivided, delineating approximately 400 units, each between 10 and 80 hectares, with an average unit area of approximately 19 hectares. Units are assigned IDs using a nomenclature of numbers and letters roughly corresponding to the unit’s location. Unit extents and locations are based on a combination of factors: tree ages within a unit are homogenous, soils classes within a unit are typically similar, slopes across all units may not be in excess of 40%, and buffer strips

are established to prevent movement of biosolids particles to water via overland flow. Each unit has received between 1 and 5 biosolids applications since treatments began in 1988. The location and extent of the plantation and composite units are shown in Map 1.



Map 1: Location map of the study site and plantation units.

## PLANTATION TREE SPECIES & UNDERSTORY

Douglas-fir (*Pseudotsuga menziesii*) is the tree species planted for harvest. There are also various volunteer tree species in some of the stands. These volunteers include red alder (*Alnus rubra*), Cottonwood (*Populus* sp.) and hemlock (*Tsuga* sp.). The understory is highly varied from unit to unit but typically includes fireweed (*Chamerion angustifolium*), salal (*Gaulthoria shallon*), sword fern (*Polystichum* sp.), deer fern (*Blechnum* sp.), bracken fern (*Pteridium aquilinum*), vine maple (*Acer circinatum*), lupin (*Lupinus* sp.), scotch broom (*Cytisus scoparius*), evergreen blackberry (*Rubus laciniatus*), thimbleberry (*Rubus parviflorus*), salmonberry (*Rubus spectabilis*), and elderberry (*Sambucus* sp.). The plantation is primarily composed of soils in the Barneston, Klaus, Tokul and Blethen Gravelley Loam soil series as well as Skykomish Outwash soils. The plantation's soils are therefore inherently low in nutrients and well drained. A satellite map and subset of underlying soil series are shown in Map 2 on page 27.

## B. BIOSOLIDS PROGRAM & PLANTATION MANAGEMENT

### APPLICATION TIMING

Biosolids are applied to each unit at rates specific to that unit in a given year using soil analysis and stand data reports collected up to several months prior (principles and methodologies for calculating biosolids application rates are described in the following section). Biosolids are applied using a side cast spreader mounted on a log forwarder chassis. Applications begin when the trees are 5 years old and continue every 3 to 4 years on a given unit until canopy closure (which typically occurs when the stand is approximately 15 to 18 years old). Upon reaching canopy closure, applications cease due to the inability of the biosolids to reach the target soils. When the stand is approximately 30 to 35 years old, the stand is thinned. Applications then resume and continue until harvest (generally when the stand is between 35 and 50 years old). With a 4-year application interval, each unit would receive 3 applications pre-thinning, and 2 to 3 applications post-thinning, for a total of 5 to 6 applications per unit with each rotation prior to harvest. This study includes units with stand ages between roughly 4 and 45 years old. An annual record of biosolids applications for the plantation is illustrated in Map 3.

### DETERMINATION OF BIOSOLIDS APPLICATION RATES

The application rates of biosolids are determined unit-by-unit, for each application, using a worksheet tool developed by researchers at the University of Washington (Henry et al., 1999).

For the duration of the plantation's application history, application rates have been prescribed by two researchers at the University of Washington and contracted by King County WTD. An example of the worksheet is shown below in Figure 2. One week to several months prior to each biosolids application, site assessments are conducted at each plantation unit in order to calculate an appropriate agronomic application rate. Site assessments include two components: 1) a visual assessment of the stand, understory, and soils; and 2) soil sampling. Collectively, this information is used to complete the rate calculating worksheet for each unit prior to an application which determines an appropriate biosolids application rate. The parameters that are used to determine the biosolids application rate in this plantation include:

- An estimate of the N-requirement of the plantation and understory (based on the age of the stand and ecological characteristics of the site)
- Soil NO<sub>3</sub> concentrations in both the A and B horizons (based on soil sampling)
- The depth of the A and B soil horizons
- An estimate of the amount of biosolids-N that will be immobilized by the soil
- An estimate of the mineralization rate of the organic N in the biosolids coupled with the % NH<sub>4</sub> concentration of the biosolids
- An estimate of the percentage of ammonia likely to volatilize

The concentration of nitrate in a given unit's soil is a significant component of this calculation. Nitrate concentration is a measure of available N for tree growth, and also functions as a metric to monitor the likelihood of the soil to leach nitrate into proximate waterbodies. General stand health and understory species assemblages are also used as an indication of nutrient availability. For example, the dominance of nitrogen-fixing species in the understory, or chlorotic needles within the stand, are indicators of N deficiency. In contrast, a biodiverse understory and long

leaders within the stand would be indicative of a nutrient rich soil. A combination of all these factors is used to come to a final determination of the biosolids application rate for a given unit.

While these worksheets are used to develop the prescribed application rate for a given unit, the actual amount applied on site can deviate slightly due to the logistics and mechanics of transporting and spreading the biosolids on a given unit. However, regulations require that actual application rates are no more than 15% greater than what is prescribed.

# Sample Worksheet For Site Assessments and Determination of Application Rates

Unit #: 24-08-06

**Nitrogen Budget Sheet for Determining Agronomic N Rate for Biosolids**

**Calculations for Determining Residual Soil N from Soil Testing**

Sample Date	LMS #	Depth of layer (in)	Nitrate Conc. (ppm)	Residual Soil N (lb/2.0)
Top	174390-43	4	0.35	0.422
Next	174390-44	20	0.00	0.000
Next				
<b>Total</b>				<b>0</b>

**Site Notes**

Evaluation Date	4/15/20
Unit Name/No.	Unit #: 24-08-06
Application history	2006 - 5,2
Stand age	40
Understory	sword fern, salmon berry
Soils	Tokul Gravelly Loam
Comments	Post thinning application? Nice A horizon in the soil, very low residual N

Supplied by Sally Brown, UW

From KC Environmental Lab and WTD Biosolids Quality Report

From KC Environmental Lab collection and analysis of site soil sample

**1. Total N requirements for crop grown**

1.1 Uptake for crop or forest understory	50 lb/ac
1.2 Uptake for trees	100 lb/ac
<b>Total</b>	<b>150 lb/ac</b>

**2. Nitrogen available from the soil**

2.1 N from previous applications (4 yrs ago)	0 lb/ac
2.2 Residual soil nitrate	0 lb/ac
<b>Total</b>	<b>0 lb/ac</b>

**3. Estimate of the amount of N immobilized by soil**

**Net amount needed by crop/soil**

Plant available nitrogen from biosolids	1.2 %	2.5 lb/t
4.1 NH4+ concentration in biosolids	20 %	-5 lb/t
4.2 Amount available N per dry ton Percent ammonia volatilization	5.7 %	4.5 lb/t
4.3 Amount available N per ton volatilized Organic N concentration in biosolids	40 %	-7 lb/t
4.4 Percent Organic N mineralized Amount available N per ton added	1.0 %	59 lb/t
4.5 Percent denitrification Amount available N per ton denitrified		
<b>Net Plant Available Nitrogen</b>		<b>5.6 t/ac</b>

Figure 3: Sample worksheet developed by University of Washington that is used to determine the recommended application rate for a given unit. In this case, the unit number is 24-08-06. Soil samples are taken and results for each horizon are reported in the upper left corner of the worksheet. Notes from the ecological assessment are reported in the bottom left corner of the worksheet. The right side of the worksheet requires a number of numeric inputs in order to calculate a recommended application rate. These include an estimate for the nitrogen requirements of the crop and how much will be lost after application as determined by the project manager/project scientist, the pool of nitrogen already available in the soil based on the soil sampling results, and the amount of nitrogen supplied by the biosolids themselves based on the quality reports from the WWTP.

## UNIT SITE ASSESSMENTS

Site assessments of units are conducted throughout the year. However, site access is often difficult in winter and soils tend to be saturated, so in practice sampling tends to take place primarily in the spring, summer, and fall. A general assessment of stand health and species composition of the understory is recorded on a worksheet that registers site-specific data collected for each unit prior to every application of biosolids. Any evidence of chlorotic needles or abnormally long or short leaders on stand trees is reported, in addition to a species list of the dominant plants in the understory. An approximation of stand age (based on stand height and DBH) is also recorded during this ecological survey. Both will inform the estimate of the N needs of the crop.

## SOIL SAMPLING & ANALYSIS

Soil sampling is conducted prior to each application of biosolids for a given unit. One or two soil sampling pits are dug within each unit by excavator, the number and location of which is dependent on the variability of conditions within a given unit (e.g. abnormally rocky or wet conditions). General observations of the soils' physical characteristics (color, texture, drainage, etc.) are recorded for each pit. The depth of the A and the B horizons are measured and recorded on the unit worksheet. Samples are taken by collecting material from the A and B horizon, separately. Analysis for soil nitrate has been conducted through the history of the program at the King County Environmental Laboratory. Nitrate<sup>-</sup> concentration is determined by a 2M KCl

extraction, and the resulting solution concentration determined by autoanalyzer (Kalra and Maynard, 1991, and Plumb, 1998). Total carbon and nitrogen concentrations have been included in the unit soil analysis since 2017. Soil samples are sent to the Kansas State University Soil Testing lab for analysis by combustion. Because soil sampling occurs prior to each biosolids application, the very first soil sample taken in a unit is conducted prior to the first biosolids application on the site. The first record therefore reflects the conditions before any effects of biosolids can be seen and reports the soil chemistry of the unit at  $t_0$ . Subsequent soil sampling for the applications that follow would effectively report soil chemistry at  $t_1$ ,  $t_2$ ,  $t_3$ , etc., creating a chronological record of the effect of biosolids on plantation soil chemistry.

## DATASET CONSOLIDATION

For this study, worksheets containing soil metrics, site assessments and recommended application rates were collected and consolidated at the King County WTD offices, in addition to records of reports of the actual rate applied on units. At the start of the program in 1988, soil sample results and application reports were maintained only as hard-copy records. In more recent years, field data was initially recorded on hard-copy worksheets, later transcribed and combined with lab results, and the entire application record stored digitally. In order to create a complete database of the study, all hard-copy reports were collected and transcribed into a central database and combined with existing digital records.

The dataset consisted of approximately 800 application records for just over 400 units. The dataset was reviewed to identify incomplete records and, when possible, the incomplete records

were completed using a range of digital and hard-copy records at King County, as well as soil sampling records supplied by the King County Environmental Laboratory. For complete records that included both the prescribed application rate as well as the rate actually applied on the site, the actual rate was used in further analyses. However, for records that had only one record (i.e. the prescribed rate or the actual rate) that rate was used. After multiple reviews attempting to complete partial records using the supplementary datasets available, records that remained incomplete were excluded if they lacked soil sampling data, or if they had neither a prescribed nor actual application rate and/or application date. This tabular data provided the study with the soil and application rate history for each unit number that existed over the course of the program. However, the spatial record of these applications were not yet incorporated into this dataset.

The spatial extent of these applications is complicated slightly by the fact that the unit boundaries for a given unit ID number is re-evaluated prior to every application. Additionally, the unit ID number was changed in some cases to describe a slightly different extent, but one that overlapped with the original ID number. These inconsistencies meant that the extent over which each application occurs only very roughly corresponds to the unit ID number, and that the ID number could not be used to consistently determine the spatial extent of the application. In order to develop a historic record of successive applications over the same spatial extent in the plantation, each application for every unit over the entire application history of the plantation would need to correspond to its own spatial data record.

King County WTD had a pre-existing GIS database that contained the spatial extent of every unit where the plantation applied biosolids, but those records were not complete. Each feature class for a given unit in the GIS database included only the location of the unit, its size, and the unit's unique identifier. The unique identifier was composed of the unit ID number and the year of the

application. This unique unit ID number was also used in the tabular dataset, along with the complete record of the application and soil metrics. A join of the spatial and tabular datasets was therefore possible using this shared data field—the spatial data for each unit’s feature class could be linked with the application rate and soil sampling data collected for each application event.

Geospatial data processing and management were performed using a combination of QGIS and Esri ArcGIS software, and all maps were generated in QGIS (QGIS Development Team, 2022; Redlands CESRI, 2011). The dataset was joined to the geospatial database, which contained the spatial extent and location data of each plantation unit that was treated with biosolids during the entire duration of recorded applications in the unit. The product of this geospatial operation contained a feature class for every unit applied with biosolids for every application a unit received during the observational period, and each feature class was linked to the associated application and field data for each completed application. This data included the application rate, date of application, soil analysis prior to application, soil horizon depth, approximate stand age, and ecological observations of understory and stand health. This geospatial operation produced a database where each unit had a feature class (and associated field data) for each application year, and where each feature class had a potentially different spatial extent and location from other applications that shared the same unit name (i.e. the spatial extent of the applications from year to year, and independent of the unit name). Map 1 was created using this joined dataset and shows the location of these units.

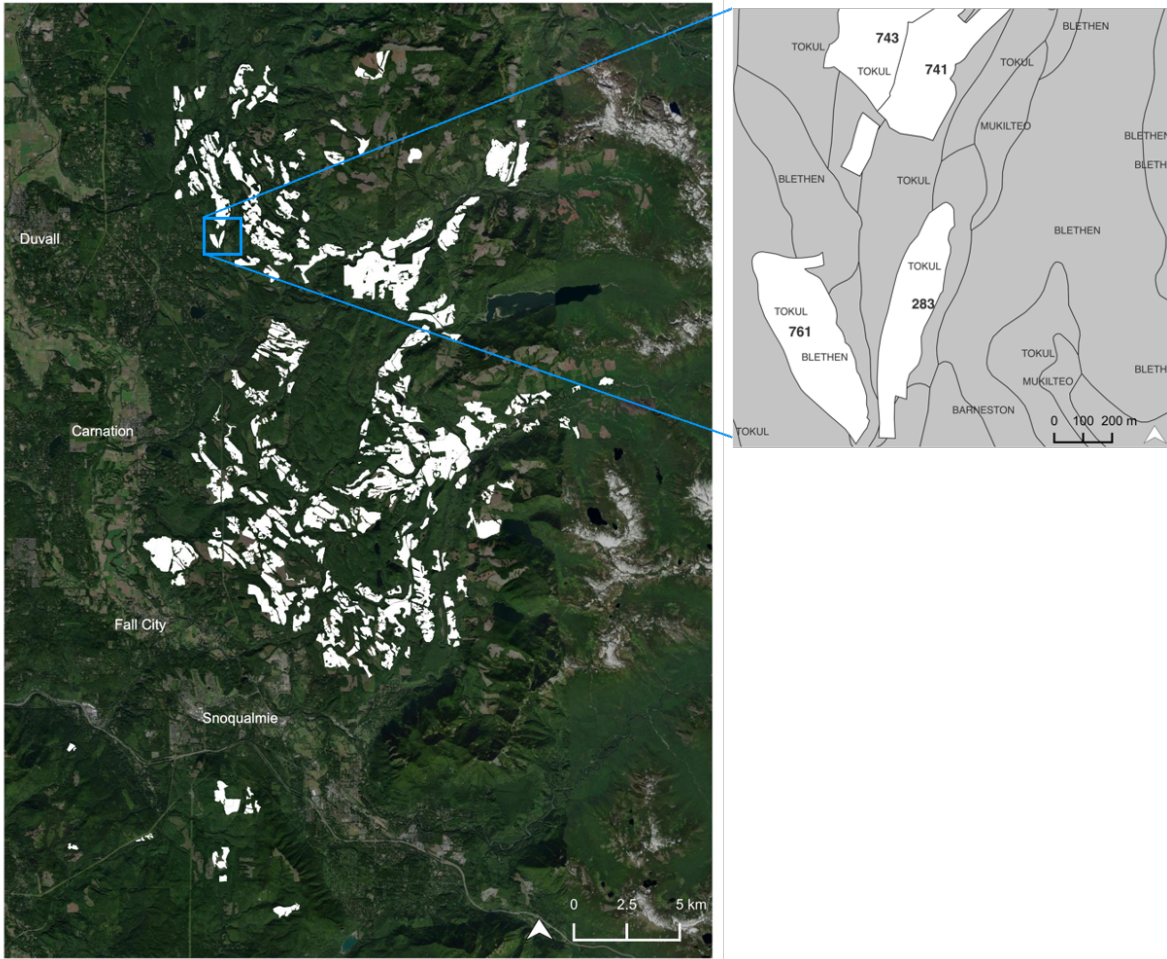
## C. DATA MANAGEMENT & ANALYSIS

The prior data compilation and consolidation produced a spatial dataset where each feature class was affiliated with the application rate, date, and soil sampling data for a given application over the land-area mapped by the feature class, creating a patchwork of overlapping units in terms of space and time in the plantation. Map 3 shows this annual record of biosolids with overlapping extents. In order to produce a single feature class record that contained the entire application history of that spatial extent, we needed to compress these overlapping feature classes. A “join by spatial extent” was performed in GIS—overlapping spatial extents were identified, and the data associated with the resulting spatial extent was compressed into a single record. For the purposes of this study, only units with 2 or more complete application records, and greater than 2 acres in extent, were included in any further analyses, which eliminated the need to remove “slivers” from the dataset. Application records that spanned multiple spatial extents were retained as long as the spatial extent for each included one or more different and/or additional applications. The final dataset was composed of 239 individual application units, each with an application history record spanning at least 2 applications. Table 2 on page 30 summarizes the number of units in the final dataset by the number of applications each unit received.

The resulting data subset was reviewed for outliers in the reported concentrations of nitrate, total carbon, and total nitrogen in the A and B horizon. Simply eliminating values would make the application history incomplete and invalidate the record. It was critical that as many records were included in the study as possible in order to have enough statistical power. So to maintain the integrity of the application record, concentrations greater than 3 standard deviations from the

horizon's mean for a given soil metric were removed. The horizon's mean was then recalculated and used to replace the removed values.

The 20-year average of rainfall and precipitation was determined for the calendar month in which the sample was taken using monthly averages from 2008 to 2019 measured at the North Bend, WA weather station (47.4957°N, 121.787°W) (U.S. Climate Data.). The dominant soil series for each unit was determined by overlaying and clipping the NRCS soil survey map to the geospatial data of the plantation units (Soil Survey Staff, U.S. NRCS). Once the dominant soil series for each unit was determined, the estimated soil organic carbon stock (SOC stock) and soil organic matter (SOM) content typical of each soil series observed in the plantation was extracted from the SoilWeb soil series database (U.C. Davis & U.S. NRCS). Map 2 shows the satellite imagery and underlying soil series of the plantation.



Map 2: Satellite imagery of study site and subsection of underlying soil series established by the U.S. Soil Survey.

## STATISTICAL ANALYSIS

The dataset, once compiled and processed, was assessed by examining trends in soil nitrate, as well as determining the impact of variables that had the potential to influence N-dynamics. Analyses were conducted both in R Studio and in SPSS (IBM Corp, 2020; R Core Team, 2016). A description of each of the variables considered is shown below. Main effect means for numerical variables (such as application rate and temperature) were tested by ANOVA and

performed using R. ANOVAs were all one-way tests of significance with the threshold of significance  $p > 0.05$ . Several R-generated graphing tools were also used in this analysis. Scatter plots and linear regressions were used to identify any linear trends in quantitative variables relative to soil nitrate concentrations using the regression slope and  $R^2$  statistic. Bar graphs were also used to illustrate differences in nitrate concentration against several categorical variables. All tables report the mean ( $\bar{x}$ ), standard deviation (SD), standard error (SE) and population size (n) of the result. Averages are reported with the associated standard error (e.g.  $\bar{x} \pm SE$ ). Summary tables of soil nitrate concentrations relative to each variable were produced to identify trends and initial dependencies. In order to determine the statistical significance of these observed trends, categorical variables (such as sample month and soil series) were tested against soil nitrate concentrations by Waller-Duncan post-hoc test using SPSS.

## DESCRIPTION AND NOMENCLATURE OF VARIABLES

- Soil horizons: Soil metrics are reported for the A and B horizons of the soil profile, separately. Metrics for the A horizon are reported as nitrate\_A, total carbon\_A, total nitrogen\_A and C/N\_A. Metrics for the B horizon are reported as nitrate\_B, total carbon\_B, total nitrogen\_B, and C/N\_B.
- Single Application Data: Soil chemistry metrics are analyzed as independent soil samples across all applications and units.
- Delta Application Data ( $\delta$ ): Soil chemistry metrics are analyzed as the difference between any two subsequent applications for a given unit. For example,  $\delta$  nitrate\_A is the change

in nitrate\_A concentration between the first and second application of biosolids, or the second and third application, or third and fourth application. These variables are included to demonstrate incremental change over the course of the entire recorded history of applications.

- Grand Delta Application Data ( $\Delta$ ): Soil chemistry metrics are analyzed as the change between the first and last sampling. For example,  $\Delta$  nitrate\_B is the difference between the nitrate\_B concentration prior to any biosolids treatment, and the nitrate\_B concentration measured in the most recent soil sample on record. These variables are included to demonstrate the plantation's total response to successive biosolids applications over the course of the entirety of the recorded history of biosolids applications in the plantation.
- $\delta$  Year and  $\Delta$  Year: The number of years that have passed between the soil samples being reported.  $\delta$  Year would be the number of years between any two successive soil sample being reported as a  $\delta$  soil metric for a given unit.  $\Delta$  Year is the number of years between the first application and the last application (i.e. most recent) for a given unit. This metric is also representative of the number of years that span between the first and last soil sample taken for a given unit.
- Application Number and Grand Total Application Number: The number of applications a unit received prior to soil sampling, and the total number of applications a unit received over the entire duration of recorded applications on the unit, respectively. "Application number" includes a "0" application value because a sampling was conducted prior to the first application. Subsequent "application number" values reflect the number of

applications on a unit prior to soil sampling. “Grand total application number” is simply a number count of applications on a given unit at the end of the observational period.

- Treated/Untreated: Soil metrics of samples taken prior to the first biosolids application for a given unit are reported as “untreated” soils. Samples taken thereafter are reported as “treated” soils.
- Application Rate (Mg/ha): For a given soil sample, the application rate reports the biosolids application rate on the unit prior to that soil sample being taken.
- Net Application Rate (Mg/ha): For a given soil sample, the net application rate is the total application rate applied on the unit prior to that sample being taken.
- Grand Total Application Rate (Mg/ha): For a given soil sample, the grand total application rate is the total application rate applied on the unit over the course of the entire observational period.
- Sample Month: The month in which the soil sample was taken for a given unit.
- Climate: For a given soil sample, the Sample Month Mean Temperature (°C ) and Sample Month Mean Precipitation (cm) for the month in which the soil sample was taken. The mean for each calendar month was determined using monthly averages from 2008–2019 measured at the North Bend, WA weather station (47.4957°N, 121.787°W) (U.S. Climate Data).
- Sample Depth (cm): The total depth of the soil sample taken from a given soil horizon. During soil sampling, the A and B horizon are exposed, soil is collected from the entire depth/length of each horizon, separately, and analyzed. The total depth of each horizon is recorded as the sample depth.

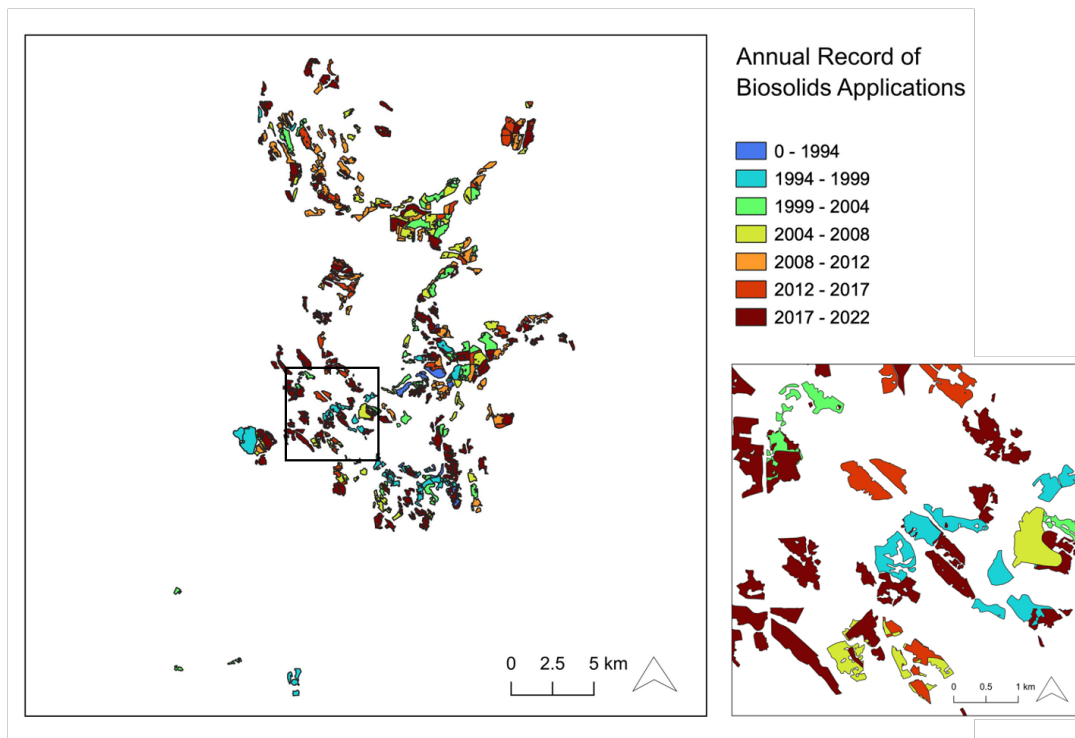
- Soil Series: The dominant soil series within a given plantation unit. Identification of the dominant soil series in a given unit was carried out using the USDA Soil Survey dataset (Soil Survey Staff, U.S. NRCS) and ArcGIS. A summary of characteristics for each soil series discussed in this study is provided in Appendix B(ii) and includes the Soil Organic Carbon Stock (SOC) (kg/m<sup>2</sup>) and Soil Organic Matter (SOM) (%) (U.C. Davis & U.S. NRCS).
- Unit Area (ha): Reports the number of hectares in a given plantation unit.

### III. RESULTS

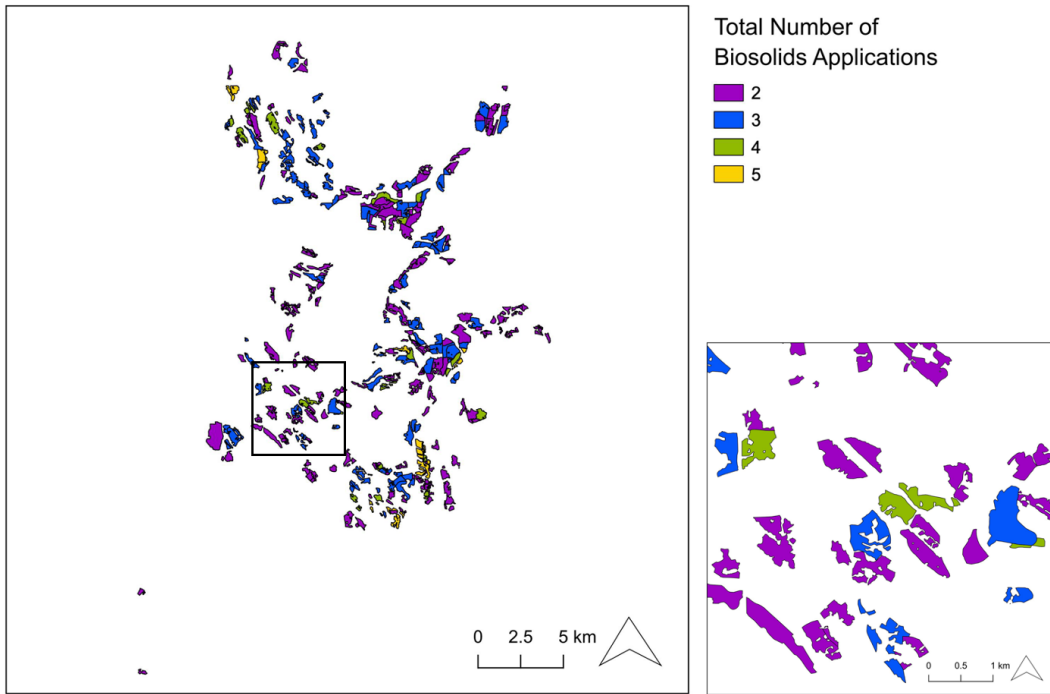
The final dataset, once compiled and processed, included a total of 239 units, all between 1 and 65 hectares in size, with each unit receiving between 2 and 5 applications (i.e. each unit corresponding to between 2 and 5 site assessment and soil sampling records) throughout the course of the study. This yielded a total of 623 records, each with an associated application rate, date, and soil sampling data. (Note: while all soil sampling data records included soil nitrate concentrations, not all records included total carbon and total nitrogen concentration). The table below summarizes the number of plantation units included in the study, and the total number of biosolids applications (and associated site assessment records) each of those units received over the course of the entire study. Map 4 illustrates the final selection of units with the total number of applications each unit received at the conclusion of the study. The average concentration of nitrate in the soil's A horizon across all units and applications was  $4.9 \pm 0.26$  mg/kg. The average concentration of nitrate in the B horizon was  $1.46 \pm 0.09$  mg/kg. The following sections summarize the results of this study, first reporting observations of trends in soil nitrate, total carbon, and total nitrogen in the A horizon against an array of variables, followed by trends observed in the B horizon.

Total Number of Application Records Per Unit	Number of Plantation Units Included in Analysis	Total Number of Application Records Included in Analysis
2	129	
3	84	
4	17	
5	9	
TOTAL	239	623

Table 2: Summary of records included in the study and classified by the number of applications received on a given unit.



Map 3: Annual record of biosolids application within the plantation. Dataset includes units prior to the join by spatial extent.



Map 4: Final selection of units following GIS analysis and record of the grand total number of biosolids applications each unit received at the conclusion of the study.

## A. A HORIZON

### NITRATE

#### Application Number:

The concentration of nitrate in the A horizon was found to be the most responsive metric of soil chemistry tested in this study. A one-way ANOVA showed that nitrate\_A was significantly dependent on the application of biosolids, when compared to the soil chemistry of the unit prior to any application of biosolids ( $p < 0.001$ ) (Appendix B(iii)). The mean nitrate\_A concentration of treated soils is higher than that of untreated soils ( $\bar{x} = 5.84 \pm 0.34$  mg/kg and  $2.65 \pm 0.26$  mg/kg, respectively) (Table 3) indicating that the application of biosolids increased nitrate concentrations in the surface horizon of plantation soils (Figure 3a).

Treated/Untreated	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Treated	5.84	5.43	0.34	261	1.58	1.77	0.11	251
Untreated	2.65	2.53	0.26	96	1.15	0.98	0.10	92

Table 3: Nitrate table of means comparing treated and untreated soils in the A and B horizon. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was also significant ( $p < 0.05$ ).

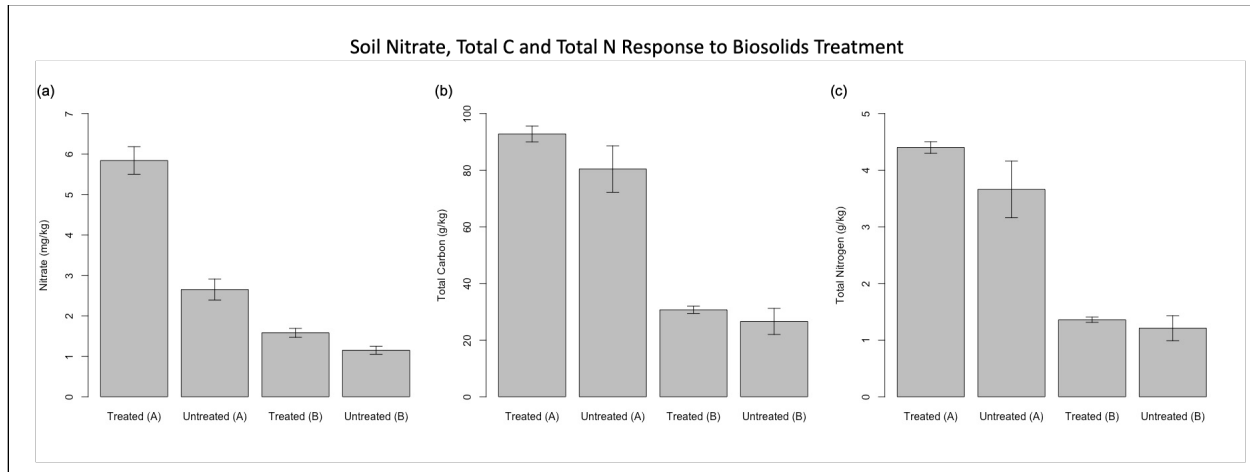


Figure 4: Nitrate, Total C and Total N concentrations in treated and untreated soils. Error bars show standard error. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was also significant ( $p < 0.05$ ). ANOVA of Total Carbon\_A and Total Carbon\_B were not significant. ANOVA of Total N\_A was trending towards significance ( $p < 0.053$ ). ANOVA of Total N\_B was not significant.

Given that nitrate\_A was found to be significantly dependent on the presence or absence of biosolids, it was subsequently determined by one-way ANOVA that the number of applications was also significant ( $p < 0.001$ ) (Appendix B(iii)). The summary table of means (Table 4) reaffirms that the nitrate\_A concentration in units with zero applications of biosolids is lower than that of those that received applications of biosolids, and that there is a potential increasing trend in mean nitrate\_A concentration with additional applications of biosolids. To quantify this trend a Waller-Duncan Post-Hoc test was used to determine whether there were significant differences in nitrate\_A concentration with increasing application number. It was found that nitrate\_A concentration in soils with zero applications was statistically lower than soils that received 2 or more applications. However, the mean soil nitrate concentration of soils that received only 1 application was not statistically different from soils that had not received biosolids (Waller-Duncan harmonic mean sample size = 23.148, MSE = 21.298). Nitrate\_A

concentrations in soils that had received 2 or more biosolids applications were statistically similar. Mean nitrate<sub>A</sub> in soils receiving 2 or more applications measured  $7.50 \pm 1.05$  mg/kg, while the mean nitrate<sub>A</sub> in soils that had received one or fewer applications measured  $4.00 \pm 0.35$  mg/kg (Figure 5). These post-hoc results indicate that the soil nitrate concentrations in the A horizon remain statistically unchanged after one application of biosolids, increase after 2 applications, and then plateau after subsequent applications. These results are of particular importance in that they demonstrate that the forest soils do not continuously accumulate nitrate over time with more applications. If this were the case, the nitrate concentration would be seen to continue to increase after 2 applications of biosolids. Instead, we see nitrate concentrations levelling off after 2 applications, indicating that the nitrogen addition to the soil is largely being removed. Potential mechanisms for the observed plateau of nitrate can be attributed to assimilation by the plantation as intended, immobilization by soil microbes, or leaching out of the soil horizon. Mineralization, nitrification and plant uptake would be indicated by a number of metrics. Increased microbial biomass N (MBN) has been established as a good measure of the mineralized-N pool (Soon et al., 2007) and has been shown to increase with biosolids applications (Villa & Ryals, 2021). Tree biomass and increased foliar N concentrations are also indicators of soil nitrate uptake. Wang et al. (2004) used <sup>15</sup>N isotope to trace the fate of biosolids-N in a *Pinus radiata* plantation, finding increased biomass and foliar N concentrations. If instead, N immobilization was the primary sink for biosolids-N rather than plant uptake, we would observe increases in total N concentrations of the A horizon, which we did not (Figure 3c). Similarly, nitrate leaching would be evidenced by an increasing concentration of nitrate in the B horizon, which we also did not observe (Table 3).

To further investigate if biosolids-N was largely being mineralized and taken up by the plantation biomass, the change in nitrate\_A concentration was also tested by one-way ANOVA against the number of applications, both as an incremental change in nitrate\_A ( $\delta$  nitrate\_A) following an application for a given unit and as a total change in nitrate\_A ( $\Delta$  nitrate\_A) over the course of the entire history for a given unit. Neither variable was found to have a significant dependence on application number, reaffirming the previous conclusion that nitrate\_A is not increasing over time, the applied biosolids are not accumulating, and are likely being assimilated by the forest system (Table 5).

Application Number	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
0	2.65	2.53	0.26	96	1.15	0.98	0.10	92
1	4.92	4.83	0.41	142	1.44	1.70	0.14	138
2	6.61	5.67	0.62	85	1.86	1.80	0.20	80
3	7.42	6.38	1.28	25	1.75	2.21	0.45	24
4	8.60	7.01	2.34	9	0.79	0.49	0.16	9

Table 4: Table of means reporting nitrate\_A and nitrate\_B concentrations following successive biosolids applications. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was not significant.

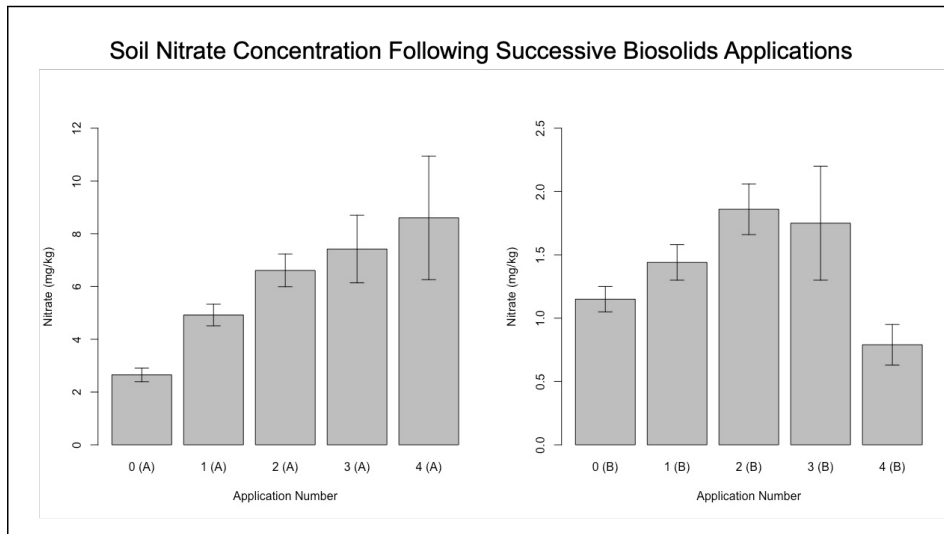


Figure 5: Soil nitrate concentrations for “(A)” and “(B)” soil horizons following successive biosolids applications. Error bars show standard error. ANOVA of nitrate\_A was significant ( $p < 0.001$ ). ANOVA of nitrate\_B was not significant.

Grand Total Application Number	Δ Nitrate_A (mg/kg)				Δ Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
2	1.53	4.64	0.69	45	0.44	2.18	0.33	43
3	3.31	5.84	0.81	52	0.81	2.32	0.35	44
4	4.44	8.77	2.26	15	0.96	3.71	0.99	14
5	3.27	6.11	2.036	9	-1.04	1.39	0.46	9

Table 5: Table of means reporting the total change in nitrate ( $\Delta$  nitrate\_A and  $\Delta$  nitrate\_B) concentration at with the total number of applications for a given unit at the completion of the study period. ANOVA of  $\Delta$  nitrate\_A and  $\Delta$  nitrate\_B were not significant.

### Application Rate:

Subsequently, the effect the biosolids application rate on nitrate\_A was investigated. A one-way ANOVA of nitrate\_A showed a statistical dependence on application rate ( $p < 0.001$ ) (Appendix B(iii), Table 6) while the summary table of means showed that low application rates (0 – 5.6 Mg/ha) yield lower concentrations of nitrate\_A ( $\bar{x} = 2.88 \pm 0.30$  mg/kg), while higher application

rates (>5.6 Mg/ha) yield higher nitrate<sub>A</sub> concentrations ( $\bar{x} = 5.85 \pm 0.64$  mg/kg). These results are further illustrated by scatter plot and linear regression, which show only a weakly positive correlation between nitrate<sub>A</sub> and application rate (Figure 6a) and a low goodness-of-fit ( $R^2 = 0.0476$ ). This indicates that the relationship between nitrate<sub>A</sub> and application rate is not likely to be linear or directly correlated, and rather has one or more interacting variables that influence the soil's geochemical response to biosolid applications. Similarly, one-way ANOVA indicated that  $\delta$  nitrate<sub>A</sub> was found to be significantly dependent on application rate ( $p < 0.01$ ), but linear regression and scatter plot of the data showed only a weak positive correlation and low goodness-of-fit ( $R^2 = 0.0377$ ) (Figure 6b). One-way ANOVA showed no statistical dependence of  $\Delta$  nitrate<sub>A</sub> on the grand total application rate, and the linear regression and scatter plot revealed that the  $\Delta$  nitrate<sub>A</sub> had essentially no correlation, positive or negative, to the total application rate and very low goodness-of-fit ( $R^2 = 0.00259$ ) (Figure 6c). Cumulatively, these results show the application rate of biosolids has no linear correlation to the nitrate concentrations measured in the A horizon prior to application, incrementally ( $\delta$ ), or over the entire course of recorded applications at this site ( $\Delta$ ). If biosolids were being applied in excess, one would expect higher application rates to correlate with higher nitrate<sub>A</sub> concentrations. Instead, we see that there is no strong positive correlation in nitrate<sub>A</sub> with the application rates being used throughout the period in question. This suggests that the nitrate being applied is being taken up by the forest system, and that the plantation may be able to accommodate higher biosolids application rates without increasing the risk of overapplication and nitrate leaching. Villa & Ryals (2021) observed a similarly non-linear relationship between biosolids application rate and microbial biomass-N (MBN), which they used as a proxy for quantifying the relative size of the mineralized-N pool in agricultural soils applied with biosolids repeatedly, at varying

rates over a 20-year period. While Villa and Ryals (2021) observed the biggest increase in MBN at sites that received the highest application rate, they also saw no significant change in soils that received moderate and low application rates, and even reported a decrease in the mean MBN in 0–10 cm soils that received the lowest application rate. This study supports our findings in so far as we see no positive trend in nitrate\_A concentration with application rate, and it is therefore likely that the application rate has not exceeded the capacity of the plantation to mineralize, nitrify, and assimilate the applied biosolids-N at current rates.

Application Rate (Mg/ha)	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
16.8 - 22.4	5.75	6.61	2.09	10	2.28	2.80	0.88	10
11.2 - 16.8	5.97	5.51	0.47	140	1.59	1.81	0.16	136
5.6 - 11.2	5.71	5.26	0.52	102	1.55	1.64	0.17	96
0 - 5.6	2.89	2.98	0.30	105	1.15	0.95	0.10	101

Table 6: Table of means reporting nitrate (nitrate\_A and nitrate\_B) concentration following a biosolids application, at a range of application rates. ANOVA of nitrate\_A and nitrate\_B were both significant (  $p < 0.001$  and  $p < 0.05$ , respectively).

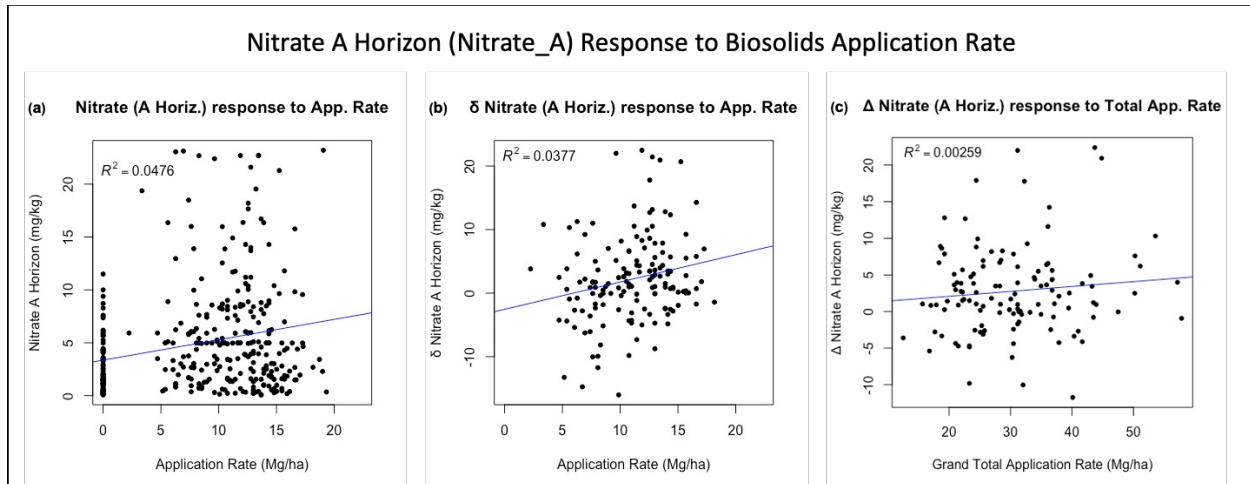


Figure 6: Scatter plots of nitrate\_A (a),  $\delta$  nitrate\_A (b) and  $\Delta$  nitrate\_A (c) concentrations relative to application rate and grand total application rate with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of nitrate\_A and  $\delta$  nitrate\_A were significant ( $p < 0.001$  and  $0.01$ , respectively). ANOVA of  $\Delta$  nitrate\_A was not significant.

### Soil Series:

It has previously been observed that N mineralization is influenced more strongly by soil type than biosolids composition and application rate (Garau et al., 1986). For example, highly acidic soils leach less nitrate due to the restriction of nitrifying bacteria and conversion of ammonia-N to soluble nitrate-N, while soils with low anion exchange capacity (AEC) (i.e. low clay content) are more susceptible to nitrate leaching due to their low electrochemical affinity for nitrate anions. Given the large expanse and range of soil types included in this study, the effect of soil series was investigated as a proxy for the chemical and physical properties of the soils included in this study. Nitrate\_A concentration,  $\delta$  nitrate\_A and  $\Delta$  nitrate\_A were tested against soil series by one-way ANOVA and none were found to be significant. Subsequently, the NRCS soil series standards for soil organic carbon (SOC) stock ( $\text{kg/m}^2$ ) and soil organic matter (SOM) (%) were identified for each of the soil series observed in the plantation (Appendix B(ii)). These values were plotted against the experimental mean nitrate\_A concentration for each observed soil series

(Table 7, Figure 7). Both the SOC Stock and SOM exhibited a relatively high goodness-of-fit ( $R^2 = 0.585$  and  $0.661$ , respectively) when plotted against nitrate\_A (Figure 8). Additionally, the mean nitrate concentration for each soil series observed in the plantation was moderately inversely correlated with the NRCS series averages for SOC and SOM, indicating that soil series with lower SOC and SOM concentrations exhibited higher nitrate A concentrations. In a study conducted in the same plantation as this study, it was found that the total N storage following biosolids application was higher in Tokul series soils when compared to Klaus series soils (Tokul being a generally lower quality soil relative to Klaus). However, this previous study did not investigate how nitrate-N concentrations varied by soil series (Leonard et al., 2021). Wang et al. (2003) investigated the varying effects of soil quality on N mineralization and nitrification. Wang et al. found that N mineralization was significantly higher in “brown” soils relative to “volcanic” soils following biosolids applications in a New Zealand plantation, but nitrification activity in brown-type soils took much longer to establish. The brown-type soils were characteristically higher in  $\text{NH}_4\text{-N}$ , and lower in total N, C, and pH, relative to the volcanic-type soils. It was suggested that the nitrification capacity of the soils was slower in brown-type soils due to its lower pH—that soil conditions with lower soil pH limits the number of nitrifying microbes in the soil, and thus limits nitrification and concentration of plant-available nitrate (Wang et al., 2003). While the brown- and “volcanic”-type soils used in Wang et al.’s study do not directly correspond to the soils series included in this study, it is clear that the variable response of nitrate\_A in a range of soil series is consistent with previous findings, i.e. that soil properties can have a significant effect on the geochemical cycling of nitrate in biosolids-amended soils. However, the major parameters that control nitrate concentration in these

amended soils (e.g. soil carbon content, and mineralization and nitrification rates) are complex and remain difficult to quantify.

Soil Series	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Barneston	3.78	4.29	0.44	96	1.34	1.48	0.16	90
Blethen	5.11	5.84	0.93	39	1.25	1.06	0.18	35
Elwell-Olomount ROC	0.46	-	-	1	0.21	-	-	1
Kaleetan	5.62	5.77	2.58	5	2.23	1.72	0.77	5
Klaus	6.3	5.29	0.91	34	2.41	2.47	0.42	34
Menzel Variant	8.76	7.93	3.54	5	1.86	0.78	0.39	4
Nargar	3.91	3.82	2.21	3	1.6	2.27	1.31	3
Norma	2.21	1.71	1.21	2	1.8	0.85	0.6	2
Olomount	4.67	1.05	0.75	2	0.62	0.24	0.17	2
Skykomish	3.89	3.44	0.86	16	1.08	0.7	0.18	15
Snoqualmie	8.01	7.32	2.77	7	1.13	0.67	0.25	7
Tokul	5.35	5.05	0.48	146	1.4	1.6	0.13	144
Winston	4.98	-	-	1	1.29	-	-	1

Table 7: Table of means reporting nitrate\_A and nitrate\_B concentrations for each soil series observed in the study. ANOVA of nitrate\_A and nitrate\_B were not significant.

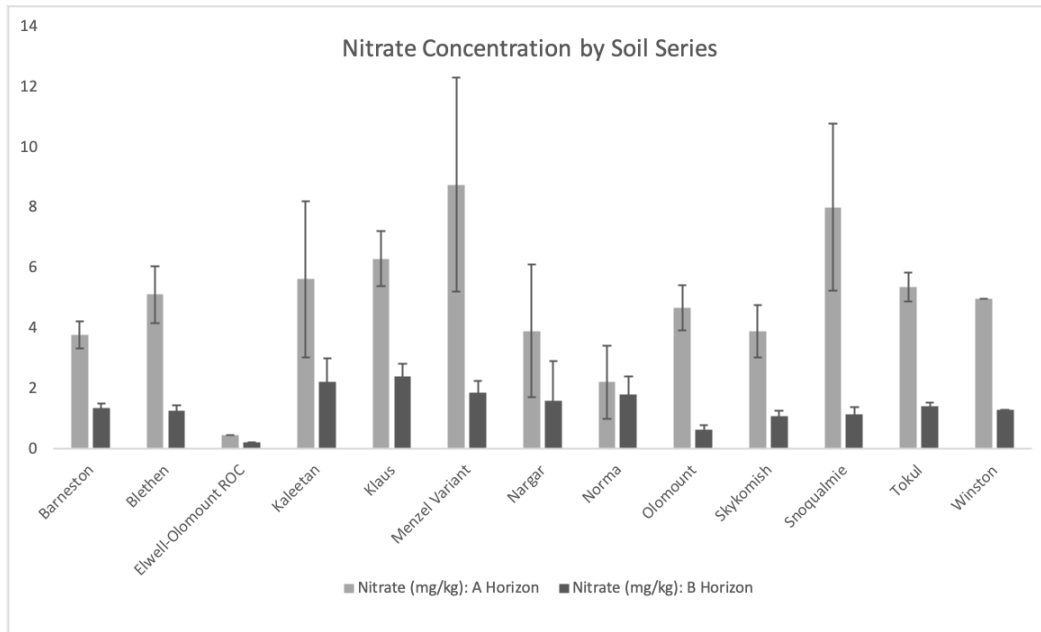


Figure 7: Bar graph reporting nitrate\_A and nitrate\_B concentrations  $\pm$  SE for each soil series observed in the study. ANOVA of nitrate\_A and nitrate\_B were not significant.

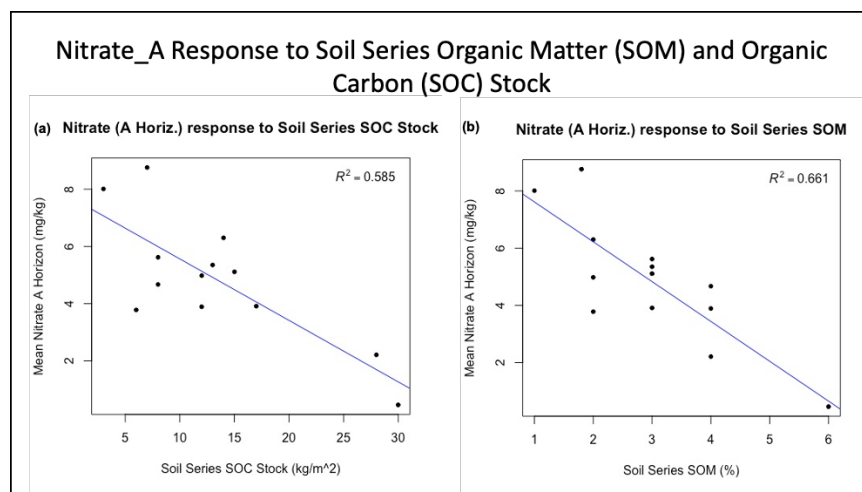


Figure 8: Scatter plot of the mean nitrate\_A concentration of each observed soil series against the soil organic carbon (SOC) stock (kg/m<sup>2</sup>) (a) and soil organic matter (SOM)(%) (b) concentrations corresponding to the NRCS Soil Survey series metrics with linear regression in blue and R<sup>2</sup> correlation coefficient.

## Climate:

Nitrate\_A concentration was also tested against several climate variables by one-way ANOVA to identify any dependence on the time of year soils were sampled. While the calendar month of the soil sample collection was not significant, the mean temperature and precipitation of the sample month in the A horizon were significant ( $p > 0.01$  and  $p > 0.05$ , respectively) (Appendix B(iii)). A Waller-Duncan post-hoc test of nitrate\_A and calendar month shows that samples taken in December ( $\bar{x} = 1.23 \pm 0.38$  mg/kg,  $n = 11$ ) are statistically lower in nitrate than those taken in June, July, August, and October ( $\bar{x} = 6.81 \pm 1.19$  mg/kg,  $n = 33, 20, 24$  and  $35$ , respectively). However, nitrate\_A concentrations of soils sampled in January, February, March, April, May, September, and November are not distinctly different ( $\bar{x} = 4.85 \pm 1.11$ ,  $n = 18, 14, 35, 9$  and  $17$ ) (Waller-Duncan harmonic mean sample size = 16.00, MSE = 26.76). These results indicate that nitrate\_A concentrations were statistically higher in soil samples taken in the summer and early fall (June, July, August, and October), than those samples taken in December. This suggests that lower reported nitrate concentrations from soils sampled in December may not report reduced soil nitrate concentrations if samples were instead taken during the summer months (Table 8, Figure 9). It should be noted that the  $n$  of samples from September is low relative to the  $n$  from other months, which may explain the lack of placement in the homogenous group of means with samples taken in June, July, August, and October. In examining the scatter plots and linear regression of nitrate\_A relative to temperature and precipitation, there is a weak negative correlation in nitrate\_A with increasing precipitation, and a weak positive correlation in nitrate\_A with increasing temperature. However, both plots report poor goodness-of-fit ( $R^2 = 0.0173$  and  $0.0366$ , respectively) (Figure 10).

Continuously high soil moisture typically tends to favor low-oxygen soil conditions. Low-oxygen and low-temperature soil conditions inhibit microbial activity, as well as the nutrient and water absorption capacity of plants (Nyle C. Brady, 1984). Nitrate mineralization and immobilization by the soil’s microbial community would therefore also be inhibited in low-temperature and low-oxygen conditions, resulting in lower soil nitrate concentrations. Given that Washington soils are typically drier and warmer in summer months, wetter and colder in winter months, and more moderate in spring and autumn, climate is a likely contributor to the trends we see in the nitrate\_A concentrations of soil samples, and may have the potential to confound trends in soil nitrate concentrations from biosolids applications. Therefore, in future management of biosolids applications and soil monitoring, soil sampling should ideally be carried out during more temperate climate periods (i.e. spring and autumn months).

Sample Month	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
January	5.70	3.71	0.77	23	2.14	2.63	0.56	22
February	3.72	3.51	1.01	12	1.63	1.67	0.48	12
March	3.78	3.27	0.77	18	2.05	2.74	0.67	17
April	5.05	5.09	1.36	14	0.85	0.77	0.21	14
May	5.34	5.05	0.87	34	1.33	1.04	0.19	31
June	6.13	4.41	0.77	33	1.08	0.78	0.14	32
July	7.19	7.86	1.76	20	1.32	1.60	0.39	17
August	6.40	6.39	1.30	24	1.31	1.29	0.27	23
September	5.40	6.85	2.80	6	0.84	0.63	0.24	7
October	7.51	5.96	1.01	35	1.86	2.08	0.36	34
November	4.28	4.32	1.05	17	0.89	0.63	0.16	16
December	1.23	1.26	0.38	11	1.84	2.30	0.87	7

Table 8: Table of means reporting nitrate\_A and nitrate\_B concentrations for each calendar month soil samples were collected. ANOVA of nitrate\_A and nitrate\_B were not significant.

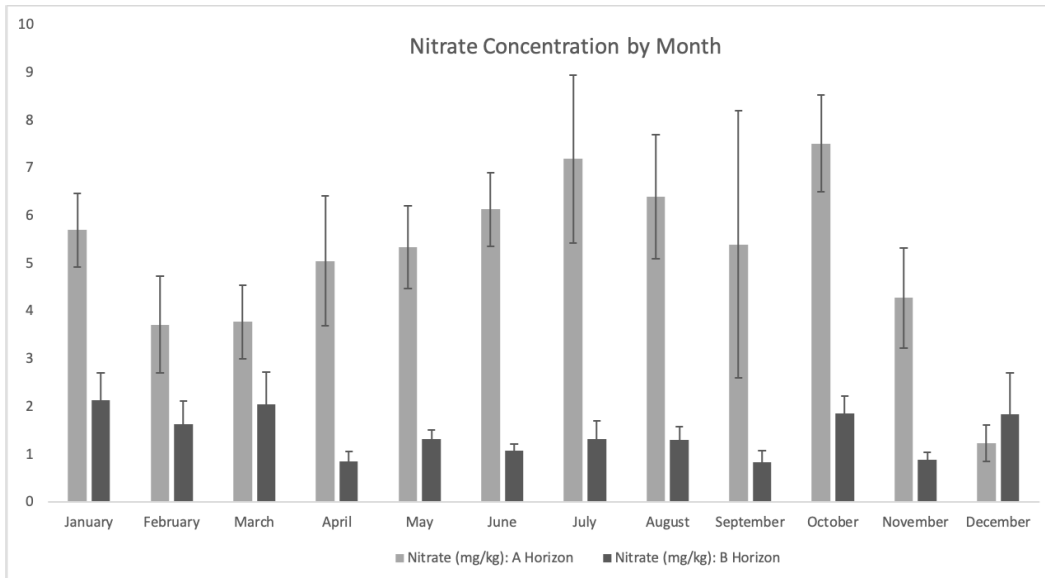


Figure 9: Bar graph of mean nitrate\_A and nitrate\_B concentration  $\pm$  SE for each calendar month soil samples were collected. ANOVA of nitrate\_A and nitrate\_B were not significant.

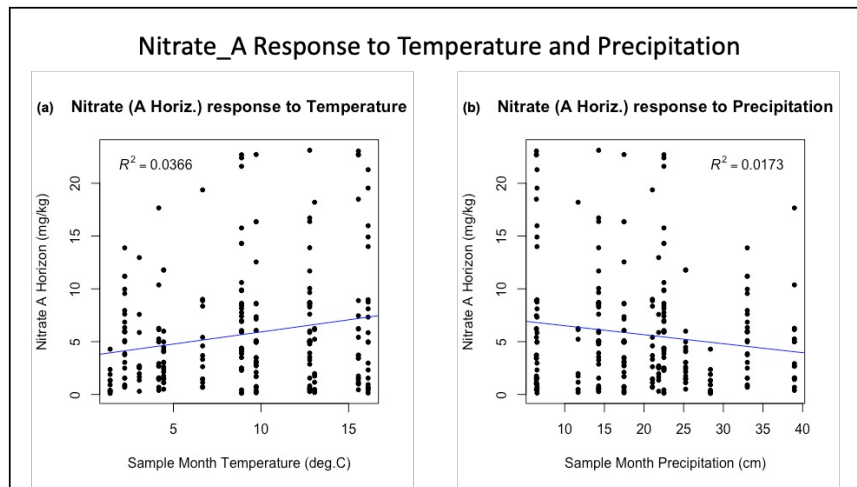


Figure 10: Scatter plot of the nitrate\_A concentration relative to the 20-year average temperature (a) and precipitation (b) of the month in which the soil sample was collected, with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of temperature and precipitation in the A horizon was significant ( $p > 0.01$  and  $p > 0.05$ , respectively).

### Sample Depth:

As part of the soil sampling process, the depth of both the A and B horizons is reported in site assessments. Depth of the A horizons varied across samples ranging from 1.27 to 43.2 cm ( $\bar{x} = 15.5 \pm 0.41$  cm). Depth of the B horizon ranged from 2.54 to 76.2 cm ( $\bar{x} = 46.4 \pm 0.64$  cm). Nitrate\_A was found to be significantly dependent on horizon depth ( $p < 0.001$ ) based on the results of a one-way ANOVA (Appendix B(iii)). However, inspection of the table of means of both nitrate\_A and  $\delta$  nitrate\_A revealed no clear trend (Appendix B(iv)). Scatter plots of both variables relative to horizon depth yield a poor goodness of fit ( $R^2 = 0.0279$  and  $0.00251$ , respectively) (Figure 11). Magesan et al. (1998) reported decreasing soil nitrate stock with increasing soil depth, independent of the application rate of sewage effluent in a New Zealand *Pinus radiata* plantation. Additionally, the soil nutrient effect of biosolids applications was shown to decrease with increasing depth. Nitrate stock at the 0–5 cm depth was 1.9 kg/ha higher in effluent-treated soils relative to untreated soils, while 15–20-cm-depth soils exhibited only a 1.1 kg/ha increase in nitrate stock (Magesan et al., 1998). Villa & Ryals (2021) also observed decreasing nitrate concentrations with depth independent of increasing rates of biosolids. While the method of soil sampling used in this study limits our ability to observe finer depth intervals, the general decreasing trend seen in Figure 6(a) is supported by the findings discussed here. Sampling a deeper A horizon would include soil material from deeper soil depths, where nitrate is typically lower and experiences less effect from biosolids amendments. Therefore, it is expected that in this study we would observe lower nitrate concentration with increasing horizon depth.

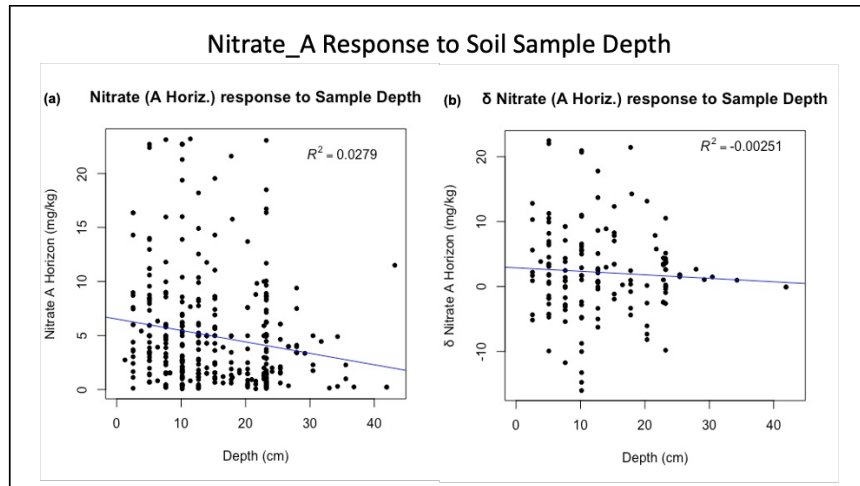


Figure 11: Scatter plot of nitrate\_A concentration (a) and  $\delta$  nitrate\_A concentration (b) relative to the depth at which each soil sample was taken, with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of both nitrate\_A and  $\delta$  nitrate\_A were not significant.

### Unit Area:

Nitrate\_A,  $\delta$  Nitrate\_A and  $\Delta$  Nitrate\_A were tested by one-way ANOVA for dependencies on the unit area. None were found, indicating that the size of the unit over which each biosolids treatment is applied does not influence the variability seen in the soil chemistry of the unit (Appendix B(iii) and Appendix B(iv)). The delineation of a unit's extent is drawn so as to minimize physical and ecological variability within and between units. This is done to standardize, as much as possible, the environmental parameters that affect the prescribed application rate and response of application in the units. Minimizing the environmental variability between units becomes increasingly difficult the larger the units are, as it becomes more likely that the unit extent contains a wider range of environmental factors such as changes in elevation and topography. These results indicate that the soil nitrate response to nitrate is independent of the unit size. The result of these ANOVAs is important for the future management of the plantation in that it points to the homogeneity of soils across units, regardless

of the size of the unit itself, and that the variability between units is being successfully minimized.

### **Sample Year:**

Nitrate\_A,  $\delta$  nitrate\_A and  $\Delta$  nitrate\_A were tested by one-way ANOVA for dependencies on the number of years between applications (i.e.  $\delta$  or  $\Delta$  soil metrics were tested against  $\delta$  years and  $\Delta$  years, respectively) and none were found to be significant. These findings indicate that the frequency of biosolids applications does not account for variability in nitrate\_A concentrations. If we were to have seen higher nitrate\_A concentrations with higher frequencies of applications (i.e. smaller  $\delta$  years and  $\Delta$  years), it may have indicated that the applications were too frequent, and that the nitrate from applied biosolids was not fully assimilated by the trees and understory. Instead, we see no correlation in nitrate\_A with the frequency of application, indicating that it is likely that the current frequency of application for the plantation is not exceeding the rate of nitrate assimilation, thereby lessening the likelihood of excess nitrate and nitrate leaching in treated soils. It should be noted, however, that these results may be influenced by a lack of observations for longer sampling intervals. The application intervals ranged from 1 to 25 years, but the vast majority of observations were between 1 and 7 years between applications ( $n = 119$ ), while the number of observations for application intervals between 8 and 25 years totaled only  $n = 34$  (Appendix B(iii) and Appendix B(iv)). This sampling bias may compromise the observed result.

## TOTAL CARBON

### **Application Number:**

One-way ANOVA of total C<sub>A</sub>, unlike nitrate<sub>A</sub>, was not significantly dependent on the application of biosolids when compared to the soil chemistry of the unit prior to any application of biosolids. The mean total carbon<sub>A</sub> concentration of treated soils is slightly higher than that of untreated soils ( $\bar{x} = 92.8 \pm 2.80$  g/kg and  $80.4 \pm 8.19$  g/kg, respectively) (Table 9, Figure 4b).

The application number was also not significant when tested against total C<sub>A</sub>,  $\delta$  total C<sub>A</sub> and  $\Delta$  total C<sub>A</sub>. Similarly, post-hoc Waller-Duncan tests revealed no separation of means by application number, indicating that total C<sub>A</sub> concentrations remain largely unchanged regardless of the number of applications on a given unit (Table 10). Collectively, these results show that successive biosolids applications are not measurably affecting the total carbon concentration of treated forest soils in the A horizon, neither incrementally nor over the course of the entire recorded history of applications (Table 11). These results contradict past findings on this plantation, where total carbon was found to be higher in soils that had been treated with biosolids (Leonard et al., 2021). This study did however find that carbon storage was higher in soils with lower initial organic matter content, and varies greatly within a small geographic area—factors that may confound any larger trends we might see in this study. In addition, Leonard et al. (2021) measured carbon and bulk density at discrete depth intervals, rather than by horizon, as was done in the present study. This difference in methodology and density of sampling may also explain the lack of any observable increase in total C<sub>A</sub> in this study. In addition, the absence of bulk density measures in this dataset may have masked any changes in soil C as a result of biosolids application.

Treated/Untreated	TotalC_A (g/kg)				TotalC_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Treated	92.8	27.6	2.8	97	30.7	12.8	1.3	97
Untreated	80.4	24.6	8.19	9	26.6	14.5	4.6	10

Table 9: Total carbon table of means comparing treated and untreated soils in the A and B horizon. ANOVA of Total Carbon\_A and Total Carbon\_B were not significant.

Application Number	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
0	80.4	24.6	8.20	9	26.6	14.5	4.60	10
1	91.4	28.5	4.25	45	30.6	13.3	1.98	45
2	96.6	27.3	4.91	31	33.0	12.9	2.32	31
3	77.6	20.1	5.81	12	23.9	8.31	2.40	12
4	108	25.1	8.36	9	32.6	13.4	4.45	9

Table 10: Table of means reporting Total C\_A and Total C\_B concentration following successive biosolids applications. ANOVA of Total C\_A and Total C\_B were not significant.

Grand Total Application Number	$\Delta$ Total C_A (g/kg)				$\Delta$ Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
2	17.4	26.8	89.3	9	2.37	15.8	5.01	10
3	27	57.1	28.6	4	13.1	5.26	2.63	4
4	-0.849	56.1	32.4	3	-10.6	18.7	10.8	3
5	-	-	-	0	-	-	-	0

Table 11: Table of means reporting the total change in Total C ( $\Delta$  Total C\_A and  $\Delta$  Total C\_B) concentration at the completion of the study period. The total change was calculated as the final carbon concentration minus the initial carbon concentration prior to biosolids application. ANOVA of  $\Delta$  Total C\_A and  $\Delta$  Total C\_B were not significant.

### **Application Rate:**

One-way ANOVA of total C<sub>A</sub>,  $\delta$  total C<sub>A</sub> and  $\Delta$  total C<sub>A</sub> yielded no significant dependencies on application rate, net application rate, and grand total application rate, respectively. Scatter plots of these variables had no clear trends and linear regressions showed weak goodness-of-fit: total C<sub>A</sub> vs. application rate  $R^2 = -0.008$ ,  $\delta$  total C<sub>A</sub> vs. application rate  $R^2 = -0.016$ ,  $\Delta$  total C<sub>A</sub> vs. grand total application rate  $R^2 = -0.027$  (Figure 12). As was seen in the total C<sub>A</sub> response to application number, there is little evidence to show that higher biosolids application rates result in higher or lower total carbon<sub>A</sub> concentrations in plantation soils. Similarly, in a long-term study conducted in a radiata pine plantation, Xue et al. reported significant increases in total C in surface soil following five successive biosolids applications at a standard and high application rate (300 kg N/ha and 600 kg N/ha, respectively) relative to control soils (0 kg N/ha), but no significant difference between the standard and high application rates (Xue et al., 2015). Similar to our own findings, these results show that total C response to varying biosolids application rates is often confounded by other factors, but may be evidenced instead by increases in tree growth and microbial biomass C, as was the case in Xue et al. (2015). The differences in soil series, depths of the A horizon, and the absence of bulk density measures are additional factors that complicate this analysis.

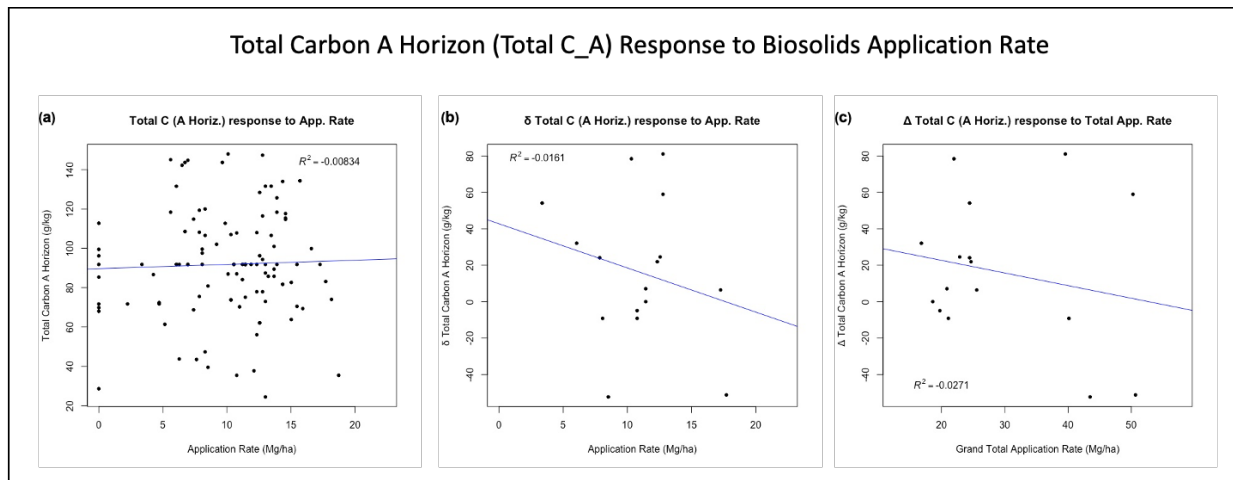


Figure 12: Scatter plots of total C\_A (a),  $\delta$  total C\_A (b) and  $\Delta$  total C\_A (c) concentrations relative to application rate and grand total application rate with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of total C\_A and  $\delta$  total C\_A and  $\Delta$  total C\_A were not significant.

### Soil Series:

One-way ANOVA of total C\_A,  $\delta$  total C\_A and  $\Delta$  total C\_A yielded no significant dependencies on soil series. SOC stock and SOM for each soil series (derived from the NRCS Soil Survey dataset) were plotted against the mean total C\_A measured within each observed soil series. While SOM had no identifiable correlative relationship with total C\_A, SOC stock had a slight inverse trend (i.e. we observed higher total C\_A in soil series with lower SOC soil). However, both SOC stock and SOM plots yielded poor goodness-of-fit to a linear regression ( $R^2 = -0.0168$  and  $-0.0108$ , respectively) (Appendix A(ii)). This conclusion is supported by the findings of Leonard et al. (2021), which showed significant increases in total C concentration following long-term biosolids applications within a plantation's Tokul series soils. Klaus series soils, a generally higher quality soil, exhibited no such pattern, indicating that the physical and chemical properties of the soils themselves affect the storage translocation of carbon in plantation soils.

**Unit Area:** One-way ANOVA of Total carbon\_A yielded no significant dependencies on unit area Appendix B(iii) and Appendix B(iv)). As was the case regarding nitrate\_A and total N\_A concentration, this finding points to the homogeneity between units, regardless of unit size.

**Sample Year:** One-way ANOVA of  $\delta$  and  $\Delta$  total C\_A yielded no significant dependencies on the  $\delta$  years and  $\Delta$  years, respectively (Appendix B(iii) and Appendix B(iv)). As was the case regarding soil nitrate\_A and total N\_A concentrations, this finding points to the likelihood that the current frequency of biosolids applications is not exceeding the rate of biosolids assimilation by soils.

## TOTAL NITROGEN

### **Application Number:**

One-way ANOVA showed that total N\_A was tending towards a significant dependence on the application of biosolids, when compared to the soil chemistry of the unit prior to any application of biosolids ( $p < 0.053$ ) (Appendix B(iii)). The mean total N\_A concentration of treated soils is slightly higher than that of untreated soils ( $\bar{x} = 4.40 \pm 0.10$  g/kg and  $3.66 \pm 0.50$  g/kg, respectively) (Table 12, Figure 4c). Similarly, in a 20-year study of agricultural biosolids application rates and effects in central California, Villa and Ryals (2021) found a significant change in total N in two-thirds of study sites in the 0–30 cm soil profile, and that sites with the

higher number of applications over a 20-year period observed the greater increase in total N. Sites that were applied annually saw an increase in total N from  $1 \pm 0.1$  to  $3 \pm 0.3$  after 20 years, while sites that were applied every 5 years saw no significant increase. In the present study, given that total N<sub>A</sub> was found to be higher in soils applied with biosolids (independent of application number or rate), it was tested by one-way ANOVA to determine if the number of applications was also significant (Appendix B(iii)). This test revealed no significant dependencies for total N<sub>A</sub>,  $\delta$  total N<sub>A</sub> and  $\Delta$  total N<sub>A</sub> on application number. Similarly, a post-hoc Waller-Duncan test revealed no separation of means by application number, indicating that total N<sub>A</sub> concentrations remain largely unchanged regardless of the number of applications, in total or incrementally, on a given unit (Table 13, Table 14). Collectively, these results show that while the treatment of soils seems to elevate total N<sub>A</sub> concentrations, successive biosolids applications are not incrementally increasing the total nitrogen concentration of treated forest soils. Rather, total N<sub>A</sub> increases after the very first biosolids application, and does not increase further in subsequent applications.

Treated/Untreated	TotalN <sub>A</sub> (g/kg)				TotalN <sub>B</sub> (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Treated	4.40	0.98	0.10	92	1.36	0.45	0.05	92
Untreated	3.66	1.41	0.50	8	1.21	0.63	0.22	8

Table 12: Total nitrogen table of means comparing treated and untreated soils in the A and B horizon. ANOVA of Total N<sub>A</sub> was trending towards significance ( $p < 0.053$ ). ANOVA of Total N<sub>B</sub> was not significant.

Application Number	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
0	3.66	0.65	0.50	8	1.21	0.63	0.22	8
1	4.37	0.78	0.15	43	1.28	0.39	0.06	43
2	4.48	1.15	0.22	28	1.53	0.50	0.10	28
3	4.18	0.97	0.22	12	1.21	0.25	0.07	12
4	4.53	1.42	0.22	9	1.46	0.62	0.21	9

Table 13: Table of means reporting Total N\_A and Total N\_B concentration following successive biosolids applications. ANOVA of Total N\_A and Total N\_B were not significant.

Grand Total Application Number	Δ Total N_A (g/kg)				Δ Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
2	1.27	1.36	0.481	8	0.0349	0.518	0.183	8
3	0.781	1.49	7.44	4	0.711	0.0947	0.0473	4
4	1.63	2.18	1.26	3	0.472	0.635	0.367	3
5	-	-	-	0	-	-	-	0

Table 14: Table of means reporting the total change in Total N (Δ Total N\_A and Δ Total N\_B) concentration at the completion of the study period. ANOVA of Δ Total N\_A and Δ Total N\_B were not significant.

### Application Rate:

One-way ANOVA of total N\_A, δ total N\_A and Δ total N\_A yielded no significant dependencies on application rate, net application rate, and grand total application rate, respectively. Scatter plots of these variables had no clear trends and linear regressions showed weak goodness-of-fit: total N\_A vs. application rate  $R^2 = -0.008$ , δ total N\_A vs. application rate  $R^2 = -0.08$ , Δ total N\_A vs. grand total application rate  $R^2 = -0.07$  (Figure 13). As was seen in the total N\_A response to application number, there is little evidence to show that higher biosolids application rates result in higher or lower total N\_A concentrations in plantation soils. (Leonard et al., 2021) also did not observe a relationship between total N and application rate. In Leonard

et. al, the 20–30 cm soil depth there was no measurable difference in % Total N of soils relative to control soils. Both the control and the treated Tokul soils measured  $3.2 \pm 1.3$  g/kg total N, and treated Klaus soils were only  $1.1 \pm 0.29$  g/kg higher in total N than control soils. Similarly, in Villa & Ryals (2021), researchers observed little relation in total N with application rate in the upper 30 cm of soil of approximately 30% of sites. However, the study did observe a significant trend in application rate in all sites for the 0–100 cm soil profile, highlighting the importance of sample depth in determining total N concentration, and the risk of underestimating the effects of biosolids on total N by sampling only shallow soils.

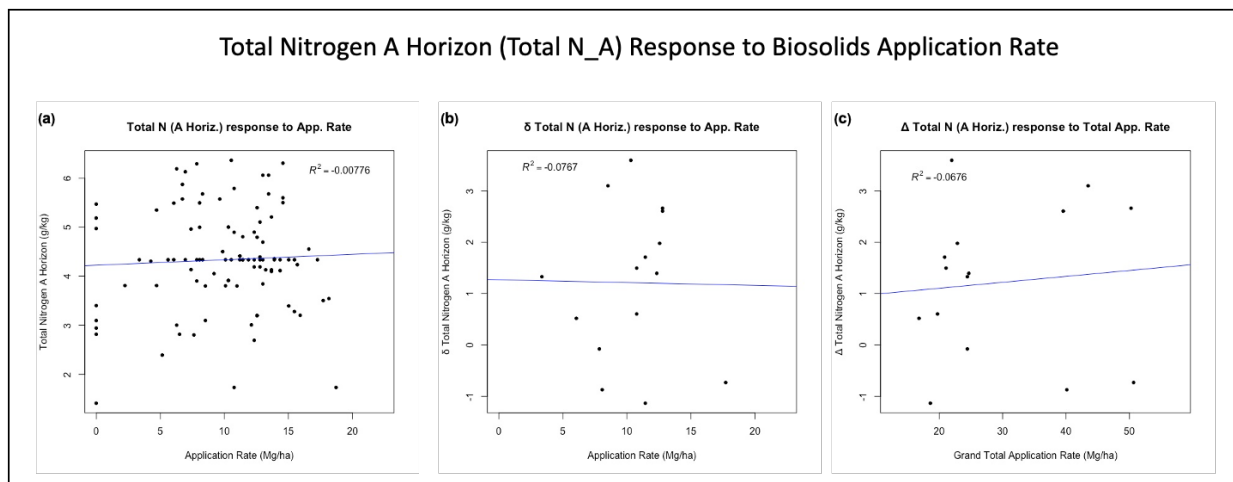


Figure 13: Scatter plots of total nitrogen\_A (a),  $\delta$  total nitrogen\_A (b) and  $\Delta$  total nitrogen\_A (c) concentrations relative to application rate and grand total application rate with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of total N\_A and  $\delta$  total N\_A and  $\Delta$  total N\_A were not significant.

### Soil Series:

Unlike nitrate\_A and total C\_A, one-way ANOVA of total N\_A was found to be significantly dependent on soil series ( $p < 0.05$ ). Subsequent ANOVAs of  $\delta$  total N\_A and  $\Delta$  total N\_A,

however, yielded no significant dependencies. The observed significance on total N<sub>A</sub> may simply have been due to differences in soil series properties rather than biosolids addition, so, to rule out this possibility, SOC stock and SOM for each soil series were plotted against the mean total N<sub>A</sub> measured within each observed soil series. Both SOC stock and SOM had a minor inverse trend with total N<sub>A</sub> (i.e. we observed higher total N<sub>A</sub> in soil series with lower SOM and SOC stock concentrations). However, both SOC stock and SOM plots yielded poor goodness-of-fit to a linear regression ( $R^2 = 0.0198$  and  $-0.0216$ , respectively) (Appendix B(iii)). If we had instead observed a significant trend and/or goodness-of-fit with SOC or SOM, the properties of the soil series themselves would likely have influenced the observed total N<sub>A</sub> concentrations rather than the biosolids applications. Rather, we see a poor correlation in total N<sub>A</sub> with the established soil series metrics, strengthening the conclusion that the statistical significance seen in total N<sub>A</sub> and soil series is evidence of the series having variable capacities of total N storage. This conclusion is supported by Leonard et al. (2021), where there were significant increases in total N concentration observed following long-term biosolids applications within a plantation's Tokul series soils. Klaus series soils, a generally higher quality soil, exhibited no such pattern, indicating that the physical and chemical properties of the soils themselves affect the storage and transport of biosolids-N in plantation soils.

**Unit Area:** One-way ANOVA of Total N<sub>A</sub> yielded no significant dependencies on unit area (Appendix B(iii) and Appendix B(iv)). As was the case regarding nitrate<sub>A</sub> and total C<sub>A</sub> concentration, this finding points to the homogeneity between units, regardless of unit size.

**Sample Year:** One-way ANOVA of  $\delta$  and  $\Delta$  Total N<sub>A</sub> yielded no significant dependencies on the  $\delta$  years and  $\Delta$  years, respectively (Appendix B(iii) and Appendix B(iv)). As was the case regarding soil nitrate<sub>A</sub> and total C<sub>A</sub> concentrations, this finding points to the likelihood that the current frequency of biosolids applications is not exceeding the rate of nitrogen assimilation by soils.

## C/N

One-way ANOVA of the ratio of total C to total N (C/N) revealed no significant dependencies in the A horizon. However, the B horizon was found to be significantly dependent on application rate and soil series ( $p < 0.05$  and  $p < 0.01$ , respectively). In comparing C/N in soils treated with biosolids, relative to control soils, our results are consistent with the trend observed in Xue et al. (2015). In the A horizon, we see a decrease in the C/N ratio of treated soils relative to control soils ( $\bar{x} = 21.4 \pm 0.53$  and  $22.3 \pm 1.25$ , respectively), while in the B horizon we see an increase in C/N relative to control soils ( $\bar{x} = 23.2 \pm 0.83$  and  $21.1 \pm 1.33$ , respectively) (Table 15). Xue et al. posited that C/N ratios in the B horizon may be related to increased root production and root C turnover as an effect of biosolids applications. However, in our study it should be noted that the sample size of the untreated soils is limited ( $n = 9$ ) relative to treated soil samples ( $n = 92$ ), which may affect these observed trends.

In examining the effect of application number on the C/N ratio of plantation soils, there appears to be no clear trend in either horizon with increasing application number or application rate (Table 16, Figure 14, Figure 15). In surface soils with a C/N ratio greater than 30, we would

expect to see amended nitrogen immobilized by soil microbial biomass. However, in soils where the C/N is below 20, mineralization is likely to occur. The C/N ratio of soils sampled in this study ranged between 17 and 24 in the A horizon, and it is likely that the rate of mineralization and immobilization will likely balance (Henry et al., 1999). Given that we see little change in C/N and nitrate with increasing application number, these observations provide further evidence that it is unlikely that nitrogen is being leached from the soil.

Treated/Untreated	C/N_A				C/N_B			
	n	SD	SE	Mean	n	SD	SE	Mean
Treated	92	5.05	0.53	21.4	92	7.93	0.83	23.2
Untreated	8	3.54	1.25	22.3	8	3.76	1.33	21.1

Table 15: C/N table of means comparing treated and untreated soils in the A and B horizon. ANOVA of C/N\_A and C/N\_B were not significant.

Application Number	C/N_A				C/N_B			
	n	SD	SE	Mean	n	SD	SE	Mean
0	8	3.54	1.25	22.3	8	3.76	1.33	21.07
1	43	3.61	0.55	21.5	43	8.39	1.28	24.56
2	28	7.05	1.33	21.9	28	7.41	1.40	22.3
3	12	3.10	0.89	18.4	12	5.58	1.61	19.8
4	9	4.54	1.51	23.7	9	9.31	3.10	24.18

Table 16: Table of means reporting C/N\_A and C/N\_B concentration following successive biosolids applications. ANOVA of C/N\_A C/N\_B was not significant.

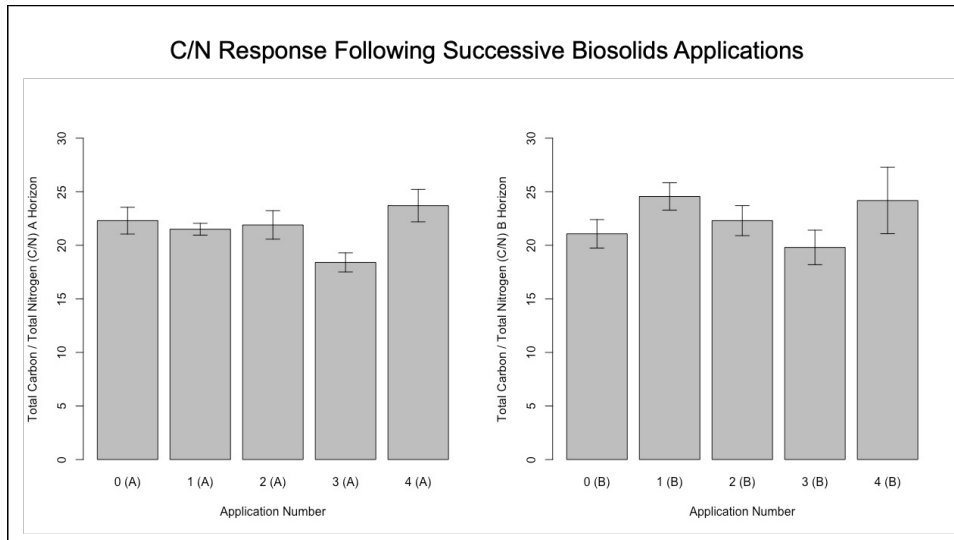


Figure 14: C/N for “(A)” and “(B)” soil horizons following successive biosolids applications. Error bars show standard error.

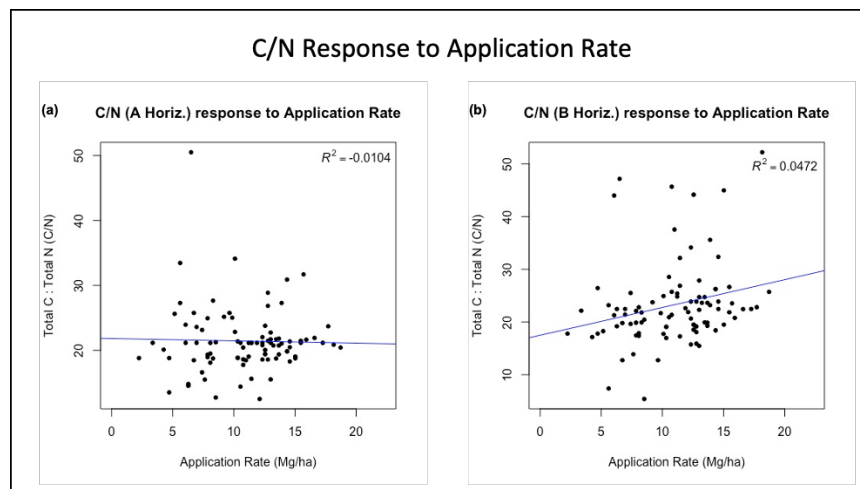


Figure 15: Scatter plots of C/N\_A (a) and C/N\_b (b) relative to application rate with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of total C/N\_A was not significant. ANOVA of C/N\_B was significant ( $p < 0.05$ ).

## B. B HORIZON

### NITRATE

#### **Application Number:**

A one-way ANOVA showed that nitrate\_B was significantly dependent on the application of biosolids, when compared to the soil chemistry of the unit prior to any application of biosolids ( $p < 0.05$ ) (Appendix B(iii)). The mean nitrate\_B concentration of treated soils was higher than that of untreated soils ( $\bar{x} = 1.58 \pm 0.11$  mg/kg and  $1.15 \pm 0.10$  mg/kg, respectively) (Table 3), indicating that the application of biosolids increased nitrate concentrations in soils (Figure 4a). It should be noted that while this increase was statistically significant, it is a small enough increase to have minimal environmental impact. Given that nitrate\_B was found to be higher in soils applied with biosolids (independent of application number or rate), it was subsequently tested by one-way ANOVA to determine if the number of applications was also significant. This test revealed no significant dependencies on application number for nitrate\_B,  $\delta$  nitrate\_B and  $\Delta$  nitrate\_B. Post-hoc Waller-Duncan tests showed that the mean soil nitrate\_B is statistically lower in soils that received 4 applications than the mean of soils that received 2 applications ( $\bar{x} = 0.805 \pm 0.16$  mg/kg and  $1.86 \pm 0.20$  mg/kg, respectively). Soils that received 0, 1 or 3 applications are not statistically different from each other ( $\bar{x} = 1.36 \pm 0.19$  mg/kg) (Waller-Duncan harmonic mean sample size = 22.510, MSE = 2.280). Review of the table of means and bar plot (Table 4, Figure 5) indicates that the nitrate\_B concentration of soils that received 1, 2 or 3 applications are higher than soils that were not treated at all ( $\bar{x} = 1.61 \pm 0.21$  mg/kg and  $1.15 \pm$

0.98 mg/kg, respectively). However, soil nitrate in soils that received 3 applications are not significantly higher than in soils that only received 2 applications ( $\bar{x} = 1.75 \pm 0.45$  mg/kg and  $1.86 \pm 0.20$  mg/kg, respectively). These results are of particular importance in that, while treated soils are statistically higher in nitrate\_B than untreated soils, there appears to be no linear trend in nitrate\_B with successive applications, or cumulatively (Table 5). Similarly, Cogger et al. (2013) saw little relationship between application number and nitrate concentrations at the 30–60 cm soil depth (which roughly corresponds to the depth of the B horizon in this study). In an agricultural trial of biosolids application rates, soil nitrate did not have any clear increasing or decreasing trend with successive applications at a range of application rates between 24 and 45 Mg/ha. This demonstrates that nitrate in the A horizon is not leaching into the B horizon and/or that nitrate is not accumulating over time with more applications. If this were the case, the nitrate concentration would be seen to increase with more applications. Instead, these results point to the likely removal of nitrate from the A horizon before it reaches the B, and the reduced likelihood of nitrate leaching from the soil system.

**Application Rate:** Subsequently, the effect of the biosolids application rate on nitrate\_B was investigated. A one-way ANOVA of nitrate\_B showed a statistical dependence on application rate ( $p < 0.05$ ) (Appendix B(iii)), while the summary table of means showed that low application rates (0–5.6 Mg/ha) yield lower concentrations of nitrate\_B ( $\bar{x} = 1.15 \pm 0.09$  mg/kg), while higher application rates (>5.6 Mg/ha) yield higher nitrate\_B concentrations ( $\bar{x} = 1.60 \pm 0.24$  mg/kg) (Table 6). These results are further illustrated by scatter plot and linear regression, which show no correlation between nitrate\_B and application rate (Figure 16a) and a low goodness-of-fit ( $R^2 = 0.0149$ ). These indicate that the relationship between nitrate\_B and application rate is

not likely to be linear or directly correlated, and rather has one or more interacting variables that influence the soil's geochemical response to biosolid applications.

As was the case in  $\delta$  nitrate\_A, a one-way ANOVA indicated that  $\delta$  nitrate\_B was found to be significantly dependent on application rate ( $p < 0.01$ ). However, a regression analysis showed no correlation and low goodness-of-fit ( $R^2 = 0.0473$ ) (Figure 16b). A one-way ANOVA showed no statistical dependence of  $\Delta$  nitrate\_B on the grand total application rate, and the linear regression and scatter plot revealed that the  $\Delta$  nitrate\_B had essentially no correlation, positive or negative, to the total application rate with a very low goodness-of-fit ( $R^2 = -0.0075$ ) (Figure 16c).

Cumulatively, these results show the nitrate concentration in the A and B horizon of the soils had no linear correlation to the nitrate concentrations measured in the A or B horizons prior to applications, incrementally ( $\delta$ ), or over the course of the application period studied ( $\Delta$ ).

Similarly, Cogger et al. (2013), found only marginally higher nitrate concentrations (4.53 mg/kg) in soils treated with higher biosolids applications rates (45 Mg/ha) relative to soils treated at significantly lower application rates (3.45 mg/kg at an application rate of 24 Mg/ha) (Cogger et al., 2013). Additionally, it should be noted that the study conducted in Cogger et al. was undertaken using much higher application rates than were used in this study, where the majority of units received between 11 and 16 Mg/ha of biosolids in a single application. Additionally, Cogger et al. was conducted in agricultural soils where crops were periodically harvested (and soils were more often disturbed than in plantation soils). Higher application rates and frequent soil disturbances would increase the likelihood of observing high soil nitrate concentrations if biosolids were being applied in excess. In overapplied soils, one would expect higher application rates to correlate with higher nitrate\_A and nitrate\_B concentrations. Instead, in both Cogger et al. and the present study, we see that there is no strong positive correlation in nitrate\_A or

nitrate\_B with the application rates being used throughout the recorded history of applications. This suggests that the nitrate being applied is being taken up by the forest system. This data could also be interpreted as an indication that the plantation may be able to accommodate higher biosolids application rates without increasing the risk of overapplication and nitrate leaching.

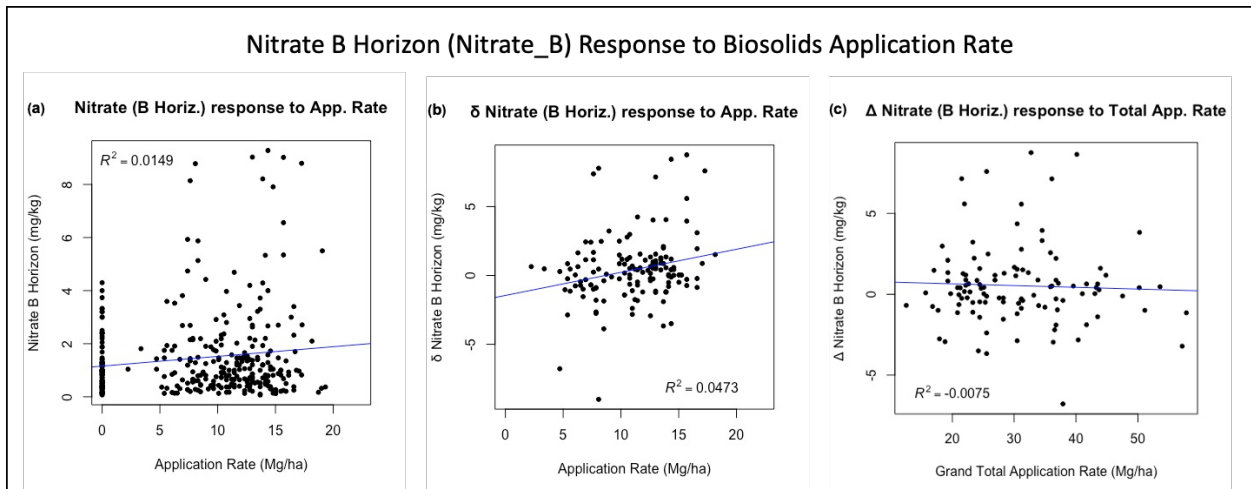


Figure 16: Scatter plots of nitrate\_B (a),  $\delta$  nitrate\_B (b) and  $\Delta$  nitrate\_B (c) concentrations relative to application rate and grand total application rate with linear regression in blue and  $R^2$  correlation coefficient. ANOVA of nitrate\_B and  $\delta$  nitrate\_B were found to be significant ( $p < 0.05$  and  $p < 0.01$ , respectively). ANOVA of  $\Delta$  nitrate\_B was not significant.

**Soil Series:** Nitrate\_B concentration,  $\delta$  nitrate\_B and  $\Delta$  nitrate\_B were tested against soil series by one-way ANOVA and none were found to be significant. Subsequently, the NRCS soil series standards for soil organic carbon (SOC) stock (kg/m<sup>2</sup>) and soil organic matter (SOM) (%) were identified for each of the soil series observed in the plantation (Appendix B(ii)). These values were plotted against the experimental mean nitrate\_B concentration for each observed soil series (Table 6, Figure 7). While SOM exhibited a weakly inverse trend and moderate goodness-of-fit

( $R^2 = 0.23$ ), SOC stock had no obvious trend and very poor goodness-of-fit ( $R^2 = -0.0427$ ), when plotted against nitrate\_A (Figure 17).

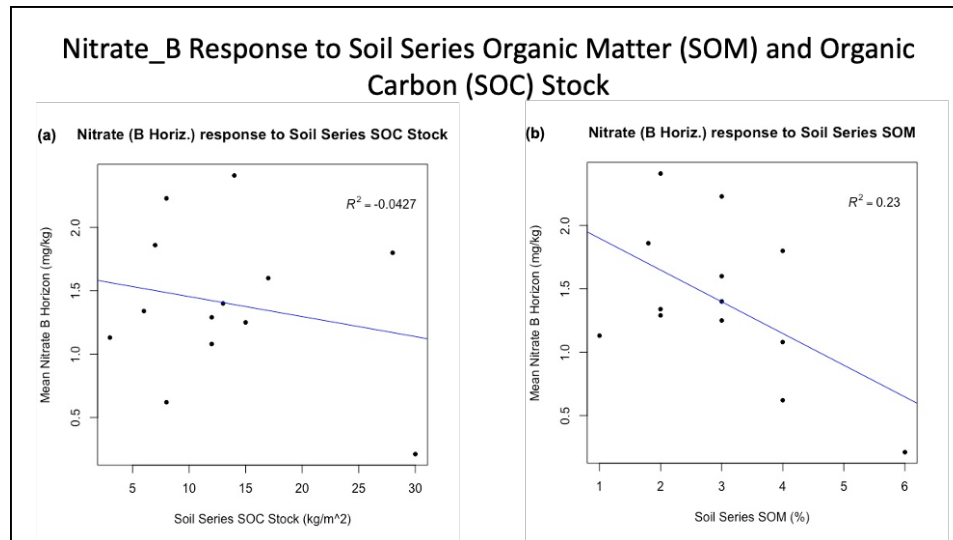


Figure 17: Scatter plot of the mean nitrate\_B concentration of each observed soil series against the soil organic carbon (SOC) stock (kg/m<sup>2</sup>) (a) and soil organic matter (SOM)(%) (b) concentrations corresponding to the NRCS Soil Survey series metrics with linear regression in blue and  $R^2$  correlation coefficient.

**Climate:** Nitrate\_B concentration was also tested against the month of soil sampling and several climate variables by one-way ANOVA to identify any dependence on the time of year and climate in which the soils were sampled. Nitrate\_B concentration was trending towards a significant dependence on temperature ( $p < 0.061$ ) but not on sample month or precipitation. Subsequently, the results of a post-hoc Waller-Duncan test illustrated that the mean nitrate\_B concentrations were not significantly different from month to month. Scatter plots of nitrate\_B against the mean sample month temperature and precipitation yielded no clear trend and poor goodness-of-fit ( $R^2 = 0.011$  and  $0.004$  respectively) (Figure 18). This lack of climatic response seen in nitrate\_B is likely due to the fact that the B horizon is largely insulated by the A horizon

to temperature changes. The upper 15 cm of soil is warmer than the air at every season of the year, while the subsoil is warmer in autumn but cooler in spring and summer because of its insulation from the surface and lag in conduction (Nyle C. Brady, 1984). However, while seasonal temperature variation has little impact on the B horizon, seasonal changes in precipitation can cause seasonal transport of dissolved nutrients from the A horizon into the B horizon. The absence of any seasonal increase in nitrate in the B horizon in our data is further evidence that nitrate in applied biosolids has largely remained in the A horizon, rather than leached out.

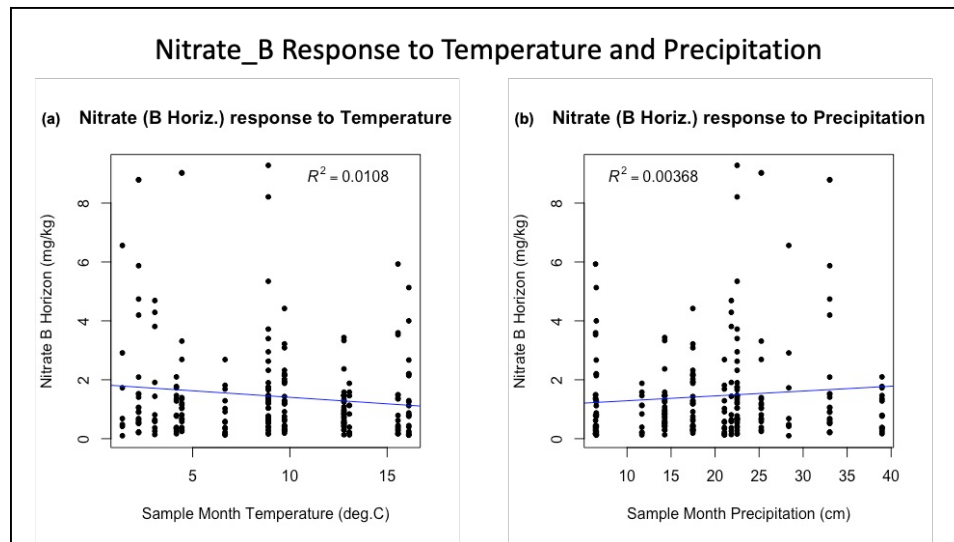


Figure 18: Scatter plot of the nitrate\_B concentration relative to the 20-year average temperature (a) and precipitation (b) of the month in which the soil sample was collected, with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of temperature was trending towards significance ( $p < 0.061$ ). ANOVA of precipitation was not significant.

**Unit Area:** One-way ANOVA of nitrate\_B yielded no significant dependencies on unit area (Appendix B(iii) and Appendix B(iv)). As was the case regarding nitrate\_A concentration, this finding points to the homogeneity between units, regardless of unit size.

**Sample Year:** One-way ANOVA of nitrate\_B yielded no significant dependencies on the  $\delta$  years and  $\Delta$  years, respectively (Appendix B(iii) and Appendix B(iv)). As was the case regarding soil nitrate\_A concentrations, this finding points to the likelihood that the current frequency of biosolids applications is not exceeding the rate of nitrate assimilation by soils.

## TOTAL CARBON

### **Application Number:**

One-way ANOVA of total C\_B, unlike nitrate\_B, was not significantly dependent on the application of biosolids, when compared to the total C concentrations of the unit prior to any application of biosolids. The mean total C\_B concentration of treated soils is slightly higher than that of untreated soils ( $\bar{x} = 30.7 \pm 1.30$  g/kg and  $26.6 \pm 4.60$  g/kg, respectively) (Table 9, Figure 4b). The application number was also not significant when tested against total C\_B,  $\delta$  total C\_B and  $\Delta$  total C\_B. Similarly, post-hoc Waller-Duncan tests revealed no separation of means by application number, indicating that total C\_B concentrations remain largely unchanged regardless of the number of applications on a given unit (Table 10). Collectively, these results

show that successive biosolids applications are not measurably affecting the total carbon concentration of treated forest soils in the B horizon, neither incrementally, nor over the course of the observational period.

**Application Rate:** One-way ANOVA of total C<sub>B</sub>,  $\delta$  total C<sub>B</sub> and  $\Delta$  total C<sub>B</sub> yielded no significant dependencies on application rate, net application rate, and grand total application rate, respectively. Scatter plots of these variables had no clear trends and linear regressions showed weak goodness-of-fit: total C<sub>B</sub> vs. application rate  $R^2= 0.0114$ ,  $\delta$  total C<sub>B</sub> vs. application rate  $R^2= -0.0658$ ,  $\Delta$  total C<sub>B</sub> vs. grand total application rate  $R^2= -0.0588$  (Figure 19). As was seen in the total C<sub>B</sub> response to application number, there is little evidence to show that higher biosolids application rates result in higher or lower total C<sub>B</sub> concentrations in plantation soils. Ouimet et al. (2015) observed similar trends in total C. In this long-term plantation study of biosolids application rates, at the 0–5cm soil depth, total C was positively correlated with application rate ( $58 \pm 5$  g/kg at the low application rate of 130 m<sup>3</sup>/ha, increasing to  $64 \pm 6$  g/kg and  $71 \pm 9$  g/kg at the medium and high application rates of 200 and 400 m<sup>3</sup>/ha, respectively). However, as was seen in the present study, Ouimet et al. (2015) did not find a significant change in total C at lower soil depths with increasing application rates ( $23 \pm 3$  g/kg at the 10–20cm depth for all application rates, and between 13 and  $14 \pm 4$  g/kg at the 20–40 cm soil depth at all application rates).

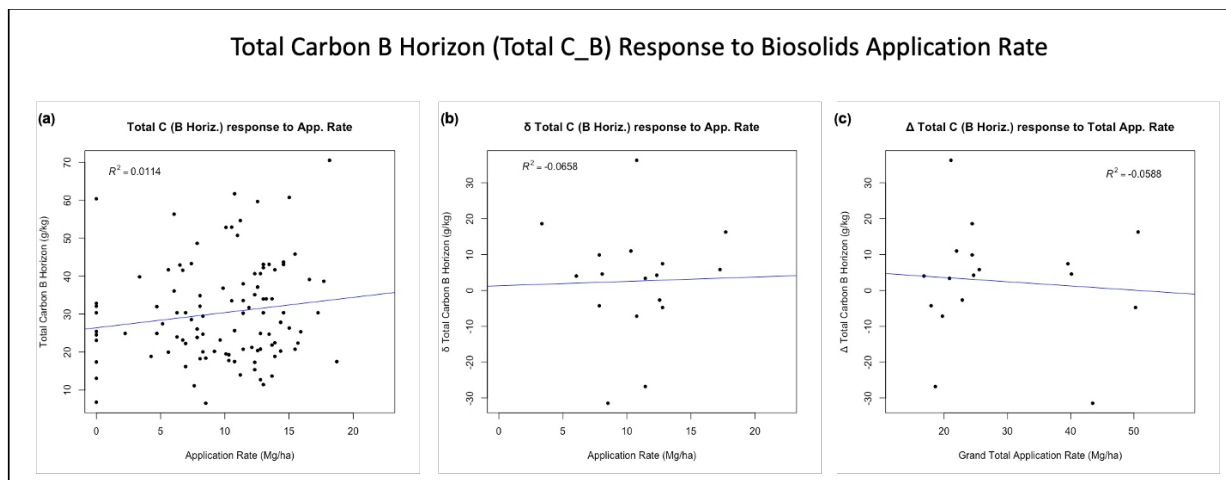


Figure 19: Scatter plots of total carbon<sub>B</sub> (a),  $\delta$  total carbon<sub>B</sub> (b) and  $\Delta$  total carbon<sub>B</sub> (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of total carbon<sub>B</sub>,  $\delta$  total carbon<sub>B</sub>, and  $\Delta$  total carbon<sub>B</sub> was not significant.

**Soil Series:** Total C<sub>B</sub> concentration,  $\delta$  total C<sub>B</sub> and  $\Delta$  total C<sub>B</sub> were tested against soil series by one-way ANOVA, but only total C<sub>B</sub> was found to be significant ( $p < 0.001$ ). Subsequently, the NRCS soil series standards for soil organic carbon (SOC) stock (kg/m<sup>2</sup>) and soil organic matter (SOM) (%) were identified for each of the soil series observed in the plantation (Appendix B(ii)). These values were plotted against the experimental mean total C<sub>B</sub> concentration for each observed soil series (Appendix C(iv)). Scatter plots of SOC stock and SOM exhibited no obvious trends and very poor goodness-of-fit ( $R^2 = -0.0651$  and  $-0.0739$  respectively), when plotted against total C<sub>B</sub> (Appendix A(ii)).

**Unit Area:** One-way ANOVA of Total C<sub>B</sub> yielded no significant dependencies on unit area (Appendix B(iii) and Appendix B(iv)). As was the case regarding nitrate<sub>B</sub> and total N<sub>B</sub> concentration, this finding points to the homogeneity between units, regardless of unit size.

**Sample Year:** One-way ANOVA of  $\delta$  and  $\Delta$  Total C<sub>B</sub> yielded no significant dependencies on the  $\delta$  years and  $\Delta$  years, respectively (Appendix B(iii) and Appendix B(iv)). As was the case

regarding soil nitrate<sub>B</sub> and total N<sub>B</sub> concentrations, this finding points to the likelihood that the current frequency of biosolids applications is not exceeding the rate of biosolids assimilation by soils.

## TOTAL NITROGEN

### **Application Number:**

One-way ANOVA of total N<sub>B</sub>, unlike nitrate<sub>B</sub>, was not significantly dependent on the application of biosolids, when compared to the total N concentrations in units prior to any application of biosolids. The mean total N<sub>B</sub> concentration of treated soils is slightly higher than that of untreated soils ( $\bar{x} = 1.36 \pm 0.47$  g/kg and  $1.21 \pm 0.22$  g/kg, respectively) (Table 12, Figure 4c). The application number was also not significant when tested against total N<sub>B</sub>,  $\delta$  total N<sub>B</sub> and  $\Delta$  total N<sub>B</sub>. Similarly, post-hoc Waller-Duncan tests revealed no separation of means by application number, indicating that total N<sub>B</sub> concentrations remain largely unchanged regardless of the number of applications on a given unit (Table 13). Collectively, these results show that successive biosolids applications are not measurably affecting the total nitrogen concentration of treated forest soils in the B horizon, neither incrementally, nor over the course of the entire recorded history of applications.

### **Application Rate:**

One-way ANOVA of total N<sub>B</sub>,  $\delta$  total N<sub>B</sub> and  $\Delta$  total N<sub>B</sub> yielded no significant dependencies on application rate, net application rate and grand total application rate, respectively. Scatter plots of these variables had no clear trends and linear regressions showed poor goodness-of-fit: total N<sub>B</sub> vs. application rate  $R^2= 0.00785$ ,  $\delta$  total N<sub>B</sub> vs. application rate  $R^2= -0.0702$ ,  $\Delta$  total N<sub>B</sub> vs. grand total application rate  $R^2= 0.128$  (Figure 20). As was seen in the total N<sub>B</sub> response to application number, there is little evidence to show that higher biosolids application rates result in any changes in the total N<sub>B</sub> concentrations in plantation soils. Ouimet et al. (2015) saw a similar lack of correlation in total N with application rate at lower depths. At the 0–5 cm soil depth, total N was found to be marginally higher, by 0.4 m/kg, following high biosolids application rate relative to the low application rate (130 and 400 m<sup>3</sup>/ha, respectively). Total N was entirely unchanged at the 20–40 cm soil depth with a total N concentration of  $0.9 \pm 0.3$  g/kg at all application rates.

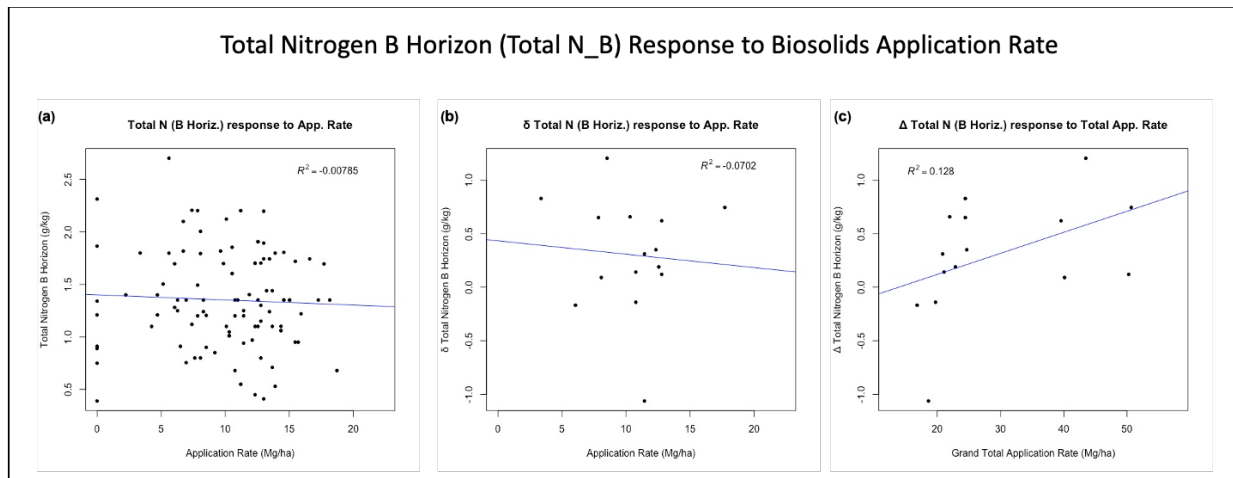


Figure 20: Scatter plots of total nitrogen<sub>B</sub> (a), δ total nitrogen<sub>B</sub> (b) and Δ total nitrogen<sub>B</sub> (c) concentrations relative to application rate and grand total application rate with linear regression in blue and R<sup>2</sup> correlation coefficient. ANOVA of total nitrogen<sub>B</sub>, δ total nitrogen<sub>B</sub>, and Δ total nitrogen<sub>B</sub> was not significant.

**Soil Series:** Total N<sub>B</sub> concentration, δ total N<sub>B</sub> and Δ total N<sub>B</sub> were tested against soil series by one-way ANOVA, but only total N<sub>B</sub> was found to be significant ( $p < 0.01$ ). Subsequently, the NRCS soil series standards for soil organic carbon (SOC) stock (kg/m<sup>2</sup>) and soil organic matter (SOM) (%) were identified for each of the soil series observed in the plantation (Appendix B(ii)). These values were plotted against the experimental mean total N<sub>B</sub> concentration for each observed soil series. Scatter plots of SOC stock and SOM exhibited a weakly inverse trend and poor goodness-of-fit ( $R^2 = 0.0983$  and  $0.199$  respectively), when plotted against total C<sub>B</sub> (Appendix A(ii)).

**Unit Area:** One-way ANOVA of Total N<sub>B</sub> yielded no significant dependencies on unit area (Appendix B(iii) and Appendix B(iv)). As was the case regarding nitrate<sub>B</sub> concentration, this finding points to the homogeneity between units, regardless of unit size.

**Sample Year:** One-way ANOVA of  $\delta$  and  $\Delta$  Total N<sub>B</sub> yielded no significant dependencies on the  $\delta$  years and  $\Delta$  years, respectively (Appendix B(iii) and Appendix B(iv)). As was the case regarding soil nitrate<sub>B</sub> concentrations, this finding points to the likelihood that the current frequency of biosolids applications is not exceeding the rate of nitrogen assimilation by soils.

## IV. CONCLUSIONS

This study has found there is little evidence to suggest that biosolids applications pose a risk to groundwater quality at the rates and frequencies used during the 30-year study period. While the application of biosolids increased soil nitrate concentration relative to untreated soils, nitrate\_A concentration was found to increase as the number of applications increased, but nitrate\_A had no linear relationship to the application rate in the A horizon. Nor did nitrate\_B have any linear correlation with application number or rate. If biosolids were being applied in excess, one would expect to find the added nitrate moving from the A horizon into the B horizon, and a gradual increase in both with increasing application number and rate.

Additionally, the results of this study demonstrate that the nitrate concentration in the A and B horizons showed little change in concentration incrementally ( $\delta$ , i.e. between applications), or over the course of the application period studied ( $\Delta$ , i.e.  $t_0$  to  $t_n$ , where  $n$  is the total number of applications a given unit received during the study period). The study also did not find any statistically significant incremental or total change in total C or total N. If biosolids application rates were exceeding the assimilative capacity of the plantation, we would have expected to observe increases in these soil metrics over time. Collectively, these results suggest that the nitrate being added to plantation soils in biosolids applications is largely being taken up by the forest system, rather than being lost to leaching.

This study also found evidence to support the conclusion that the plantation may be able to accommodate more frequent applications without increasing the risk of overapplication and nitrate leaching. None of the soil metrics evaluated were statistically dependent on the  $\delta$  years,

indicating that the soils response to the biosolids applied had no dependence on the amount of time that passed between applications ( $\delta$  years). If applications were too frequent and did not allow enough time for the complete assimilation of nitrogen, nitrogen would accumulate as nitrate or total N, and would be statistically dependent on the amount of time between applications, with higher concentrations observed with more frequent applications (i.e. lower  $\delta$  years).

This study also observed that soil series have variable capacities for the storage and transport of nitrate. SOC and SOM moderately inversely correlated with soil nitrate concentrations, most notably in the A horizon. However, the specific parameters that influence these dynamics are not clear. Factors such as drainage rate and soil pH were not included in this study—parameters known to affect the microbial activity required in nitrification and mineralization of nitrogen. Without a better understanding of these soil characteristics in each unit of the study site, it is difficult to come to any conclusions on the mechanisms by which soil series influences nitrogen dynamics in the plantation. Including soil pH measurements in future analyses may provide useful insight for developing an answer to this question.

Finally, this study found that climate can have a significant effect on soil nitrate concentrations. Periods of high precipitation and cold temperatures can inhibit the mineralization and immobilization of nitrogen by the soil's microbial community, resulting in lower soil nitrate concentrations than would be found in drier and warmer conditions. In order to prevent obtaining samples with artificially low nitrate concentrations, thus potentially masking excess increases in nitrate, this study indicates that sampling should be carried out during the more temperate periods in spring and autumn months.

These findings point to several possibilities for future studies. Monitoring surface and groundwater nitrate concentrations directly, and over a long period of time at variable application rates, would provide more insight into the nitrogen-dynamics of land-applied biosolids.

Additionally, the use of  $^{15}\text{N}$  isotopes has proved to be an especially useful tool in developing a mass-balance of nitrogen, and quantifying the movement of biosolids-N through the plant-soil system. This tool would be of particular use in furthering our understanding of how variable application rates of biosolids-N change these dynamics. Currently, the determination of biosolids application rates uses estimate

es for mineralization and denitrification rates, as well as plant and understory N-requirements.

Developing a detailed mass balance of the plantation's nitrogen cycling would provide more exact values for these parameters, increase the accuracy of the application rates calculated for the site, and further reduce the risk of overapplication of nitrate and groundwater contamination.

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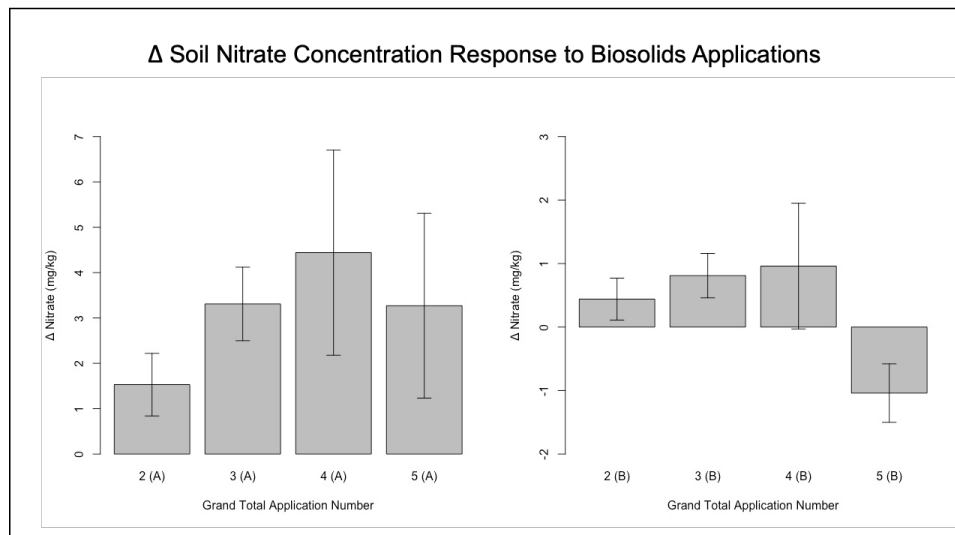
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<https://doi.org/10.1016/j.scitotenv.2015.05.096>

# VI. APPENDIX

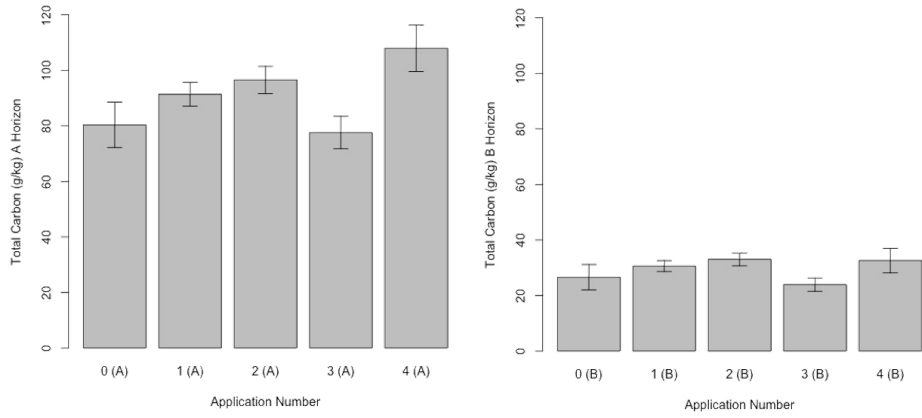
## A. FIGURES

### i. BAR CHARTS

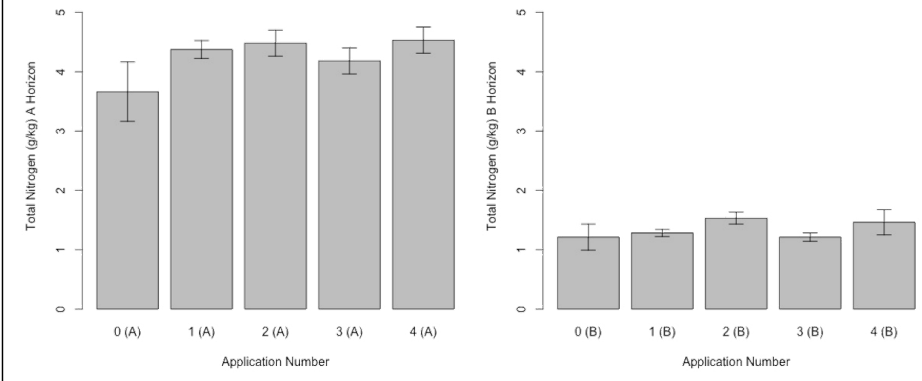
#### Application Number



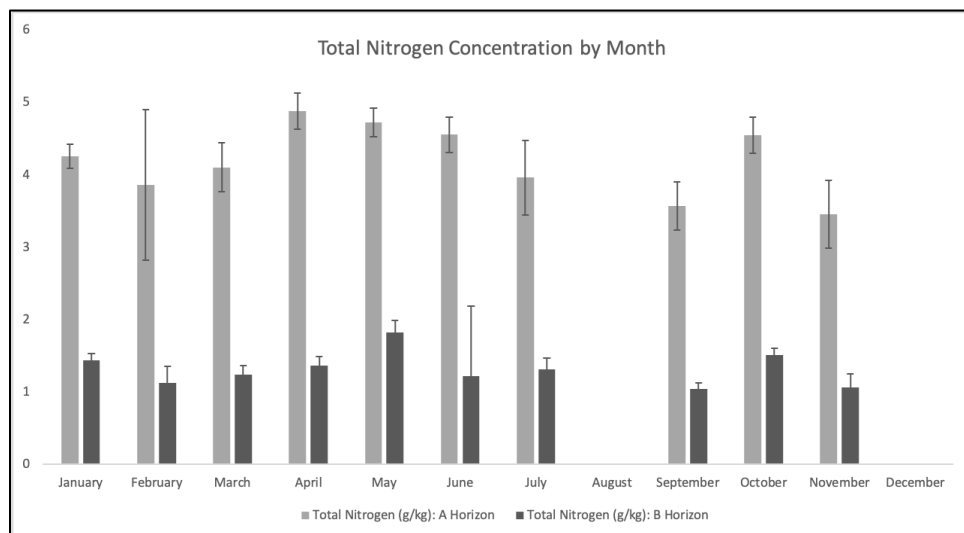
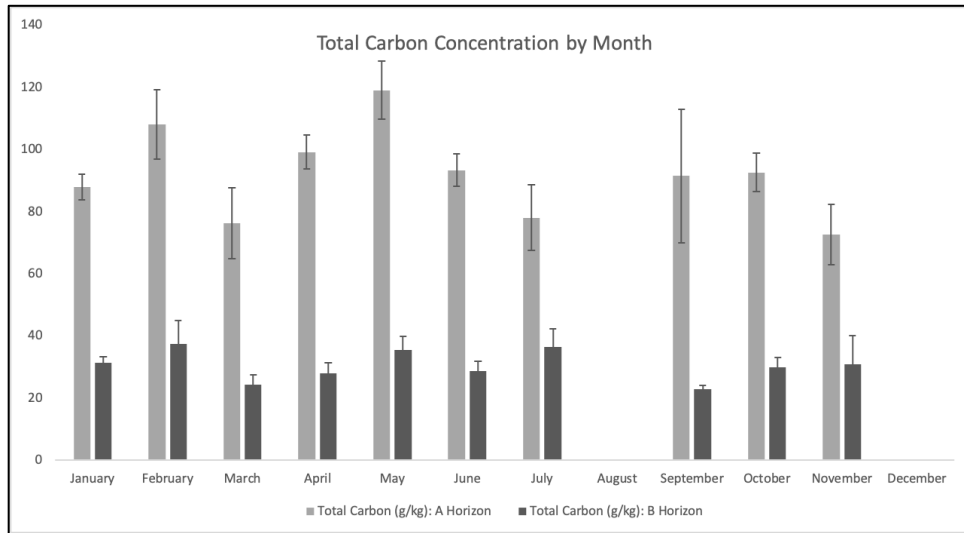
### Soil Total Carbon Concentration Following Successive Biosolids Applications



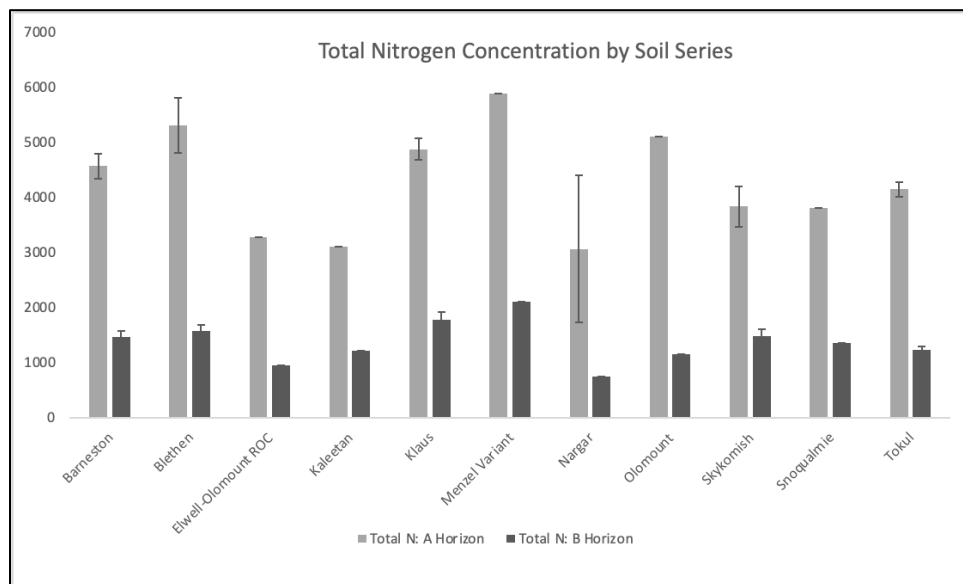
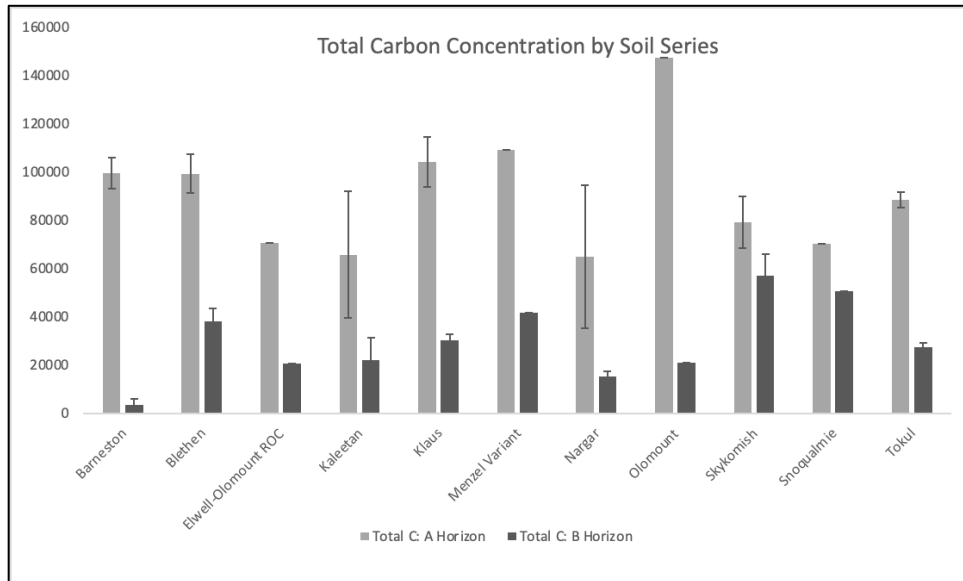
### Soil Total Nitrogen Concentration Following Successive Biosolids Applications



## Month/Climate

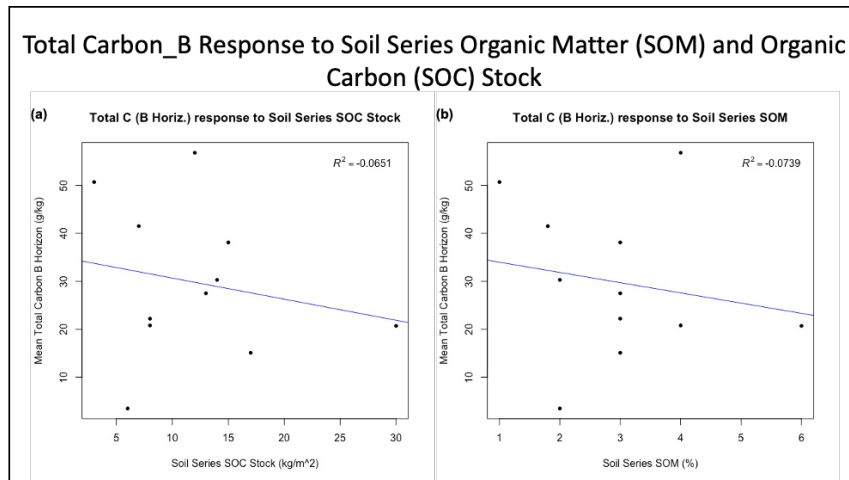
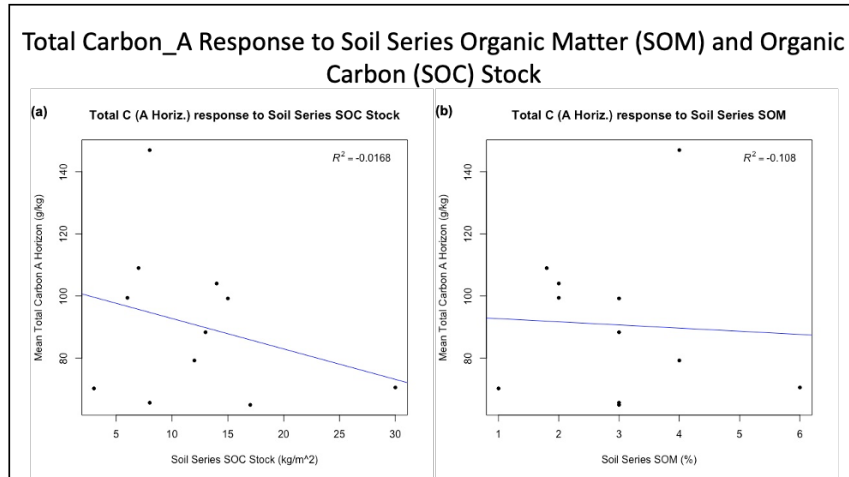


## Soil Series

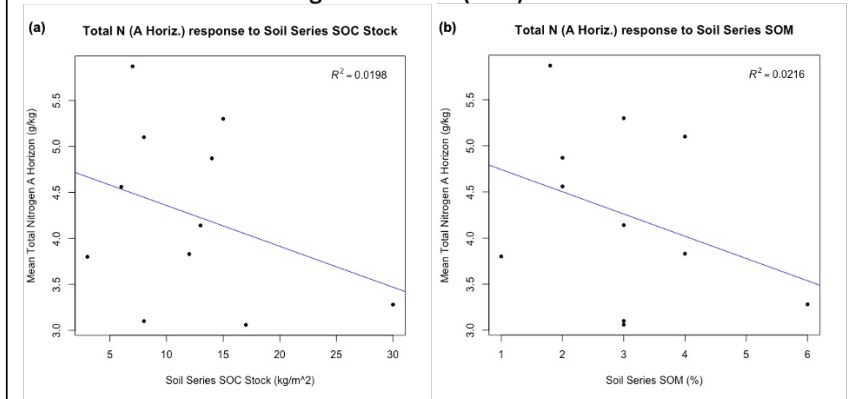


ii. SCATTER PLOTS

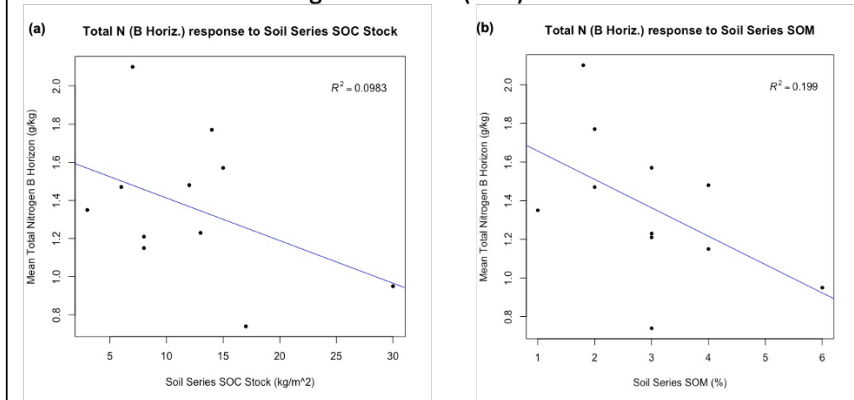
Soil Series



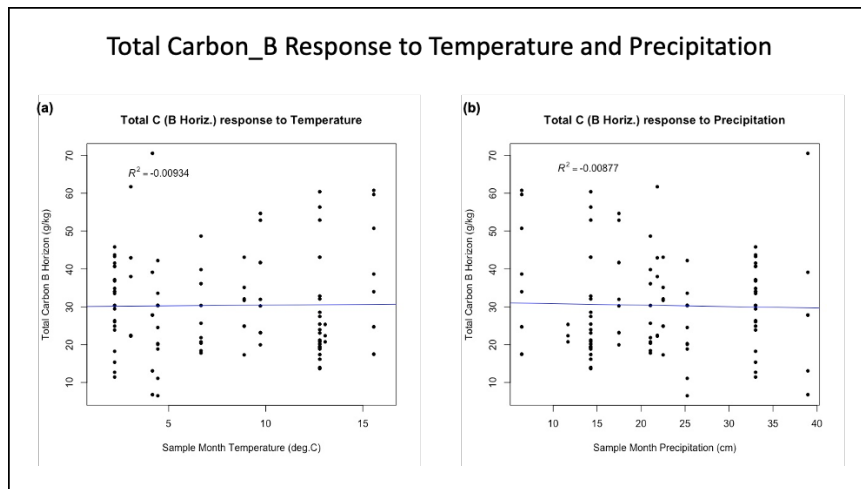
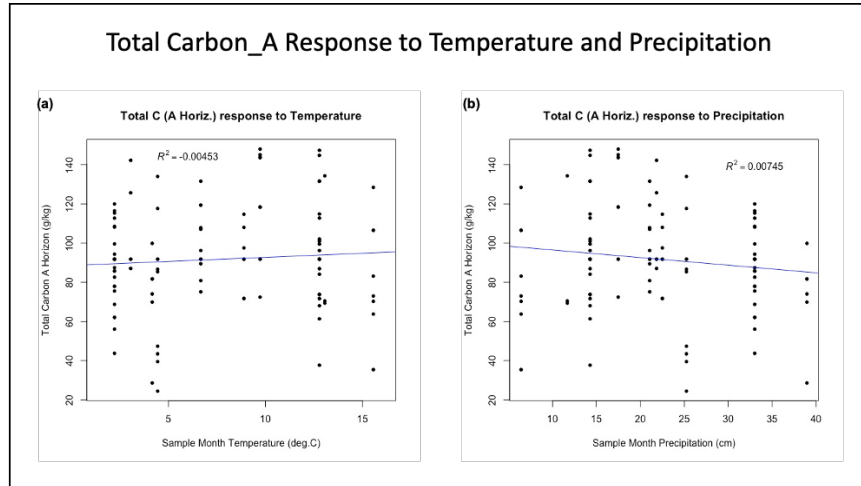
### Total Nitrogen\_A Response to Soil Series Organic Matter (SOM) and Organic Carbon (SOC) Stock



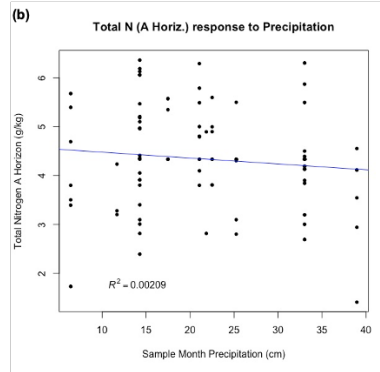
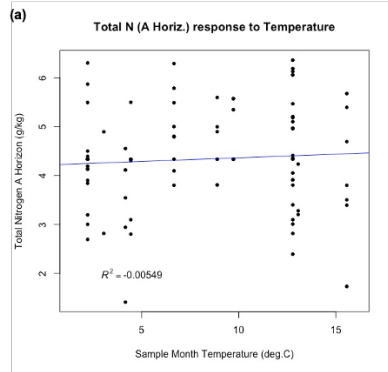
### Total Nitrogen\_B Response to Soil Series Organic Matter (SOM) and Organic Carbon (SOC) Stock



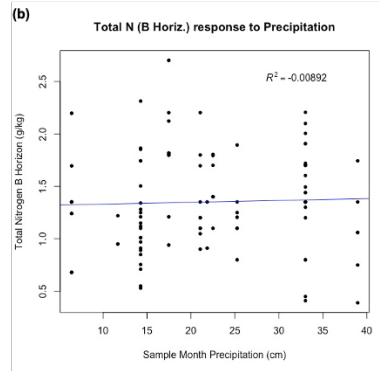
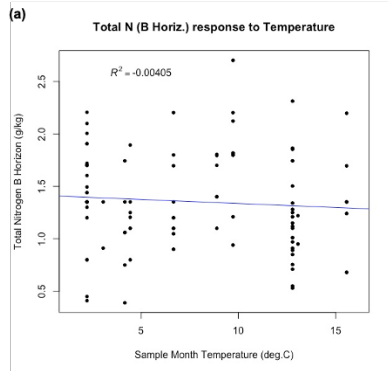
# Climate



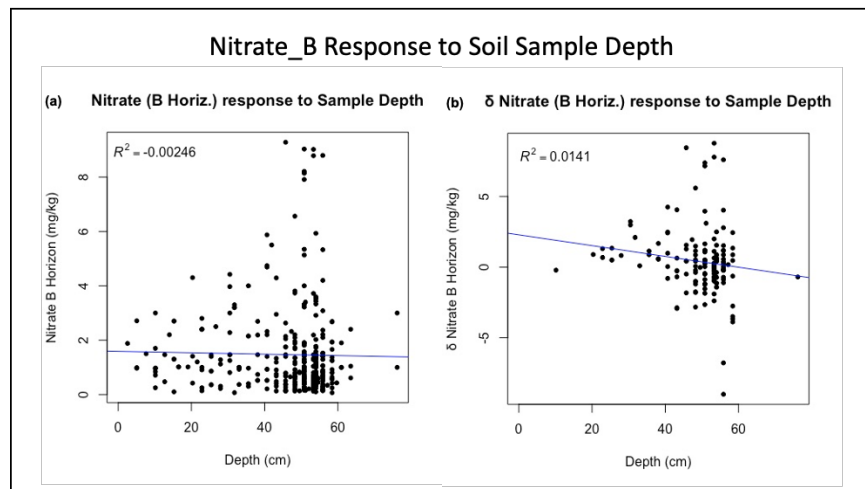
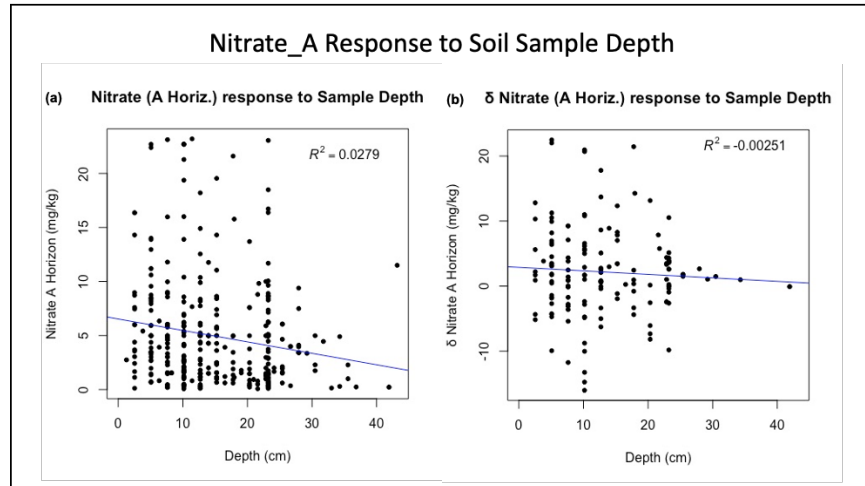
## Total Nitrogen\_A Response to Temperature and Precipitation



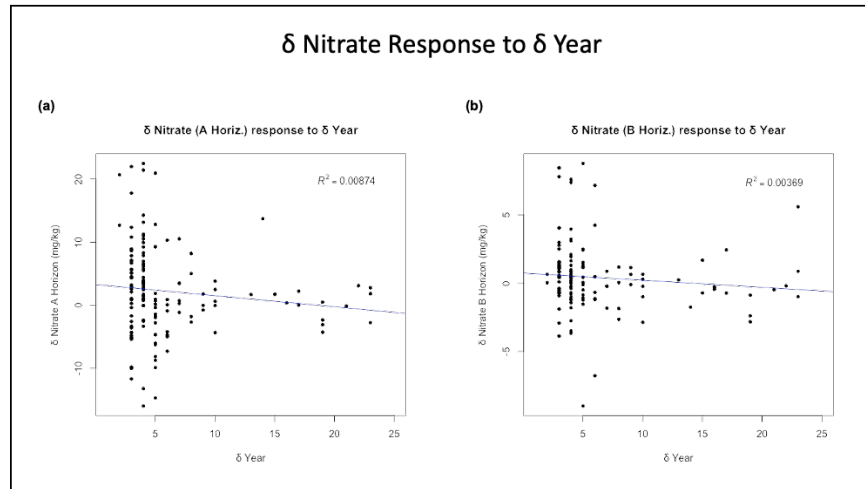
## Total Nitrogen\_B Response to Temperature and Precipitation



# Sample Depth



Year



## B. TABLES

### i. BIOSOLIDS COMPOSITION

**Summary of Biosolids Composition**

Biosolids Analyte	Unit	Brightwater Plant		South Plant		West Point Plant		U.S. EPA Limit
		Mean	SD	Mean	SD	Mean	SD	
Total Solids	%	20.62	0.01	23.34	0.01	27.39	0.01	
Total Volatile Solids	%	58.93	2.92	62.48	1.93	68.84	4	
pH		9.1	0.32	9	0.15	8.9	0.42	
Total Kjeldahl-N	mg/kg	76,930	4788	72218	5966	64892	6302	
Ammonia-N	mg/kg	9727	1033	11189	1240	8476	725	
Organic-N	mg/kg	67191	4986	61125	5977	56009	6085	
Total P	mg/kg	16106	3518	24041	3869	15190	2129	
Total K	mg/kg	1141	131	1725	249	1142	202	
Total Sulfate	mg/kg	375	237	179	99	194	102	
As	mg/kg	4	0.35	6	0.56	6	1.13	41
Ba	mg/kg	155	14	170	12	187	56	
Be	mg/kg	0.19	0.02	0.11	0.01	0.14	0.03	
B	mg/kg	60	9	17	4	11	1.38	
Cd	mg/kg	1	0.07	2	0.2	2	0.22	39
Ca	mg/kg	22080	1615	25855	2447	17577	2470	
Cr	mg/kg	17	1.64	23	2	28	7	
Cu	mg/kg	272	24	341	27	372	43	1500
Fe	mg/kg	9440	744.35	18702	3861	15698	3909	
Pb	mg/kg	12	1.33	22	4	63	14	300
Mg	mg/kg	6952	3052	7850	1901	5186	1097	
Mn	mg/kg	887	99	408	73	741	377	
Hg	mg/kg	0.46	0.16	0.56	0.26	0.7	0.33	17
Mb	mg/kg	7	0.59	7	1.02	11.1	2	
Ni	mg/kg	16	1.38	17	2	22	5	420
Se	mg/kg	6	0.74	6	0.62	5	0.84	100
Ag	mg/kg	3	0.35	4	2	3	0.61	
Zn	mg/kg	779	90	967	112	887	111	2800
Fecal Coliform	org/g dry	1292135		90.368		205737		
Salmonella	org/4g dry	89.3		21.1		54.1		

*Citation: King County Wastewater Treatment Division. 2021 Loop Quality Data Summary. <https://kingcounty.gov/services/environment/wastewater/resource-recovery/loop-biosolids/leading-with-science.aspx>. accessed 1/13/23.*

ii. SOIL SERIES DESCRIPTIONS

Description of Soil Series			
<b>Series Name</b>	<b>Barneston</b>	<b>Blethen</b>	<b>Elwell</b>
<b>Depth (cm)</b>	150	150	150
<b>Texture</b>	ashy-skeletal over sandy or sandy skeletal	ashy-skeletal	medial
<b>Precipitation (cm)</b>	152	165	152 - 203
<b>Temperature (C)</b>	8.9	8.3	7.2
<b>Slope (%)</b>	0 - 65	5 - 65	3 - 90
<b>Elevation (m)</b>	25 - 610	60 - 550	245 - 760
<b>Drainage</b>	excessively well drained	well drained	moderately well drained
<b>Parent Material</b>	volcanic ash and loess over glacial outwash	colluvium, slope alluvium, volcanic ash	glacial till, volcanic ash, loess
<b>Organic Carbon Stock (kg/m<sup>2</sup>)</b>	6	15	30
<b>Organic Matter (%)</b>	2	3	6
<b>Clay (%)</b>	8	8	10
<b>Sand (%)</b>	61	71	21
<b>Series Name</b>	<b>Elwell-Olomount Rock Outcrop Complex (ROC)</b>	<b>Kaleetan</b>	<b>Klaus</b>
<b>Depth (cm)</b>	moderately deep	150	158
<b>Texture</b>	50% silty-loam, 25% gravelly-loam, 10% ROC	loamy-skeletal	medial over sandy or sandy skeletal
<b>Precipitation (cm)</b>		280	203
<b>Temperature (C)</b>		6.7	10
<b>Slope (%)</b>	30 - 60	8 - 65	0 - 65
<b>Elevation (m)</b>		490 - 855	215 - 430
<b>Drainage</b>	moderately well drained	well drained	well drained
<b>Parent Material</b>	glacial till and volcanic ash	volcanic ash, colluvium, breccia, glacial till	volcanic ash overlying glacial outwash
<b>Organic Carbon Stock (kg/m<sup>2</sup>)</b>		8	14
<b>Organic Matter (%)</b>		3	2
<b>Clay (%)</b>		10	3
<b>Sand (%)</b>		67	86
<b>Series Name</b>	<b>Menzel Variant</b>	<b>Nargar</b>	<b>Norma</b>
<b>Depth (cm)</b>	150	150	150
<b>Texture</b>	coarse-loam	fine sandy loam	coarse-loam
<b>Precipitation (cm)</b>	102 - 178	165	89 - 152
<b>Temperature (F)</b>	9.4	8.3	10
<b>Slope (%)</b>	0 - 3	0 - 90	0 - 3
<b>Elevation (m)</b>	60 - 155	15 - 370	0 - 305
<b>Drainage</b>	well drained	well drained	poorly drained
<b>Parent Material</b>	alluvium and volcanic ash	volcanic ash and glacial outwash or alluvium	old alluvium
<b>Organic Carbon Stock (kg/m<sup>2</sup>)</b>	7	17	28
<b>Organic Matter (%)</b>	1.8	3	4
<b>Clay (%)</b>	12	7.5	17
<b>Sand (%)</b>	43	63	55
<b>Series Name</b>	<b>Olomount</b>	<b>Skykomish</b>	<b>Snoqualmie</b>
<b>Depth (cm)</b>	80	150	150
<b>Texture</b>	medial-skeletal	sandy-skeletal	sandy-skeletal
<b>Precipitation (cm)</b>	178	152	165
<b>Temperature (C)</b>	7.2	7.2	8.9
<b>Slope (%)</b>	3 - 90	0 - 65	0 - 8
<b>Elevation (m)</b>	243 - 550	125 - 550	430
<b>Drainage</b>	well drained	excessively well drained	excessively well drained
<b>Parent Material</b>	glacial till, volcanic ash, colluvium	glacial outwash and volcanic ash	alluvium
<b>Organic Carbon Stock (kg/m<sup>2</sup>)</b>	8	12	3
<b>Organic Matter (%)</b>	4	4	1
<b>Clay (%)</b>	10	8	3
<b>Sand (%)</b>	45	75	88
<b>Series Name</b>	<b>Tokul</b>	<b>Winston</b>	
<b>Depth (cm)</b>	150	150	
<b>Texture</b>	medial	coarse-loam	
<b>Precipitation (cm)</b>	150	140	
<b>Temperature (C)</b>	48	48	
<b>Slope (%)</b>	0 - 90	0 - 65	
<b>Elevation (m)</b>	50 - 460	50 - 580	
<b>Drainage</b>	moderately well drained	well drained	
<b>Parent Material</b>	loess, volcanic ash over cemented glacial till	glacial outwash, loess, volcanic ash.	
<b>Organic Carbon Stock (kg/m<sup>2</sup>)</b>	13	12	
<b>Organic Matter (%)</b>	3	2	
<b>Clay (%)</b>	7	8	
<b>Sand (%)</b>	53	63	

Citation: Web Soil Survey: Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. <http://websoilsurvey.nrcs.usda.gov/>. accessed 11/1/22

iii. ANOVA ANALYSIS RESULTS

ANOVA Analysis: Single Application Data								
	Nitrate_A	Nitrate_B	Total C_A	Total C_B	Total N_A	Total N_B	C/N_A	C/N_B
<b>Treated/Untreated</b>								
Pr(>F)	6.25E-08 ***	2.77E-02 *	0.195	0.338	5.27E-02	0.366		
F	30.6	4.885	1.701	0.93	3.85	0.82		
DF	1	1	1	1	1	1		
Residuals	355	341	104	105	98	98		
<b>Application Number</b>								
Pr(>F)	2.13E-10 ***	0.06	0.26	0.86	0.32	0.26	0.94	0.28
F	42.8	3.60	1.27	0.03	1.00	1.28	0.01	1.20
DF	1	1	1	1	1	1	1	1
Residuals	355	341	104	105	98	98	90	90
<b>Application Rate</b>								
Pr(>F)	1.91E-05 ***	1.34E-02 *	0.72	0.14	0.63	0.63	0.81	2.12E-02 *
F	18.80	6.17	0.13	2.22	0.24	0.23	0.06	5.51
DF	1	1	1	1	1	1	1	1
Residuals	355	341	104	105	98	98	90	90
<b>Net Application Rate</b>								
Pr(>F)	7.13E-11 ***	9.67E-03 **	0.14	0.64	0.48	0.19	0.48	0.40
F	45.20	6.77	2.20	0.23	0.50	1.72	0.52	0.72
DF	1	1	1	1	1	1	1	1
Residuals	355	341	104	105	98	98	90	90
<b>Soil Series</b>								
Pr(>F)	0.17	0.14	0.12	7.44E-04 ***	1.46E-02 *	9.76E-03 **	0.56	1.31E-03 **
F	1.38	1.44	1.61	3.41	2.39	2.54	0.88	3.28
DF	12	12	10	10	10	10	10	10
Residuals	344	330	95	96	89	89	81	81
<b>Sample Month</b>								
Pr(>F)	0.61	0.27	0.55	0.89	0.38	0.20	0.79	0.35
F	0.26	1.21	0.37	0.02	0.79	1.67	0.07	0.87
DF	1	1	1	1	1	1	1	1
Residuals	245	230	103	104	97	97	89	89
<b>Sample Month Mean Temperature</b>								
Pr(>F)	1.44E-03 **	6.12E-02	0.47	0.89	0.50	0.44	0.92	0.46
F	10.39	3.54	0.53	0.09	0.46	0.60	0.01	0.55
DF	1	1	1	1	1	1	1	1
Residuals	246	231	104	105	98	98	90	90
<b>Sample Month Mean Precipitation</b>								
Pr(>F)	2.16E-02 *	0.17	0.18	0.78	0.28	0.73	0.59	0.52
F	5.35	1.86	1.79	7.80E-02	1.21	0.125	0.29	0.42
DF	1	1	1	1	1	1	1	1
Residuals	246	231	104	105	98	98	90	90
<b>Sample Depth</b>								
Pr(>F)	9.1E-04 ***	0.69	0.49	0.73	0.25	0.71		
F	11.2	0.16	0.47	0.12	1.36	0.141		
DF	1	1	1	1	1	1		
Residuals	354	340	104	105	98	98		
<b>Unit Area</b>								
Pr(>F)	0.91	0.87	0.60	0.73	0.84	0.74		
F	0.01	0.03	0.28	0.12	0.04	0.11		
DF	1	1	1	1	1	1		
Residuals	355	341	104	105	98	98		

Significance codes: \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05

**ANOVA Analysis: Delta Application Data (  $\delta$  )**

	$\delta$ Nitrate_A	$\delta$ Nitrate_B	$\delta$ Total C_A	$\delta$ Total C_B	$\delta$ Total N_A	$\delta$ Total N_B	$\delta$ C/N_A	$\delta$ C/N_B
<b>Application Number</b>								
Pr(>F)	0.68	0.63	0.64	0.48	0.87	0.12	0.72	0.31
F	0.17	0.23	0.23	0.53	0.03	2.98	0.14	1.1
DF	1	1	1	1	1	1	1	1
Residuals	151	140	14	15	13	13	13	13
<b>Application Rate</b>								
Pr(>F)	9.22E-03 **	5.38E-03 **	0.40	0.92	0.96	0.78	0.42	0.95
F	6.96	8.00	0.76	0.01	0.00	0.08	0.69	0.00
DF	1	1	1	1	1	1	1	1
Residuals	151	140	14	15	13	13	13	13
<b>Net Application Rate</b>								
Pr(>F)	0.72	0.31	0.68	0.66	0.84	0.20	0.87	0.34
F	0.13	1.05	0.18	0.21	0.04	1.81	0.03	0.98
DF	1	1	1	1	1	1	1	1
Residuals	151	140	14	15	13	13	13	13
<b>Soil Series</b>								
Pr(>F)	0.96	0.93	0.11	0.06	0.24	0.23	0.95	0.46
F	0.35	0.41	2.58	3.49	1.64	1.69	0.05	0.84
DF	9	9	2	2	2	2	2	2
Residuals	143	132	13	14	12	12	12	12
<b>Unit Area</b>								
Pr(>F)	0.69	0.55	0.88	0.29	0.80	0.90		
F	0.16	0.36	0.02	1.18	0.07	0.02		
DF	1	1	1	1	1	1		
Residuals	151	140	14	15	13	13		
<b><math>\delta</math> Year</b>								
Pr(>F)	0.13	0.22	0.56	0.79	0.30	0.88		
F	2.34	1.52	0.36	0.08	1.18	0.03		
DF	1	1	1	1	1	1		
Residuals	151	140	14	15	13	13		

Significance codes: \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05

**ANOVA Analysis: Grand Delta Application Data (Δ )**

	Δ Nitrate_A	Δ Nitrate_B	Δ Total C_A	Δ Total C_B	Δ Total N_A	Δ Total N_B	Δ C/N_A	Δ C/N_B
<b>Grand Total Application Number</b>								
Pr(>F)	0.12	0.44	0.64	0.48	0.87	0.11	0.72	0.31
F	2.48	0.60	0.23	0.53	0.03	2.98	0.14	1.10
DF	1	1	1	1	1	1	1	1
Residuals	119	108	14	15	13	13	13	13
<b>Grand Total Application Rate</b>								
Pr(>F)	0.25	0.67	0.45	0.74	0.74	0.10	0.55	0.35
F	1.31	0.19	0.61	0.11	0.11	3.05	0.38	0.96
DF	1	1	1	1	1	1	1	1
Residuals	119	108	14	15	13	13	13	13
<b>Soil Series</b>								
Pr(>F)	0.78	0.82	0.11	0.06	0.24	0.23	0.95	0.46
F	0.62	0.57	2.58	3.49	1.64	1.69	0.05	0.84
DF	9	9	2	2	2	2	2	2
Residuals	111	100	13	14	12	12	12	12
<b>Unit Area</b>								
Pr(>F)	0.59	0.56	0.88	0.29	0.80	0.90		
F	0.29	2.11	0.02	1.18	0.07	0.02		
DF	1	1	1	1	1	1		
Residuals	119	108	14	15	13	13		
<b>Δ Year</b>								
Pr(>F)	0.78	0.47	0.52	0.30	0.18	0.25		
F	0.08	0.53	0.43	1.15	2.05	1.47		
DF	1	1	1	1	1	1		
Residuals	119	108	14	15	13	13		

Significance codes: \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05

iv. SUMMARY TABLE OF MEANS

**Application Rate**

Application Rate (Mg/ha)	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
16.8 - 22.4	71.1	24.9	12.4	4	39.3	22.6	11.3	4
11.2 - 16.8	93.1	25.4	3.71	47	31.0	12.0	1.76	47
5.6 - 11.2	97.3	30.6	4.83	40	30.0	13.3	2.10	40
0 - 5.6	78.6	19.9	5.14	15	27.1	12.0	3.01	16

Application Rate (Mg/ha)	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
16.8 - 22.4	3.28	1.10	0.55	4	1.27	0.43	0.21	4
11.2 - 16.8	4.42	0.81	0.12	44	1.32	0.45	0.07	44
5.6 - 11.2	4.54	1.08	0.17	38	1.42	0.48	0.08	38
0 - 5.6	3.81	1.21	0.32	14	1.29	0.49	0.13	14

Grand Total Application Rate (Mg/ha)	Δ Nitrate_A (mg/kg)				Δ Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
46.4 - 57.8	4.23	4.09	1.55	7	-0.11	2.14	0.81	7
35.2 - 46.4	3.33	7.21	1.36	28	0.26	2.91	0.56	27
23.9 - 35.2	2.91	5.79	0.80	53	0.72	2.51	0.38	44
12.6 - 23.9	1.80	5.25	0.91	33	0.65	2.04	0.36	32

Grand Total Application Rate (Mg/ha)	Δ Total C_A (g/kg)				Δ Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
46.4 - 57.8	3.86	77.9	55.1	2	5.76	14.9	10.5	2
35.2 - 46.4	6.52	68.0	39.2	3	-6.49	21.7	12.5	3
23.9 - 35.2	26.6	19.9	9.97	4	9.63	6.44	3.22	4
12.6 - 23.9	18.3	30.5	11.5	7	1.71	17.9	6.32	8

Grand Total Application Rate (Mg/ha)	Δ Total N_A (g/kg)				Δ Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
46.4 - 57.8	0.97	2.40	1.70	2	0.43	0.44	0.31	2
35.2 - 46.4	1.61	2.16	1.25	3	0.64	0.56	0.32	3
23.9 - 35.2	0.88	0.83	0.48	3	0.61	0.24	0.14	3
12.6 - 23.9	1.25	1.47	0.56	7	-0.01	0.54	0.20	7

## Soil Series

Soil Series	Total C A Horizon (g/kg)				Total C B Horizon (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Barneston	99.4	25.91	6.48	16	3.49	9.22	2.55	16
Blethen	99.2	21.2	8.01	7	38.1	9.22	5.37	7
Elwell-Olomount ROC	70.5	-	-	1	20.7	-	-	1
Kaleetan	65.6	37	26.2	2	22.2	9.22	9.22	2
Klaus	104	36	10.4	12	30.3	8.2	2.37	12
Menzel Variant	109	-	-	1	41.5	-	-	1
Nargar	64.9	41.7	29.5	1	15.1	3.37	2.38	2
Olomount	147	-	-	1	20.8	-	-	1
Skykomish	79.2	18.6	10.7	3	56.8	9.22	9.22	3
Snoqualmie	70.2	-	-	1	50.7	-	-	1
Tokul	88.3	25	3.22	60	27.5	9.22	1.53	61

Soil Series	Total N A Horizon (g/kg)				Total N B Horizon (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Barneston	4.56	0.90	0.23	16	1.47	0.41	0.10	16
Blethen	5.3	1.33	0.50	7	1.57	0.31	0.12	7
Elwell-Olomount ROC	3.28	-	-	1	0.95	-	-	1
Kaleetan	3.1	-	-	1	1.21	-	-	1
Klaus	4.87	0.63	0.20	10	1.77	0.45	0.14	10
Menzel Variant	5.87	-	-	1	2.1	-	-	1
Nargar	3.06	1.88	1.33	2	0.74	0.09	0.06	2
Olomount	5.1	-	-	1	1.15	-	-	1
Skykomish	3.83	0.63	0.36	3	1.48	0.23	0.13	3
Snoqualmie	3.8	-	-	1	1.35	-	-	1
Tokul	4.14	0.96	0.13	57	1.23	0.45	0.06	57

Soil Series	Δ Nitrate_A (mg/kg)				Δ Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
Barneston	3.16	4.62	0.82	32	1.02	2.27	0.44	27
Blethen	3.87	6.63	2.1	10	0.11	2.19	0.73	9
Elwell-Olomount ROC	-	-	-	0	-	-	-	0
Kaleetan	1.22	-	-	1	-1.39	-	-	1
Klaus	1.1	5.98	1.6	14	0.45	3.67	0.98	14
Menzel Variant	1.54	7.28	5.15	2	-1.12	0.6	0.43	2
Nargar	7.6	-	-	1	3.83	-	-	1
Norma	-2.42	-	-	1	-1.2	-	-	1
Olomount	-	-	-	0	-	-	-	0
Skykomish	-0.32	6.21	2.54	6	0.6	1.18	0.53	5
Snoqualmie	-0.02	6.62	4.68	2	-0.11	1.4	0.99	2
Tokul	3.34	6.53	0.91	52	0.46	2.38	0.34	48
Winston	-	-	-	0	-	-	-	0

## Climate

Sample Month	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
January	87.8	20.2	4.11	24	31.3	9.87	2.01	24
February	108	24.8	11.1	5	37.5	16.4	7.33	5
March	76.2	36.0	11.4	10	24.4	10.4	3.12	11
April	99.1	17.4	5.49	10	28.0	10.5	3.31	10
May	119	28.3	9.42	9	35.5	12.9	4.30	9
June	93.3	24.9	5.31	22	28.8	13.8	2.93	22
July	78.0	31.9	10.6	9	36.5	17.1	5.70	9
August	-	-	-	-	-	-	-	-
September	91.4	37.2	21.5	3	22.8	2.33	1.35	3
October	92.5	16.5	6.22	7	29.9	8.34	3.15	7
November	72.6	23.9	9.75	6	30.9	22.6	9.22	6
December	-	-	-	-	-	-	-	-

Sample Month	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
January	4.25	0.81	0.17	24	1.44	0.47	0.10	24
February	3.86	1.47	1.04	2	1.13	0.31	0.22	2
March	4.10	0.90	0.34	7	1.24	0.34	0.13	7
April	4.88	0.79	0.25	10	1.36	0.41	0.13	10
May	4.72	0.59	0.20	9	1.82	0.52	0.17	9
June	4.55	1.16	0.25	22	1.22	0.46	0.10	22
July	3.96	1.54	0.51	9	1.31	0.47	0.16	9
August	-	-	-	-	-	-	-	-
September	3.57	0.58	0.33	3	1.04	0.16	0.09	3
October	4.54	0.66	0.25	7	1.51	0.26	0.10	7
November	3.45	1.14	0.47	6	1.06	0.47	0.19	6
December	-	-	-	-	-	-	-	-

Sample Month Mean Temperature (°C)	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
12 - 17	6.61	6.27	0.68	84	1.17	1.13	0.13	80
6 - 11	6.21	5.51	0.60	83	1.47	1.58	0.18	79
1 - 6	4.07	3.69	0.41	81	1.74	2.19	0.26	74

Sample Month Mean Temperature (°C)	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
12 - 17	90.7	28.9	4.89	35	29.9	14.4	2.44	35
6 - 11	104	23.7	4.64	26	31.1	11	2.15	26
1 - 6	85.4	26.45	3.94	45	30.3	13	1.92	46

Sample Month Mean Temperature (°C)	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
12 - 17	4.33	1.26	0.21	35	1.22	0.44	0.07	35
6 - 11	4.73	0.68	0.13	26	1.56	0.45	0.09	26
1 - 6	4.08	0.92	0.15	39	1.33	0.45	0.07	39

Sample Month Mean Precipitation (cm)	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
28 - 39	4.26	3.91	0.55	51	1.65	2.12	0.32	45
17 - 28	5.56	5.12	0.48	113	1.58	1.81	0.17	108
6 - 17	6.61	6.27	0.68	84	1.17	1.13	0.13	80

Sample Month Mean Precipitation (cm)	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
28 - 39	84.8	21.4	3.91	30	31.2	12.9	2.35	30
17 - 28	97.8	29.3	4.58	41	30.1	11.9	1.84	42
6 - 17	90.7	28.9	4.89	35	29.9	14.4	2.44	35

Sample Month Mean Precipitation (cm)	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
28 - 39	4.09	0.92	0.17	30	1.37	0.49	0.09	30
17 - 28	4.56	0.80	0.13	35	1.47	0.44	0.08	35
6 - 17	4.33	1.27	0.21	35	1.22	0.44	0.07	35

Net Annual Precipitation (cm)	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
5250 - 7000	4.12	5.31	1.37	15	1.05	1.64	0.44	14
3500 - 5250	4.14	4.40	1.10	16	0.78	0.83	0.20	17
1750 - 3500	4.61	4.04	0.66	37	1.42	1.71	0.28	37
0 - 1750	6.35	5.68	0.41	193	1.73	1.83	0.14	183

Net Annual Precipitation (cm)	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
5250 - 7000	98.5	39.4	11.9	11	30.0	14.7	4.43	11
3500 - 5250	86.7	25.6	7.39	12	26.6	10.4	3.01	12
1750 - 3500	78.6	28.7	6.95	17	32.0	10.6	2.57	17
0 - 1750	97.3	23.8	3.15	57	31.4	13.5	1.79	57

Net Annual Precipitation (cm)	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
5250 - 7000	4.29	1.51	0.46	11	1.43	0.61	0.18	11
3500 - 5250	4.00	1.18	0.34	12	1.20	0.48	0.14	12
1750 - 3500	3.97	0.65	0.17	14	1.50	0.52	0.14	14
0 - 1750	4.61	0.82	0.11	55	1.35	0.38	0.05	55

## Sample Depth

Sample Depth (cm)	Nitrate_A (mg/kg)			
	Mean	SD	SE	n
34.3 - 45.7	2.58	4.44	1.81	6
22.9 - 34.3	4.42	4.42	0.49	83
11.4 - 22.9	4.38	4.35	0.40	116
0 - 11.4	5.94	5.66	0.46	152

Sample Depth (cm)	Nitrate_B (mg/kg)			
	Mean	SD	SE	n
58 - 76.5	1.24	0.80	0.16	26
39.5 - 58	1.48	1.80	0.12	241
21 - 39.5	1.54	1.14	0.17	48
2.5 - 21	1.44	0.96	0.18	27

Sample Depth (cm)	Total C_A (g/kg)			
	Mean	SD	SE	n
34.3 - 45.7	-	-	-	0
22.9 - 34.3	125	23.0	11.5	4
11.4 - 22.9	83.6	23.1	11.5	20
0 - 11.4	91.9	27.0	3.07	77

Sample Depth (cm)	Total C_B (g/kg)			
	Mean	SD	SE	n
58 - 76.5	37.0	9.43	3.33	8
39.5 - 58	29.7	13.4	1.40	91
21 - 39.5	30.5	9.63	3.40	8
2.5 - 21	-	-	-	0

Sample Depth (cm)	Total N_A (g/kg)			
	Mean	SD	SE	n
34.3 - 45.7	-	-	-	0
22.9 - 34.3	-	-	-	0
11.4 - 22.9	4.20	1.10	0.25	19
0 - 11.4	4.38	1.07	0.13	70

Sample Depth (cm)	Total N_B (g/kg)			
	Mean	SD	SE	n
58 - 76.5	2.12	-	-	1
39.5 - 58	1.34	0.46	0.05	92
21 - 39.5	1.43	0.45	0.18	6
2.5 - 21	1.50	-	-	1

## Unit Area

Unit Area (ha)	Nitrate_A (mg/kg)				Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
49.8 - 66.0	6.17	7.70	2.72	8	1.68	1.87	0.66	8
33.6 - 49.8	4.35	4.27	0.79	29	1.31	1.57	0.30	28
17.4 - 33.6	4.82	5.07	0.49	106	1.48	1.58	0.16	101
1.2 - 17.4	5.11	5.00	0.34	214	1.47	1.62	0.11	206

Unit Area (ha)	Total C_A (g/kg)				Total C_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
49.8 - 66.0	131	-	-	1	43.1	-	-	1
33.6 - 49.8	82.9	36.9	13.04	8	24.0	15.1	5.32	8
17.4 - 33.6	94.7	29.1	5.39	29	31.9	13.4	2.45	30
1.2 - 17.4	91.0	25.5	3.09	68	30.2	12.4	1.51	68

Unit Area (ha)	Total N_A (g/kg)				Total N_B (g/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
49.8 - 66.0	6.06	-	-	1	1.74	-	-	1
33.6 - 49.8	4.02	1.33	0.47	8	1.13	0.52	0.19	8
17.4 - 33.6	4.31	1.01	0.19	27	1.39	0.50	0.10	27
1.2 - 17.4	4.36	0.99	0.12	64	1.36	0.44	0.06	64

Unit Area (ha)	Δ Nitrate_A (mg/kg)				Δ Nitrate_B (mg/kg)			
	Mean	SD	SE	n	Mean	SD	SE	n
49.8 - 66.0	4.29	3.43	1.98	3	1.03	0.93	0.54	3
33.6 - 49.8	1.15	2.83	0.90	10	0.12	2.77	0.92	9
17.4 - 33.6	1.82	7.66	1.26	37	-0.07	2.54	0.46	31
1.2 - 17.4	3.45	5.18	0.62	71	0.84	2.39	0.29	67

## Sample Year

<b>δ Year</b>	<b>δ Nitrate_A (mg/kg)</b>				<b>δ Nitrate_B (mg/kg)</b>			
	<b>Mean</b>	<b>SD</b>	<b>SE</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>SE</b>	<b>n</b>
19 - 25	-0.51	2.73	0.91	9	-0.16	2.61	0.92	8
13 - 19	3.06	4.76	1.80	7	0.05	1.37	0.49	8
7 - 13	1.63	3.72	0.88	18	-0.38	1.25	0.30	17
1 - 7	2.44	7.36	0.67	119	0.60	2.58	0.25	109

<b>Δ Year</b>	<b>Δ Nitrate_A (mg/kg)</b>				<b>Δ Nitrate_B (mg/kg)</b>			
	<b>Mean</b>	<b>SD</b>	<b>SE</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>SE</b>	<b>n</b>
23 - 30	3.70	7.22	1.66	19	0.13	2.64	0.62	18
16 - 23	2.15	6.08	1.43	18	0.82	3.70	0.87	18
9 - 16	2.53	3.63	0.97	14	0.24	2.41	0.67	13
2 - 9	2.74	5.93	0.71	70	0.63	1.94	0.25	61