RELATIONSHIP BETWEEN BIOSOLIDS APPLICATION AND UNDERSTORY DEVELOPMENT IN DOUGLAS-FIR (*Pseudotsuga menziesii* (Mirbel) Franco) STANDS IN WESTERN WASHINGTON

by

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A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science

University of Washington

1998

Approved by ____________________
Chairperson of Supervisory Committee

Program Authorized
to Offer Degree College of Forest Resources

Date August 21, 1998

Master's Thesis

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Abstract

Relationship between biosolids application and understory development in Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco) stands in western Washington

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Biosolids, the residuals from primary and secondary treatment of wastewater, are routinely applied as a fertilizer and soil amendment to Douglas-fir forests in western Washington. This study explored changes in understory composition and abundance, Douglas-fir sapling growth and survival, and differences in understory nitrogen accumulations in response to the land application of biosolids to a variety of sites in western Washington.

Heavy rates of biosolids application (> 50 dry Mg ha $^{-1}$) have significantly changed understory composition. In older stands red elderberry and stinging nettle became dominant understory species, but were absent in the adjoining untreated areas. Biosolids treatment significantly reduced salal cover by about 50%, increased sword fern cover, but had no effect on Oregon grape. However, current biosolids applications at lower rates of 11 Mg ha $^{-1}$ every 5 years, may not change species composition and abundance in the same way.

Biosolids applied to stands of 2-year-old Douglas-fir saplings did not change diameter or height increment in the 12 months after application. Sapling mortality was greater in treated stands, with smallest trees the most affected.

In general, understory biomass and N content increased with biosolids applications. However, these increases were dependent upon stand conditions. Understory biomass and N content in 2-year-old stands treated with biosolids treated were significantly greater than in adjoining control plots by 3.5 Mg ha^{-1} and 73 kg N ha^{-1} respectively. Understory N content in treated 9-year-old stands increased by 18 kg N ha^{-1} relative to control plots. The untreated 9-year-old plots had substantial understory N accumulation of 81 kg N ha^{-1} yr^{-1} showing that substantial N reserves are already present on this site. In the 68 year-old stands dominated by salal and Oregon grape understory, no substantive change in understory biomass or N content occurred with biosolids treatment.

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Acknowledgments

I wish to acknowledge all those who have supported and helped in my studies. In particular, special thanks to Chuck Henry, for his professional and financial support. I appreciate the many and varied work, educational and social opportunities, and the contacts you have provided. Chuck, thanks for understanding my learning needs and making my time in Washington a valuable career experience and a great time.

To my committee, Chuck, Bob Edmonds and Charles Halpern: thanks for their assistance, criticism and support.

To the Australian Academy of Science, I am grateful for financial support through the Wal Gentle Scholarship. To Marcia Lambert, thanks for both encouraging and helping me pursue this opportunity, and to John Turner for smoothing a few corners.

To State Forests Of NSW, thanks for financial and career support. I appreciate the organization and the management's long term commitment to being the best forest managers.

To the Peggy Leonard, Doug Newlands and Roberta King at King County your assistance with application sites and advise was invaluable. Thanks Mark Grey, Mark Cullington, Maryann Baird, Daniel Thompson and Dan Bennett for your assistance.

Dedication

The author wishes to dedicate this thesis to Rosemary for her continual understanding, support and love. Thanks Sam, Anita, Tom, Mum, Big B, John and Amanda. This is for all of us.

Chapter 1:

INTRODUCTION

Biosolids, the residuals from primary and secondary treatment of wastewater, is a synonym for sewage sludge that suggests beneficial reuse, rather than disposal (U.S. EPA 1995). Biosolids are useful for fertilization because they are rich in macro and micronutrients and partially decomposed organic matter.

In Washington, land application of biosolids is a social and legal requirement. Ocean disposal and landfilling of biosolids are banned and little public support exists for new incineration plants. Producers are seeking and creating economic and environmentally sensitive ways of using year round the output from sewerage treatment works. Major uses include application on agricultural and forest lands, land reclamation and the production of compost. Currently 20% of biosolids produced in King County, Washington are applied in second growth and plantation Douglas-fir forests. These forests are found on nitrogen (N) poor soils found on the western side of the Cascade Mountains near Snoqualmie.

Land application of biosolids is controlled by 40 CFR Part 503 (U.S. EPA 1994) and Washington State Best Management Guidelines (WDOE 1994). These specify site criteria that need to be evaluated in the planning phase of an operation, maximum cumulative trace metal loading rates from the biosolids, buffer distances that application are required to maintained from creeklines, and practices to minimize the pathogen risk to human health. The maximum annual rate of biosolids applied to a site is based on: the amount of additional N or phosphorus that plants on the site can uptake, the nutrient concentration in the biosolids, the background levels of nutrients in the soil and the acceptable limits on leachate from the site.

Due to improved waste management in U.S. cities, most biosolids meet the U.S. EPA pollutant concentration limits for metals. Thus for King County, there is no specific cumulative site application rate if trace metal concentrations are below a specified level defined within 40 CFR Part 503 (U.S. EPA 1994). Only if these metal concentrations are exceeded are annual loadings monitored to ensure the cumulative site loadings are not exceeded. In most cases, excess nutrient availability limits rate of application. Forest application rates are normally based on the agronomic N rate of the site. Application at agronomic rate supplies the N requirement of the site with negligible nitrate leaching. The N requirement for a forest site is "the sum of the N uptake by trees and understory and soil immobilization of N" (U.S.EPA. 1995).

Just as environmentally responsible management is the key to ongoing biosolids land application, so sustainable forest management is the key to long term forestry operations. Landscape based forest management must view forest ecosystems as complex arrays of vegetational, biological and physical components within an economic and social setting. To manage these areas, modern, multi-dimensional silvicultural practices are used. Interactions commonly exist between different practices, e.g., fertilization and thinning, establishment techniques and weed control. Forest managers needs to review the effects of these silvicultural practices on all ecosystem components to ensure the maintenance of long term site productivity and biodiversity.

Traditional fertilization strategies have looked at increasing tree growth, with management often seeking to maximize the net present value of the stands being treated (Knott et al. 1995). Since the 1960s, N fertilization of Douglas-fir in western Washington has significantly improved tree growth and stand value (Chappell et al. 1992). Henry and Cole (in press) have shown that biosolids are as or more effective as urea and other inorganic N fertilizers in increasing stand growth.

Today in some forests, increased timber value is no longer the sole focus of silvicultural practice. On public forest lands on the Olympic Peninsula in Washington, where stand structural diversity on a landscape scale has been reduced, adaptive management strategies propose thinning and fertilization in certain regrowth stands to enhance more rapid development of overstory / understory structures characteristic of late seral forests (Lippke et al. 1996). Hayes et al. (1997), when looking at wildlife responses to young stands in western Washington, states that ".. management activities can dramatically change stand structure and the rate and direction of ecological succession, with (positive) ramifications to the presence and absence of wildlife". In the Mountains to Sound Greenway project in the I-90 corridor east of Seattle, biosolids fertilization is being used to increase tree growth for visual amenity, enhanced stand development and for greater timber production.

Whether biosolids fertilization is used to increase timber values or other forest management goals, it is essential to understand the interactions between fertilization and all stand components. This study is an investigation of the specific interactions between biosolids and understory plants. Biosolids fertilization may cause :

- Increased understory competition with tree saplings in young stands that have not achieved crown closure. Alternatively, decreased competition from understory plants can arise where canopy closure is accelerated.
- Altered understory species composition, abundance and diversity, which affect wildlife habitat, food sources and stand aesthetics.

- Accelerated recycling of nutrients in young stands by understory vegetation. These nutrients, unless accumulated by understory plants, might otherwise be lost from the system.
- Increased ground cover that changes hydrologic parameters (e.g., rate of water runoff) on the site. Increased soil cover, will generally reduce erosion potential of soil on the site and diminish the rate of overland flow, thereby reducing transportation of soil materials and organic amendments from the site.

The constituents of the understory of a forest stand can be defined in various ways. Turner (1979) looking at fertilizer effects on understory described "understory of older stands as trees less than breast height, shrubs, ferns, herbs and mosses, and in younger stands as anything not the desired tree species." The EPA Process Design Manual (U.S. EPA 1995) in defining biosolids application rate calculation, only has two general plant uptake categories, crop trees and understory. I have defined understory as non-conifer species in Douglas-fir stands with heights less than 10 m. This definition is consistent with Pojar and Mackinnon (1994) descriptions of shrubs, wildflowers, graminoids and ferns in their field guide "Plants of the Pacific Northwest Coast."

The goal of this research was to investigate the interactions of biosolids and understory dynamics in typical operational sites in Douglas-fir stands in western Washington. By understanding these relationships, recommendations for management of potential beneficial or adverse impacts can be developed.

Chapter 2:

STUDY OBJECTIVES AND HYPOTHESES

The general objectives of this study were to:

- examine the effects of biosolids on forest ecosystems, particularly understory dynamics, in Douglas-fir sites in which biosolids are typically applied, and
- determine the level of N uptake in the understory after biosolids application. Measurements of the changes in the rate of N accumulation in the understory component of different aged Douglas-fir stands assists managers in quantifying the role understory has in determining biosolids applications at an agronomic rate.

Specific objectives were to:

1. assess the effects of past applications of biosolids on understory composition and abundance,
2. determine the effect of biosolids application on the growth and survival of young Douglas-fir saplings in stands with substantial understory growth, and
3. quantify understory N accumulation rate.

I tested the following general hypotheses:

Hypothesis 1. Application of biosolids to Douglas-fir stands changes understory composition and abundance.

Understory composition and abundance is not uniform across Douglas-fir stands. It is affected by edaphic and climatic factors that alter site productivity, as well as stand age and disturbance history that affect successional development. The application of biosolids changes edaphic factors, and alters the availability of soil resources within a forest site.

Biosolids alter a site's productivity by changing soil moisture, nutrient availability and forest floor characteristics. These edaphic changes variably affect the growth rate and survival of different understory species. Understory development after biosolids application should follow different successional pathways depending on disturbance history, species availability, and on edaphic and microsite factors.

Hypothesis 2. Application of biosolids to Douglas-fir stands that do not have overstory canopy closure, will increase understory biomass during the initial growing season after application.

Nutrients from biosolids applications are available to both overstory and understory plants. If light is not a limiting through canopy closure, and understory plants are present, biosolids application to forests on nutrient deficient soils, should improve levels of soil nutrients and moisture holding capacity to enhance and thus understory growth. Additionally, different responses to additional nutrients among understory species may alter the relative dominance of a site.

Hypothesis 3. Application of biosolids to Douglas-fir stands increases understory N content accumulation over the initial growing season after application.

Increases in the nutrient pool of a nutrient-deficient soil should lead to increases in the N concentration in stems and foliage of understory plants. Combined with a predicted increase in understory biomass following biosolids application, increased understory N accumulations should occur. Biosolids applications can also increase nutrient concentrations beyond "the critical level" or the "adequate level of nutrient concentration" (Carter 1992) into luxury consumption if biomass does not increase further. Preferential growth response by "nitrogen-loving" species after biosolids application, may further increase average nutrient concentration of the site.

Hypothesis 4. Application of biosolids to young 2-year-old Douglas-fir saplings in stands where understory is present, increases competition from understory plants and thus adversely affects growth and survival of planted Douglas-fir saplings.

Interspecific competition for above-ground and below-ground resources is a major factor affecting relative growth of individuals. Biosolids provide adequate nutrients for both seedlings and understory plants. Any change in the relative dominance of the individuals by uneven increases in growth can affect the supply of other limiting resources. Massive biomass growth by understory relative to saplings can increase shading and decrease the availability of soil moisture for saplings. The application of biosolids to stands within the first two years after planting would be expected to favor the understory plants that tend to grow more vigorously than do planted Douglas-fir saplings.

Chapter 3:

LITERATURE REVIEW

3.1 Productivity of Douglas-fir stands in western Washington

In western Washington 20% of King County biosolids are applied in second growth and plantation Douglas-fir forests. Natural Douglas-fir stands and plantations are common over a range of soil types from sea level to 1000 m elevation. Depending on site factors and past disturbance history, natural stands exist as either pure stands or in combination with hemlock, true firs and often red alder and big leaf maple. Alternatively, plantations are primarily monocultures of planted Douglas-fir.

In Douglas-fir forests, Steinbrenner (1979) summarized site factors affecting productivity in his development of forest soil productivity relationships. Site index (expressed as stand height at a given age) is the standard measure of site productivity. Site quality (SQ) classes are combinations of site index, with SQ1 stands containing the highest stand height and representing the most fertile sites, while SQ V are the shortest stands growing on the poorest sites. The factors that affect the productivity of Douglas-fir include: elevation, precipitation, soil parent material, profile characteristics, effective and total soil depth and clay content. Other studies (Edmonds et al. 1994) have shown relationships between site productivity and soil organic matter and soil N content.

Seventy percent of coastal Douglas-fir sites show significant growth response to N fertilization (Chappell et al. 1992), with basal area response highly correlated with site index (Carter 1992). Assuming light and moisture are adequate, greatest responses occur on poor sites, with smaller positive responses in stands on higher site indexes (Chappell et al. 1992).

3.2 Factors affecting understory development in Douglas-fir stands

Understory composition in Douglas-fir stands is affected by independent variables that affect site productivity, as well as stand age and disturbance history. Within the range of the Douglas-fir stands, different understory species occupy certain niches. These niches, represent the interrelated environmental factors affecting the species ability to survive, grow and reproduce. Environmental factors include morphological (edaphic, topographic and climatic) or mode of action (light, moisture, heat and nutrients) factors. Morphological factors can partially compensate one factor for another, while no compensation exists in environmental factors based on mode of action (Klinka et al. 1989).

Klinka et al. (1989), utilizes the non compensatory nature of "mode of action" factors when describing indicator plants in coastal British Columbia. They describe the niche for a species by an creating "ecological amplitudes" that represents the upper and lower limits of that environmental factor where the species will survive. Once the ecological amplitude of a plant species is determined from detailed physical and chemical measurements, the ability of a species on a particular site can be determined by knowing the level of the factor on the site. Alternatively, the presence and abundance of a species on a site, allows a prediction to be made of the level of the factor on the site."

These amplitudes describe the environmental attributes of sites that will permit certain species to survive and flourish. Klinka et al. (1989), has placed species along environmental gradients developed for climate, soil moisture, soil nitrogen and ground surface materials. The last three environmental factor gradients are particularly important in this study of the impacts biosolids have on understory composition.

Environmental factors that correlate with understory abundance and composition in Douglas-fir stands include:

(a) Soil Moisture

Different understory communities exist over the moisture gradient where Douglas-fir exists. Sword fern (*Polystichum munitum* (Kaulfuss) K.Presl) and Redwood sorrel (*Oxalis oregana* Nutt) communities typify very moist sites. Oregon grape (*Berberis nervosa* Pursh) and Pacific rhododendron (*Rhododendron macrophyllum* D.Don ex G.Don) or alternatively sword fern and salal (*Gaultheria shallon* Pursh) dominate sites of intermediate soil moisture. Salal or oceanspray (*Holidiscus discolor* (Pursh) Maxim) dominates understory vegetation at the dry end of the spectrum (Franklin and Dryness 1988). Franklin and Dryness (1988) state that "understory composition is useful in recognizing 5 site quality types (with different growth rates) in western British Columbia, Washington and Oregon."

Klinka et al. (1989), classified soil moisture regimes which characterized plant-water relationships. They defined amplitudes for species according to the period of water deficit and depth of groundwater table. The application of biosolids may affect soil waterholding capacity, and therefore, change the water deficit status of the site. This change may alter the sites location on the soil moisture gradient to a location that is less suitable for the existing species on the site.

(b) Soil nitrogen

Klinka et al. (1989) describes understory species that are indicators of poor, moderate and rich soil N. Indicators of N poor soil (which include salal and various huckleberries) are found on soils with annual mineralizable N ranging from <10 to 30 kg N ha^{-1} and total soil

N less than 2500 kg N ha^{-1}. These species generally inhabit acid substrates with pH less than 4.5. Species indicative of moderate N soils include hardhack (*Spirea douglasii ssp. douglasii* Hook) , baldhip rose (*Rosa gymnocarpa* Nutt.), trailing blackberry (*Rubus ursinus* Cham. and Schlecht.), Oregon grape and oceanspray. These are found on soils with annual mineralizable N ranging from 30 to 70 kg N ha^{-1} and total soil N between 2500- 3500 kg N ha^{-1}. Indicators of rich and very rich N soils including fireweed (*Epilobium angustifolium* L.), Himalayan blackberry (*Rubus discolor* Weihe and Nees), red elderberry (*Sambucus racemosa* L.) and stinging nettle (*Urtica dioica* L.), are found on substrates containing readily available N from strong nitrification, with annual mineralizable N ranging from >70 kg N ha^{-1} and total soil N > 3500 kg N ha^{-1}.

(c) Ground surface (litter layer) materials

Understory plants obtain substantial nutrients and water from the surface soil layer, making this layer important in influencing species establishment and growth. Klinka et al. (1989) created 5 indicator classes for ground surface materials. As biosolids add decomposed organic matter to the forest floor, the two most relevant classes when examining the effect of biosolids are those classes with organic matter either present or absent. Klinka et al. (1989) have salal and huckleberries as indicator species in the *Vaccinium parvifolium* Sm. group. They are found on Mor humus forms, consisting of 5 cm or more compacted organic matter overlying mineral soils. Sword fern, bedstraw (*Galium triflorum* Michx.) and red elderberry are in the *Polystichum munitum* group. These indicator series are found on Moder or Mull humus forms, consisting of less than 5 cm of friable organic materials.

(d) Stand growth stage

Stand age, growth stage and disturbance intensity and frequency impact on understory abundance and composition. Plantations can generally be considered as even aged stands developed from one major disturbance, while in natural forest stands depending on the number and intensity of disturbance, single or multiple cohort stands can develop.

The development of a single cohort stand can be described by four stages (Oliver and Larson 1996):

- Stand Initiation stage. After fire or clearcutting, all overstory trees are removed but forest floor herbs, advance regeneration, buried seeds and roots may remain. A new stand re-initiates with trees seedlings, shrubs and herbs colonizing the available growing space.
- Stem exclusion stage. The fully stocked stand moves towards crown closure. The overstory foliage layer rises, with understory, and subdominant or suppressed trees in the canopy often dying. Individual species number in the stand generally declines, with the forest floor often bare of herbaceous plants.
- Understory reinitiation stage. As the overstory ages, understory herbs and shrubs capable of living under low light intensity appear in the forest floor. The understory species are often not be the same species present in the stand initiation stage.
- Old-growth stage. The overstory trees are still predominant, but the death of some old trees is causing irregular gaps, were understory and advanced growth can flourish.

Within a chronosequence of Douglas-fir stands of given SQ that would represent the first two growth stages described above , Turner and Long (1978) showed vascular plant abundance decreased with age after a hiatus at about age 20. Between age 5 and 22 the developing overstory, "provided the degree of canopy under which salal with a reduction in light intensity and evaporative demand, achieves its best development" (Long 1975). Salal abundance then decreased with age, while Oregon grape and twinflower (*Linnaea borealis* L.) abundance increased until major shading occurred. Non vascular plants, i.e., lichens and mosses increase in abundance as the stand age.

Understory presence is heavily dependent on the stage of overstory development and crown closure. Understory biomass as a percentage of total biomass is highest in the first 10 years of stand development (Turner et al. 1975). As the stand enters the overstory stem exclusion phase (between ages 15 and 35 depending on site), overstory shading and underground resource competition significantly reduces the abundance and density of understory species. By 60 years in many forests, pure unthinned Douglas-fir stands have poor understory development. Thinning of Douglas-fir stands will reverse this trend, providing sufficient light and below ground resources exist for understory reinitiation.

In Douglas-fir stands, Halpern (1989) examined early successional changes in understory plants for 21 years after clearcutting and burning. Within two years after disturbance most understory species had either survived or were re-established on the sites. The extent of colonization of the site by invading species is dependent on the scale and intensity of the disturbance and the availability of reproductive material for vegetative expansion. Persistent species were often initially present in low abundance, but over time these species gradually recovered or expanded. In the first two years after burning, understory herbs and shrubs (salal, Oregon grape and sword fern) abundance decreases, while fugitive annuals rapidly peaked. Over time, dominant understory species mix changed gradually from annual (e.g., *Epilobium paniculatum* Nutt.ex.Torr. & Gray) to herbaceous biennial and perennial (e.g., *Cirsium* P.Mill, *Epilobium angustifolium* L.) to woody perennials (*Ceanothus* L. *spp.*, *Rubus parviflorus* Nutt.) (Halpern 1988).

In managed stands, overstory thinning can change the stand's growth stage depending on timing and intensity of the operation. The growth stages where understory can be affected by biosolids or fertilizer application is in the stand initiation and understory reinitiation stages. Understory is also major component in the old-growth stage of forest development, although it is very unlikely that biosolids would be applied to such stands.

3.3 Growth strategies of understory plants in relation to nutrition

Figure 3.1 is a "schematic illustration of the relationship between nutrient concentration in plant tissue and growth responses to added nutrients (Landsberg and Gower 1997). In the initial nutrient limitation phase, the addition of limiting nutrients increases plant growth. In the luxury consumption phase, no significant growth response occurs to increasing nutrient concentrations, while in the third phase nutrients become toxic and growth declines.

Chapman-King et al. (1986) examined growth response of plants from application of biosolids and wastewater. Fertilized plants grow "not simply because a deficient nutrient has been supplied, but because of the plants genetic capacity and suitable environmental conditions allow them to convert these raw inputs into dry matter." Variability in growth exists among individuals within and among species, as well as in response to changing environmental conditions (e.g., periods of drought or wet weather.) Different growth patterns, survival strategies, and genetic constraints characterize all species. Different base levels and threshold concentrations for different species help explain why nutrient concentrations can affect species abundance and composition.

Tilman's resource ratio hypothesis predicts that species composition within a community is determined by the relative availability of limiting resources (Tilman 1984). The relationships between essential resources such as nutrients, moisture and space, are hyperbolic growth isoclines that show that no amount of one resource can completely substitute for a lack of another, but one resource can partially substitute for another at intermediate levels of both resources. This view conflicts with Klinka et al.'s view that no compensation exists between "mode of action" environmental factors. Tilman states that only the best competitors for open space, can survive in habitats with low disturbance rates. Once a site is disturbed, it can be "invaded by other species that are superior competitors for other resources (nutrients, water), but inferior competitors for open space."

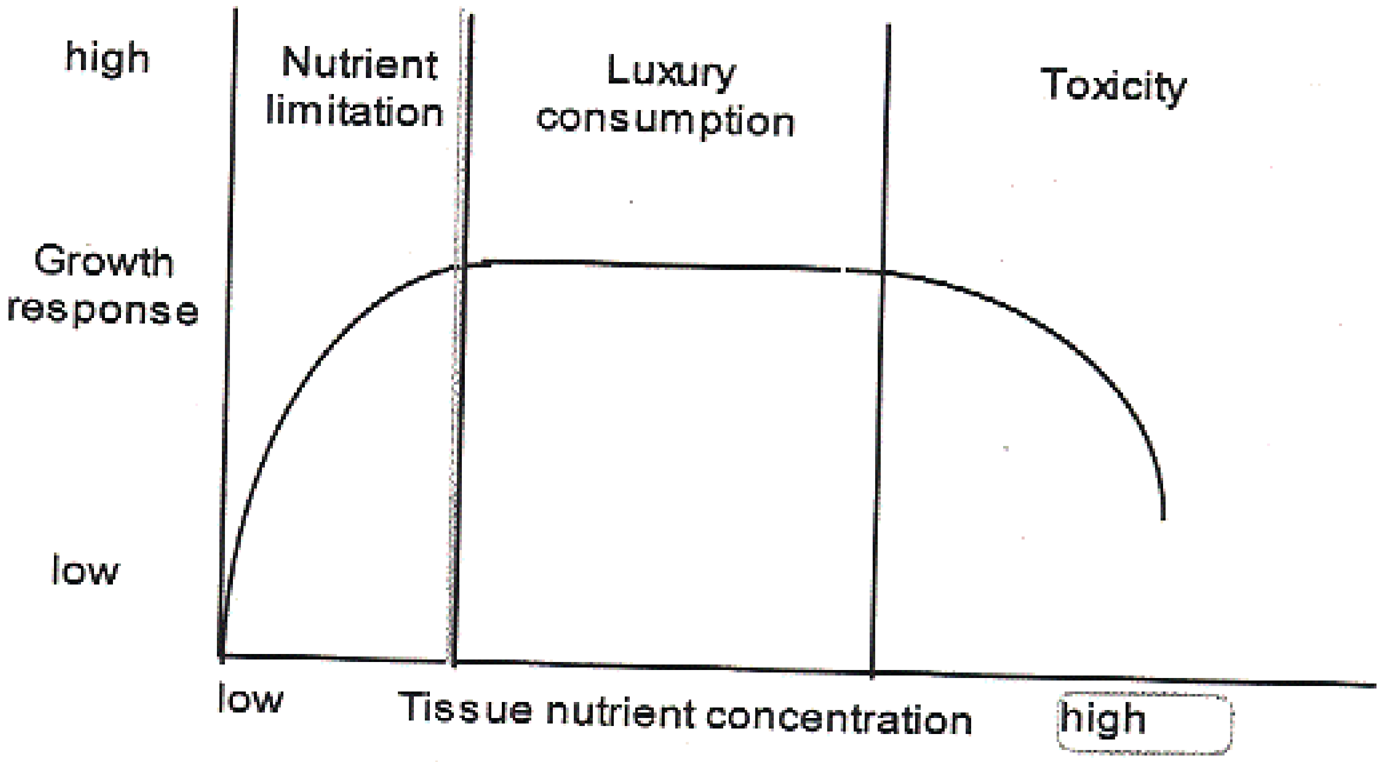

Figure 3.1. Schematic diagram modeling the general pattern between nutrient concentrations in tissues and plant growth responses to added nutrients (Landsberg and Gower 1997).

In plant communities where disturbance supplies light and possible nutrients (thinning and biosolids), "disturbance is a process that influences the relative supply rates of the resources for which competition occurs. The species composition of a light and nitrogen limited community should be strongly influenced by the ratio of the disturbance rate to the supply rate of nitrogen."

The nutrient concentration of plants are often indicative of their life history strategies. Plants that require higher rates of nutrients (i.e., have high tissue concentrations) to produce their biomass are commonly early successional species (Pastor and Bockheim 1984). Although no physiological explanation exists, alternative theories offer reasons why early successional plants have high nutrient concentrations. Pastor and Bockheim 1984, cite Chapin and Vitousek that although nutrient usage is inefficient, these species can achieve more rapid production and nutrient uptake and greater nutrient retention in perennial tissues than late successional species. These early successional species reduce the pool of available nutrients that would be available for competing species, even if they don't need to use it. Alternatively these early successional species may be considered as very efficient plants. They have higher concentration of chlorophyll and more efficient photosynthesis per leaf, so they will fix more C per gram of leaf and also have a higher N concentration.

Chapin (1983) in black spruce stands in Alaska noted that nutrient efficient trees and shrubs with low N concentration and turnover have advantages in when nutrient s are limiting, but "may not exploit flushes as thoroughly as high turnover species during times of rapid nutrient release." The impact of interplant competition during these flush periods, may force a change in species' characteristics that favors fast growing species. For example applications of biosolids to semiarid grassland in New Mexico increased fungal (Fresquez and Dennis 1990) and vegetation (Fresquez et al. 1990) abundance and biomass, but decreased the diversity of species present.

Prescott et al. (1993a) and Coward (1993) noted in coniferous stands that had been fertilized, that shifts occurred in the understory composition from low N species e.g., salal to species richer in N and P. Prescott et al. (1995) also noted reduced cover of ericaceous species (*Kalmia* L. and *Vaccinium* L.) and replacement with species requiring higher N with repeated applications of N or NPK, where total N exceeded 672 kg N ha^{-1}. High concentrations of ammonium and nitrate are often felt to inhibit ericaceous plants like salal (Prescott et al. 1993a). The ericoid mycorrhizae of salal are adept at assimilating complex organic N and P (Read 1983), but are suppressed by application of available N (ammonium) and simple organic N. The colonization rate of salal plants inoculated with four species of ericoid mycorrhizal fungi, was similar for treatments with N sources as glutathione (a peptide), bovine serum albumin (a protein) and N free controls. Treatments with N sources of ammonium and glutamine (an amino acid) significantly reduced colonization rates (Xiao 1994, Prescott and Weetman 1994).

Alternatively, Stanek et al. (1979) showed a combination of moderate fertilization and heavy thinning treatments increased salal cover and biomass. The thinning regime allowed release of the salal, while the level of fertilizer application boosted this response compared with unfertilized thinned sites. The concentration of N must not have exceeded the critical threshold, thereby allowing increased salal growth without causing species composition change.

3.4 Previous studies of N accumulation in response to application of biosolids

Major gaps exist in our knowledge of the N accumulation rate of most overstory and understory species, as well as on the variability in accumulation rates for plants of different sizes, growth stage or vigor. Research into the N accumulation of forest crops following fertilization is sparse. Dyck et al. (1984) calculated the N accumulation rate for both 6 and 55-year-old Douglas-fir after biosolids application of 101 and 28 kg-N ha^{-1} respectively, while Henry (1989) calculated N uptake rate for young sapling sized poplars of 78 and 132 kg-N ha^{-1}.

Understory shrubs are frequently omitted from forest nutrient cycling studies (Cole and Rapp 1981; Chapin 1983), due to the complexity in determining sampling strategies for a range of deciduous and evergreen plants found in the understory layer. Deciduous species differ from evergreens in showing more rapid movement of nutrients into leaves in spring and a pulse of nutrient loss in autumn (Chapin 1983). The seasonal growth patterns for biomass and nutrients in blue spruce forests near Fairbanks in Alaska, showed new growth of *Vaccinium* L. began in early June with maximum mass of leaves achieved in mid July and by current stems in mid August. *Vaccinium* L. stems increased in biomass throughout the summer, but act as a source of N and P in spring and began to accumulate N and P only late in summer, first through plant uptake and in autumn through translocation from leaves. The evergreen *Ledum* L. continued both current leaf and current stem growth until mid August.

Most fertilizer studies consider only above ground biomass, because of ease of measurement and the difficulty in separating and identifying the different roots under field conditions. However, Heilman and Gessel (1963) showed that below ground biomass can contain between 5 and 33% of the total biomass in 30-to 52-year-old Douglas-fir stands. Prescott and Weetman (1994), in young Cedar Hemlock stands in British Columbia, showed that roots contain on average 43% of total understory N content.

Previous studies have shown increases in understory biomass and N content following biosolids application. Brockway (1983) observed a 132% increase in understory biomass and 100 to 200% increase in N concentration following biosolids application to 36-year-old red pine stands in Michigan. This followed an initial decline in biomass after two months due to smothering effects of the application. He also found that increases in understory foliar N content were greater than the overstory, but because of differences in biomass production between overstory and understory, the understory contributed relatively little to the change in N uptake of the site.

Mcleod et al. (1986) in newly established loblolly pine plantations observed a 260% increase in total understory biomass where biosolids with 800 kg N ha^{-1} were disc incorporated with no herbicide treatment. Understory biomass increased in treated areas in 3-, 9- and 27-year-old plantations, competing severely with trees in the 3 and 9-year-old stand. Herbs showed the greatest response, but increases in the shrub-vine and woody components indicate a potential long term effect.

Aschmann et al. (1990,1992), in an eastern mixed hardwood (*Quercus* L., *Carya* Nutt.) forest showed significant increases in N concentration with increasing sludge rate from 0-800 kg N ha^{-1} for *Lonicera* L., *Parthenocissus* Planch., *Viburnum* L., *Lindera* Thumb., *Rubus* L., *Berberis* L. and *Circaea.* L. Although foliar N increases were greater in the understory than the overstory, due to the small scale of biomass production in understory compared to trees, the understory contribution to the total N uptake of the forest was small. They estimated increase in the total forest uptake between the control sites and those applied at 800 kg N ha^{-1} was 50 kg N ha^{-1}. The increase in understory between treatment and control represented a maximum of 12.5 kg N ha^{-1} with the total N absorbed by all vegetation on the site less than 100 kg N ha^{-1}.

Chapter 4:
EFFECT OF BIOSOLIDS APPLICATIONS ON SPECIES COMPOSITION

4.1 Introduction

Biosolids application changes both resource availability and the physical environment. Biosolids improve soil water holding capacity, increase nutrient availability and alter forest floor characteristics (Henry et al. 1993). These edaphic changes differentially affect growth and survival of understory species.

Prior to this study, I hypothesized that past biosolids application to 68 and 75 year-old Douglas-fir stands at Pack Forest changed understory composition and abundance. Between 3000 and 8000 kg N ha^{-1} were applied in biosolids to the study sites over a 12-19 year period. These high levels of N and organic matter, should increase both nitrophytic and moisture loving understory species.

4.2 Study Areas

The research sites are located on the University of Washington's Charles Lathrop Pack Forest near Eatonville, Washington. Pack Forest has a mean annual temperature of 10° C, with a maritime climate producing mean annual rainfall of 1200 mm (Cole 1988). Two existing biosolids trials in Douglas-fir stands, the Silvicultural Demonstration Site (SDS) and the Highway Thinning Trial (HTT) were used. Characteristics of these trials are described in Table 4.1.

The treated HTT stands received twice the application rate of biosolids of the SDS. The HTT Site is a very low SQ stand, with lower basal area and crown density than similarly thinned and unthinned sites in the SDS.

Table 4.1. Characteristics of research sites at Pack Forest used to investigate understory species composition and abundance.

	Silvicultural Demonstration Site (Yonaka et al. 1983)	Highway Thinning Trial (Cole 1988)
Soil type	Residual soils from andesite. Plots in Baumgart (High SQ) and Pheeny (Low SQ) series	Barneston coarse gravely outwash
Elevation (m)	550-600	200
Stand Age (yr)	68	75
Site Quality	Low SQ (31m at age 50) High SQ (34 m at age 50)	Low SQ (27 m at 50 years breast height.)
Topography	Gentle slopes <15° on ridgelines and S-SE facing upper slopes	Relatively flat lower slopes.
Thinning history	Low SQ 1984 High SQ 1985	routine thinning 1977 salvage thinning of dying trees 1996
Biosolids history	1985-1986 ridges 47 Mg ha^{-1} 1987 lower slopes 47 Mg ha^{-1} 1991 all site 11 Mg ha^{-1} 1996 all site 11 Mg ha^{-1}	1977-78 95 Mg ha^{-1} 1980 47 Mg ha^{-1} 1995 10 Mg ha^{-1}

4.2.1 Previous vegetation

The SDS Management Plan (Yonaka et al. 1983) lists understory species and maps understory plant associations. On both high and low SQ, salal was the indicator species in the understory association. The species present in the SDS, prior to biosolids application are listed in Table 4.2.

Table 4.2. List of vegetation species in Silvicultural Demonstration Site (Yonaka et al. 1983) prior to biosolids application and thinning.

More than 10% of canopy or ground cover on site	
Douglas-fir	*Pseudotsuga menziesii* (Mirbel) Franco
Oregon grape	*Berberis nervosa* Pursh
salal	*Gaultheria shallon* Pursh
sword fern	*Polystichum munitum* (Kaulfuss) K. Presl
Widely distributed but with less than 10% cover	
red alder	*Alnus rubra* Bong.
western red cedar	*Tsuga heterophylla* (Raf.) Sarg.
bracken fern	*Pteridium aquilinum* (L.) Kuhn
deerfern	*Blechnum spicant* (L.) Roth
kinnickinick	*Arctostaphylos uva-ursi* (L.) Spreng.
red huckleberry	*Vaccinium parvifolium* Sm.
Scattered occurrences over much of site	
bigleaf maple	*Acer macrophyllum* Pursh
black cottonwood	*Populus balsamifera* ssp. *trichocarpa* (Torr. &Gray ex Hook.) Brayshaw
bitter cherry	*Prunus emarginata* (Dougl. ex Hook.) Walp.
lady fern	*Athyrium filix-femina* Roth
oceanspray	*Holidiscus discolor* (Pursh) Maxim
salmonberry	*Rubus spectabilis* Pursh
squashberry	*Viburnum edule* (Michx.) Raf.
trailing blackberry	*Rubus ursinus* Cham. and Schlecht.
baldhip rose	*Rosa gymnocarpa* Nutt.
starflower	*Trientalis latifolia* (Hook.) Hulten
Few occurrences	
Pacific madrone	*Arbutus menziesii* Pursh
evergreen huckleberry	*Vaccinium ovatum* Pursh
maidenhair fern	*Adiantum pedatum* L.
cowparsnip	*Heracleum lanatum* Michx.
foamflower	*Tiarella trifoliata* L.
solomonplume	*Smilacina racemosa* (L.) Desf.

4.2.2 Treatments present on existing research areas

Existing biosolids research areas were used because individual treatment areas were well defined and replication of treatments had occurred.

On both SQ areas in the SDS, the following treatments existed:

1. stand thinned and biosolids applied.
2. stand thinned and no biosolids applied.
3. stand unthinned and no biosolids applied.

At the HTT, the following treatments existed:

1. stand thinned and biosolids applied.
2. stand thinned and no biosolids applied.
3. stand unthinned and biosolids applied
4. stand unthinned and no biosolids applied.

4.3 Methods

Measurements of abundance and composition of current vegetation in treated and untreated stands were made, with the differences assumed to be caused by the biosolids and thinning treatments. A pilot survey of tallest understory vegetation was made in summer 1996. A total of 29 transects were randomly located in treatments as per Table 4.3.

The percent cover of the tallest understory vegetation was measured using the line-intercept method (Mueller-Dombois and Ellenberg, 1974). A transect consisted of four 10 m long sub-sections separated by 3 m intervals down the slope. The tallest understory overtopping the vertical projection of the transect was recorded with measurements of the extent of the understory crown vertically above the tape made to the nearest 10 cm. At 2 meters intervals along the transect, all understory species and their height were recorded.

Table 4.3. Distribution of transects per treatment in the summer 1996 pilot study.

	Demonstration Site		Highway Thinning
Treatment	Low SQ	High SQ	Low SQ
Thinned/Biosolids	4	6	2
Thinned/No biosolids	4	4	2
Unthinned/Biosolids	-	-	2
Unthinned/No biosolids	3	1	2

The choice of measuring only the tallest understory species crossing the transect ignored lower layers of vegetation. Although this methodology does not describe understory occupancy, it is useful for characterizing changes in the visual appearance of the site.

On biosolids treated sites, red elderberry and stinging nettle generally overtopped salal and Oregon grape, while on untreated sites salal was the tallest understory. To supplement these earlier measurements, the percent cover of major evergreen understory was measured using the line-intercept method (Mueller-Dombois and Ellenberg, 1974). This assessment was undertaken in March 1997, with transects laid out in an identical manner to the pilot study, but measurements were made of cover of all evergreen species present, regardless of relative species height.

In March 1997, both trials were surveyed to investigate differences in evergreen vegetation indicated by the summer study. Forty transects were established as per Table 4.4.

Table 4.4. Distribution of transects per treatment in March 1997 study of major evergreen understory.

Treatment	Demonstration Site		Highway Thinning
	Low SQ	High SQ	Low SQ
Thinned/Biosolids	7	7	3
Thinned/No biosolids	7	7	3
Unthinned/Biosolids	-	-	3
Unthinned/No biosolids	-	-	3

Although the SDS has treated over 100 hectares, the treatments are not replicated within a Site Quality. As such, the 7 transects per treatment and SQ are considered subsamples. An ANOVA of cover percentage data for evergreen species (transformed by the arcsine square root transformation to normalize data) has been conducted on the two true replicates within the SDS.

Cole (1988), describes the experimental design of the HTT as four treatments replicated three times on 0.06 ha plots. The experiment is a factorial design of thinning and biosolids treatment on vegetation growth. One transect in this study was measured in each of the 12 plots. As the experiment was replicated, SAS was used to conduct individual two way ANOVA of cover percentage data (transformed by the arcsine square root transformation to normalize data) for each evergreen species. A Tukey's Studentized Range Test was then used for multiple comparison.

4.4 Results

4.4.1 Pilot study

The following trends were observed:

1. In thinned, biosolids treated stands on the SDS (Table 4.5), red elderberry was the tallest understory species over 41% and 24.8% of the transect length on high SQ and

low SQ sites, respectively. In biosolids treated stands on the HTT Site (Table 4.6) red elderberry was the tallest understory over 22.3% and 28.4% of the transect length in thinned stands and unthinned stands, respectively. In untreated stands on both sites red elderberry did not occur.

2. In the thinned, biosolids treated stands in high and low SQ sites in the SDS, stinging nettle was the tallest understory species 27.7% and 13.4% of the transect length, respectively . In untreated sites stinging nettle did not occur.
3. In thinned, biosolids treated stands on the HTT Site, stinging nettle was the tallest understory over 11.4% of total transect length. In unthinned sites and untreated sites stinging nettle was not present.
4. Salal as the tallest understory plant, increased with thinning on the SDS in stands not treated with biosolids. Salal increased with thinning from 28.7% to 58.7% of the transect length on high SQ and from 0.6% to 51% on low SQ sites. On the low SQ and lower basal area stands at the HTT site, this trend did not occur.
5. Salal coverage as tallest understory species decreased with biosolids treatment. In treated thinned sites in the SDS, salal was tallest on less than 1% of transect length on high SQ sites and 8.2% on low SQ sites. Again at the HTT, salal as tallest understory decreased with biosolids application.

Table 4.5. Cover (%) of tallest understory species in Silvicultural Demonstration Site pilot study transects in summer 1996.

Common name	High SQ			Low SQ		
	Biosolids thinned	No biosolids thinned	No biosolids unthinned	Biosolids thinned	No biosolids thinned	No biosolids unthinned
grass				0.0	0.0	1.0
moss	0.0	0.4	3.1	0.0	0.0	17.4
sweet scented bedstraw				0.0	0.0	1.5
twinflower	0.0	3.6	13.2	0.0	1.2	0.0
red alder				0.0	3.5	0.0
forest floor	1.0	0.2	27.0	0.5	0.4	71.1
baldhip rose				0.6	0.4	0.0
salmonberry				0.7	0.0	0.0
Himalayan blackberry	2.4	0.0	0.0	1.0	0.0	0.0
dovefoot geranium				1.2	0.0	0.0
trailing blackberry	7.3	2.8	0.1	1.8	2.9	0.0
oceanspray	1.0	1.9	4.3	1.9	2.0	0.0
Oregon grape		0.3	14.6	4.7	9.5	6.6
bracken fern	16.8	21.8	0.9	5.0	12.1	0.4
salal	0.6	58.7	28.7	8.2	51.0	0.6
vine maple				2.2	0.4	0.0
red huckleberry	7.5	5.1	2.7	3.3	5.5	0.0
stinging nettle	27.7	0.0	0.0	13.4	0.0	0.0
sword fern	8.3	3.4	4.2	14.5	10.9	1.4
red elderberry	24.8	0.0	0.0	41.0	0.0	0.0

Table 4.6. Cover (%) of tallest understory species in Highway Thinning pilot study transects in summer 1996.

Common name	Biosolids- thinned	Biosolids-unthinned	No biosolids thinned	No biosolids unthinned
		%		
twinflower	0.0	0.0	5.0	1.5
forest floor	0.3	2.8	5.2	10.2
dovefoot geranium	0.4	4.0	0.0	0.0
red huckleberry	0.8	7.1	0.8	2.4
Oregon grape	1.1	16.7	16.1	19.6
baldhip rose	1.3	1.5	2.0	0.0
snowberry	3.3	0.0	0.0	0.0
sword fern	5.2	18.2	1.0	6.3
grass	5.8	0.0	0.4	0.0
salal	7.8	10.8	43.3	48.0
red alder	8.3	0.0	1.7	1.1
oceanspray	9.6	3.2	9.6	7.8
stinging nettle	11.4	0.0	0.0	0.0
trailing blackberry	18.4	5.0	7.7	0.5
red elderberry	22.3	28.4	0.0	0.0

4.4.2 Evergreen species analysis

4.4.2.1 Salal

In the March sampling of thinned stands in the SDS (Table 4.7), cover of salal was significantly reduced from 69% on untreated sites to 14% on biosolids treated areas. On thinned high SQ stands, salal cover was from 63% on untreated sites and 19% on biosolids treated areas. On thinned low SQ stands, salal cover was 75% on untreated sites and 10% on biosolids treated sites.

In the March sampling of all stands in the HTT (Table 4.8), cover of salal was significantly reduced with biosolids application, but not affected by thinning. On thinned stands, salal cover reduced from 61% on untreated sites to 10% on biosolids treated areas. On unthinned stands, salal cover significantly reduced from 51% on untreated sites to 26% on biosolids treated sites. A significant interaction exists between biosolids and thinning. In thinned treated sites the reduction in salal cover was greater after biosolids application than in neighboring unthinned sites. Although salal cover was reduced on biosolids treated sites, the species was still present. Visual observations showed salal was often present on raised ground around rotting logs, old stumps or around tree stems.

Table 4.7. Average cover (%) of major evergreen species in March 1997 in the Silvicultural Demonstration Site at Pack Forest (with SD in brackets)

Species		High site	Low site	Combined
salal	Treated	19 (20)	10 (11)	14 (16)^{a}
	Untreated	63 (24)	75 (.4)	69 (18)^{b}
Oregon grape	Treated	18 (12)	4 (8)	11 (8)^{a}
	Untreated	21 (18)	4 (3)	13 (15)^{a}
sword fern	Treated	40 (17)	24 (10)	32 (16)^{a}
	Untreated	4 (4)	11 (8)	7 (7)^{a}

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
ANOVA -SALAL						
Between Groups	1434.449498	1	1434.449498	69.7808223	0.01402971	18.5127647
Within Groups	41.11300068	2	20.55650034			
Total	1475.562499	3				
ANOVA- OREGON GRAPE						
Between Groups	3.425539496	1	3.425539496	0.03094944	0.87655404	18.5127647
Within Groups	221.3635559	2	110.6817779			
Total	224.7890953	3				
ANOVA - SWORD FERN						
Between Groups	414.9430486	1	414.9430486	8.67480954	0.09853285	18.5127647
Within Groups	95.66620381	2	47.83310191			
Total	510.6092524	3				

Same letter for a species indicates no significant difference exists at $p< 0.05$

Table 4.8. Average cover (%) of major evergreen species in March 1997 in the Highway Thinning Trial at Pack Forest (SD in brackets)

		Thinned	Unthinned
salal	Treated	10 (6)^{a}	26 (8)^{a}
	Untreated	61 (11)^{b}	51 (10)^{b}
Oregon grape	Treated	10 (9)^{a}	35 (20)^{b}
	Untreated	11 (8)^{a}	33 (9)^{b}
sword fern	Treated	41 (9)^{a}	32 (23)^{a}
	Untreated	11 (10)^{b}	3 (5)^{b}

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
ANOVA -SALAL						
Model	2061.304	3	687.101	20.24	0.004	3.61
Error	271.603	8	33.950			
Total	2332.908	11				
Source						
Biosolids	1767.741	1	1767.741	52.07	.0001	4.86
Thinning	41.277	1	41.277	1.22	.3022	4.86
Biosolids*Thinning	252.287	1	252.287	7.43	.0260	4.86
ANOVA- OREGON GRAPE						
Model	1089.366	3	363.122	3.30	0.0784	3.61
Error	879.147	8	109.893			
Total	1968.514	11				
Source						
Biosolids	3.676	1	3.676	0.03	0.8594	4.86
Thinning	1078.712	1	1078.712	9.82	0.0140	4.86
Biosolids*Thinning	6.978	1	6.978	0.06	0.8074	4.86
ANOVA - SWORD FERN						
Model	2231.589	3	743.863	5.46	0.0245	3.61
Error	1089.778	8	136.222			
Total	3321.367	11				
Source						
Biosolids	2020.709	1	2020.709	14.83	0.0049	4.86
Thinning	202.175	1	202.175	1.48	0.2578	4.86
Biosolids*Thinning	8.704	1	8.704	0.06	0.8068	4.86

Same letter for a species indicates no significant difference exists at $p< 0.05$

4.4.2.2 Oregon grape

In the March sampling of thinned stands in SDS (Table 4.7), Oregon grape cover percentage was not significantly affected by biosolids application. In the HTT site (Table 4.8), Oregon grape was significantly less prevalent in thinned sites, but its cover percentage was not affected by biosolids application. On treated and untreated thinned sites cover percentage was 10 ± 9% and 11 ± 8%, compared with 35 ± 20% and 33 ± 9% on unthinned plots. No significant interaction existed between biosolids and thinning.

4.4.2.3 Sword fern

In the March sampling in the SDS, sword fern cover was not significantly affected at $p<.05$ by biosolids treatment. Sword fern cover on treated sites was 32 ± 16% and 7 ± 7% on untreated. In the HTT site, sword fern was significantly greater on treated sites but unaffected by thinning. On treated thinned and unthinned sites cover was 41 ± 9% and 32 ± 23%, compared with 11 ± 10% and 3 ± 5% respectively on untreated plots.

4.5 Discussion

The original SDS Management Plan species list included all species found in the current transect surveys (Table 4.4) with the exception of red elderberry and stinging nettle. Both of these species are now common in treated areas.

A number of species previously listed were not found on transects during the current survey. These included black cottonwood, lady fern, squashberry, Pacific madrone, evergreen huckleberry, maidenhair fern and foamflower. All of these species are classified as scattered or rare. Some of the reasons they were not found could include:

- Control transects were not located in creek lines, where some of these species are present. Creeks were not sampled as controls because in biosolids application, creek lines are buffered and no biosolids are applied.
- The pilot study only recorded the tallest plant that crossed the transect line. Full measurement of all understory species only occurred at 2-m intervals along the transect. This method inadequately sampled rare and scattered plants.

Coward (1993) using a similar method as the pilot study on the HTT reported decreases in salal, twinflower and moss and increases in trailing blackberry, red elderberry and bedstraw. In 4- ,10- and 16-year-old stands in British Columbia, she showed the overall effect of biosolids treatment after 2 years was to decrease shrub cover, particularly salal. Fireweed and bracken fern cover both increased. Her method did not quantify percentage cover of sub-dominant understory plants.

4.5.1 Potential explanations for species change

Plants have different requirements of temperature, nutrients, light and moisture for growth and survival. Natural variability in these requirements occur between individual plants within species, while substantial variability occurs among species. Klinka et al. (1989) described the ecological amplitudes for plants in coastal British Columbia. These amplitudes describe environmental attributes of sites that will give competitive advantages for certain species to survive and flourish. Two important gradients, soil moisture and soil nitrogen for major species applicable to this study are shown in Figure 4.1 and 4.2. Red elderberry and stinging nettle are examples of nitrophytic species that require soils with higher soil nitrogen. Alternatively, salal grows best in lower soil nitrogen (Klinka et al. 1989). Tilman (1987) showed, in the recolonization of old croplands, that species more characteristic of N-rich sites are more common after N fertilization. In addition to the soil moisture and soil nitrogen gradients, Klinka et al. (1989) also describes how the presence of both stinging nettle and red elderberry indicating that the site has been disturbed.

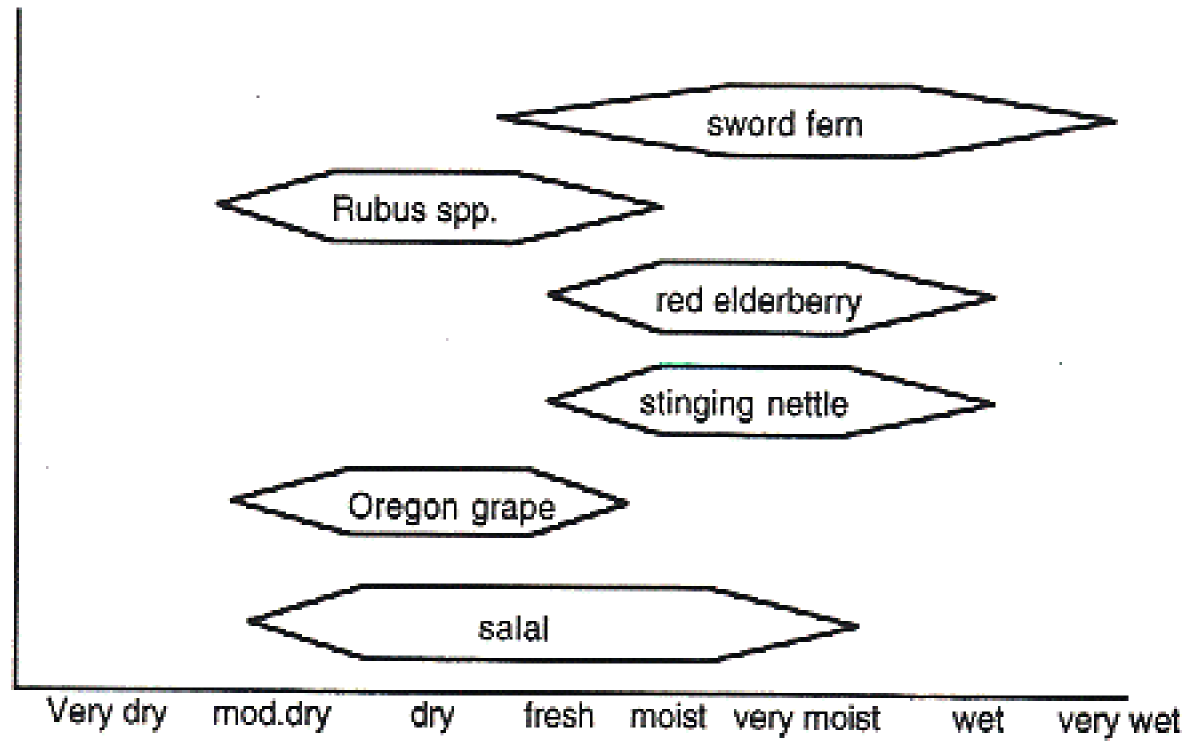

Figure 4.1. Schematic ecological amplitudes for main species found in the study areas as a function of soil moisture (from Klinka et al. 1989).

Simplistic models like that in Figure 4.3, modified from Chapman-King et al. (1986), keep other environmental variables constant and vary the critical environmental gradient (N levels) that will most affect growth . Species relative biomass (as a surrogate for species relative abundance) increases until the optimum N level is reached. This would occur at point A1 (Figure 4.3) for salal (a low N species) or A2 for red elderberry (a nitrophytic species). Assuming other plant needs are not limiting, species biomass remains constant until higher threshold concentrations at B1 or B2, respectively, are reached. After this higher threshold level is reached, growth impairment can occur from either nutrient toxicity of the plant or associated mycorrhizal associations, or increased physical competition from species that are better suited to the environment. This simplistic model, partially explains why nutrient concentrations affect species biomass and composition.

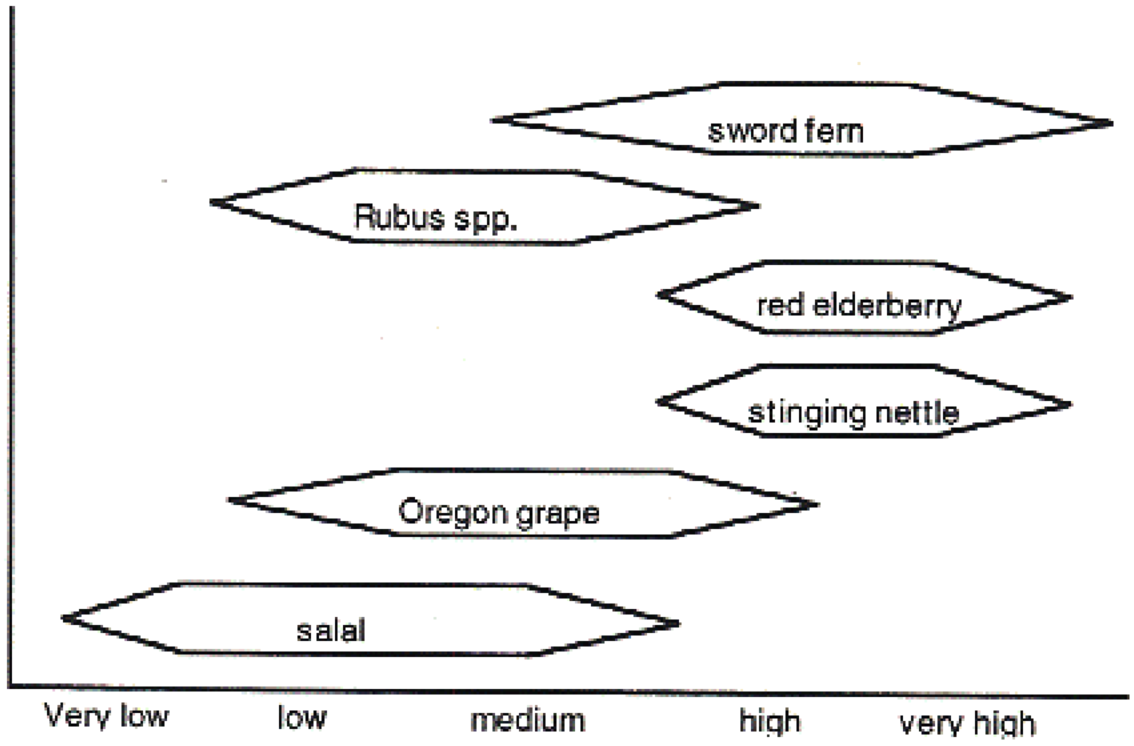

Figure 4.2. Schematic ecological amplitudes for main species found in the study areas as a function of mineralizable soil nitrogen (from Klinka et al. 1989).

The increase in red elderberry on treated sites probably resulted from either increased moisture, nutrients, site disturbance or combinations of these. However, the substantial growth of red elderberry in the unthinned stands in the HTT possibly infers that increasing light penetration to the understory may not be the significant process in the re-initiation of this species. Instead the biosolids may have caused an edaphic change, with increasing nitrogen and moisture allowing species development.

The increased moisture holding and soil N content after biosolids appears ideal for stinging nettle introduction. The patterns observed in this study confirm those described by Klinka (1989); stinging nettle preferring moder and mull forms of humus with "layers less than 5 cm of friable (partly fragmented or comminuted) organic materials overlying mineral soil."

This humus layer must allow airborne seed to germinate and emerge. This situation is prevalent after biosolids applications, especially where biosolids accumulate in small microsite depressions.

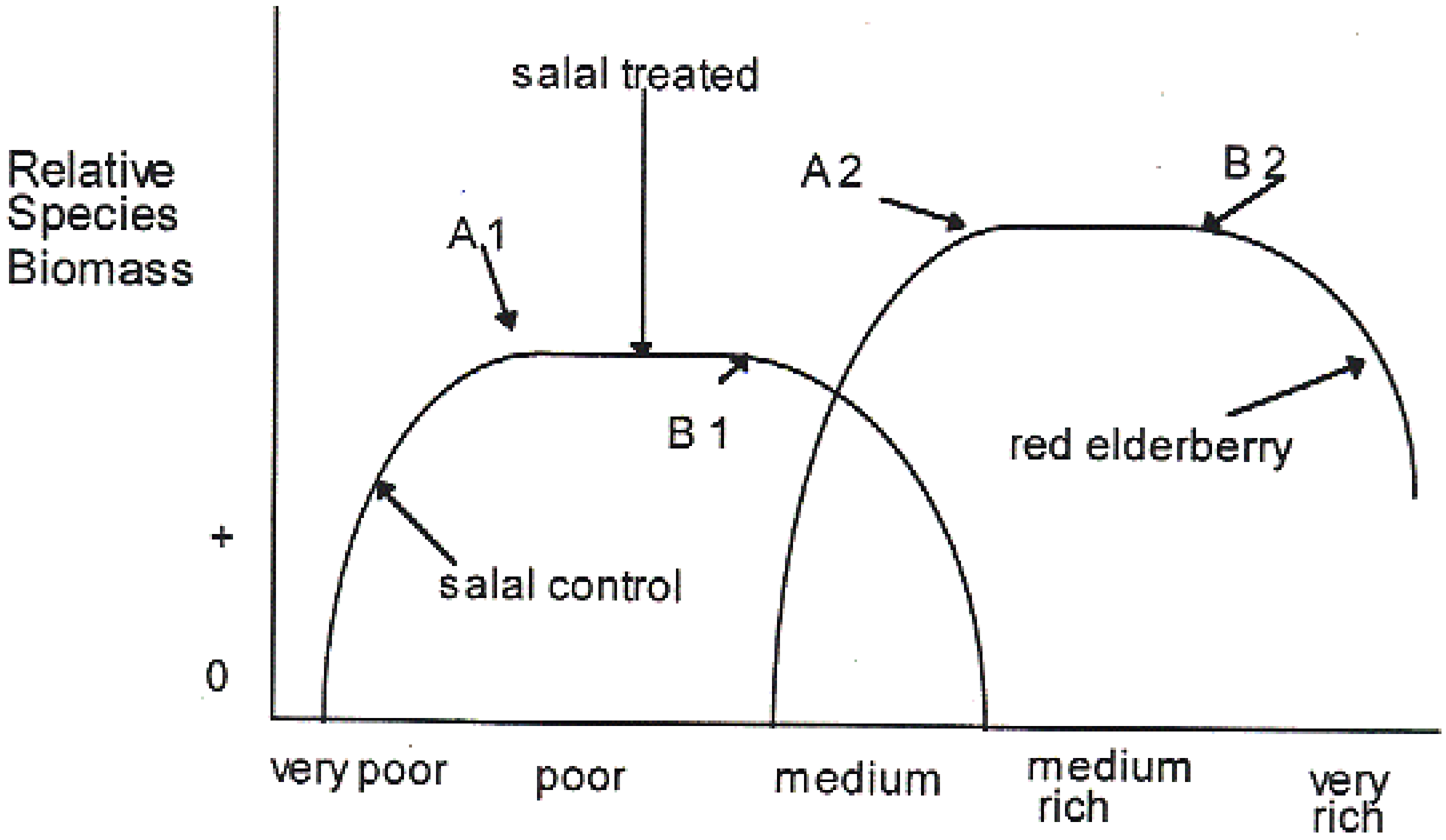

Figure 4.3. Conceptual relative species biomass response of salal and red elderberry at Pack Forest to increased soil nitrogen levels and consequent plant N concentrations [modified from Chapman-King (1986)]. A is the optimum level of N concentration for a particular species, B is the threshold concentration level, beyond which growth is impaired.

The absence of nettle in unthinned sites in the HTT where biosolids was applied, may show that stinging nettle requires primarily higher light levels, characteristic of a thinned site. This must be combined with increased soil moisture, moder and mull humus, nitrogen additions and the presence of a seed source.

The reduction in salal in treated sites follows trends in Klinka's schematic representation. Heilman and Gessel (1963) noted understory shifts from salal to species richer in N and P after fertilization. Prescott et al. (1994) found in the adjoining Gessel Unit trials at Pack Forest that salal was absent in fertilized blocks, with snowberry (*Symphoricarpos albus* (l.) Blake) and sword fern (both species with characteristically higher N concentrations) present instead.

Possible mechanisms for reduction in salal cover by reducing salal growth and survival include:

- Ammonia volatilization, coupled with desiccation of the understory foliage. Up to 20% of ammonium in the biosolids is volatilized in the first weeks after application (Grey 1996). Highest ammonia concentrations are at ground level thereby affecting understory more than overstory. For burning of foliage to occur, other environmental factors such as exceptionally hot, dry summer conditions may also be required.
- N toxicity of ericoid mycorrhizae associated with salal. Increased ammonium and nitrate concentration inhibit ericaceous plants in the genus *Gaultheria* L., *Kalmia* L. and *Vaccinium* L.(Prescott 1993a). The ericoid mycorrhizae symbiotic relation with salal is effective in assimilating complex organic N and P (Read 1983), but is suppressed by application of available N and simple organic N. Xiao (1994) found mycorrhizal colonization of salal roots was less in plants fertilized with inorganic N forms than when fertilized with organic N, e.g., peptides (Prescott and Weetman 1994). Prescott et al. (1995) noted that reduction in *Kalmia* L. and*Vaccinium* L., and their replacement with higher N species occurred where N or NPK was applied at greater than 672 kg N ha^{-1}. The SDS has received 3000 kg N ha^{-1}, and the HTT 8000 kg N ha^{-1}.

Generalizations that forests or even plantations are homogeneous units, still does not capture the spatial or stand variability present. Within treated sites in the SDS variability in salal is associated with other stand factors or application practices. Microsites with higher salal present normally had noticeably less nettle and red elderberry. Possible reasons could include:

- a lack in the uniformity and intensity of thinning has failed to achieve the minimum level of canopy reduction in these microsites needed to allow understory reinitiation of early successional understory plants. Heavier thinning increases the available space and, if adequate nutrients and moisture are present, allows establishment of early successional species.
- Thinning did not occur simultaneously over the whole site, thereby allowing earlier thinned sites more time to establish understory before biosolids were applied. Where salal is currently established on thinned untreated high SQ in the SDS, it occupies over 63% of the area, in a dense 1m tall shrub layer. The extent to which biosolids affects the growth and survival of established understory, should differ substantially from the impact on less developed, recently released plants. If currently unthinned areas in the SDS were thinned, the size of established salal plants would generally be less than 40 cm tall (from plot notes) and cover percentage much sparser than the 29% of total forest floor shown in Table 4.5.
- Salal is often associated with small microsite variations, e.g., raised ground around rotting logs, old stumps or around tree stems. These areas have higher levels of organic matter and often biosolids flow off these areas into adjoining microsite depressions. The growth of salal on raised ground and organic debris suggests that effects on mycorrhizae impacts could be more significant than the toxic effect of ammonia on foliage.

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- Thinning did not occur simultaneously over the whole site, thereby allowing earlier thinned sites more time to establish understory before biosolids were applied. Where salal is currently established on thinned untreated high SQ in the SDS, it occupies over 63% of the area, in a dense 1m tall shrub layer. The extent to which biosolids affects the growth and survival of established understory, should differ substantially from the impact on less developed, recently released plants. If currently unthinned areas in the SDS were thinned, the size of established salal plants would generally be less than 40 cm tall (from plot notes) and cover percentage much sparser than the 29% of total forest floor shown in Table 4.5.
- Salal is often associated with small microsite variations, e.g., raised ground around rotting logs, old stumps or around tree stems. These areas have higher levels of organic matter and often biosolids flow off these areas into adjoining microsite depressions. The growth of salal on raised ground and organic debris suggests that effects on mycorrhizae impacts could be more significant than the toxic effect of ammonia on foliage.

- Biosolids application is not always even within sites. Variation in application rates can occur in areas of variable road intensity (i.e., road intersections) unless extreme care is taken in application technique. Dewatered biosolids applied at current application rates presumably do not move substantial distances after applied. However, if sufficient liquid biosolids were applied (as was the case in the early 1980s operation), some movement down hill could occur with accumulation in microsite depressions. To prevent movement off-site, buffers were left on roadsides and drainage depressions. These road side buffers for the liquid biosolids operations are still heavily dominated with salal. These sites probably received little biosolids from the initial liquid applications, then routine rates in the subsequent dewatered application (cumulative rates of available N at approximately 600 kg ha^{-1}). The effect of light application rates of biosolids on salal growth is similar to the result obtained at Shawnigan Lake on Vancouver Island where "a combination of urea fertilization and thinning treatments benefited the salal undergrowth, with heaviest thinning and moderate fertilization being the most beneficial" (Stanek et al. 1979).

Oregon grape is present on all sites at Pack Forest and its cover is not affected by biosolids application. Oregon grape is generally less than 60 cm tall and survives under competition from salal and other understory plants. It has significantly greater relative coverage in unthinned stands where light is limited. Being a slow growing plant, it does not appear to have the capacity to rapidly increase biomass and outcompete more rapidly growing early successional plants. However it also appears that Oregon grape is not detrimentally affected by any added nutrients in biosolids, nor adversely impacted by increased competition from plants with accelerated growth from fertilization.

Sword fern is an indicator species of higher SQ Douglas-fir stands (Franklin and Dyrness 1988) and has significantly greater coverage on the treated sites. The increased moisture and nutrients from biosolids should improve site quality and generate a favorable edaphic environment for sword fern. Sword fern is commonly over 1m tall and in spreading clumps which outcompetes any species present under its fronds. Although overtopped by some deciduous plants, the evergreen nature and longevity of this plant makes it ideal to increase biomass over time in biosolids treated areas.

4.5.2 Limitations of this study

This study primarily looked at relative situation between understory plants composition and abundance in previously treated biosolids treated and control plots. It was an exploratory study that examined the results of past applications. Detailed statistical analysis of the SDS is limited by the number of true replicates present. It would also have been better to monitor species composition and abundance change with permanent plots replicated over a range of stand age, stand densities and sites. These permanent plots would have had a known starting point, allowing more definitive analysis of understory changes. The major problem with long-term monitoring using permanent plots is the high resource cost and long lead time to get results. Options presented by modern high resolution remote sensing techniques may make periodic monitoring more efficient (Landsberg and Gower 1997).

Alternatively, increasing the knowledge of the mechanisms that cause individual species response to biosolids, may allow biosolids managers to model the results of biosolids application, rather than relying on the results of site monitoring on a range of sites to determine future outcomes. This study did not set out to explore the physiological mechanisms by which biosolids affect understory plants. Research to determine such mechanisms could be conducted in a controlled laboratory or green house environment and could initially include:

1. determination of the actual N concentrations that understory plants reached for optimum growth and the level of N that caused growth decline through nutrient toxicity. This would need to be done for understory plants of different initial biomass.
2. determination of the impact of different rates of biosolids application on the mycorrhizal associations in different plants.

4.6 Conclusions

Repeated or heavy biosolids applications to Douglas-fir stands at Pack Forest caused a significant change in understory species. On these sites the relative density of nitrophytic plant species, such as red elderberry and stinging nettle which where formerly absent, increased substantially. Biosolids decreased the abundance of the low N species salal, increased sword fern and had no affect on the cover of Oregon grape.

Chapter 5:

EFFECT OF BIOSOLIDS APPLICATION ON THE GROWTH AND SURVIVAL OF 2 YEAR-OLD DOUGLAS-FIR SAPLINGS IN STANDS WITH SUBSTANTIAL UNDERSTORY PRESENT

5.1 Introduction

Routine urea fertilization of Douglas-fir stands in western Washington normally occurs when trees are 10-15 years old and able to effectively compete for nutrients. Fertilization is generally not practiced in very young forest stands even though recent trials measuring growth response from N and P fertilization at time of planting have shown positive results (Chappell et al. 1992). Plantation managers have general concerns that early stand fertilization:

- increases competition from understory plants to target crop trees (Turner 1981),
- increases leader damage from greater deer browsing of more palatable saplings (Rochelle 1981),
- produces lower internal rate of return on fertilizer investments, as fertilizer costs need to be carried forward longer to the final crop harvest, before they are amortized.

For these reasons, biosolids applications in Douglas-fir plantations on Weyerhaeuser's Snoqualmie Tree Farm previously have not occurred until stands are approximately 5 years old. Most biosolids applications then occur between ages 5 and 15, after a thinning operation, or when the canopy is high enough to apply underneath it. However, since applications to young stands are very easy, investigation of the effects of biosolids on survival and growth of stands younger than 5 years is important for practical reasons of biosolids management. In particular, it is important to assess the negative impact of increased understory growth. Increased understory can be expected when higher nutrient levels are present. In the case of young stands the response is immediate after biosolids application (as shown in this research), but in stands fertilized prior to clearfelling, the response is delayed and becomes most pronounced once the canopy is removed.

Herbaceous weed control in the initial 2-3 years of plantation re-establishment is not standard practice in Douglas-fir stands in western Washington, so the effects of increased nutrients from biosolids on weed development are unchecked. Inspections of young trees on the Weyco Site at Pack Forest show irregular growth and survival, due to grass and herbaceous weed competition (Edmonds and Cole 1980). The stand on this site was treated with biosolids prior to harvest and the increased soil nutrient values still exist (Smith and Brallier 1991).

The objective of this study was to see whether biosolids applied to a two-year-old Douglas-fir stand adversely affected the growth and survival of planted saplings.

5.2 Study site

Weyerhaeuser's Snoqualmie Tree Farm is 50 km east of Seattle and is an area of major forest biosolids applications associated with the Mountains to Sound Greenway Biosolids Program. The study was conducted in the 25-08-33B Unit (Sections 32, 33 Township 25N Range 8E) at Snoqualmie Tree Farm, on the same site used for the N accumulation trial (Chapter 6). This is a unit of two-year-old planted Douglas-fir saplings. The unit is 33 ha in size on flat to gentle topography at 260-270 m elevation on a Barneston gravely sandy loam soil. Temperatures are moderate with annual average precipitation of 1500 mm (USDA SCS 1992). The previous second growth Douglas-fir stand had biosolids applied in 1987 prior to being clearfelled in 1993. The site was not burned after harvesting and large amounts of woody debris remained when replanted in 1994.

The site had low stocking of 0.2 m to 1.5 m tall saplings. The average initial stocking at age 2 was 570 (SD 84) stems ha $^{-1}$, with spacing generally irregular as large piles of woody debris from previous harvesting made site conditions in many places unfavorable for seedling establishment. Herbaceous and woody understory on the site consisted mainly of fireweed, vine maple, grass, red elderberry, bracken fern and trailing blackberry.

5.3 Methods

In the unit, 5 randomly selected control blocks (60 X 60 m) were marked prior to the 1996 biosolids application. The rest of the site (excluding the controls) was routinely applied with Renton dewatered biosolids in April 1996 at a rate of 8.3 dry Mg ha^{-1}. A pair of plots as shown in Figure 5.1, were randomly established in each of the 5 control and adjoining treatment blocks.

Approximately 30 saplings in each 12.6 m radius plot (0.05 ha) were mapped and marked so they could be relocated. These plots were established in March 1996 and remeasured in March 1997 to assess changes in the growth and survival of Douglas-fir saplings in the year after biosolids application. Measurements were made of:

- Sapling diameter at 15 cm above ground level using a micrometer.
- Sapling height using a measuring stick.
- Sapling damage levels from browsing, was ordinally classified as: 0 for no sapling damage, 1 for minor damage to lateral branches; 2 for major damage including browsing to the main leader.

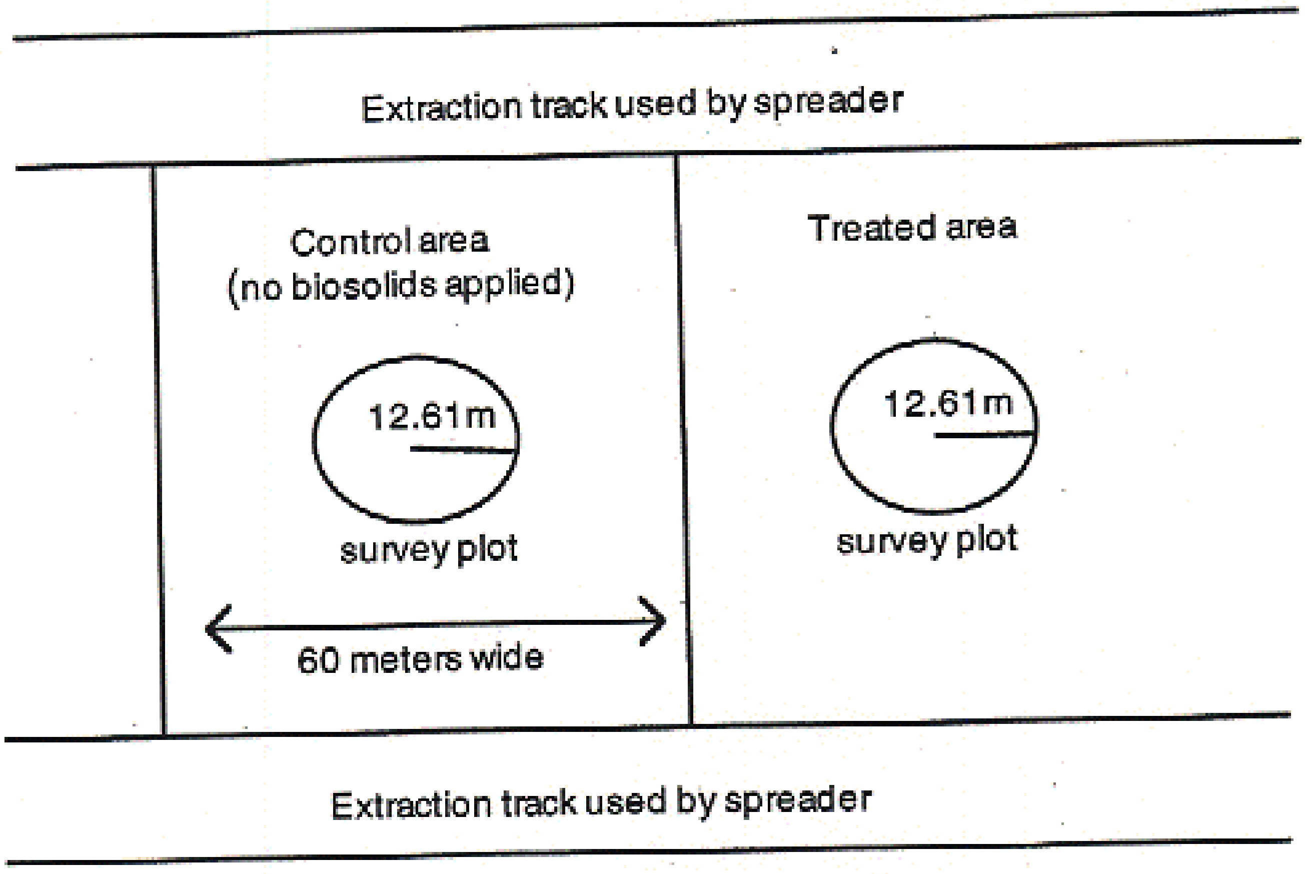


Figure 5.1. Paired plot layout for in 2-year-old stands to compare growth and survival of Douglas-fir saplings.

Average diameter increment, average height increment and mortality (the percentage of dead trees per plot) over the year between 1996 and 1997 were compared by a paired t test between the biosolids applied and control plots. Mortality was transformed by the arcsine square root transformation to normalize data. Survival percentage (the percentage of trees remaining out of the original sample) was calculated for each 10 cm initial height class.

Two factor Analysis of Covariance was performed by general linear models procedure (SAS 1995). In the two factor analyses of height increment with biosolids treatment, the continuous variable of initial height and the ordinal variable of main leader browsing were used as concomitant variable (covariates). For the analyses of diameter increment, initial diameter was substituted for initial height in the model. All covariates were measured from the start of the study and as such are not influenced by treatments (Neter et al. 1990).

5.4 Results and discussion

5.4.1 Sapling height and diameter increment

Biosolids application had no significant impact on either diameter or height increment. The results of the paired t test on average sapling height and diameter increment per plot are summarized in Table 5.1.

Table 5.1. Average sampling diameter and height increment per plot of 2-year-old Douglas-fir saplings on biosolids treated and control plots at Snoqualmie Tree Farm. SD in brackets, and results of paired t test.

	n	Control	Treated	S_d^2	t	p
Average tree diameter increment (mm yr^{-1})	5	2.3 (1.0)	2.2 (0.8)	120.53	-0.2176	0.42
Average tree height increment (cm yr^{-1})	5	16.8 (8.6)	17.9 (8.6)	1.246	0.2264	0.43

Figures 5.2 and 5.3 show average height increment by 10 cm height classes. A substantial number of saplings in the < 80 cm classes in both treated and control plots showed height loss during the 12 month analyses period. This is mainly associated with increased leader browsing on trees less than 80 cm, as shown in Figure 5.4. Results of a 2 factor ANCOVA for height increment are shown in Table 5.2. The ANCOVA showed both initial height and tree browsing were significant covariates, while biosolids treatment was not a significant variable. Height increment increased with larger initial height and decreased if the tree was browsed.

The results of a 2 factor ANCOVA for diameter increment are shown in Table 5.3. No significant relationships at $p<0.05$ existed for either the treatment variable (biosolids application), or the covariates (initial diameter or browsing) . However at the 90% confidence limit, a similar trend is present to that for height increment. The biosolids treatments is still not a significant variable, but both covariates are significant. Finding stronger relations with height than diameter in young trees is a typical result. In early growth plots for conifers less than 3 years-old, height is generally the variable measured for reasons of ease of measurement, larger changes and a strong physiological relationships

Table 5.2. Results of ANCOVA of height increment with biosolids treatment, with initial height and main leader browsing as covariates.

Source of Variation	SS	df	MS	F	P-value
Model	24439.686	3	8146.562	29.45	0.0001
Error	67766.338	245	276.597		
Cor. Total	92206.024	248			
Source					
Treatment	22.254	1	22.254	0.08	.7769
Initial Height	17038.297	1	17038.297	61.60	.0001
Browse	7379.135	1	7379.135	26.68	.0001

Table 5.3. Results of ANCOVA of diameter increment with biosolids treatment, with initial diameter and main leader browsing as covariates.

Source of Variation	SS	df	MS	F	P-value
Model	36.694	3	12.231	2.14	0.0955
Error	1398.769	245	5.709		
Cor. Total	1435.463	248			
Source					
Treatment	4.221	1	4.221	0.74	.3907
Initial diameter	17.473	1	17.473	3.06	.0815
Browse	15.000	1	15.000	2.63	.1063

with plants growth (SFNSW 1995). Although diameter was measured with a micrometer, the incremental changes were very small and the chance of significant errors in measurement more pronounced.

Biosolids had no influence on growth of 2-year-old saplings. This could be due to the following reasons:

1. One year could be too short a time to monitor growth change. The saplings may have been accumulating nutrients in root growth for a flush of growth the following year. Fertilizer - growth experiments generally last longer than one year, but some initial change in growth is evident in the first year. This analysis could be improved by looking at trends over a longer time, monitoring changes in foliar N concentration to see if the saplings increased nutrient uptake, or examining changes in root biomass.
2. Maximum annual biomass in late summer on both sites was dominated by understory plants regardless of treatment, thus trees in both biosolids and untreated plots were likely to be suppressed. Treated sites had increased understory growth after biosolids application and had an average understory biomass of 6.7 Mg ha^{-1} compared with 4.1 Mg ha^{-1} on untreated sites (see Chapter 6). Understory on both sites was dominated by herbaceous shrubs which rapidly occupied the site, but also died back at the end of the growing season. This seasonal increase in understory growth, prevented rapid growth by saplings during the spring-summer period, but did not affect growth once the herbaceous flush had died back.

In future tests of the effects of understory on growth of young stands, experiments using split plots where understory is removed on half the treated and control plots, would allow further delineation of the role of understory competition in Douglas-fir sapling growth.

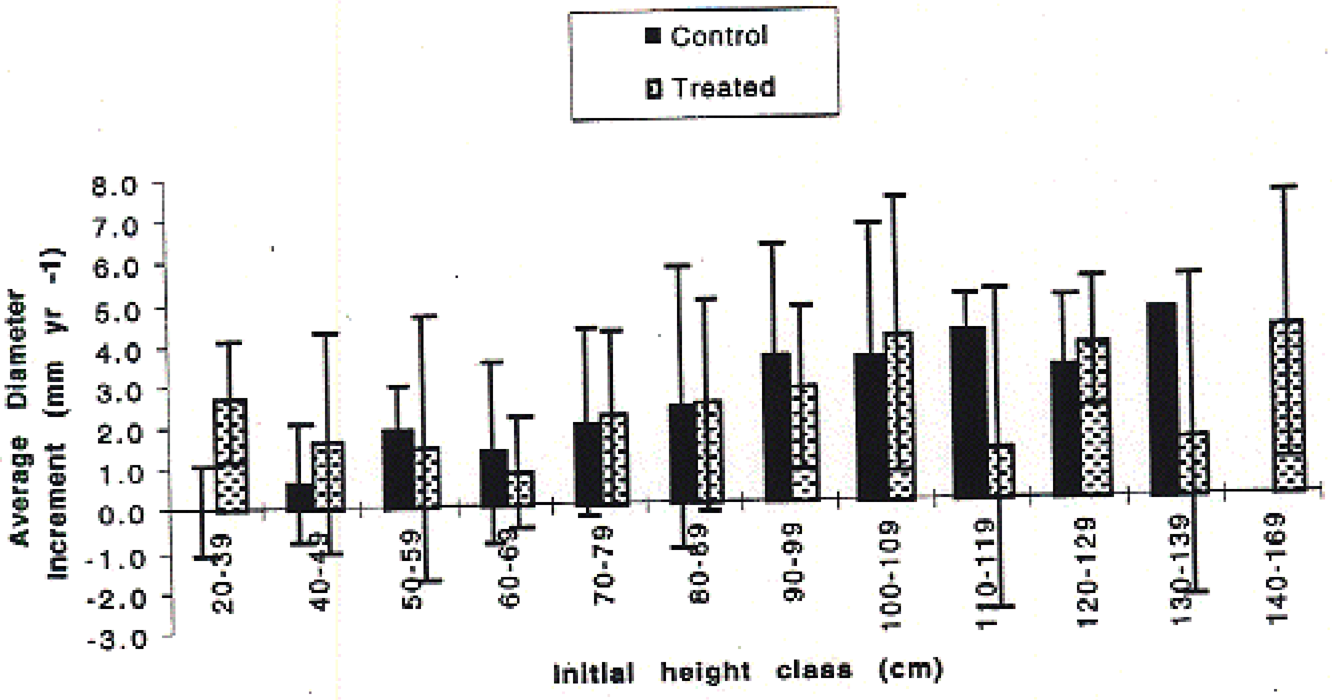


Figure 5.2. Two-year-old Douglas-fir seedling annual average diameter growth by initial tree height class at Snoqualmie Tree Farm.

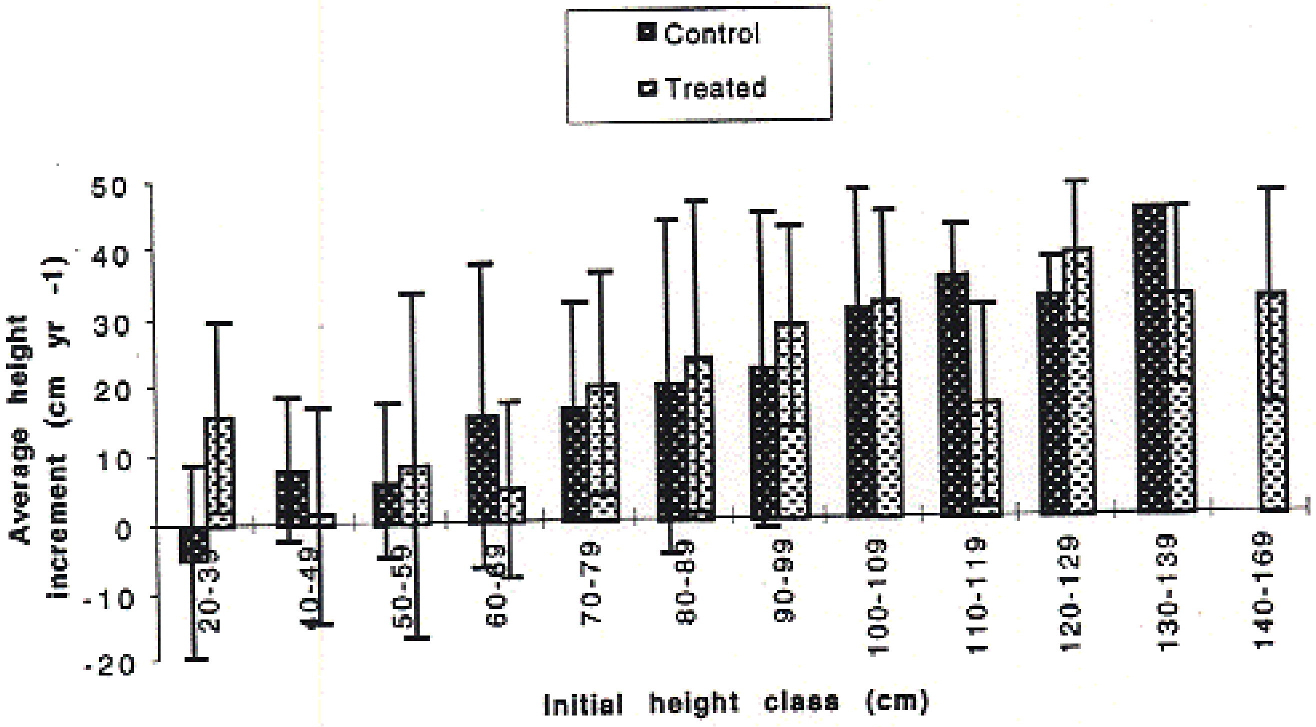
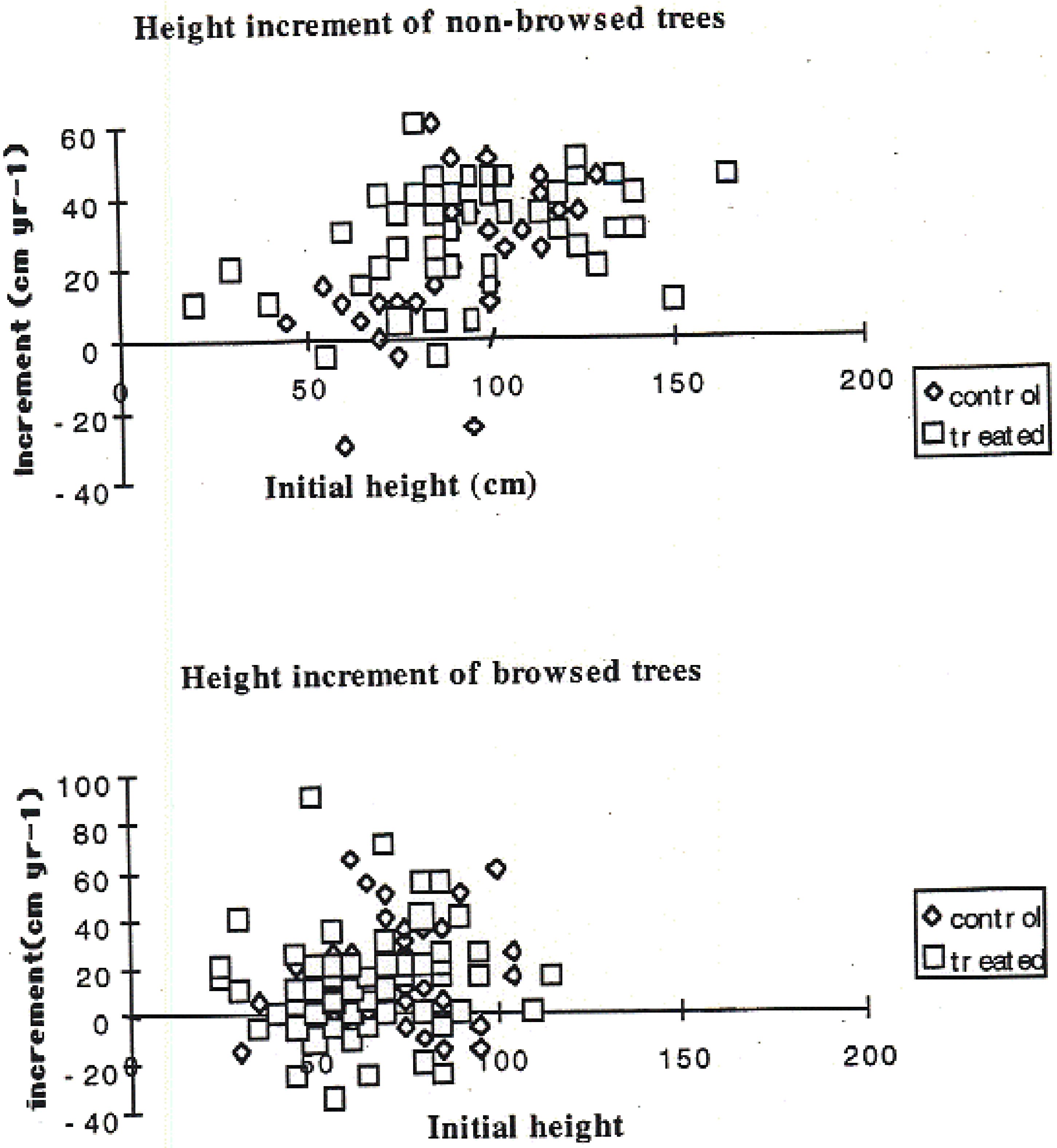


Figure 5.3. Two-year-old Douglas-fir seedling annual average height growth by initial tree height class at Snoqualmie Tree Farm.


Height increment of non-browsed trees

Height increment of browsed trees

Figure 5.4. Height increment as a factor of initial height, biosolids treatment and additional browsing, in a 2-year-old Douglas-fir stand at Snoqualmie Tree Farm between March 1996 and March 1997.

The relationship between sapling increment and initial height, shows that larger saplings have competitive advantages that allow for greater growth. Trees can gain competitive advantages over similar aged trees of the same species from microsite variation, genetic composition or spacing (Oliver and Larson 1996). Whether the competitive advantage of taller saplings was related to surrounding understory could only be tested if pairs of matched trees of similar size were compared in split plots.

5.4.2 Sapling survival and mortality

The effect of biosolids treatment on sapling mortality was not significant (p=0.14), but trends suggest greater mortality in treated sites. Fifteen percent of the saplings died in biosolids treated plots compared with 9% in the adjoining untreated plots.

Figure 5.5 shows mortality by height classes. Mortality in the 40-49 cm and 50-59 cm class was 50% and 24% in treated stands and 25% and 12% for the control. The higher survival of saplings in the 20-39 cm height class was probably related to these trees being mainly larger diameter saplings that had been previously heavily browsed by deer. These saplings still have their previous root area. This increased root area possibly gives greater access to below ground nutrients and moisture, allowing greater survival rates than for a smaller diameter seedling of equivalent size.

To determine whether increased mortality was due to increased understory competition would again require split plots in treated and control sites with the understory removed from half the sub-plots.

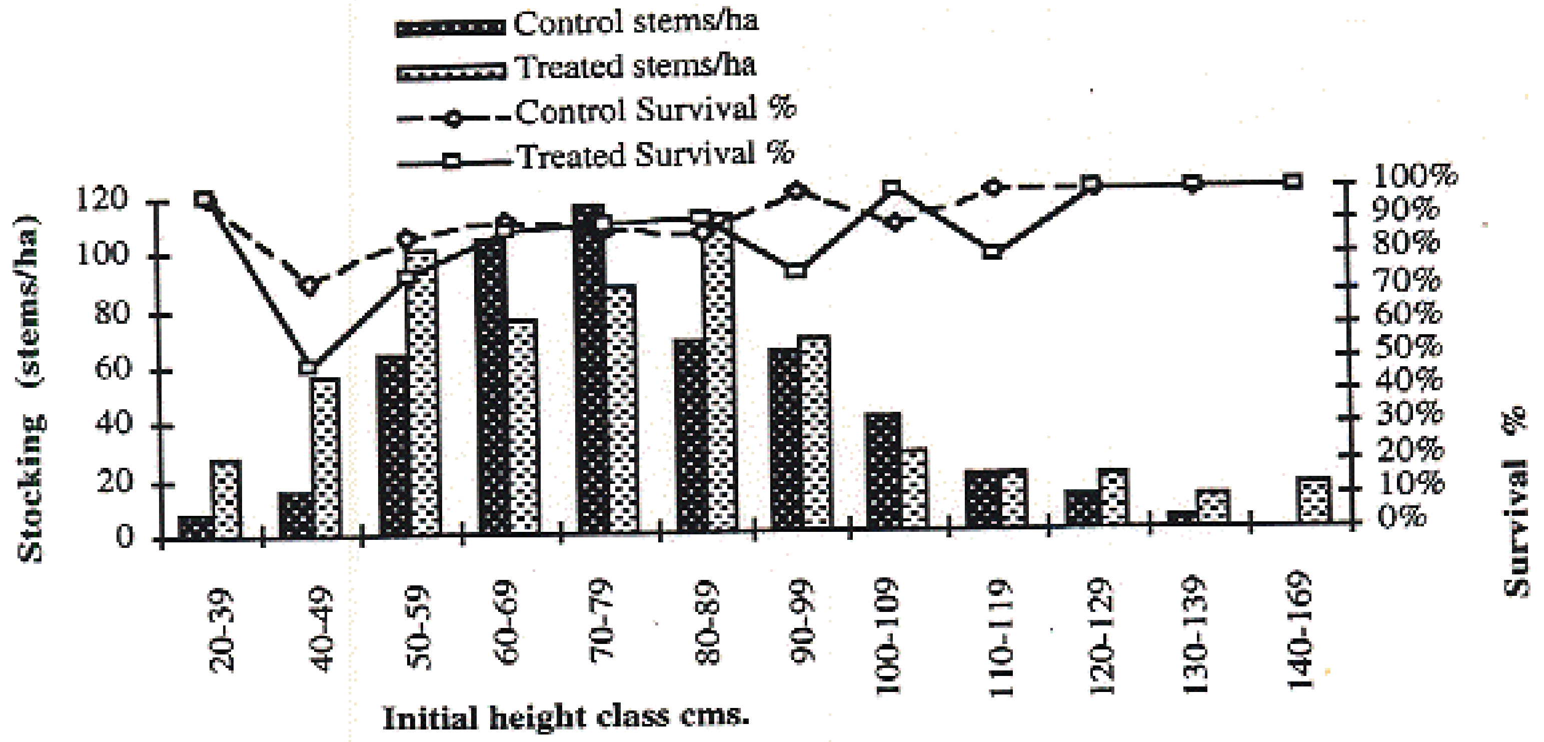


Figure 5.5. Stocking and survival % by initial height class of Douglas-fir stands in 2-year-old stands at Snoqualmie Tree Farm between March 1996 and March 1997.

5.5 Discussion on implications to biosolids management

Management of future biosolids applications must recognize that target trees need to be a minimum size to effectively compete with understory vegetation in their use of added nutrients. If no weed control is planned, this study suggests that the smallest tree should be taller than 2 m before application of biosolids to minimize mortality and achieve a beneficial growth response. Application of biosolids appears better targeted to slightly older stands with greater site occupancy. Increased height and crown area of trees and spatial occupancy are important. Due to the irregular spacing and low stocking on this stand, full site occupancy by target Douglas-fir trees will take longer to achieve. Low inter-tree competition and irregular stand growth could reduce the apical growth of individual trees in preference to increased branch expansion. This would also have possible negative effects on future timber quality.

5.6 Conclusion

Biosolids applied to 2-year-old stands did not affect Douglas-fir sapling diameter or height increment. Larger saplings had greater average height increment, but had no substantive increase over control saplings of similar size from biosolids fertilization. Mean mortality was greater in smaller saplings in the stand, less than 1.2 m tall. Mortality was 15% in treated stands and 9% in control sites, but this increase in treated stands was not significant.

Chapter 6:

NITROGEN ACCUMULATION BY UNDERSTORY PLANTS AFTER BIOSOLIDS APPLICATION

6.1 Introduction

Biosolids applications to Douglas-fir stands on N-deficient soils aim to improve stand productivity by supplying a site's N requirement, without increasing nitrate levels in the groundwater. Biosolids application rate calculations based on N, match inputs from biosolids to the assimilative capacity of the site (U.S. EPA 1995). The generalized model is stated in Equation 6.1.

Equation 6.1 Generalized agronomic application rate model.

$$\text{Application rate} = \frac{\text{Net N required by plants and soil}}{\text{Net plant available N}}$$

where:

Net N required by plants and soil

= N accumulation by trees
+ N accumulation by understory vegetation
+ N immobilization by soil microorganisms
- Soil residual nitrate and mineralizable N from previous applications

Net plant available N

= NH_4 from biosolids less volatilization
+ N mineralized from organic N in biosolids
- Denitrification losses.

N supplied by biosolids is available to all plants that have roots in the area where increased nutrient is present. Although understory biomass decreases with increasing crown closure, substantial understory generally exists in Douglas-fir stands up to crown closure or when canopy cover is reduced by thinning (Turner 1975). If understory is present, increased nutrients from biosolids should increase understory nutrient content.

Plant N accumulation rates have traditionally used gross above ground N accumulation in the season after biosolids application. Nitrogen accumulation for evergreen plants is calculated as the change in N content (biomass multiplied by respective N concentration) over a year; while with deciduous and annual plants, it is calculated as the maximum accumulation of N at any time during the growing season.

The objective of this study was to compare changes in above-ground understory biomass and N content after biosolids application. A range of different aged sites around in north-western Washington where forest application occurs, were sampled over the 1996 growing season.

6.2 Methods

My primary objective was to quantify N accumulation in both treated and untreated understory plots for three different aged stands. Statistical analysis comparing stands of different ages was not attempted due to differences in biosolids application rates, past silvicultural and treatment history, and site factors.

Biomass and N content of understory plants was sampled at estimated start and end of the growth phase (i.e., mid summer and end of winter). In mid August, herbaceous plants were assumed at both maximum biomass and N content, and woody evergreen and deciduous plants at maximum N content.

6.2.1 Study areas

Two study areas were established: one in young stands at the Weyerhaeuser Snoqualmie Tree Farm, and the second in older, thinned stands at Pack Forest. All of these study areas were operational biosolids sites.

6.2.1.1 Snoqualmie study area

At Snoqualmie, trials were established on separate sites in both 2- and 9-year-old plantations. General site characteristics are previously described in Chapter 5.

The 2-year-old plantation in unit 25-08-33B (Figure 6.1) lies at elevation 260-270 m on a Barneston gravely sandy loam soil. The previous mature Douglas-fir stand on the site had biosolids applied in 1987 and was clearfelled in 1993. The site was replanted with Douglas-fir seedlings in 1994. Biosolids from Renton WWTP (properties in Table 6.1) were applied by a Rottne forwarder fitted with an Aerospread applicator at 8.3 dry Mg ha^{-1} in early April 1996.

The 9-year-old plantation in unit 24-08-14E (Figure 6.2) lies on gentle slopes in Section 14 Township 24N Range 8E at elevation 300-320 m. The soil type is also a Barneston gravely sandy loam soil. The 4- to 10- m-tall Douglas-fir saplings were starting to achieve canopy closure and to fully occupy the site. The existing stand had biosolids applied in 1992. Additional biosolids were applied at 12.8 dry Mg ha^{-1} between mid March and early April 1996.

6.2.1.2 Pack Forest study area

The plots at Pack Forest were established in the low and high SQ, thinned 68-year-old stands in the SDS. The characteristics of these stands are described in Chapter 4.

Understory in previously untreated areas is now dominated by salal, Oregon grape, twinflower, sword fern and red huckleberry. The plots are in the buffers surrounding overstory growth plots or in non drainage-line buffers. The soils are low fertility, coarse sandy loams derived from residual andesite.

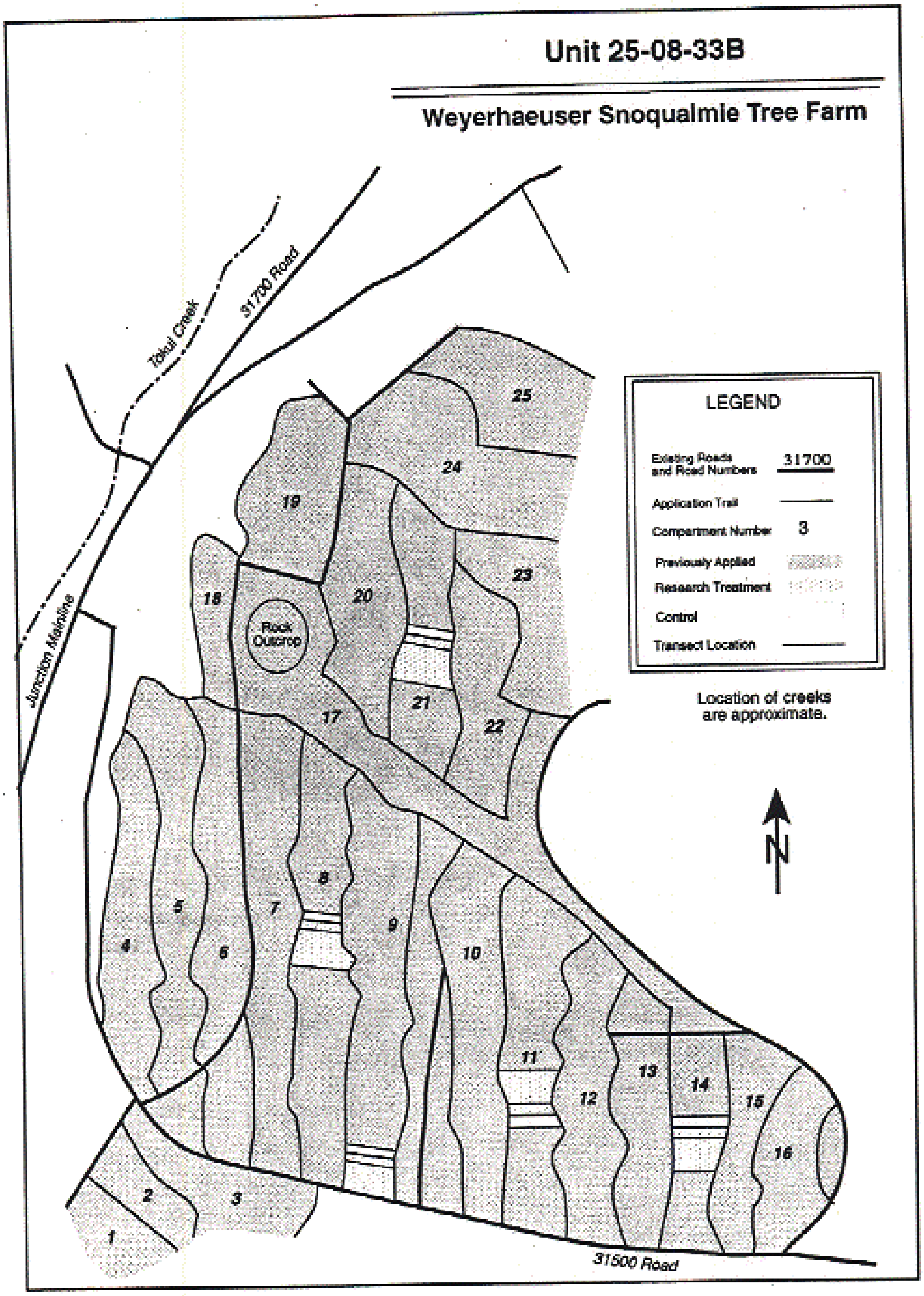
Table 6.1. Analyses of anaerobic dewatered biosolids from different treatment plants used in trials.

Location	Total Organic N	Ammonium-N	Solids %
	----------------% of dry wt	----------------	
Renton	5.4	1.1	21.5 - 22.6
West Point	5.2	1.0	21

The Pack Forest sites were hand applied on 24-26 March, 1996 with West Point anaerobic dewatered biosolids (properties in Table 6.1) at an average of 11 dry Mg ha-1. Biosolids were spread by shovel as uniformly as possible over 1 m^2 treated sub-plots.

6.2.2 Plot establishment and sample collection

In both Snoqualmie trials five, 60 x 60 m blocks were randomly selected as controls where no biosolids were applied. As shown in Figure 6.3, paired transects perpendicular to application rows were located in controls and adjacent treated areas. Five sets of 2 m x 2 m plots were established at 10 m intervals along each transect. Each plot was divided into 1 m2 subplots. All understory vegetation covering the central 0.5 x 0.5 m area was harvested in either late March or mid August. The vegetation was cut at ground level and stored in paper bags.



Location of creeks are approximate.

Figure 6.1. Site of N accumulation trials in the 2-year-old plantation 25-08-33B

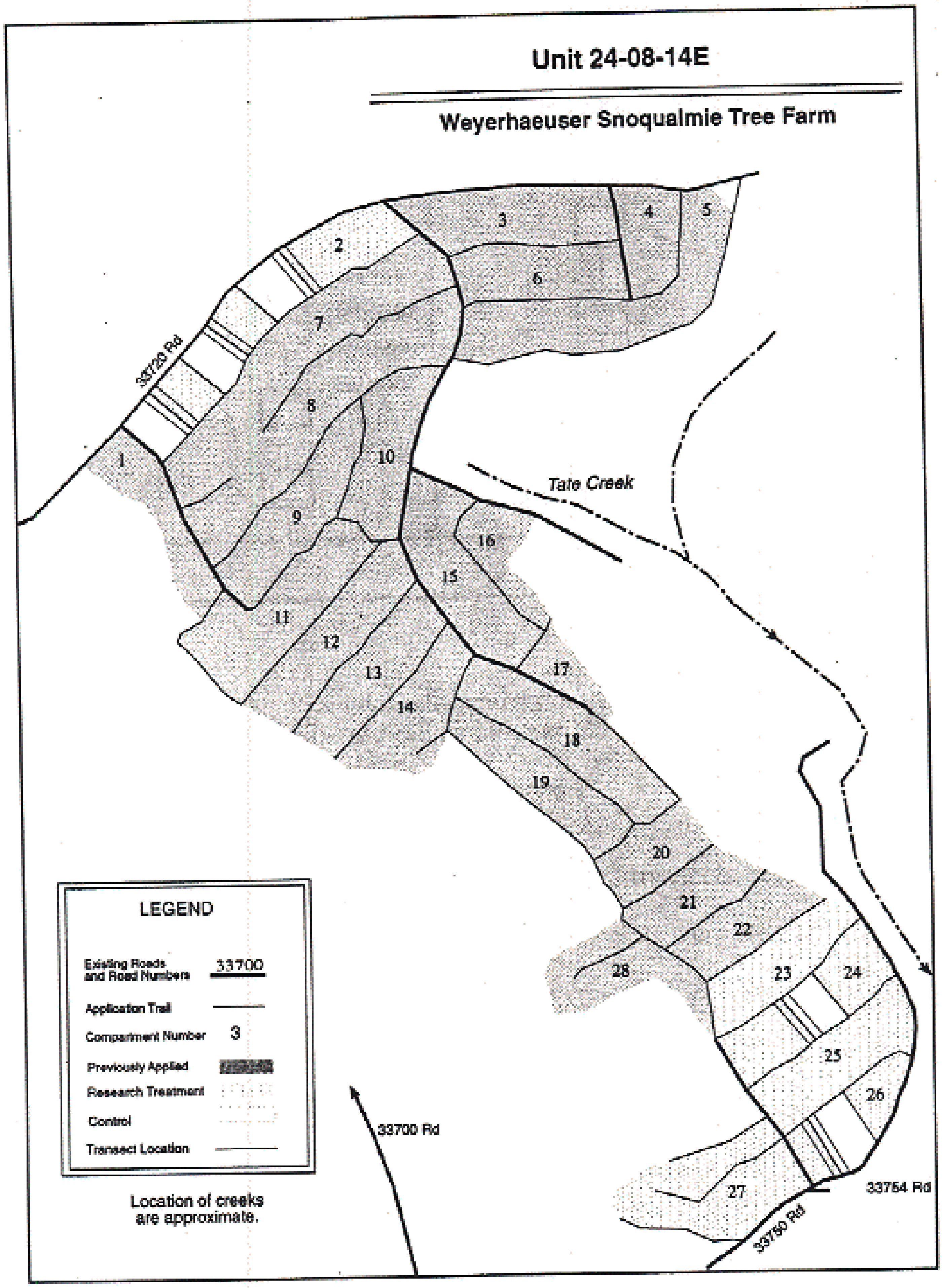


Figure 6.2. Site of N accumulation trials in the 9-year-old plantation 24-08-14E

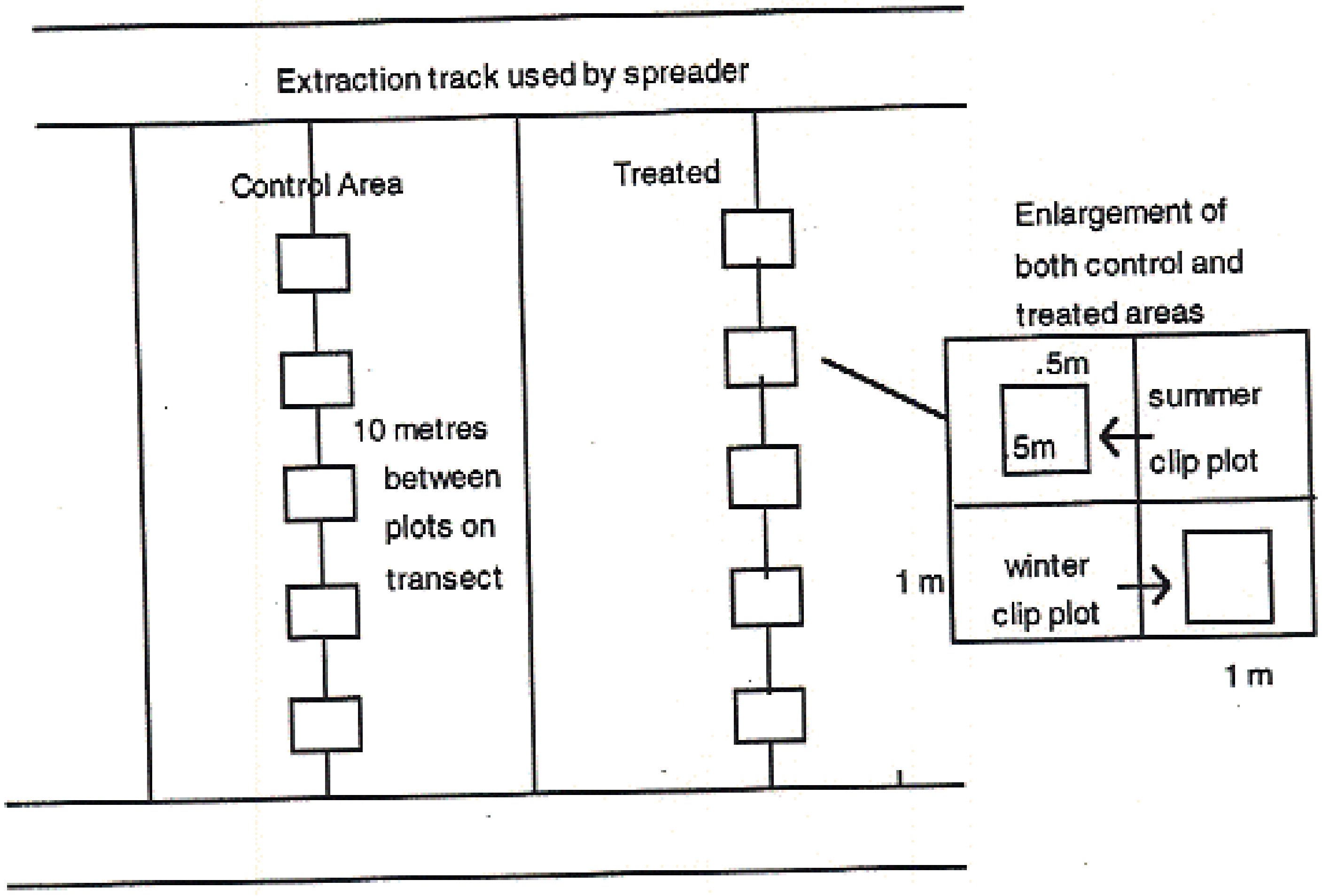


Figure 6.3. Plot layout for understory biomass sampling in the 2 -year-old and 9-year-old units

In the high and low SQ stands at Pack Forest, 4 and 5 transects, respectively, were randomly located. As shown in Figure 6.4, 2 x 2 m plots were created at 10 m intervals along the transects. The plot was subdivided into two 1 m^2 subplots. One subplot was treated with biosolids in March. A control subplot was established 4 m to the right of the central peg. All understory plants covering the central 0.5 x 0.5 m area of each initial subplot were clipped and collected in late March. The treated subplots and controls were sampled in late August. All vegetation was cut at ground level and stored in paper bags.

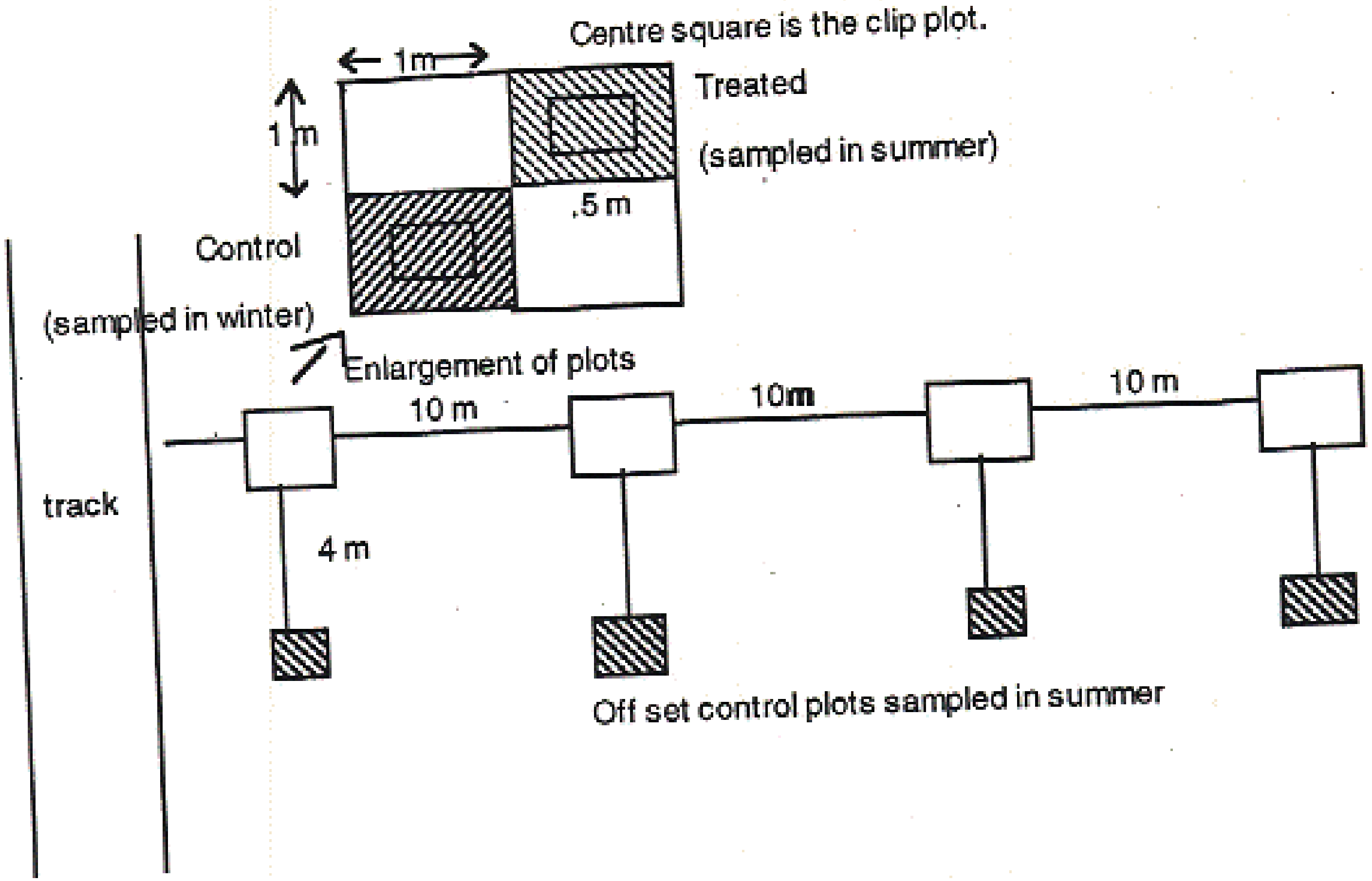


Figure 6.4. Plot layout for understory biomass sampling in the 68 -year-old stands at Pack Forest

6.2.3 Sample preparation

All samples were separated into leaf and stem material by species. Components were oven dried at 70°C for 48 hours and then weighed. Biomass was weighed to the nearest 0.1 g. The total leaf or stem samples were ground using a Wiley mill and a 20 µm mesh. Analysis for total N concentration of major species was determined by dry combustion (Perkin-Elmer CHN Analyzer Model 2400). On average, 5 samples of both leaf and stem material for major species were analyzed for any treatment and stand age. Nitrogen content for every sample was determined by multiplying the sample's biomass by the average N concentration for that species, plant part, treatment history and transect location.

6.3 Statistical Analyses

Statistical analyses were performed on transect-level data. Values for transects were derived by summing the subplots in each transect. The summation of subplots to transects captured variability within a transect and provided a larger sample area to facilitate comparisons between transects. All data manipulation was done using Excel 5.0 Pivot analysis with statistics also calculated using Excel 5.0 Statistical options (Microsoft 1994).

Treated and untreated transects in both the 2- and 9-year-old stands were compared prior to biosolids application, for both understory biomass and N content. A paired t test (see results section 6.6.2) showed no significant initial difference between transects pairs in either the 2-year or 9-year-old stands existed. In the older stand, an ANOVA was conducted to determine whether initial understory biomass and N content from transects in high and low SQ stands, represented different populations. As no significant difference existed all transect data in the older stands were pooled.

Paired transects were considered a randomized block design. Differences in biomass and N content were compared using two-factor ANOVA without replication. A post-hoc Tukey Studentized range test was used to determine means that were significantly different.

Differences in N concentration of foliage after biosolids application was compared using analysis of variance (ANOVA). Separate ANOVAs were conducted for leaf and stem material, for each major understory species on each stand age. On samples, where ANOVA showed a significant difference, a Tukey Studentized range test was used to determine which means were significantly different. No statistical comparison was conducted between species on different aged stands.

6.4 Results

6.4.1 N concentration by species

The N concentration (N conc.) for major understory species are presented in Tables 6.2 to 6.4. In all cases, the N conc. was higher in the leaf than in the stem component.

In the 2-year-old stand (Table 6.2) the only substantial pretreatment samples were stems from vine maple (*Acer circinatum* Pursh) and red elderberry (no leaf material was present in March) and grass. The average stem N conc. of vine maple and red elderberry in March was not significantly different than August concentrations on both untreated and treated plots, although N conc. appeared to increase with biosolids treatment. Nitrogen concentration of grass in March on the treated plots was significantly greater than both August and March values on untreated sites. In August N conc. of stem and leaf material from bracken fern and fireweed stems was not different between biosolids treated and control plots. However, a substantial (though not statistically significant) increase in leaf N conc. with biosolids application exists for both red elderberry and vine maple.

In the 9-year-old stand (Table 6.3) only vine maple stems, blackberry and grass were prevalent in March. The average stem N conc. for vine maple and average stem and leaf N conc. for blackberry in March was not significantly different than August untreated and treated values. Grass N conc. in March was greater than August untreated sites and less than treated sites. Grass N conc. between treated and untreated samples in August was significantly different, with the critical value of the Tukey Studentized range test of 3.261 less than the q value of 4.15 . However, at least one potential Type II error exists due to an overlap in comparisons between treated and pre-untreated samples, and pre-untreated and post-untreated samples.

Table 6.2. Average total N concentration (and statistical output of ANOVA analyses) of major understory species found in 2-year-old Douglas-fir stands at Snoqualmie Tree Farm.

Common name	Pre untreated	Post untreated	Post treated	df	MSE	F	p	F crit
	Average stem N concentration							
vine maple	1.1 (0.1)a	0.9 (0.1)a	1.4 (0.5)a	12	0.146	2.067	0.177	4.103
bracken fern	-	1.7 (0.5)a	1.5 (0.5)a	9	0.253	0.228	0.646	5.318
fireweed	-	1.2 (0.3)a	1.2 (0.5)a	8	0.193	0.001	0.992	5.591
red elderberry	1.4 (0.6)a	1.7 (0.5)a	2.2 (0.5)a	11	0.286	1.891	0.206	4.256
blackberry	1.5 (0.1)a	1.5 (0.6)a	2.1 (0.5)a	12	0.216	1.488	0.271	4.103
	Average leaf N concentration							
vine maple	-	1.7 (0.3)a	2.4 (0.7)a	9	0.311	3.412	0.100	5.318
bracken fern	-	3.0 (0.4)a	3.0 (0.7)a	9	0.277	0.001	0.980	5.318
fireweed	-	2.2 (0.3)a	2.5 (0.5)a	8	0.162	1.393	0.276	5.591
grass	2.4 (0.6)a	1.2 (0.1)a	3.1 (0.1)b	9	0.111	22.930	0.001	4.73
red elderberry	-	3.7 (0.8)a	4.9(1.1)a	8	0.897	3.542	0.102	5.592
blackberry	1.5 (0.3)a	2.2 (0.7)a	2.9 (0.8)a	6	0.355	0.789	0.415	6.608

"Pre-treated" is N concentration in March, all others in mid August .
Average concentration for a plant part and species with the same letter indicates no significant difference at p=0.05.

The August N conc. for fireweed stem and leaf material was greater in treated plots but not significantly different. However, a statistically significant difference in N conc. due to biosolids application exists for bracken fern stem and leaf, and hardhack leaf. Salal had very little variation between all treatments, with average N conc. the lowest for any species, ranging between 0.7% and 1.2%. Oregon grape had no significant change in N conc., with minimal variation in stem samples and leaf N conc. showing a slight increase on treated sites. The leaf N conc. of twinflower, sword fern and trailing blackberry significantly increased with biosolids application in the August sample.

Table 6.3. Average total N concentration (and ANOVA output) of major understory species found in 9-year-old Douglas-fir stands at Snoqualmie Tree Farm.

Common name	Pre untreated	Post untreated	Post treated	df	MSE	F	p	F crit
	Average stem N concentration							
vine maple	1.1 (0.2)a	1.4 (0.6)a	1.6 (0.6)a	16	0.150	2.371	0.130	3.741
bracken fern	-	1.0 (0.3)a	1.7 (0.5)b	10	0.149	8.939	0.015	5.110
fireweed	-	1.4 (0.3)a	1.7 (0.2)a	11	0.073	4.382	0.063	4.962
hardhack	-	1.4 (0.4)a	2.0 (0.6)a	8	0.238	4.294	0.077	5.591
blackberry	1.9 (0.8)a	1.8 (0.1)a	2.2 (0.8)a	27	0.626	0.151	0.860	3.381
	Average leaf N concentration							
vine maple	-	2.4 (0.2)a	3.2 (0.9)a	6	0.327	3.025	0.143	6.608
bracken fern	-	2.4 (0.5)a	3.7 (0.7)b	10	0.360	12.798	0.005	5.110
fireweed	-	3.3 (0.8)a	3.7 (0.5)a	11	0.447	0.773	0.399	4.965
grass	2.8 (0.7)b	2.2 (0.6)a	3.4 (0.4)b	15	0.397	4.323	0.036	3.806
hardhack	-	2.2 (0.5)a	3.6 (0.7)b	9	0.377	13.519	0.006	5.318
blackberry	2.4 (0.5)a	2.6 (0.1)	3.1 (0.4)a	8	0.169	2.641	0.150	5.143

"Pre-treated" is N concentration in March, all others in mid August .
Average concentration for a plant part and species with the same letter indicates no significant difference at p=0.05.

Understory in the 68-year-old stand was dominated by herbaceous, non deciduous plants. CHN samples from both low and high site quality plots were pooled and the results presented in Tables 6.4.

Within age classes, substantive changes in species average N concentration were often noted, but the differences were not significant. The small sample sizes in many species contributed to this trend, and significant differences may have been found by analyzing more samples.

Table 6.4. Average total N concentration (and statistical output of ANOVA analyses) of major understory species found in the 68-year-old Douglas-fir stands at Pack Forest.

Common name	Pre untreated	Post untreated	Post treated	df	MSE	F	p	F crit
	Average stem N concentration							
bracken fern	-	0.9 (0.3)a	0.8 (0.2)a	10	0.058	0.355	0.566	5.110
twinflower	1.4 (0.4)b	1.0 (0.3)a	1.3 (0.3)b	25	0.089	5.369	0.012	3.422
Oregon grape	1.5 (0.4)a	1.4 (0.5)a	1.5 (0.4)a	31	0.226	0.115	0.892	3.330
salal	0.65 (0.2)a	0.65 (0.1)a	0.8 (0.3)a	47	0.080	2.238	0.118	3.204
sword fern	0.6 (0.1)a	1.0 (0.1)b	1.2 (0.1)b	12	0.007	39.101	0.0002	4.103
trailing blackberry	1.5 (0.5)a	1.1 (0.3)a	1.2 (0.2)a	12	0.111	2.336	0.147	4.103
red huckleberry	1.2 (0.4)a	1.0 (0.1)a	1.0 (0.2)a	24	0.061	0.656	0.528	3.443
	Average leaf N concentration							
bracken fern	-	2.2 (0.4)a	2.1 (0.4)a	11	0.161	.0135	0.721	4.960
twinflower	1.9 (0.6)b	1.3 (0.3)a	2.2 (0.5)b	29	0.186	15.910	0.0001	3.350
Oregon grape	1.4 (0.3)a	1.8 (0.6)a	2.2 (0.4)a	30	0.310	3.304	0.064	3.340
salal	1.1 (0.5)a	1.1 (0.3)a	1.2 (0.3)a	47	0.134	0.413	0.664	3.204
sword fern	1.6 (0.2)a	1.5 (0.0)a	1.9 (0.1)a	11	0.047	2.219	0.165	4.256
trailing blackberry	2.1 (0.1)a	2.1 (0.2)a	2.4 (0.2)b	13	0.625	6.030	0.017	3.980
red huckleberry	-	1.8 (0.1)a	1.8 (0.4)a	17	0.096	0.152	0.860	3.683

"Pre-treated" is N concentration in March, all others in mid August .
Average concentration for a plant part and species with the same letter indicates no significant difference at p=0.05.

6.4.2 Biomass and N content of understory

6.4.2.1 2 year-old stand

In the March sampling, average understory aboveground biomass (UAB) ranged between 936 ± 431 and 1789 ± 1473 kg ha^{-1} and understory N content (UNCT) between 19 ± 11 and 24 ± 17 kg N ha^{-1} (Table 6.5). No significant difference existed in UAB or UNCT between paired transects prior to biosolids application. UAB and UNCT were mainly stem material from vine maple and red elderberry, and all foliage from grass and trailing blackberry. Understory with higher N conc. (e.g., red elderberry) had a greater relative percentage of total UNCT than their corresponding UAB percentage. Due to the dieback of aboveground parts of many herbaceous species in winter, many subplots had minimal biomass in March.

In August, UAB and UNCT on treated sites (6739 ± 2454 kg ha^{-1} and 141 ± 49 kg N ha^{-1}) was significantly greater than control sites (4095± 1412 kg ha^{-1} and 73 ± 31kg N ha^{-1}). UAB at the end of the growing season was dominated by herbaceous species (fireweed, grass and bracken fern) and vine maple. Treated sites had both significantly more UAB and an increased average understory N conc. from 17.8 to 21.1 kg N Mg^{-1}.

The average increase in N accumulation in understory applied with biosolids over the growing season was calculated by comparing for each pair of transects:

$(UNCT_{\text{post control}} - UNCT_{\text{pre control}})$ versus $(UNCT_{\text{post treatment}} - UNCT_{\text{pre treatment}})$

Nitrogen accumulation by understory in biosolids treated stands was 73 kg N ha^{-1} more than in untreated sites. This was a significant increase at $p= 0.001$.

6.4.2.2 9 year-old stand

No significant difference existed in UAB or UNCT between paired transects prior to biosolids application. Average understory N conc. was 12.3 kg N Mg^{-1} (Table 6.6). UAB and UNCT were mainly stem material from deciduous shrubs (vine maple, hardhack, oceanspray), sword fern and trailing blackberry. Understory with higher N conc. (e.g. red elderberry) had a greater relative percentage of total UNCT than their corresponding UAB percentage. Subplots that were heavily overtopped by Douglas-fir trees, appeared to have lower biomass consisting mainly of fern and blackberry. Where plots occurred beneath canopy gaps of Douglas-fir, woody shrubs between 2 and 4 m tall often occurred in conjunction with a lower herbaceous layer.

Table 6.5. Average biomass and N content of understory plants in 2-year-old Douglas-fir plantation treated with biosolids.

Common name	Pre Treated	Pre Untreated	Post Treated	Post Untreated	Pre Treated	Pre Untreated	Post Treated	Post Untreated
	Average biomass (kg ha $^{-1}$)				Average N content (kg N ha $^{-1}$)			
Average all understory	936 a	1789 a	6739 b	4095c	19 a	24 a	141 b	73 c
Standard Deviation	431	1473	2454	1412	11	17	49	31
	---------------------% of biomass ------				---------------% of N content-----------------			
fireweed	-	-	54	41	-	-	47	39
vine maple	40	52	16	10	39	41	15	6
grass	16	3	6	14	19	6	8	10
red elderberry	11	15	2	15	17	15	4	23
bracken fern	-	-	10	5	-	-	12	8
Oregon grape	7	4	2	2	7	6	2	2
trailing blackberry	11	4	2	2	9	5	2	2
other species	15	23	8	11	9	27	9	11

"Pre" is biomass in March, "Post" is biomass in mid August . Average biomass with same letter indicates no significant difference exists at p = 0.05.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
TOTAL COR	1.4E+08	19				
Treatments	1E+08	3	33827751	36.726	>.0001	3.490
Blocks (paired plots)	3E+07	4	7613362	8.2657	.002	3.259
Error	1.1E+07	12	921077			
Tukey HSD	Mean	n	q(Post treat)	q(Post untreat)	q(Pre untreat)	q CV.
Pre treated	936	5	13.52*	7.36*	1.99	
Pre untreated	1789	5	11.53*	5.37*		
Post untreated	4095	5	6.16*			
Post treated	6739	5				4.2

Average N content with same letter indicates no significant difference exists at p=0.05 from two-factor ANOVA with replication

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
TOTAL COR	62458	19				
Treatments	47574	3	15858	34.70	>.0001	3.490
Blocks (paired plots)	9400	4	2350	5.14	0.012	3.259
Error	5484	12	457			
Tukey HSD	Mean	n	q(Post treat)	q(Post untreat)	q(Pre untreat)	q CV.
Pre treated	19.00	5	12.70*	5.61*	0.55	
Pre untreated	24.29	5	12.14*	5.05*		
Post untreated	72.65	5	7.09*			
Post treated	140.41	5				4.2

* means that are significantly different.

In August UAB was greater (but not significantly) on treated sites (6.5 Mg ha^{-1}), compared with 5.8 Mg ha^{-1} on control sites. UNCT on treated sites (163 Kg N ha^{-1}) was significantly different from UNCT of 115 Kg N ha^{-1} on control sites. UAB at the end of the growing season was half herbaceous species (bracken fern, fireweed and grass), and half woody deciduous perennials (vine maple, hardhack and trailing blackberry and red elderberry). Although UNCT differed significantly from March values, no significant increase occurred between treated and control stands. The significant increases in N conc. for the major biomass components of bracken fern leaf and stems, grass and hardhack leaf material caused a substantial increase in August UNCT in treated plots compared to controls. A comparison of accumulation by understory over the year showed accumulation in biosolids treated stands was only 27 Mg N ha^{-1} more than untreated sites. This was a not a significant increase, with $p = 0.367$.

Table 6.6. Average biomass and N content of understory plants in 9-year-old Douglas-fir plantation treated with biosolids.

Common name	Pre Treated	Pre Untreated	Post Treated	Post Untreated	Pre Treated	Pre Untreated	Post Treated	Post Untreated
	Average biomass kg ha $^{-1}$				Average N content kg N ha $^{-1}$			
Average all understory	4960 a	3161 a	6519 a	5945a	61a#	40a	163 b	117 b
Standard Deviation	5292	4264	1341	2384	59	48	32	48
	% of biomass				% of N content			
vine maple	60	38	23	5	52	32	19	4
bracken fern	-	-	33	35	-	-	38	27
oceanspray	16	38	1	2	13	29	1	1
hardhack	4	0	9	16	3	-	8	18
trailing blackberry	10	10	14	9	16	16	4	11
fireweed	-	-	8	16	-	-	8	18
grass	-	-	4	9	-	-	6	12
sword fern	4	7	3	2	6	11	2	2
other species	6	7	3	5	10	13	14	7

Average biomass with same letter indicates no significant difference exists at p = 0.05 from two-factor ANOVA without replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
TOTAL COR	2.5E+08	19				
Treatments	3.3E+07	3	10842215	1.177	0.359	3.490
Blocks (paired plots)	1E+08	4	26046026	2.827	0.073	3.259
Error	1.1E+08	12	9212235			

Average N content with same letter indicates no significant difference exists at p=0.05 from two-factor ANOVA with replication.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
TOTAL COR	82518	19				
Treatments	46191	3	15397	10.330	0.001	3.490
Blocks (paired plots)	18440	4	4610	3.092	0.057	3.259
Error	17886	12	1490			
Tukey HSD	Mean	n	q(Post treat)	q(Post untreat)	q(Pre treated)	q CV.
Pre untreated	39.6	5	7.11*	4.46*	1.23	
Pre treated	61.0	5	5.88*	3.22#		
Post untreated	116.6	5	2.65#			
Post treated	162.4	5				4.2

* means that are significantly different.
# overlap of pairwise comparisons, showing at least one Type II error.

6.4.2.3 65 year-old stand

In March, no significant difference existed in UAB or UNCT between paired transects prior to biosolids application (Table 6.7). UAB and UNCT was mainly composed of woody evergreen perennials with salal and Oregon grape dominating both sites. The salal was generally greater than 1m tall and the Oregon grape 60 cm tall. Red huckleberry was the major deciduous species, with early successional species (e.g., fireweed) noticeably absent. Average understory N conc. was 11.2 kg N Mg^{1}.

In August UAB and UNCT was not significantly different between treatments. Foliage composition was from the same species present in March. No significant difference existed in UAB and UNCT between the start and end of the study. No N accumulation by understory in combined SQ biosolids occurred on treated stands, while untreated sites lost 10 Kg N ha^{-1} over the period of the sampling. This was not a significant decrease in N accumulation, with P= 0.399.

6.4.3 N content per plant part.

Total N content per stand is important for determining the level of N removal from the soil. The part of the plant that N is stored in is important to the annual amount of N cycling by understory. Plants were aggregated into plant types representative of their life-cycles. Herbaceous plants returned all or most aboveground biomass to the site annually. Deciduous plants annually returned all leaves and a small amount of branches, while evergreen plants maintained leaves for more than one growing season. The ratio of N content between leaf and stem components, in species that had a large enough sample was similar for all stands. The ratio was not substantially changed by biosolids application, although the understory biomass was increased. Table 6.8 summarizes the ratio of N content in stems and leaves of main species.

Table 6.7. Average biomass and N content of understory in 68-year-old Douglas-fir plantation treated with biosolids.

Species	High			Low		
	Pre Untreated	Post Treated	Post Untreated	Pre Untreated	Post Treated	Post Untreated
	kg ha^{-1}					
Average biomass SD	6661a (2508)	5673a (3013)	5264a (1203)	6826a (2716)	6772a (1818)	6530a (2057)
	% of biomass					
salal	56	71	62	60	72	70
Oregon grape	22	17	24	23	5	8
sword fern	7	2	8	5	-	3
red huckleberry	5	4	3	4	18	11
twinflower	2	1	0	4	2	4
Other species	8	5	2	4	3	5
	kg N ha^{-1}					
Average N content SD	76 a (31)	82 a (42)	66 a (16)	75 a (20)	67 a (17)	66 a (21)
	% of N content					
salal	47	62	49	48	63	61
Oregon grape	28	24	37	30	9	11
sword fern	7	2	8	5	-	4
red huckleberry	4	2	3	4	20	12
twinflower	5	3	-	4	3	5
other species	10	6	3	4	5	7

Average biomass with same letter indicates no significant difference exists at $p = 0.05$ from two-factor ANOVA without replication.

Source of Variation	SS	df	MS	F	P-value	F crit
TOTALCOR (High)	54602336	11				
Treatments	4127132	2	2063566	1.002	0.421	5.143
Blocks (paired plots)	38119355	3	12706452	6.170	0.029	4.757
Error	12355848	6	2059308			
TOTALCOR (Low)	59918882	14				
Treatments	239300	2	119650	0.034	0.967	4.459
Blocks (paired plots)	31295447	4	7823861	2.2051	0.158	3.838
Error	28384134	8	3548016			

Average N content with same letter indicates no significant difference exists at $p=0.05$ from two-factor ANOVA with replication.

Table 6.7. (cont.)

Source of Variation	SS	df	MS	F	P-value	F crit
TOTALCOR (High)	9561.2	11				
Treatments	486.7	2	243.4	0.767	0.505	5.143
Blocks (paired plots)	7172.3	3	2390.7	7.540	0.018	4.757
Error	1902.3	6	317.1			
TOTALCOR(Low)	4821.0	14				
Treatments	276.1	2	138.0	0.550	0.597	4.459
Blocks (paired plots)	2538.4	4	634.6	2.530	0.123	3.838
Error	2006.6	8	250.8			

Table 6.8 Relative N content (%) in biosolids treated and untreated controls in 2-, 9- and 68-year-old Douglas-fir stands separated by plant type.

	2YO			9 YO			68YO			
	Leaf	Stem	Total	Leaf	Stem	Total	Leaf	Stem	Total	Mean Leaf:stem
Untreated	%	%	kg N ha^{-1}	%	%	kg N ha^{-1}	%	%	kg N ha^{-1}	
herbaceous										
bracken fern	10	2	5.8	37	11	37				3.5:1
fireweed	38	22	28.8	17	10	20.8				1.7:1
grass	15	0	7.2	15	0	11.6				-
other	10	3	6.2	7	3	7.8				2.7:1
sub-total	73	27	48	76	24	77	79	21	7	3.0:1
deciduous										
oceanspray	-	-	-	2	2	1.5				1.0:1
hardhack	-	-	-	19	2	8.0				9.5:1
trailing b-berry	4	2	1.5	24	29	20.1				0.9:1
red elderberry	43	25	16.3							1.7:1
vine maple	7	11	4.3	4	10	5.3				0.5:1
other	4	4	1.9	1	7	3.1				0.4:1
sub-total	58	42	24	50	50	38	39	61	6	1.1.1
evergreen										
salal							45	22	36.4	2.0:1
Oregon grape							19	8.5	14.7	2.2:1
other							3	2	2.9	1.5:1
sub-total	71	29	1	53	47	0.1	67	33	54	2.0:1
Total	68	32	73	67	33	115	66	34	66	
Treated										
herbaceous										
bracken fern	12	3	16.3	47	16	61.3				3.1:1
fireweed	41	21	66.4	9	4	13.4				1.9:1
grass	11		11.7	10		9.6				-
other	11	1	12.6	10	4	12.7				4.1:1
sub-total	74	26	107	76	24	97	81	19	3	3.0:1
deciduous										
oceanspray				1	.5	1.1				2.0:1
hardhack				11	10	13.7				1.1:1
trailing b-berry	6	3	2.9	6	3	6.1				2.3:1
red elderberry	11	6	5.4							1.7:1
vine maple	30	40	21.6	22	25	31.2				0.8:1
other	1	3	1.1	11	10	13.4	31	69	11	0.4:1
sub-total	48	52	31	52	48	65.5	31	69	11	1.0:1
evergreen										
salal							48	28	46.0	1.7:1
Oregon grape							15	6	12.3	2.6:1
other							3	-	1.7	-
sub-total	77	23	3	71	29	0.5	66	34	60	2.0:1
Total	68	32	141	66	34	163	61	39	74	

6.5 Discussion

6.5.1 Effect of stand factors on understory composition

Fireweed's rapid dominance in the 2-year-old stand, substantial biomass contribution in the 9-year-old stand and absence in the 68-year-old stand, follows the trend for disturbed forests in the Tsuga heterophylla zone. That trend is, as stated by Franklin and Dryness (1988), that perennial invading herbaceous species such as fireweed and bracken fern rapidly build up their populations until the fourth or fifth year when their populations decline. Bracken fern appeared a little slower than fireweed developing in the 2-year-old stand, but was the major biomass contributor in the 9-year-old stand. It occupied small gaps, with height on treated sites up to 2 m. In bracken fern, the biomass of subterranean parts exceeds that of growth of above ground parts, which rhizomes often penetrating deeper than other understory plants (Stanek et al. 1979). This rhizomal development possibly allows it to maintain its biomass in the 9 year stand. Bracken fern was present in the older stand, but it was not a significant species. Stanek et al. (1979), in thinned Douglas-fir stands at Shawnigan Lake, developed a relationship between bracken fern cover percentage and light intensity. An increase in light intensity by 1 microEinstein effected a corresponding cover increase of 0.05%. The decreased available light in older stands would be expected to have a inverse effect on cover percent.

Crown density in stands was not uniform due to planting failures, woody debris piles, logging extraction tracks and microsite variability. Generally, the greater understory was present as the crown gap increased, except where plant growth was reduced through compaction of harvesting tracks or lack of growing area, due to large piles of logging slash. In the 9-year-old stand the effect of crown closure was most obvious. On areas where Douglas-fir trees were shading the ground, the most prevalent understory species

was *Rubus spp.* In intermediate areas where woody understory had established, the combined effects of Douglas-fir and tall understory shaded out shorter herbaceous species. In the larger opening, greatest variability in type of understory and higher prevalence of ferns and herbaceous plants existed.

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6.5.2 Effect of stand factors on understory biomass and N content

Further information on the impact of stand factors on understory vegetation biomass and N content on typical sites Douglas-fir sites is gained by combining data from Turner (1975) with those from this study. Understory biomass and N content in a range of low SQ Douglas-fir sites in western Washington are shown in Figure 6.5 and 6.6, respectively. Turner's sites (aged 9- ,22- ,30- ,42- ,73- and 95-years-old) were natural, fully stocked, salal dominated stands that do not contain the array of other early successional species found in this study. The 60-year-old stand in Turner's work was an unthinned stand with greatly reduced understory due to dense overstory cover.

The differences in understory biomass in the 9-year-old stands are small. However, substantial differences in understory N content exist. Nitrogen content in understory appears strongly related to species composition and to the history of biosolids application. Both stand age and time since last disturbance and disturbance intensity, affect species composition and development. Stands dominated by herbaceous and woody deciduous understory have much higher N, compared with salal dominated stands. This change in N content is further accentuated when the young stands are fertilized with biosolids. Nitrogen content of herbaceous and deciduous plants increase to a much greater extent than does salal from similar levels of biosolids fertilization.

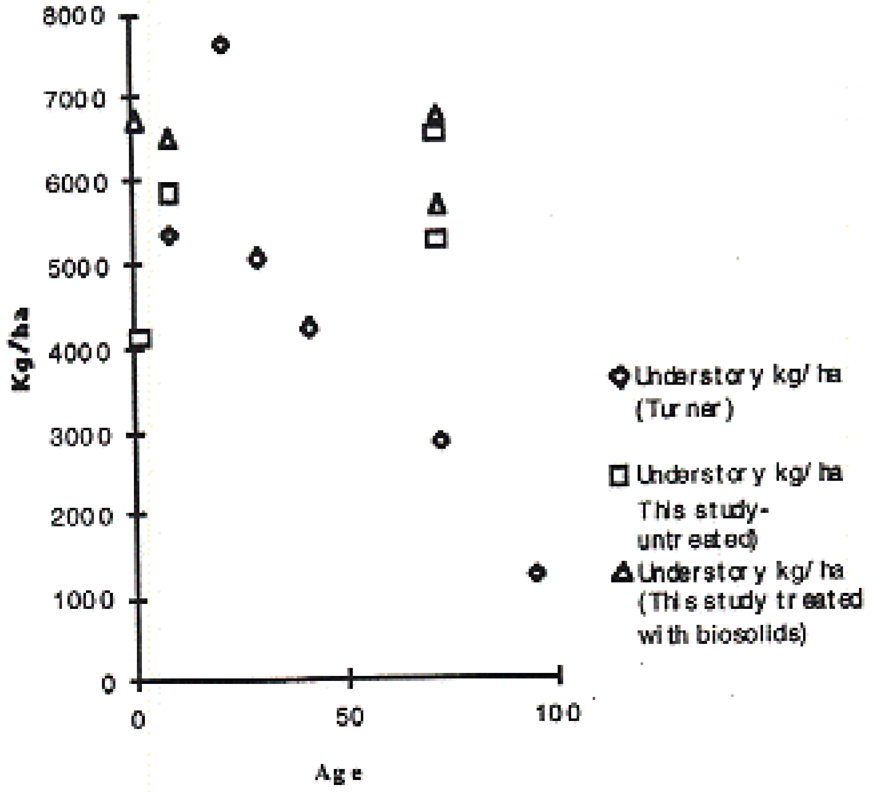

Figure 6.5 Chronosequence of biomass in understory on various low site quality Douglas-fir stands. Includes Turner et al. (1975) plots in the Cedar River watershed and this recent study.

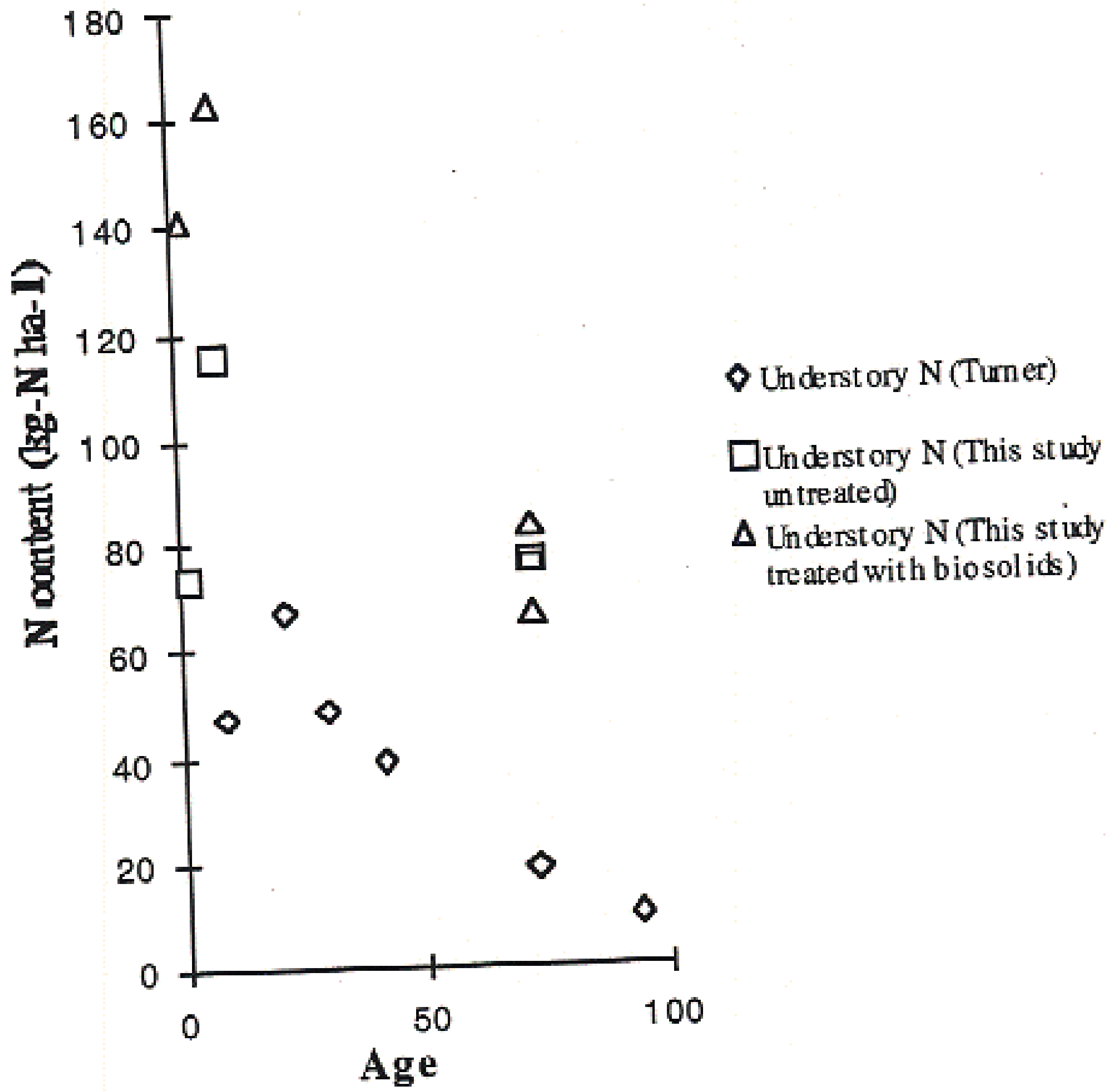

Figure 6.6 Chronosequence of N content in understory on various low site quality Douglas-fir stands. Includes Turner et al. (1975) plots in the Cedar River watershed and this recent study.

6.5.3 Understory N concentration (N conc.)

Nitrogen concentration varies both between species and within species depending on plant age and the cultural history. Evergreen shrubs generally retain varying amounts of prior years foliage. Turner (1975) showed salal current leaves had 20% greater N conc. than did older foliage. Prescott and Weetman (1994), plotting N concentration of salal plants over a 9 year period after site disturbance on unfertilized sites in British Columbia, showed a substantial decrease in N conc. over time. This could be due to an increased proportion of older foliage, or the dilution of the limited available N from the soil across the increasing stand biomass of foliage and woody tissue.

In my experimental sites, the leaf N conc. in the control plots in the 9-year-old stand were higher than for similar species in the 2-year-old stand, with bracken fern in the untreated stand the only exception. Based on the results from Prescott and Weetman (1994), it would be expected that the older understory vegetation present on similar sites would have lower average foliar N concentration. This is not the case, so it is assumed that the 9 year-old site had a higher available N pool. As control areas were untreated in 1996, the residual N pool from previous biosolids application may still be affecting the base fertility on this site. Comparing the results from the treated 2 and 9 year-old stands in 1996, the greater N conc. in the 9 year-old stands could be due to both a larger initial soil N pool and the higher biosolids application rate used.

As this study did not use a range of biosolids rates on any site, we do not know the range of N conc. that could be achieved in foliage from different application rates.

6.5.4 The recycling potential of understory N content after biosolids application

Many important nutrients (particularly carbon, nitrogen, sulfur and phosphorus) are cycled in the soil between the soil organic matter and the inorganic nutrient pool. Nutrient cycling involves the activities of plants, microbes and animals (Killham 1994). A full study on the application of biosolids on understory N recycling would require consideration of the impact of biosolids on all parts of the cycle.

An important facet of N and C cycling explored in this study is the effect of biosolids on understory growth. The increased growth of short-lived herbaceous shrubs and woody deciduous foliage in young sites after biosolids application, should improve both litter quality and quantity.

To estimate the extent of N returned to the soil in litterfall, it was assumed that 20% of N content in herbaceous foliage, and 50% of N content in deciduous foliage is translocated within the plant before abscission. Chapin (1983) described a 68% translocation of N from *Vaccinium* leaves prior to abscission while Turner et al. (1976) estimated that redistribution of N from old foliage before abscission was 35% of maximum foliar N content in a bracken fern, sword fern and Oregon grape understory. Based on these assumptions, the deciduous or annual foliage component in the 2-year-old stand in this study would annually return to the soil in litterfall 35 kg N ha^{-1} in untreated stands and 71 kg N ha^{-1} in treated stands. The 9-year-old stand would return 56 kg-N ha^{-1} in untreated stands and 76 kg-N ha^{-1} in treated sites. Evergreen foliage and litterfall from branches were not considered to be a significant source of recyclable N in either stand, and were not included in the above calculations.

In these young stands, biosolids increased potential litterfall N by 35% on the 9-year-old site and 102% on the 2-year-old stand. In the CHN analysis conducted for this study, C was also measured but not reported previously. Carbon % concentration of understory leaf material tested did not vary substantially between species, and averaged between 40-50%. The resultant low carbon:nitrogen (C:N) ratios (15-30:1) for all herbaceous and woody deciduous litterfall (even taking account for substantial N translocation from leaf material), should still provide high quality litter. These low C:N ratio should allow major nutrient cycling through rapid decomposition of organic matter and accelerated mineralization of N.

In the 68-year-old stand, evergreen foliage and stems from low N concentration species like salal and Oregon grape are the major understory biomass. Turner (1975) showed that second year leaves have 20 % lower N concentration than current foliage. Assuming that leaf abscission is only from second year or older leaves, only 10 to 15 kg N ha^{-1} would be returned from understory in litterfall annually. Klinka et al. (1989) also describes the foliage and roots from salal as resistant to decay and thus reduce the decomposition of forest floor materials. Alternatively, in the other 68-year-old stands in the Silvicultural Demonstration site that received heavy biosolids application in the 1980s and early 1990s, salal has been replaced with deciduous, nitrophytic species (e.g., stinging nettle and red elderberry). This should change the low nutrient recycling potential found on evergreen salal-Oregon grape sites to levels more similar of the 2- and 9-year-old sites examined in this study.

These biosolids application studies show a range of short term fertilizer responses and predicted long-term responses in different stand types. The past site history, fertilization rate, edaphic and vegetation factors all affect the variability of the response. If the understory is actively recycling nutrients in the stand, as assumed in the young stands in this study, the duration of fertilization effects could be prolonged. Alternatively, if the stand is dominated by mature evergreen shrubs that do not respond substantially to added nutrient, the longevity of the fertilizer effect could be minimal. However, any conclusion must consider the range of other edaphic and cultural factors affecting the response.

6.5.5 Implication of understory on application rate calculations

In the 2- year-old stand, the untreated site, although accumulating significantly less N in understory than the treated area, still increased its N content by 50 kg N ha^{-1} over the growing season. Since negligible residual nitrate was present on this site in pre-application assessments (King Co. 1995), most plant available N must have been mineralized from the active and stable organic N pools in the soil.

In the treated 2 year-old sites, an additional 72 kg N ha^{-1} was removed from the soil by understory vegetation. As the N in the biosolids was not traced with isotopes, the exact source of N accumulated in the plant is not measurable. However, it is likely that the available inorganic N in the biosolids was the first source accumulated, followed by the easily mineralized N from the organics in the biosolids.

In the 9- year-old stand the average N accumulation was 99 kg N ha^{-1} after biosolids application which represented only a small increase compared to that found on untreated sites. Substantial available N still appears in the system as understory in untreated plots accumulated 81 kg N ha^{-1} over the growing season, with no readily visual negative growth effects on overstory (note that measurements of overstory growth and N concentration were not made). The previous application in 1992 has already primed deciduous and herbaceous understory growth. It is expected that large amounts of N are annually returned to the soil from litterfall with high N conc. and that significant mineralization is occurring. It is unlikely that additional biosolids applications will cause significant net immobilization in these N enriched sites, so additional N should be readily available for overstory accumulation.

The minimal accumulation of N in the understory of the 68 year-old stand are obviously limited by the small scale of the application plots and the single growing season which results were monitored. Minimal understory accumulation could suggest:

- that the soil is heavily N deficient and that the bulk of the applied inorganic and mineralized organic N from the biosolids was immobilized into the soil organic N pool and was not available to either the understory or the trees. This would be contrary to positive tree growth responses reported by Henry et al. (1993) on these sites at Pack Forest.

- the understory has already reached a mature growth stage and may continue in a steady state condition rather than showing accelerated growth. The mature salal understory present on this site has low relative N requirements, which it can partially meet through mycorrhizal associations.

- salal's ericoid mycorrhizae are adept at assimilating complex organic N and P (Read 1983) but are incapable of accumulating N applied as biosolids. Prescott et al. (1993a) describe how high concentrations of ammonium and nitrate (greater than 600 kg N ha^{-1}) can inhibit ericaceous plants like salal. However, inorganic N fertilizer rate applied at similar rates to Total N in this study (between 120 and 200 kg N ha^{-1}) was shown by Stanek et al. (1979) to increase salal cover and biomass. Prescott and Weetman (1994), report greenhouse studies that show that salal seedlings are capable of accumulating ammonium, nitrate and ammonium nitrate in solution. Salal should not be considered as incapable of accumulating inorganic N, but rather considered as capable of meeting its low relative N requirements partially through its mycorrhizal associations

The implications of the differing rates of understory N accumulation in determining application rate calculations for stands of differing age, understory composition and past treatment history is substantial. Young Douglas-fir stands like the 2-year-old stand which have not received recent biosolids applications and have understory composition exhibiting substantial vigorous annual growth can be expected to accumulate approximately 100 kg N ha^{-1} as estimated in the U.S. EPA Process Design Manual (1995). Young stands, like the 9-year-old-stand, have vigorous annual understory growth. However, these already have enriched levels of available N status from previous applications and enriched litter turnover, and should thus only additionally accumulate between 0 and 20 kg N ha^{-1} . This rate should be used in future re-application calculations to young stands at Weyerhaeuser. In stands like the 68 year stand, with established evergreen understory, it seems reasonable to only include minimal additional understory N accumulation in application rate calculations. Alternatively, understory N accumulation in older stands that have been recently thinned and dominated by more deciduous or early successional species, or evergreen species just starting to expand in biomass after thinning, could have a higher N accumulation of approximately 30 kg N ha^{-1} as found in the U.S. EPA Process Design Manual (1995). In summary, designers of application rates need to take not only stand age and extent of existing understory into account, but also species composition, stage of understory development and past treatment history.

6.5.6 Methodological improvements and future research options

Methodological improvement of the current experimental design would have been gained from :

1. Treating larger plot areas with biosolids in the 68-year-old stand. This would have reduced variability, and possible strong edge effects from plant roots being outside the

treated areas. Preferably the area should have been machine applied as was done in the younger series of plots. Small plots were used in the current experiment because heavy, bulky material had to be carried into the stand up to 400 m from accessible road edges.

2. The biomass and N-content plots in all areas should have been larger in area (at least 10 m x 10 m). This would have allowed larger samples to be taken, which should reduce the level of variation.
3. Plots should preferably be monitored periodically over a longer period, e.g. at least 2 growing seasons. This would have captured greater annual variation caused by climatic conditions, as well as allowing sampling at different times to further refine the seasonal changes for understory.
4. Destructive sampling of different species at different times would have ensured that all understory species were sampled at their annual maximum and minimum, rather than the estimated average extremes for all understory on the plot. Such sampling within the biomass plot would, however, have changed interplant competition. An alternative to destructive clip sampling would have needed to be used. A possible alternative would been the use of biomass functions that have regressed biomass against a measurable plant parameter. Gholz et al.(1981) has a partial set of relations for some of the species found in this study. This approach was not used due to problems with estimating small scale changes in cover following treatment, the small population size most regressions were generated from, the site specific nature of some relationships, and that relationships do not exist for all understory species found in this study..
5. Establishments trials considering a greater range of sites with different edaphic and cultural histories. The replicated application of different rates of biosolids application,

if able to be monitored over longer periods may also have shown important N accumulation relationships.

6. Determining understory N accumulation after biosolids application as part of a complete N cycling experiments, so the extent of all N pools and fluxes are quantitatively known rather than estimated.

6.6 Conclusions

Understory vegetation in young Douglas-fir stands plays a very substantial role in the N accumulation and recycling after initial biosolids application. This role should be a major contributor to calculating agronomic rate. Nitrogen accumulation by understory in 2-year-old treated with biosolids was 73 kg N ha^{-1} more than in untreated sites. Understory vegetation was dominated by herbaceous species whose foliage showed increased N concentration with biosolids application. This understory material with high N content was mainly annual deciduous growth that was returned as litter for recycling of nutrients on the site.

Understory is not a substantial contributor to calculating agronomic rate on young stands receiving second applications. Although vigorous herbaceous and deciduous woody plants on the 9-year-old stand accumulated between 81 and 99 kg N ha^{-1} over the growing season, additional N accumulation by understory in biosolids treated stands was only 18 kg N ha^{-1} more than untreated sites. Initial biosolids applications and annual recycling of high N litter from deciduous understory plants have already increased the available soil N pool for understory growth and understory N accumulation on these sites. Understory in 9-year-old Douglas-fir stands on N deficient sites that have not received previous biosolids application, would be expected to behave like the very young stands. This increased rate of

N accumulation and recycling after initial biosolids application should make substantial contributions in calculating agronomic application rate.

Understory rate on old Douglas-fir stands is not a major accumulator of N after biosolids application. In the older stand where understory is well developed with woody evergreen shrubs, no significant or substantive increase in understory biomass or N content was observed. Biosolids application to these stands needs to consider the species composition of the stand as well as the stage of understory maturity. The low N accumulation and recycling of established evergreen shrubs like salal, will obviously play less role in determining applicable biosolids application rate, than if the same stand was dominated by young vigorous herbaceous or deciduous understory. In general, older stands dominated by mature salal should assume negligible N accumulation by understory species in agronomic rate calculations.

Chapter 7:

IMPLICATIONS FOR FOREST MANAGEMENT

Although operational forest biosolids application has been prevalent in the western Cascades for the last 10 years, much of this forest application has been on private lands out of view of the general public. The arrangements under the Mountains To Sound Greenway Biosolids Program, allow for continued application of biosolids to private lands, and also increases in the application of biosolids in high visibility areas like Tiger Mountain. With these public forests being highly visible and extensively used for public recreation, quantification of the impacts on other site values other than improved timber productivity is important.

The analysis of the composition of understory vegetation helps to quantify stand visual changes after heavy liquid biosolids application. After the odor of biosolids at application time, the change in visual appearance of the site is the next most obvious impact of biosolids application. The initial visual impact of biosolids from dewatered product applications is generally short term and limited to minor adherence of biosolids to tree stems and foliage. This effect generally continues until the first major rainfall event. As most biosolids falls to the forest floor, the upper portion of the stand generally maintains its color and structure. The visual effects of dewatered biosolids applied by the aerospreader is generally only noticeable on the forest floor, which is only visible in the microscale.

Over time, the visual effect is limited to the presence of residual portions of biosolids on the forest floor, and any changes in understory vegetation. After biosolids application, the understory vegetation will generally be denser and taller. Whether further changes occur affecting species composition depend on application rate and site factors. An increase in nitrophytic species, and greater biomass of deciduous understory may exist following heavy applications on recently disturbed, low SQ sites. With lower rate of biosolids application typical of current practice, changes in understory species can still occur, but are not expected to be as major as observed on the old heavy applications at Pack Forest. Although at Snoqualmie, the main understory impact was a biomass increase in current species, small clumps of nettle were present in certain microsites. The combination of increased understory growth and the potential introduction of unfavorable species like stinging nettle to the site, makes walking in the stand more difficult and limits some potential uses of the stand for public recreation.

If management strategies are using biosolids to accelerate stand development, the introduction of weedy, nitrophytic species could move the stand into a different successional pathway and may induce different development of the forest. It is more likely, however, that nitrophytic and early successional species may be short-term occupants that are rapidly building and recycling the N pool. In the longer term (10-20 years), they will be replaced by slower growing species. Although the short-term pathway may be different, the resultant forest structure may be similar.

If biosolids application and overstory thinning are intended to improve wildlife habitat, it is essential that other silvicultural practices are implemented that provide stand heterogeneity. The modification of understory is only one critical habitat structural component that needs consideration. Dead wood, hollows, and vertical and horizontal heterogeneity are all stand components that must be developed to attract certain wildlife. The type of understory, the protection the understory offers and the food that it provides will all affect the type of wildlife introduced. It would need to be researched if early successional species provide the same habitat resources as original understory components in biosolids treated Douglas-fir stands.

The effects of biosolids application on the maintenance of forest biodiversity may vary with spatial scale. In the small scale for a particular site, increased N application from biosolids may temporally follow the trends reported by Tilman (1984) and Frequez (1990) of increased understory biomass but decreased plant density and species diversity. However, the regulation of biosolids application ensure that on a landscape scale a mosaic of treated and untreated forest generally exists. Additionally, as more land area becomes available for biosolids application, the intensity and the frequency of applications to individual sites can be managed to ensure that effects on biodiversity can be minimized.

If the introduction of nitrophytic or weedy species in adaptively managed stands are considered detrimental from any perspective (i.e. long or short term), biosolids (or any fertilizer) application will need to be conducted so that thresholds that induce species change are not crossed. This would involve:

- Applying biosolids at lower rates of N than those that appeared to change species composition in the older Pack Forests trials. Current applications at agronomic rate to older thinned stands, appear not to be changing species composition as the past heavy liquid applications have done.
- Reviewing the stage of understory species development, so that existing or desired species have competitive advantage. This may involve allowing the desired understory to establish for 2 or 5 years after thinning before biosolids are applied. Such delays may slightly increase the time overstory trees take to achieve the desired diameter, but the later biosolids application should still induce a significant growth response.

- Manipulating overstory density through lighter thinning treatments. Biosolids may increase the availability of nutrients for understory plants, but other stand factors (e.g., light intensity), may prevent compositional change from occurring. Lighter thinning would favor the slower growing species like Oregon grape that develop under lower light conditions. Again this practice would be a trade off with the time required for that the stand to reach desired individual tree diameter or overstory basal area.

As with any silvicultural practice, multiple combinations of biosolids application rates, thinning intensity and stage of understory development will also adjust the resultant stand structure, overstory tree size and understory composition and abundance.

The legislative framework of forest management needs to be considered in the development of biosolids practice. In states where harvested stands must be regenerated to certain minimum stocking and " free to grow" requirements, the very early application of biosolids may make achieving these targets more difficult. If biosolids are applied in the first two years after planting, or applied in 2-3 years before final harvest of mature stands, an increased risk of greater understory competition and increased sapling mortality exists. In western Washington acceptable stocking needs to be achieved within 3 years of harvesting, so biosolids practices which increase understory vigor and therefore detrimentally affect seedling survival and growth are of concern to forest managers.

Chapter 8:

SUMMARY AND CONCLUSION

8.1 Biosolids applications on species composition

Repeated or heavy biosolids applications to older Douglas-fir stands at Pack Forest caused a significant change in understory species composition. On these sites the relative density of nitrophytic plant species, such as red elderberry and stinging nettle which where formerly absent has increased substantially. Biosolids decreased the abundance of salal, increased sword fern and had no effect on the cover of Oregon grape.

8.2 Effect of biosolids on Douglas-fir growth and survival in young stands

Biosolids applied to 2-year-old stands did not affect Douglas-fir sapling diameter or height increment. Larger saplings had greater average height increment, but effects of biosolids addition were not significant at $p<.05$. Mortality was greater in small saplings (< 1.2 m tall). Mortality was 15% in treated stands and 9% in control sites. This change in mortality percent was not significant at $p<.05$.

8.3 Nitrogen accumulation by understory plants

Understory vegetation in young Douglas-fir stands plays a very substantial role in the N accumulation and recycling after initial biosolids application. This role should be a major contributor to calculating agronomic rate. Nitrogen accumulation by understory in 2-year-old treated with biosolids was 73 kg N ha^{-1} more than in untreated sites. Understory vegetation was dominated by herbaceous species whose foliage showed increased N concentration with biosolids application. This understory material with high N content was mainly annual deciduous growth that was returned as litter for recycling of nutrients on the site.

Understory is not a substantial contributor to calculating agronomic rate on young stands receiving second applications. Although vigorous herbaceous and deciduous woody plants on the 9-year-old stand accumulated between 81 and 99 kg N ha^{-1} over the growing season, additional N accumulation by understory in biosolids treated stands was only 18 kg N ha^{-1} more than untreated sites. Initial biosolids applications and annual recycling of high N litter from deciduous understory plants have already increased the available soil N pool for understory growth and understory N accumulation on these sites. Understory in 9-year-old Douglas-fir stands on N deficient sites that have not received previous biosolids application, would be expected to behave like the very young stands. This increased rate of N accumulation and recycling after initial biosolids application should make substantial contributions in calculating agronomic application rate.

Understory rate on old Douglas-fir stands is not a major accumulator of N after biosolids application. In the older stand where understory is well developed with woody evergreen shrubs, no significant or substantive increase in understory biomass or N content was observed. Biosolids application to these stands needs to consider the species composition of the stand as well as the stage of understory maturity. The low N accumulation and recycling of established evergreen shrubs like salal, will obviously play less role in determining applicable biosolids application rate, than if the same stand was dominated by young vigorous herbaceous or deciduous understory. In general, older stands dominated by mature salal should assume negligible N accumulation by understory species in agronomic rate calculations.

8.4 Future research needs.

The results observed in this study on understory compositional change and N accumulation are based on specific actual application rates to specific stands. Future experiments need to be designed to monitor the effects of different application rates on a range of stand ages, time since disturbance, thinning regimes, and site productivity. Alternatively, increasing the knowledge of the mechanisms that cause individual species response to biosolids, may allow biosolids managers to model the results of biosolids application, rather than relying on the results of site monitoring to determine future outcomes.

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