The longevity of decaying *Eucalyptus* hybrid roots in sub-tropical plantation forests

by

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Declaration

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Summary

The global threat posed by extreme climate change has led to an increase in the amount of climate change related research. It is now more important than ever before to accurately quantify the carbon pools in terrestrial ecosystems, in order to better understand how these pools might influence the carbon cycle. The residence time of carbon in dead coarse roots (*i.e.* roots greater than 2 mm diameter), an often-neglected carbon pool, are still not well understood. (Fine roots are known to have rapid turnover, rates and was not considered in this study). The decay rate constant of decomposing roots after clear felling in *Eucalyptus* hybrid stands was determined using a chronosequence sampling approach followed by analysis of the density and carbon contents. The results were subsequently modelled with single component negative exponential model (k = 0.1058). *Eucalyptus* hybrid root systems in sub-tropical plantations took on average 6.6 years to lose 50% density, 13.1 years to lose 75% density and 28.3 years to lose 95% density. The relationships between root decomposition and root size class (2-10 mm, 10-50 mm, >50 mm diameter roots and tree stump) as well as site productivity (in the form of mean annual increment) were also investigated. Neither root size nor site productivity had significant relationships with root decomposition rate. Coarse root carbon content did not vary with time after felling or site productivity, but rather with root size. The mean carbon concentration for each root size class was 46.8 ± 1.6% (2-10 mm), 48.6 ± 1.9% (10-50 mm), 48.8 ± 1.4% (>50 mm) and 48.6 ± 2.3% (stump). The results showed that *Eucalyptus* hybrid coarse roots in sub-tropical plantations in South Africa should be regarded as an important long-term pool of sequestered carbon. The decay model is earmarked for inclusion in a South African forestry carbon calculator that estimates the stock changes of various above- and below ground carbon pools in forest ecosystems over time.

Opsomming

Die bewuswording van die erns en spoed van klimaatverandering het gelei tot ‘n groot vermeerdering van klimaatverandering verwante navorsing. Dit is tans meer belangrik as ooit tevore om die koolstof wat vasgevang word in die verskeie ekosisteme se verskillende komponente (koolstof poele), akkuraat te kwantifiseer met die doel om hul invloed op die koolstof siklus beter te verstaan. Die potensiële bydrae van dooie houtagtige dik wortels (wortels groter as 2 mm) tot die koolstof retensie in plantasie sisteme is nog nie volledig beskryf nie. (Fyn wortels is bekend vir vinniger afbraaktempo’s en het nie deel gevorm van die navorsingsprojek nie). Die hoof doel van hierdie studie was om afbraak konstantes te bepaal vir *Eucalyptus grandis x E. urophylla* dik wortelklasse in subtropiese plantasies na kaalkapping. Dit was ook belangrik om die koolstof konsentrasie van die dikker wortelklasse te bepaal om te sien of dit verskil van waardes wat gereeld in die bedryf gebruik word. ‘n Negatiewe eksponensiële funksie was die bes passende model op die wortel afbraak data, en het ‘n afbraak konstante (k) van 0.1058 opgelewer. *Eucalyptus* hibriede se wortels het gemiddeld 6.6, 13.1 en 28.3 jaar geneem om 50%, 75% en 95% van hul digtheid te verloor. Die verhouding tussen veranderlikes soos wortelklas grootte en plantasie produktiwiteit, asook wortel afbraak is ook ondersoek. Die resultate toon dat daar geen statisties betekenisvolle verhouding tussen die veranderlikes wortel grootte en plantasie produktiwiteit, of die tempo van afbraak in dik wortelklasse is nie. Verder is daar gevind dat koolstofinhoud van dooie dik wortels nie betekenisvol verander met tyd of plantasie produktiwiteit nie, maar wel met wortel grootte. Die gemiddelde koolstofinhoud van die onderskeie wortelklasse was 46.8 ± 1.6% (2-10 mm), 48.6 ± 1.9% (10-50 mm), 48.8 ± 1.4% (>50 mm) en 48.6 ± 2.3% (stompe). Die resultate het bewys dat die dooie dik wortelklasse van *Eucalyptus* hibriede in sub-tropiese plantasies ’n belangrike bron van gesekwestreerde koolstof is. Die afbraak model is beskikbaar vir toekomstige gebruik as komponent van ’n Suid Afrikaanse bosbou koolstof rekenaar model wat veranderinge in koolstof voorraad oor tyd binne plantasie sisteme bereken.

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CHAPTER 1
INTRODUCTION

1.1 Background

In recent years South Africa has agreed to voluntary alignment and compliance with the United Nations Framework Convention on Climate Change (UNFCCC), countries Reducing Emissions from Deforestation and Forest Degradation (REDD+) and Intergovernmental Panel on Climate Change (IPCC) through the South African Department of Environmental Affairs (DEA) (IPCC, 2003; DEAT (Department of Environmental Affairs and Tourism), 2006; UNFCCC, 2009; Department of Environmental Affairs, 2010, 2011). This commitment means that the country has to implement systems to reduce greenhouse gas emissions, to mitigate the causes and effects of past emissions and to adapt to the climate change caused through past emissions (DEA, 2011; UNFCCC, 2011). For example, the South African government has decided to implement a carbon taxation system, in order to keep to this commitment. The carbon tax bill was passed in the South African parliament on 20 November 2018 and is scheduled to be implemented from June 2019.

As more scientific data and information on global warming and climate change accumulate, it is becoming more apparent that climate change perhaps might be the greatest environmental challenge of the twenty-first century (Food and Agriculture Organization of the United Nations (FAO), 2006). A great deal of climate change related research is focused on determining how much C is stored in various C pools within terrestrial ecosystems. It is important to account for the C stored in each C pool across different ecosystems since changes in C stocks influence the balance between terrestrial and atmospheric C which in turn will affect climate change (Keith *et al.*, 2010). Commercial forest plantations have the potential to help mitigate the effects of anthropogenic climate change through the sequestering of atmospheric C dioxide (CO_2) as C in tree biomass, dead organic matter and soil C (IPCC, 2006; Heath *et al.*, 2011).

Multiple carbon pools can be identified within the forest ecosystem and include above-ground biomass (AGB), below-ground biomass (BGB), litter, dead wood and soil organic matter (IPCC, 2003). It is necessary to quantify the C stored in these various pools to determine the value of this C stock and changes in C stock over time (Du Toit *et al.*, 2016). Usually, the simplest way to calculate the annual change in C stocks in forest plantations is by calculating the sum of changes in each of the living biomass, dead organic matter and soil pools (IPCC, 2006). Tier 1 methods for estimating plantations C stocks at a country wide

level lack the desired accuracy for South African C accounting and taxation systems, since these methods are international level default values. If SA is to implement a carbon taxation system progression towards higher resolution country-specific Tier 2 and regional specific Tier 3 estimates of C stocks will be essential. It is also encouraged for general reporting on C stocks (IPCC, 2006; Bird *et al.*, 2010).

Currently there is still a lack of empirical information on belowground tree biomass globally, which prevent the accurate estimations of forest C stocks and the understanding of subsequent forest C dynamics (McKinley *et al.*, 2011; Russell *et al.*, 2015). Roots have the potential to store large amounts of C and nutrients (Vogt *et al.*, 1986; Kurz *et al.*, 1996; Cairns *et al.*, 1997) and should be regarded as an important pool of C and nutrients in forest ecosystems (Palviainen and Finér, 2015). To quantify the potential of dead woody roots to store C or nutrients requires an understanding of the rate at which these roots decompose. In comparison to other woody detritus studies there have been far fewer studies focussing on root decomposition (Yavitt and Fahey, 1982; Fahey *et al.*, 1988; Zhang and Wang, 2015).

Estimations for the C stored in decomposing roots has yet to be made for South African plantation forests. Therefore, the quantification of this carbon pool could improve the accuracy of the current carbon accounting models or calculators within the South African forestry industry.

1.2 Study objectives

1.2.1 Main objective

The main objective of this study is to determine longevity of dead *Eucalyptus* hybrid roots in order to understand its potential for storing C.

1.2.2 Specific objectives

1. Develop a methodology that is most suitable for effectively sampling decomposing roots within the time and budget constraints of the study.
2. Produce decay constants for loss of density in the decomposing roots.
3. Determine if root decay rate varies with certain variables such as root size.
4. Determine the C concentration of the decomposing roots.
5. Compare the suspension method for measuring volume to the CT-scanning method.

1.2.3 Research questions

1. What is the turnover rate of clonal Eucalypt hybrid roots in the subtropical forestry region of SA?

2. Does decay rate vary significantly with root size, tree size or with site index? (Warmer and wetter sites usually have higher productivities and these factors may affect root decomposition).
3. Does root C concentration remain at more or less 50% as decay progresses?
4. Does C concentration vary significantly with root size, tree size or with site index? (Where site index is the mean height of the 80th percentile (by diameter) of trees at reference age five).
5. Is there a significant difference in volume measurements by CT-scanner method and suspension method?

CHAPTER 2
LITERATURE REVIEW

2.1 Defining the root system and its respective components

2.1.1 Terminology

From the moment plant material dies it becomes part of the dead organic matter fraction and starts to undergo the process of decomposition. Decomposition is defined as the physical or chemical breakdown of organic matter (complex organic structure) to its most basic form (mineral form) and can occur both above and within the soil (Thomas and Packham, 2007). Several terms have been used when referring to dead organic matter, but to allow for inter-study comparability it is advised to use one of the more common scientific terms such as detritus or debris (Harmon and Sexton, 1996). The term debris can be used for both above and below ground dead organic matter and debris from woody plants, can be referred to as woody debris (Harmon and Sexton, 1996).

2.1.2 Woody debris

Coarse woody debris in forest ecosystems can consist of; snags (standing dead material such as stump), logs and dead roots (Harmon and Sexton, 1996). Coarse woody debris (CWD) (including coarse roots), can serve several functions in forest ecosystems, including acting as a substrate for the decomposer the community, storing Carbon (C) and nutrients, maintaining forest biodiversity and enriching the soil with nutrients and humus through decomposition, hence influencing soil development (Harmon *et al.*, 1986; Janisch *et al.*, 2005; Jomura *et al.*, 2007; Fraver *et al.*, 2013).

Roots have the potential to store large amounts of C and nutrients (Vogt *et al.*, 1986; Kurz *et al.*, 1996; Cairns *et al.*, 1997) and should be regarded as an important pool of C and nutrients in forest ecosystems (Palviainen and Finér, 2015). To understand the potential of dead woody roots for storing C or nutrients, requires an understanding of the rate at which these roots decompose. In comparison to other woody detritus studies there have been far less studies focussing on root decomposition (Yavitt and Fahey, 1982; Fahey *et al.*, 1988; Zhang and Wang, 2015).

The lack of research can perhaps be contributed to the technical difficulties associated with root decomposition studies. Decomposing roots have to be sampled from within the soil which can be a very physically intensive and destructive process (Brunner and Godbold, 2007). Additionally, the physical condition of the decomposing roots will begin to deteriorate

as the decomposition process progresses, which can increase the difficulty of sampling. Furthermore, in order to dynamically measure root decomposition, the environment in which decomposition occurs (soil) has to be disturbed (Bloomfield *et al.*, 1996).

2.1.3 Defining the root system

Before one can conduct a study on a biomass fraction (such as the root system) one first needs to define what will constitute the root system for the particular study. There exists only a few established principles for defining or describing the root system (Silver and Miya, 2001). Some studies use ground level as a separation point between the aboveground biomass and belowground biomass. It is important to consider including the stump (the fraction of material remaining after the tree has been felled) and the root crown (biomass fraction directly below the stump) as part of the below ground root biomass even though these biomass fractions can be found above ground (Gifford, 2000; Palviainen and Finér, 2015). Therefore, separating above and below ground biomass at the stump cut can be seen as a more heuristic approach (Drexhage and Gruber, 1999; Magalhães and Seifert, 2015). The stump was included as part of the root system for the current study since it forms part of the biomass fraction that remains after clear felling plantation crops.

2.1.4 Sub dividing the root system – root components

Within a particular biomass fraction (dead roots in the case of the current study), it is important to separate components that are significantly different from one another which could lead to differences in the measurements of a specific parameter. Root components are often classified based on size, simply because size conveniently incorporates both structural and functional differences within roots (Fahey and Arthur, 1994). From a structural point of view; root surface area in relation to volume increases with decreasing root size, which has showed to increase fragmentation and decomposition rates (Zhou *et al.*, 2007). From a functional point of view; smaller roots (fine roots) generally are responsible for taking up nutrients and water from the soil rhizosphere whilst larger roots (coarse roots) are responsible for facilitating water and nutrient transport to the above ground plant system, supporting the fine root network and supporting plant structure (Tobin *et al.*, 2007). These functional differences lead to differences in nutrient concentrations and chemical composition (lignin and cellulose concentrations) of the root material which may lead to differences in the rate of decomposition (John *et al.*, 2002).

Root size for separating root components

Diameter values are used as a measure of root size and the diameter sizes used to separate two fractions from one another can be referred to as a size threshold. These size thresholds differ from one study to another which can make inter-study comparability very difficult (Harmon and Sexton, 1996). When it comes to woody detritus studies, the most important size distinction is between fine and coarse size fractions (Harmon and Sexton, 1996). Although there is some sort of consensus on fine and coarse root thresholds, there exists no standard for subdividing the coarse root fraction (Tufekcioglu *et al.*, 1999; Bolte *et al.*, 2004). A root size break point between fine and coarse roots of 2 mm is one of the most common size thresholds (Silver and Miya, 2001; Cusack *et al.*, 2009; Zhang and Wang, 2015). Other studies applied fine and coarse size thresholds at a diameter of 10 mm, (Harmon and Sexton, 1996; Chen *et al.*, 2001) (Table 2.1). However, it is recommended that some of the more common size thresholds be used to allow for inter-study comparability. This does not mean other additional size thresholds cannot be used for specific study objectives (Harmon and Sexton, 1996) (Table 2.1). Table 2.1 illustrates the nonconformity when it comes to root size threshold values by listing threshold values from a multitude of root studies. These studies either used 2 mm or 10 mm as a root size threshold for separating fine and coarse root fractions but differ depending on sub-division of the coarse root fraction. Palviainen and Finér, (2015) also included the stump as part of the root system (Table 2.1).

Table 2.1: How published root decomposition studies have subdivided roots into different size classes or fractions.

Species	Root fraction	Size threshold (mm)	Reference
Norway Spruce (*Picea abies*)	Coarse	Stump > 100 50 - 100	(Palviainen and Finér, 2015)
Sitka spruce (*Picea sithensis*), Western Hemlock (*Tsuga heterophylla*), Douglas-fir (*Pseudotsuga menziesii*), Lodgepole pine (*Pinus contorta*), Ponderosa pine (*Pinus ponderosa*)	Fine Coarse	< 10 > 10	(Harmon and Sexton, 1996; Chen *et al.*, 2001)
Pinus radiata	Coarse	10 - 50 > 50	(Garrett *et al.*, 2008)
Global Study (a range of Conifers, Broadleaf's and Graminoids species)	Fine Coarse	< 2 > 2	(Zhang and Wang, 2015)
Global Study (a range of Conifers, Broadleaf's and Graminoids species)	Fine Coarse	< 2 2 - 5 > 5	(Silver and Miya, 2001)

2.2 Approach for studying woody root decomposition (Experimental design)

There are different experimental designs or approaches that have been used to study CWD decomposition dynamics. The most commonly used approaches include: 1) the chronosequence, 2) time series, 3) decomposition-vector and 4) laboratory incubation approach (Figure 2.1). In this section each of these methods will be discussed in detail, and a brief summary of the advantages and disadvantages of applying each method will follow thereafter in Table 2.2.

2.2.1 Time series

The first method and currently the most rigorous and reliable method is time series approach. In a time series the decomposition process would be examined as it actually progresses through time (Harmon and Sexton, 1996). That is, roots are actually continuously monitored as decomposition occurs. Normally in a time series, several pieces of material are positioned and allowed to age and one would take measurements incrementally (Harmon and Sexton, 1996). It is important to measure as much as possible about the initial site and sample conditions before the process begins in order to yield the best results from the time series approach (Harmon and Sexton, 1996).

A great advantage of the time series is the increased precision and resolution by which the decomposition process can be studied (Harmon and Sexton, 1996). Mainly because the time series allows for the initial sample and site conditions to be measured, which are very important for correctly understanding decomposition (Harmon and Sexton, 1996). But the drawback of the time series approach is that it can be very costly, especially in terms of the time invested in the study (Harmon and Sexton, 1996; Garrett *et al.*, 2007). This is why a time series is rarely used to examine coarse (larger) woody debris decomposition, because it may take several years for coarse debris to lose most of its biomass.

2.2.2 Chronosequence

In a chronosequence measurements are taken from material that are in different states of decay (*i.e.* has aged for different durations) instead of measuring one piece of material as it progresses through the decomposition process (Yavitt and Fahey, 1982; Harmon *et al*., 1986; Harmon and Sexton, 1996). The chronosequence approach is different from the time series approach since it is a substitution of space for time (Harmon and Sexton, 1996). The chronosequence approach has been used in numerous coarse woody detritus studies (Grier, 1978; Means, Cromack and MacMillan, 1985; Harmon *et al*., 1987; Sollins *et al.*, 1987), but has also been used in coarse root studies (Fahey *et al.*, 1988; Chen, Harmon and Griffiths, 2001; Ludovici *et al*., 2002; Palviainen and Finér, 2015).

The age information for these different pieces of material can be obtained from past records such as thinning or harvesting records in the case of plantation forestry (Harmon and Sexton, 1996). If these records do not exist and the age of the material is not known then one can make use of decay classes (Harmon and Sexton, 1996). When using decay classes, the decomposing material is divided into different classes that represent different stages of decay (Harmon and Sexton, 1996). It is common to divide the decomposing material into three to five decay classes based on physical rather than biological indicators (Sollins *et al.*, 1987). When using decay classes, it is important that the classification process is very thorough to prevent material being classified as the wrong decay class (Garrett *et al*., 2007).

Chronosequence studies pose several problems depending on the way in which it is applied. For example, if one were to measure the rate at which mass is lost or nutrients are released it is important to know the initial measurements of the material in order to know if any material has been lost (Harmon and Sexton, 1996). This is referred to as losses due to fragmentation. If the chronosequence is used to measure changes in density or nutrient concentrations (as

in the case of the current study), only the current measurements are needed (Harmon and Sexton, 1996).

Another important consideration when using the chronosequence approach is the fact that different pieces of material are sampled at each sampling interval and if past harvest records are used to identify the age of the material instead of decay classes then there is also the differences between the sites. Sampling across different sites leads to uncertainties regarding inter-site differences (Harmon and Sexton, 1996). That being said, uncertainties due to differences in site conditions can be kept to a minimum when keeping as many variables constant as are possible. In the current study only clonal *Eucalyptus grandis* x *E. urophylla* hybrid plantations were sampled. The soil, topography and microclimatic conditions were very similar between all sites in order to further limit the potential error associated with inter-site differences.

Chronosequences are very useful for studying processes that stretch over long periods of time (Garrett *et al.*, 2007). It is well known that the decomposition of wood is primarily a function of size. Larger pieces of woody debris such as coarse roots generally have lower decay rates than smaller pieces and therefore can decompose for longer periods of time. Therefore, a chronosequence lends itself well to studying the decomposition dynamics of a material such as tree coarse roots which could take several decades to compose.

2.2.3 Decomposition Vector Approach

The decomposition-vector approach is a hybrid between the time series and chronosequence approach (Harmon and Sexton, 1996; Harmon *et al.*, 2000). The decomposition-vector approach is simply the resampling of a chronosequence and could improve the resolution of the process being studied (Harmon and Sexton, 1996; Harmon *et al.*, 2000). Harmon *et al.*, (2000) presented this new method for estimating rates of biomass, volume and density loss when they resampled a chronosequence after three years. In that study, in north western Russia, the authors sampled logs from three different species (*Pinus sylvestris* L., *Picea abies* (L.) Karst, and *Betula pendula* Roth) in a chronosequence. They concluded that the decomposition-vector method yielded similar results to that of the chronosequence method (Harmon *et al.*, 2000). Thus, resampling a chronosequence might not yield additional insight into the decomposition process, but more studies on different species and climates are needed to assess the results obtained by using this method (Harmon *et al.*, 2000).

2.2.4 Laboratory incubation

Laboratory incubation is a short term time series study done in a controlled laboratory environment *e.g.* for studying the potential effects of temperature and moisture on C respired from decomposing roots by incubating small root sections for periods of up to 4 hours (Chen *et al.*, 2000). This approach cannot produce decomposition rates, neither can it give insight to the effect of the decomposer community on the decomposition rate, but can give insight to the sensitivity of root respiration (decomposition) to abiotic factors such as changes in temperature and moisture (Chen *et al.*, 2000). Therefore, this method has limited applicability for understanding decomposition of woody debris, because decomposition reactions in a completely synthetic environment cannot be taken as prologue for *in situ* decomposition.

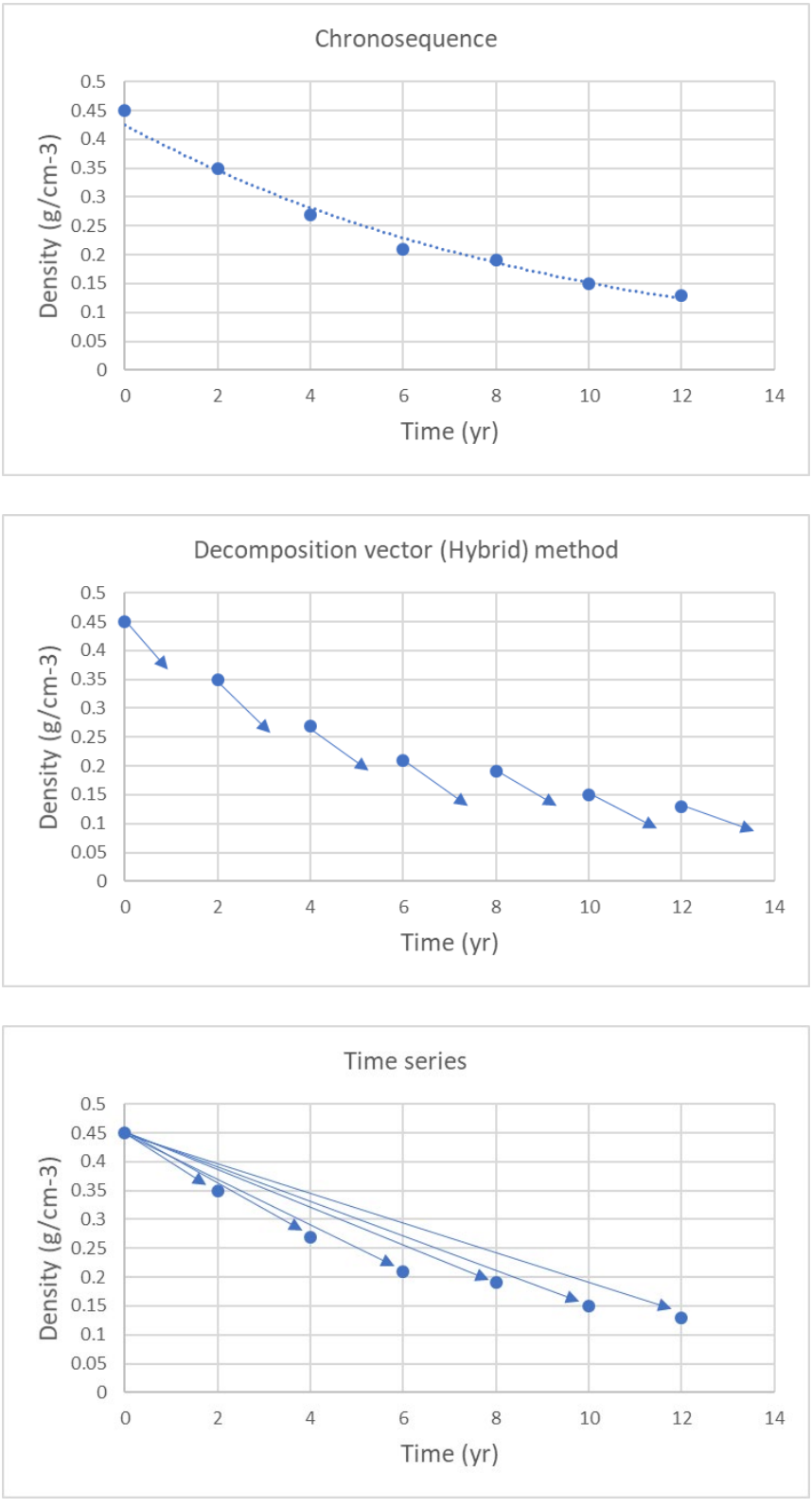

Figure 2.1: Illustration of the differences between the three general approaches (experimental designs) for studying decomposition dynamics (Chronosequence (2.1a), Hybrid (2.1b) and Time series approach (2.1c)). Adapted from Harmon and Sexton (1996).

Table 2.2: The advantages and disadvantages of the different experimental designs/approaches for studying root decomposition.

Approach	Advantages	Disadvantages	Reference
Time series	High precision of the data. High resolution of the process being studied.	High cost in terms of effort and time invested.	(Harmon and Sexton, 1996)
Chronosequence	Time efficient Lower input costs	Uncertainty about the initial condition and size of the dead trees. Differences in environment between the different ages/locations that are sampled.	(Harmon and Sexton, 1996; Harmon *et al.*, 2000; Garrett *et al.*, 2007)
Decomposition-vector approach	Could improve resolution compared to sampling a single chronosequence.	Uncertainty about the initial condition and size of the dead trees. Differences in environment between the different ages/locations that are sampled.	(Harmon and Sexton, 1996; Harmon *et al.*, 2000)
Laboratory Incubation	Can yield very precise results on decomposition dynamics, *e.g.* how decomposition rate is influenced by variables such as temperature and moisture.	Synthetic laboratory environment cannot be used as a substitute for *in situ* root decomposition.	(Chen *et al.*, 2000)

2.3 Determining decomposition constants

The rate of coarse root decomposition has been successfully described by measuring and modelling both mass (*e.g.* Ludovici *et al.*, 2002) and density (*e.g.* Chen *et al.*, 2001) loss. In the current study density loss was measured in order to determine the rate of root decomposition. When determining root decay using density loss it is necessary to accurately determine sample volume to prevent variability between density measurements (Harmon and Sexton, 1996). Once root sample volume is known the formula below can be used to calculate sample density:

$$p = \frac{M}{V}$$

where p is the density (g cm^{-3}) of the sample, M is the dry mass of the sample (g) and V is the fresh volume (cm^3) (volume after sampling *i.e.* not dried).

This section will discuss several methods that can be used to measure sample volume and conclude by considering multiple of models that could potentially be fitted to the density data.

2.3.1 Measuring sample volume

Measuring sample volume of decomposed woody debris, such as woody roots can be very difficult due to the deterioration of the root structure as decomposition progresses. There are multiple methods that can be used to accurately measure the sample volume of decomposing woody roots.

Suspension method for estimating sample volume of small samples

One of the most common methods for estimating the volume of small samples is by applying the Archimedes principle. It states that when an object is fully or partially immersed in a fluid the object is buoyed up by a force equal to the weight of the fluid that the object displaces (Hughes, 2005). Hughes, (2005) explains that there are several methods that apply the Archimedes principle *i.e.* the placing of an object in a measuring cylinder and recording the rise in the water level, immersing the object in a water filled container with an overflow spout to record the volume of overflow and the suspension technique.

The suspension technique is a popular technique for estimating the volume of biomass samples in the forestry industry. Hughes, (2005) showed that the suspension technique is more accurate than the other two more traditional water displacement methods and is more accurate than measuring volume using Vernier calliper measurements. Hughes, (2005) performed the experiments on small accurately machined PVC cylinders ranging in volume from 1.5 to 15.7 ml. Hughes, (2005) described the suspension technique as a faster, better and cheaper method of accurately measuring the volume of small objects. The suspension technique can be applied by suspending a small object below the surface of a fluid in a container placed on an electronic scale. The volume of the immersed object is simply the weight registered on the scale divided by the density of the fluid, which in the case of water, approaches unity, *i.e.*

$$V = \frac{\Delta\omega}{\rho}$$

where ρ is the measured density of the fluid, $\Delta\omega$ is the change in weight recorded by the balance when the object is suspended in the fluid and V is the unknown volume (Hughes, 2005).

X-ray Micro-Computed Tomography for estimating sample volume

Laboratory X-ray micro-computed tomography (micro-CT) has a wide range of imaging applications which can also be used to measuring wood sample volume (Du Plessis *et al.*, 2017). Micro-CT is becoming more popular in many scientific fields mainly because it allows for easy non-destructive imaging of a wide range of morphological structure (Du Plessis *et al.*, 2017). The technique involves the recording of two-dimensional X-ray images from various angles around an object, followed by a digital three-dimensional reconstruction. Following reconstruction, the 3D volume can be analysed using a variety of software tools. These software tools allow for dimensional, volumetric or other more advanced

measurements to be made (Du Plessis *et al.*, 2017). This technology has obvious benefits when trying to estimate the volume of highly decomposed samples (Du Plessis *et al.*, 2017).

Direct measurements for estimating sample volume

Volume can also be determined by directly measuring the dimensions of a cylindrically shaped material such as a log or a stump (the piece of material remaining after felling). Harmon and Sexton (1996) explains that if samples are regularly shaped, then volume estimates based on external measurements can be just as reliable as volume displacement measurements. The direct measurement technique was only used to determine the volume of the stump discs (which where cylindrically shaped).

Sample volume is calculated as the product of the cross-sectional area with the length of the sample using several methods. Huber's method commonly used and only requires a length measurement and the cross-sectional area at the mid length of the sample (Bredenkamp, 2012). Smalian's method is also popular and is the easiest to use when under-bark volume is required. Sample length and the average cross sectional of the thin and thick ends of the sample are needed to apply Smalian's method (Bredenkamp, 2012). The third method is that of Newton and was applied in the current study because it is regarded as the most accurate method of the three:

$$\mathrm{V} = (d_t^2 + 4\mathrm{d}_m^2 + \mathrm{d}_T^2) \cdot \pi/24 \cdot \mathrm{l}$$

where V is volume (cm^3), l is the length of the section of material and d the diameter; stem diameter is measured (cm) at the thin end (d_t), mid length (d_m), and thick end (d_T) of the sample; l is the length of the section of material in cm. (Bredenkamp, 2012)

2.3.2 Comparing the different methods for determining volume

The direct measurement method can be just as accurate as the suspension method, but only if samples are regularly shaped (Harmon and Sexton, 1996). For samples that are not regularly shaped the suspension or micro-computed tomography method should be considered. Since suspension method involves the displacement of a fluid, it can happen that the volume of a sample is underestimated if the fluid is absorbed instead of being displaced. Therefore, care should be taken when applying the suspension method to highly decomposed samples since it could under estimate volume (MacMillan, 1988; Fahey *et al.*, 1991; Stewart and Burrows, 1994). In order to prevent the fluid from being absorbed the sample may have to be sealed by using a wax or plastic film (Garrett *et al.*, 2007). Another alternative is to saturate the sample with water prior to submerging it. However, this

technique can only be used if it won't cause the sample to disintegrate when in contact with the water.

Using X-ray micro-computed tomography for determining volume of highly decomposed samples is an interesting method that has been gaining more attention in recent years, mainly due to its non-destructive and non-interactive method for measuring volume (Du Plessis *et al.*, 2017) and should be considered as a possible alternative to the suspension and direct measurement methods. X-ray computed tomography is able to accurately measure the volume of highly decomposed and irregularly shaped samples (Du Plessis *et al.*, 2017). Such a method can serve well when dealing with detritus fractions that are by nature very irregularly shaped such as root detritus.

2.3.3 Review of Existing Models for Describing Root Decomposition

Generally, decomposition constants are used to compare the rates at which CWD decompose and are produced by modelling the changes in density or mass loss. This section will cover some of the more popular models that have been used to model root decay. Thereafter will follow a table containing the formulas of each model (Table 2.5).

The most commonly used model for producing decay constants is the single component negative exponential model (Olson, 1963; Lambert *et al*., 1980; Wieder and Lang, 1982; Means *et al*., 1985; Harmon *et al*., 1986). Therefore, there has been a wide range of studies that have successfully modelled root decomposition using a single component negative exponential function to describe either mass or density loss in terms of time (Yavitt and Fahey, 1982; Chen *et al.*, 2001; Silver and Miya, 2001; Garrett *et al.*, 2012; Zhang and Wang, 2015). The single component negative exponential model is also used often to model decomposition of CWD in general (Shorohova *et al.*, 2008; Melin *et al.*, 2009).

Linear models have also been used to produce decay constants (Lambert *et al*., 1980; Wieder and Lang, 1982; Silver and Miya, 2001). Lambert *et al*. (1980) studied the decay of balsam fir (*Abies balsamea*) boles in an upper subalpine forest of the White Mountains, New Hampshire, USA. They showed that the linear and exponential models were equally efficient for modelling decomposition, according to their R^2 values. They decided to use the negative exponential model based on theoretical preference and visual inspection of the data. Silver and Miya, (2001) in their meta-analysis of global root decay fitted both a linear and exponential decay function to calculate root decay rates for the studies that reported only mass loss or C loss over time without a decay constant (Silver and Miya, 2001). They found

that the exponential equation provided a fit as good or better than the linear model in all cases (Silver and Miya, 2001).

The assumption that CWD is not a homogeneous substrate has led to a few studies fitting multiple exponential models (Means *et al.*, 1985; Chen *et al.*, 2001). Chen *et al.*, (2001) compared a single component negative exponential model to a double-exponential model (Table 2.3). The double exponential model was fitted for the density loss data of the species containing resin cores. They hypothesized that the occurrence of resin cores would lead to a slower decomposition rate compared to species without resin cores. This led to the addition of a second component to the double exponential model to account for the fast and slow decomposing components separately. The model indicated a better fit than the single-exponential model for woody roots with resin cores (Table 2.3). Ultimately the double exponential model was an improvement over the single component negative exponential model for the species containing resin cores. Chen *et al.*, (2001) mentioned that although the decomposition rate constant calculated from the single-exponential model is a good index of decomposition, it can be misleading if the woody roots have highly decomposition resistant resin cores.

Table 2.3: The goodness of fit (Adjusted R^2) of single and double negative exponential models used to model density loss (Adapted from Chen *et al*., 2001).

Species	Root size (mm)	Adjusted R^2 (Single component negative exponential)	Adjusted R^2 (Double negative exponential)
Sitka spruce (*Picea abies*)	10-50	0.95	0.94
	50-120	0.84	0.98
Douglas-fir (*Pseudotsuga menziesii*)	10-50	0.82	0.80
	50-150	0.90	0.93
Lodgepole pine (*Pinus contorta*)	10-50	0.64	0.97
	50-110	0.62	0.97

In a review of the methods for measuring decomposition, nutrient turnover, and stores in plant litter, Harmon *et al*., (1999) explained that CWD components should only be separated if the diameter of the material exceeds 10cm. The root systems from the current study were too small to divide into their individual components, mainly due to the fact that the rotation ages were short (7 years) to produce wood that can be used for pulping. Therefore, the structural components of the roots were not evaluated in the current study.

The sigmoidal model has also been used in previous CWD decomposition studies, but is far less popular. The logic behind using a sigmoidal model is based upon the argument that CWD decomposition has three distinct phases, 1) an initial phase characterized by low

decomposition rates when the microbes are still colonizing the substrate (Harmon *et al*., 2000; Hyvönen and Ågren, 2001), 2) a phase of rapid decomposition when microbes metabolize easily decomposable compounds such as cellulose and hemicellulose (Harmon *et al*., 2000; Berg and McClaugherty, 2003), 3) a phase of lower decomposition rates because of increasing lignin concentration, which is harder for the microbes to break down (Harmon *et al*., 2000; Berg and McClaugherty, 2003). However, it will be highly speculative to try and identify distinct phases over the decomposition time when using the chronosequence approach. This is because chronosequences are applied by measuring different pieces of material that have aged for different durations. It would be much more appropriate to study phases of decay using a time series when the same material is studied from the start of decay until the study period ends.

Palviainen and Finér, (2015) studied the decomposition dynamics of Norway Spruce in Southern Finland (Table 2.4). Palviainen and Finér, (2015) tested both the negative exponential model and the sigmoidal model. They fitted the negative exponential model to mass, density and C loss data but the sigmoidal model was only fitted to mass and C loss. They found that the negative exponential model better described stump than coarse root decomposition in terms of density and mass loss (Table 2.4). Palviainen and Finér, (2015) found that a sigmoidal model always described mass and C loss better than the exponential model for coarse woody root decomposition (Table 2.4).

Table 2.4: Goodness of fit (Adjusted R^2) of models used to describe density, mass and C loss of Norway spruce root systems in Southern Finland. (Palviainen and Finér, 2015).

Species	Site	Model	Dependant variable	Root fraction (mm)	Adjusted R^2
Norway Spruce (*Picea abies*)	Southern Finland, Boreal Forests. Trees were clear felled.	Single negative exponential	Density	Stump	0.79
				50-100	0.48
				>100	0.54
		Single negative exponential	Mass	Stump	0.82
				50-100	0.49
				>100	0.56
		Sigmoidal	Mass	Stump	0.91
				50-100	0.91
				>100	0.82
		Single negative exponential	C	Stump	0.81
				50-100	0.48
				>100	0.54
		Sigmoidal	C	Stump	0.88
				50-100	0.95
				>100	0.77

Table 2.5: Different functions used to model density, mass and C loss in root decomposition studies. The variable Y is defined as the proportion of the initial density (Y_0), and the density at time t (Y_t). Thus, $Y = Y_0/Y_t$ is defined on the interval $0 \leq Y \leq 1$. Model parameters: k_n, k_l, k_{1d}, k_{2d}, k_{1p} and k_{2p} (decay constants); C_l and A are other constants.

Model name	Model	Reference
Type I exponential (negative)	$Y = e^{-k_n t}$	(Wieder and Lang, 1982; Chen *et al*., 2001)
Linear	$Y = C_l - k_l t$	(Lambert *et al*., 1980; Wieder and Lang, 1982)
Double exponential	$Y = Ae^{-k_{1d}t} + (1 - A)e^{-k_{2d}t}$	(Wieder and Lang, 1982; Chen *et al*., 2001)
Sigmoidal (Richards type)	$Y = 1 - (1 - e^{-k_{1p}t})^{k_{2p}}$	(Palviainen and Finér, 2015)

2.4 Factors Affecting Root Decomposition

There are three main factors that govern organic matter decomposition in terrestrial ecosystems; 1) initial substrate quality, 2) the decomposer community and 3) the effects of the environment (Laiho and Prescott, 2004; Thomas and Packham, 2007). Understanding the key predictors of root decomposition is important to help explain why decomposition rates might vary between different areas and species. It seems that the factors controlling decomposition differ for coarse and fine roots, and therefore the most important controls of both fractions are discussed here.

Garrett *et al*., (2012) studied the rate of decay of *Pinus radiata* (D. Don) coarse roots in plantations forests at eight locations covering a range of climate and soil types across New Zealand (Table 2.6). They measured root density of live root material and thereafter annually over a four-year time series following felling. They concluded that in addition to the age of the material (time since death), mean annual temperature (MAT) explained most of the variation in density.

The study by Garrett *et al*., (2012) was not the only one to identify climatic variables as the main drivers of decomposition. Zhang and Wang (2015), compiled a global dataset for root decomposition in order to understand the global patterns in fine and coarse root decomposition and the factors governing this process (Table 2.6). Their extensive dataset was gathered from several sources including; the published database of Silver and Miya, (2001), various other publications and the ISI Web of Knowledge. However, this study was conducted on a multitude of species, not purely trees or woody plants. Their results showed that substrate quality (Initial lignin content) was the most important predictor of fine root decomposition, while lignin to nitrogen (lignin: N) ratio, MAT, and mean annual precipitation (MAP) were the most important factors governing coarse root decomposition. MAT was especially important for predicting coarse root decomposition.

Olajuyigbe *et al*., (2011) studied the decay dynamics of logs, stumps and coarse roots using a five-decay class system (chronosequence approach) in managed Sitka spruce (*Picea sitchensis* (Bong) Carr.) forests in Ireland. Olajuyigbe *et al*., (2011) measured the volume, mass, density loss and C:N ratios of all the CWD types (logs, stumps, and coarse roots). They categorised coarse roots into small (2–10 mm), medium (10–50 mm) and large (>50 mm) diameter classes. Olajuyigbe *et al*., (2011) found a significant correlation between changes in density and decay class in all CWD types. Density decreased by 58%, 38%, 50% and 38% for stumps, small, medium and large roots respectively when moving from decay class zero to four (with increasing decay). Their regression curves showed that there was a strong correlation between C:N ratios and density (R^2 = 0.74 and 0.93 for stumps and roots respectively). C:N ratios declined with 41%, 51%, 72% and 57% for stumps, small, medium and large roots respectively, as decay progressed from decay class zero to four (Table 2.6). Olajuyigbe *et al*., (2011) also found that the size classification of roots did not significantly affect their decay rate.

Different factors have been identified as controls of fine root decomposition as opposed to coarse root decomposition. Bachega *et al*., (2016) studied the decomposition of *Eucalyptus grandis* and *Acacia mangium* fine roots (<2 mm) in monoculture plantations in tropical conditions (Table 2.6). Their study was carried out at the Itatinga experimental station of Sao Paulo University. The litterbag technique (a time series approach) was used and root residues of each species were collected every three months form each plot over a period of 12 months. They identified litter C quality and initial litter lignin content as being the primary controls of *Eucalyptus grandis* and *Acacia mangium* fine root decomposition.

Silver and Miya (2001) conducted a global study on the effects of climate and litter quality on root decomposition (Table 2.6). Silver and Miya (2001) studied a range of plants (conifers, angiosperms or graminoids) as well as a range of climates. Root Ca concentrations and C:N ratio were the main predictors of root decomposition with latitude, MAT, MAP and actual evapotranspiration explaining a smaller percentage of the variability in root decomposition. These results identified root chemistry as the main predictor of root decomposition and climate (environmental factors) as secondary predictor.

Table 2.6: Main factors (in addition to time) affecting root decomposition for several root decomposition studies.

Reference	Species	Site information	Root size (mm)	Main factors affecting root decomposition
(Garrett *et al*., 2012)	*Pinus radiata*	Range of climates across New Zealand	10-152 (coarse)	MAT
(Bachega *et al*., 2016)	*Acacia mangium, Eucalyptus grandis*	Tropical plantations, Sao Paulo, Brazil	<2 (Fine)	C quality of substrate, [Lignin]
(Silver and Miya, 2001)	Global Study (a range of Conifers, Broadleaf's and Graminoids species)	Global study	<2, 2-5 and >5	Main variables: [Ca], C:N; Secondary variables: latitude, MAT, MAP and actual evapotranspiration
(Zhang and Wang, 2015)	Global Study (a range of Conifers, Broadleaf's and Graminoids species)	Global Study	<2 (Fine)	Initial [Lignin]
			≥2 (Coarse)	MAT, MAP, Lignin:N
(Olajuyigbe *et al*., 2011)	*Picea sitchensis* (Bong) Carr.	Ireland	Stumps, 2–10 (small), 10–50 (medium), and >50 (large)	C:N

2.4.1 Additional factors that could influence root decomposition - Burning

There are several other additional factors that could potentially affect root decomposition, such as prescribed or natural burning of the stumps. Some organizations operating in the forestry industry (Mondi plc specifically uses this technique in their plantation forests) implement prescribed burning after harvesting, to clear the surface of unwanted debris left over from the harvesting process. Burning will mostly affect the stump, but since the stump is regarded as part of the root system in many root decomposition studies it is an important component to consider. Shorohova *et al*., (2008) studied the effect of prescribed burning of stumps after clear fell compared to stumps receiving no burning along a 40-year chronosequence. The experiment was conducted on Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and Birch (*Betula* sp.) in a Southern Boreal forest in Finland. They found that the prescribed burning of stumps after clear felling led to a significant decrease in the wood decomposition of Pine wood, as well as for pine and spruce bark. This shows that prescribed or natural burning of stumps after harvest could decrease the decomposition rate of roots systems in forest ecosystems. However, since root decomposition studies are so few in number it is easy to understand that the effect of burning on root decomposition has been studied to a lesser extent.

2.4.2 Rates of decomposition from coarse roots

Decomposition rates can vary to a large degree since the factors that controls decomposition vary drastically from one ecosystem to another. Appendix A lists the decomposition rate constants (k) from multiple studies to serve as a reference of the range of root decomposition rates that may exist across different sites, species and climates. These studies were all coarse root studies that used a single component negative exponential model to produce the decay constants, and used the chronosequence approach as their experimental design. This makes the studies contained in Appendix A, comparable to the current study in some aspects.

2.4.3 Conclusions

There seems to be much variation between studies with regards to the factors controlling root decomposition (Tables 2.6 and Appendix A), pointing to the fact that there might be no single factor controlling decomposition. Means *et al.*, (1985) explained that decomposition cannot be described by a single dominant factor but rather multiple factors. It was interesting that the coarse and fine root decomposition seemed to be governed by different types of variables (Table 2.6). Garrett *et al.*, (2012) and Zhang and Wang, (2015) both wanted to determine the drivers of coarse root (>2 mm) decomposition and found that climate (MAT for Garrett *et al.*, (2012) and both MAT and MAP for Zhang and Wang, (2015)) was the main governing variable (Table 2.6).

The other studies focussing on fine root (<2 mm) decomposition, found either initial substrate quality or the decomposer community to be the main drivers of decomposition (Table 2.6). But no specific substrate was identified as being the dominant controlling factor of fine root decay across all the studies examined (Table 2.6).

The diverse findings between coarse and fine roots from the studies discussed here, points to the fact that it might be important to separate coarse and fine roots when determining the controlling factors of root decomposition. Zhang and Wang, (2015) came to the same conclusion in their meta-analysis of fine and coarse root decomposition. Furthermore, the factors controlling decomposition are not limited to constant factors such as precipitation climate etc., but also to abrupt changes in environment such as burning (either natural or prescribed). Burning was singled out in the current study since it is a regular occurrence in plantations and can cause large changes in forest ecosystem functions. In theory, burning of previously felled trees should affect decomposition because it causes changes to the substrate quality of the litter. Few studies have been published on root decomposition as a whole so even less literature is available which identifies the effects of burning on root

decomposition and how this might change across different climates. Hence more research on different climate and soil combinations need to be conducted before the effect of burning on root decomposition can really be understood. Shorohova *et al.*, (2008) showed that the prescribed burning of stumps after clear felling led to a significant decrease in the wood decomposition of Pine wood, as well as for Pine and Spruce bark in a Southern Boreal forest in Finland.

2.5 Eucalyptus and Eucalyptus hybrids

Forestry in South Africa is generally practiced in sub-par climatic regions with generally lower annual precipitation, making careful and intensive management critical for sustaining highly productive plantation forests. Additionally, the country boasts a great diversity in site conditions. Highly adaptable and productive species such as those from the genus *Eucalyptus* are used across the diverse growing conditions to accommodate these shortcomings. The genus *Eucalyptus* is comprised of approximately 746 species, suitable for dry and wet, hot and cold conditions, and high and low latitudes (Bredenkamp, 2012).

In addition to using a wide variety of *Eucalyptus* species, clonal hybrids have also been introduced to address the diverse site conditions. Combining different genetic stock (Hybridisation), has the potential of producing trees with improved genetic characteristics that are capable of improving the yield and quality of the harvestable product (Phiri, 2013). Some of the most common *Eucalyptus* clonal hybrids used in the South African forestry industry include; *Eucalyptus grandis* with *E. camaldulensis*, *E. longirostrata*, *E. nitens* and *E. urophylla* (Bredenkamp, 2012).

CHAPTER 3
MATERIALS AND METHODS

3.1 Study sites

3.1.1 Location

Eleven study sites (distinct management units/compartments) selected for this study, were located on the Zululand coastal plain in close proximity to the small town of Kwambonambi, northern KwaZulu-Natal, South Africa. Kwambonambi is located at -28° 36' 00" S and 32° 05' 00" E and falls under the King Cetshwayo District Municipality. The Zululand coastal area was once a combination of indigenous lowland coastal forest and grassland which was later converted to a commercial forestry production area (Dovey *et al*., 2011). All study sites were within a 30 km radius of Kwambonambi and similar in terms of soil type, elevation and topography (Figure 3.1). Field work was undertaken during the relatively dry winter period in that region in 2017 in the managed pulpwood plantations of Mondi and Sappi. All study sites were managed for pulpwood production leading to a fairly short average rotation length of 7-8 years (Sappi and Mondi harvest records).

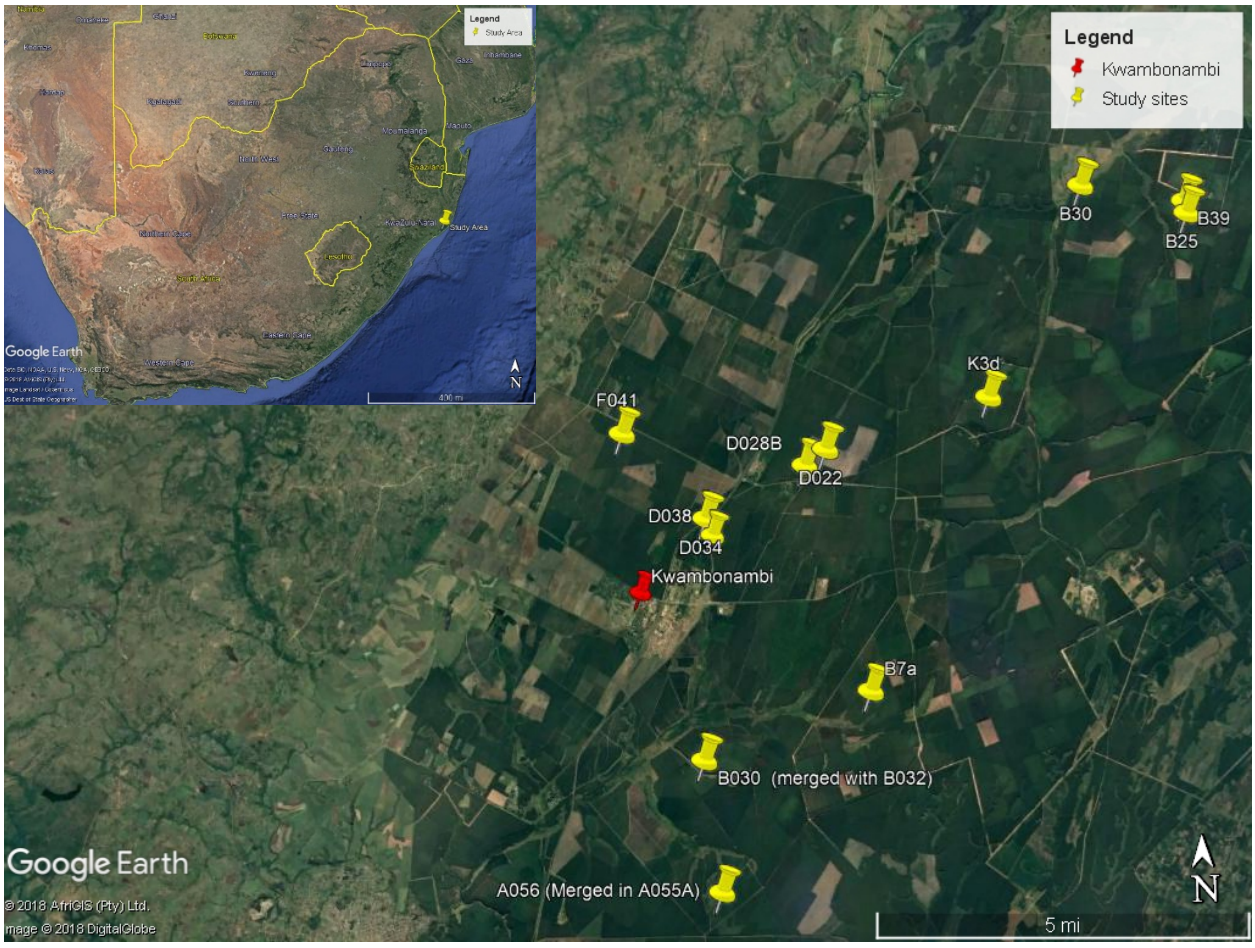

Figure 3.1: Map of study sites Kwambonambi, KwaZulu-Natal, South Africa.

3.1.2 Climate and soils

The Zululand area has a sub-tropical climate (Fey and Hughes, 2010) and is a classified as a summer rainfall region receiving a mean annual precipitation (MAP) of about 920 mm (Schulze, 1997). The area has a mean annual temperature (MAT) of 21.7 °C and an A-pan evaporation of 1814.5 mm per annum (Schulze, 1997). The soils of the study area are can be classified as sandy structureless albic arenosols (Fey and Hughes, 2010). The sandy soils (<5 % clay) are deep and free draining with a low organic C content (<1 %) (Dovey *et al.*, 2011).

3.1.3 Experimental Design

There are several potential approaches for studying woody root detritus decomposition. The most commonly used approaches include: 1) the chronosequence, 2) time series, 3) decomposition vector and 4) laboratory incubation approach (Chapter 2). The most common approaches are the time series and chronosequence approach (Harmon and Sexton, 1996; Harmon *et al.*, 1999). Since this was to be an *in situ* root decomposition study, the laboratory incubation approach was excluded. Therefore, the time-series approach, the chronosequence or a hybrid between the two approaches (decomposition vector method) were considered. The chronosequence approach was selected as the experimental design that best suited the objectives and constraints of this decomposition study; constraints included a limited study period of two years and a limited budget (Section 2.2).

Establishing the Chronosequence

The focus of this study was specifically on clear felled, planted *Eucalyptus* stands. Decomposing clonal *Eucalyptus grandis* x *E. urophylla* roots were sampled in a single chronosequence during the winter of 2017. The focus of this study was specifically on clear felled *Eucalyptus* stands; hence each age considered for the chronosequence would represent the amount of time that had passed since clear felling (*i.e.* stump ages). The chronosequence contained five different stump ages with an additional compartment (age) consisting of freshly felled trees (Table 3.1). The latter case served as a reference for the starting point of decomposition process. For each age in the chronosequence, two sites of contrasting site quality were sampled in order to get an accurate representation of decomposition of the entire area and its different quality sites.

Each compartment represented a single study site. The time that had passed since clear felling was recorded from the month when clear felling occurred (from forest management

records) until the month of sampling for the study reported in this thesis. Using accurate past harvesting records are common practice when establishing a chronosequence (*e.g.* Chen *et al.*, 2000; Ludovici *et al.*, 2002). The compartments were selected in a way that would allow the chronosequence to be a representation of the largest part of the root decomposition process as possible. This was done by conducting a survey to assess the state of decay of a range of potential sites at the study area before sampling began.

This study applies a relative rather than fixed sampling interval, as suggested by Harmon and Sexton, (1996). A fixed sampling interval is when the time between sampling intervals are kept constant (*e.g.* every 2 years), which is normally the way in which one would sample in a time series study (Harmon and Sexton, 1996). When using the chronosequence approach one has to find pieces of material that have already aged different durations, and therefore the options are usually limited (Harmon and Sexton, 1996). In this study the limited number of potential sites (material) that qualified for sampling (from past harvest records of Mondi and Sappi) inevitably led to the use of the relative sampling interval.

Using past harvesting records was the first of two potential solutions to establish a chronosequence. The second technique used decay classes for estimating the age of the decomposing material (Harmon and Sexton, 1996) (Section 2.2.2). It was hypothesized that the clonal hybrid monocultures of *Eucalyptus grandis* x *E. urophylla* would decompose fairly homogenously. Therefore, it would have been an unnecessary additional investment of time and effort to apply the decay class method since the accurate harvest records of Sappi and Mondi were readily available.

Table 3.1: Stand and site quality information for sites of different ages that had been selected for sampling in an age chronosequence

Years after clear fell	Fell date	Plantation	Compartment	Site productivity ranking	MAI at culmination (m^3 ha^{-1} $annum^{-1}$)	Site index at base age 5 (SI_5)
0	2017-07	Kwambo Timbers	B7b	High	22.	15.9
	2017-07	Kwambo Timbers	K3d	Low	17	13.6
3	2014-12	Flatcrown	D28B	High	24	16.6
	2014-12	Flatcrown	D022	Low	17	13.8
4	2013-08	Mavuya	B25	High	28	18.2
	2013-08	Mavuya	B39	High	25	16.9
6	2011-04	Canewood	B030	High	32	19.8
	2011-11	Gages	L020	Low	16	13.4
8	2009-04	Canewood	A056	High	38	22.2
	2009-08	Realisation	F041	Low	15	13.0
10	2007-04	Flatcrown	D034	High	29	18.6
	2007-04	Flatcrown	D038	High	31	19.4

3.1.4 Study area selection

Decomposing roots are sampled within the soil which can be a very physically intensive and destructive process (Brunner and Godbold, 2007). Therefore, soil type and structure were considered as important criteria for selecting the study area. In addition to soil, the study area also needed to be a dedicated pulpwood production area, producing one of the South Africa's most important pulpwood species. The most important selection criteria were the existence of accurate records of previous felling dates and the location of these compartments. This database also had to be large enough to allow for the sampling of the many different age classes that would eventually make up the chronosequence.

3.1.5 Site selection

Potential compartments were identified by applying a set of selection criteria on the harvest records. Firstly, all sites had to be planted with the same hybrid, to exclude the possibility of differences in wood density and nutrient content between hybrids or species. Clonal *Eucalyptus grandis* x *E. urophylla* was selected as the hybrid of choice due to its abundance in the Zululand area. The increased abundance of this particular hybrid compared to other species in the same area, meant representativeness, and that there would be a greater list of potential sites to choose from. It was not possible, however, to obtain only one clone/variety of *Eucalyptus grandis* x *E. urophylla*.

Since the focus of this study was on clear felled planted trees, coppiced stands had to be excluded from the data set. All potential compartments had to have been felled within the same period (within six months of each other) to qualify for selection. The last step was to identify two sites for each age in the chronosequence that were preferably of contrasting site quality (a high and low-quality site for each age category in the chronosequence). Climate and soil variation within the Zululand area creates variation in productivity between sites/compartments. Sites of contrasting quality were identified for each age of the chronosequence so that the resulting decomposition data would represent the decomposition of the entire study area (Table 3.1). Site quality was measured either by using the site index (SI) or mean annual increment (MAI) measured in m^3/ha/a. Site index is a tree growth-based measure of site quality (Dovey *et al.*, 2011). In forestry, site index (SI) is a measurement used to describe the productivity of a site. Site index reports the average height of the dominant and co-dominant trees in a stand at a specific base age (in this study age 5 years was used). The MAI is a measure of the stem volume growth of the stand per unit area divided by the age of the stand.

The site quality for all sites were measured in MAI except for site B25 and B39, which only had site index (SI) data. A conversion factor from the Institute for Commercial Forestry Research (ICFR) (2005), was used to estimate the MAI for site B25 and B39 in order to have a comparable dataset. The MAI measurements from the other sites were then used to produce a site index measurement at base age five (SI_5). MAI was deemed an acceptable measure of site quality for data analysis due to the uniformity across all sites in terms of stocking and the age at which the measurement was taken.

3.1.6 Description of experimental layout at each site

When conducting a decomposition study using the chronosequence approach one usually measures how parameters such as density or nutrient content change over time (Harmon and Sexton, 1996). There is no specific variable that should be used as the dependent variable when estimating woody root decomposition dynamics: it may vary depending on the study objective (Harmon and Sexton, 1996). Sections of root were sampled from three different trees at each of the sites in the chronosequence (Table 3.2). Multiple samples were collected from each root system and was divided into four distinct size fractions; 2-10 mm, 10-50 mm and >50 mm diameter roots and a stump sample. Density, C and nutrient content was measured for each individual root and stump sample in the laboratory.

3.2 Sampling methods

Root samples were collected for six different age classes (two sites for each age), from three trees at each compartment and were divided into four root size classes (Table 3.2).

Table 3.2: Root sample sizes sampled from each of the three trees, at each of the 12 sites.

Time after felling	Tree size	Root size (mm)
6 different ages ranging from 0 – 10 years (12 sites in total)	Small	2-10
		10-50
		>50
		Stump
	Medium	2-10
		10-50
		>50
		Stump
	Large	2-10
		10-50
		>50
		Stump

3.2.1 Stump sampling:

The stump (the fraction of material remaining after the tree has been felled) and the root crown (biomass fraction directly below the stump) was included as part of the below ground root biomass in this study (Gifford, 2000; Palviainen and Finér, 2015). It was more practical

to use the felling point as the divide between below ground biomass (root system) and above ground biomass as compared to the soil surface as the divide. Since the stump and root crown constitute a significant amount of the biomass remaining after felling it is more appropriate and practical to use the former option (Drexhage and Gruber, 1999).

An important aspect of the sampling process was correctly identifying stumps from the target crop rotation. Often, stumps were still present at the same site from multiple prior crop rotations. From the harvest records the correct rotation of stumps could easily be identified by comparing the harvest date with the condition of the stumps from that specific rotation. By using this approach, it was easy and quick to identify the correct rotation, seeing that stumps of two rotations old (if still recognisable and existing) were visibly more weathered than those only one rotation old.

Three stump samples were collected from each site and these were selected based on stump diameter which was measured at 10 cm above the soil surface. Because of the variation in condition of the material (degree of decay), two distinct methods for sampling stumps became necessary:

1. Stumps that were still in a fairly good condition that were measurable and
2. Stumps that have decomposed to a point where they were not measurable.

During sampling it became apparent that the stumps at compartment A56 was mulched using a mulching machine. Unfortunately, it was not possible to replace the compartment due to the limited number of potential sites that were available for sampling (Section 3.1.5). An assumption was made that although the stumps might be highly damaged the below ground roots would still be intact. Therefore, roots were sampled from compartment A56 but the stump densities were derived by means of regression of all other stump density measurements.

Method 1 (for stumps in generally good condition):

The soil surface around each stump was cleared to remove any debris before sampling began. The diameters of 30 stumps were measured using diameter tape within the selected compartment. Stumps with the smallest, average and largest stump diameter measurements, from the measured stumps, were selected to be sampled. These stumps were selected to represent a small, medium and large stump within their respective compartments. Stumps were grouped into small, medium and large to see if there might be a difference in the rate of decomposition (density loss) between different sized stumps/trees.

A 2-4 cm thick disc was then cut from each of the stumps using a 66 cm hand saw with fine teeth. The diameter of each disc was measured at three locations (bottom, middle and top) using diameter tape. The length (thickness) of the disc was measured at three different locations around the disc using a measuring tape. Samples were then immediately placed in labelled plastic bags and sealed.

Method 2 (for a site with highly decomposed stumps):

A grid of 50 stumps were visually assessed to give a subjective estimate of the amount of material remaining. The outcome affected the way in which the material was collected; >50% percent material remaining in more than half of the stumps within the grid or <50% material remaining in more than half of the stumps within the grid (*e.g.* Table 3.3). Nine out of the twelve stumps that were measured in Table 3.3 had more than 50% of their biomass still remaining at time of measurement.

Table 3.3: Illustration of the grid used for the subjective estimate of the amount of material remaining on sites with highly decomposed stumps.

Row 1	Row 2	Row 3
1 (70% material remaining)	5 (80% material remaining)	9 (75% material remaining)
2 (60% material remaining)	6 (90% material remaining)	10 (85% material remaining)
3 (35% material remaining)	7 (70% material remaining)	11 (45% material remaining)
4 (10% material remaining)	8 (80% material remaining)	12 (65% material remaining)

- ***More than 50% material remaining:***

When more than half of the stumps from the plot had more than 50 % material remaining, it was possible to find a small percentage of the stumps that still contained enough material to allow for an accurate diameter and stump height measurement (Figure 3.2). Diameter measurements were made using diameter tape and the smallest and largest stumps as well as the stump with a diameter nearest to the mean stump diameter were selected for sampling. A marker was used to draw a line at about five-centimeters below the top (cutting face) of the stump to indicate where the cut would be made. Disc length (thickness) and diameter measurements were then taken before cutting to serve as a backup in case the disc might break into pieces. The diameter of each disc was measured at three locations (bottom, middle and top) using diameter tape (In this case the bottom measurement being at the market cutting point. The length (thickness) of the disc was measured at three different locations from the top of the stump to the market cutting point. A 2-4 cm disc was then cut from each of the stumps using a 66 cm hand saw with fine teeth. All the length (thickness)

and diameter measurements were repeated once the stump had been successfully cut. Samples were then immediately placed in plastic bags and sealed.

Figure 3.2: A highly decomposed stump (Compartment D034, 10 years old) that had > 50 % of its biomass remaining and could still be measured.

- ***Less than 50% material remaining:***

When more than half of the stumps had less than 50 % material remaining, there were not sufficient stumps in the compartment that allowed for the necessary stump height and diameter measurements to be made (Figure 3.3). Attempting to sample an object that has less than half of its material remaining is surely questionable if some of the study assumptions are not made clear. This study set out to measure decomposition as density loss over time, not mass loss. A perfect fresh sample therefore has a set volume (if shrinkage is ignored due to soil moisture remaining fairly constant) and the decomposition is therefore defined as the mass inside that set volume decreasing over time. Therefore, when a highly decomposed sample is measured such as the one in Figure 3.4, the original sample volume is determined and not the current volume after decomposition. The remaining mass is then measured and divided by the original sample volume.

The original sample volume was determined by utilizing linear regression analysis. Since only the base diameter of these stumps were available, the middle (halfway between cutting point and base of the stump) and top (cutting point) diameters had to be estimated using a regression model. The regression model was constructed to predict stump middle and top diameters by using the base diameter and the average height of stumps from 30 freshly felled trees. Finally, stump volume could be determined with the average stump height of

the thirty stumps together with the measured base diameter and the estimated stump diameters (stump middle and top diameter) by using Newtons formula (Section 2.3.1). The remaining stump biomass of the decayed stump was then carefully sampled by making a cut at the base of the stump using a hand saw with fine teeth. Samples were then immediately placed in plastic bags and sealed. The final density of these highly decayed stumps was later determined by dividing all remaining mass (dried) with the estimated stump total volume.

The rationale was that these measurements could still serve an important function even though more than 50 % of the material had already been lost. The reasoning behind this decision is the length of the chronosequence would otherwise have been too short. Harmon and Sexton 1996 recommended that a good chronosequence be established using at least 5 decay classes.

Figure 3.3: A typical highly decomposed stump (Compartment A056, 8 years old) where the necessary stump height and diameter measurements could not be made.

Figure 3.4: Measuring the base diameter of a highly decomposed stump (Compartment A056, 8 years old).

3.2.2 Root sampling

Root sampling followed after stump sampling and these samples were cut from the same small, medium and large stumps that were selected at each compartment. A mechanical digger was used to create a single trench, 1.5 m deep and 4-5 m in length, about 20 cm from the selected stump (Figure 3.5). The root system was then exposed by hand using a geological hammer, hand shovel, paint brush and tooth brush (for brittle or fragile roots) from within the trench (Figure 3.6). This process was the slowest part of the sampling procedure, since the fragile roots had to be carefully exposed in order to retrieve a suitable sample.

Figure 3.5: Mechanical digger opening up a trench to expose the root system.

Figure 3.6: Roots being carefully exposed by hand using several tools (geological hammer, paint brushes, tooth brushes and hand shovels) following the excavation of the trench.

Roots were separated into the different size classes; 2-10 mm, 10-50 mm and >50 mm by using callipers. Samples were taken from both the lateral- as well as the vertical roots to account for possible differences between roots in different positions within the soil (Harmon and Sexton, 1996). Cross sectional cuts were made using a silky saw, pruning scissors or large pruning scissors, depending on the size of the root (Figure 3.7). Samples were carefully cleaned using small soft bristle paint brushes to remove as much of the excess soil as possible. Samples were then immediately placed in plastic bags that were marked preceding sampling with the compartment number, tree size (large, medium or small) and root size (2-10 mm, 10-50 mm and >50 mm). Holes in the plastic bags ensured that the roots stayed air dry to prevent the growth of fungus and further decomposition.

Figure 3.7: A root system of a single tree after root samples had been removed.

3.3 Laboratory procedures

3.3.1 Measurement parameters

The goal with the laboratory analysis was to use the root samples collected in field and determine the density, nutrient concentration and C concentration of each root sample collected in field. Understanding how these parameters change over time will enable the quantification of the rate of decomposition and hence longevity of these roots after they die.

3.3.2 Sample preparation

Large and irregularly shaped samples were cut into smaller and more regularly shaped pieces respectively, using a band saw. Samples were cleaned to remove any sand on the surface of the sample using hand brushes with soft bristles. Some samples had large amounts of internal sand intertwined with the decomposing biomass (Figure 3.8). As most of the sand could not be removed without damaging the sample and the sand within cavities would not affect volume determination but would affect mass measurements, the internal sand was only removed following volume determination. Live roots that penetrated the sample were carefully removed if possible (Figure 3.9).

Figure 3.8: An example of a decomposing root sample with internal sand contamination.

Figure 3.9: An example of live roots growing through a dead root sample.

3.3.3 Measuring sample volume

Sample volume measurements were used to determine sample density which would then be used to determine the rate of root decomposition. The initial goal was to measure the root sample volume of the younger material using the displacement method and the micro-computed tomography method for highly decomposed samples from the older ages in the

chronosequence. A third method, the direct measurement method, was incorporated for measuring the volume of stump disc samples. This direct measurement method was necessary to estimate the volume of highly decayed stumps. In order to save time and reduce budget costs, a decision was made to utilize this method for all stump discs. The cylindrical shape of the stump discs lent itself to easy volume estimation using Newtons formula for a truncated cone General information on all three methods are discussed in Section 2.3.1.

Both the micro-C and displacement method (methods described in detail in Sections 3.3.1.1 and 3.3.1.2 below) were used to determine root sample volume. The displacement method was used on the root samples from 'younger' ages in the chronosequence (roots that are not very decomposed) (Table 3.4). The micro-CT method was used on the older roots in the chronosequence (Table 3.4). The micro-CT method for determining volume was compared to the displacement method for determining volume. The methods were compared by using both methods to measure the sample volume of the samples from the third age in the chronosequence (4 years after felling) (Table 3.4).

Table 3.4: Method used to determine volume of root samples for the different ages of the chronosequence

Method for measuring volume	Time after felling (years)					
	0	3	4	6	8	10
Displacement Technique (2-10 mm, 10-50 mm and >50 mm roots)	x	x	x			
Micro-CT (2-10 mm, 10-50 mm and >50 mm roots)			x	x	x	X
Direct measurement method (All stump discs)	x	x	x	x	x	x

Displacement method

The displacement technique was used to calculate the volume of the samples from the first age in the chronosequence (zero years after felling) and the second and third ages in the chronosequence (three and four years after felling). A 500ml glass beaker was filled with water and placed on a Mettler PC 4400 scale. A laboratory retort stand was used in combination with a piece of rubber and a needle to keep the sample submerged in the water and to prevent any movement of the sample (Figure 3.10). The mass attained from this experiment was used to calculate the volume of each section of root using the Archimedes principle, *i.e.* by dividing the subsequent mass with the density of the fluid (water in this experiment).

Figure 3.10: Application of the displacement method for determining sample volume

Micro-computed tomography (micro-CT)

General information on the micro-computed tomography technique was discussed in section 2.3.1.2. Determining volume using the micro-computed tomography (micro-CT) included the following steps (1) sample preparation and mounting, (2) scanner set-up and parameter selection, (3) the scanning procedure, (4) image reconstruction and (5) image visualization (Du Plessis *et al.*, 2017). Samples were scanned using a General Electric Phoenix VTOMEX L240 with NF180 additional x-ray source.

Samples were stacked on phenolic foam blocks, with each block containing around 3-6 root samples from the different root size classes (Figure 3.11). The foam blocks were marked with tooth picks for orientation purposes (Figure 3.11). The layout off each stacked block was recorded before scanning in order to distinguish between roots later on in the process. The following settings were used for scanning all the root samples; voltage: 150 kV, current: 150 µA, filter: 0.1 mm Cu and resolution 300 µm. The scanned images were analysed using Volume Graphics VGStudio Max 3.1.

Figure 3.11: Stacking of root samples on phenolic foam blocks before being scanned in the CT-scanner.

Direct measurement

Stump diameter and height measurements were used to determine stump disc volumes by using Newton's formula for determining the volume of a cylinder (Section 2.3.1.3). Harmon and Sexton, (1996) explain that if samples are regularly shaped, then volume estimates based on external measurements can be just as reliable as volume displacement. Most of the stump discs were regularly shaped meaning it was perfectly appropriate to measure the volume of the discs using the direct measurement technique.

3.3.4 Sample drying and mass measurements

Samples were cleaned as best possible to remove most of the external and internal sand before measuring sample mass. After the cleaning process, roots were oven dried to a constant mass at 65°C. However, several studies recommend that woody biomass be dried at 105°C (Phiri, 2013; Seifert and Seifert, 2014). Phiri, (2013) and Seifert and Seifert, (2014), showed that drying biomass at lower temperatures than the standard resulted in overestimated biomass values. This was mainly because of the remaining water or moisture that is still bound to the biomass components (Seifert and Seifert, 2013). Some studies have used lower temperatures between 60 and 105 ºC if a focus was placed on analysing the chemical properties of the material (Montagu *et al.*, 2005; Saint-André *et al.*, 2005). Since the goal of this study was also to determine sample C, nitrogen and other nutrients it was decided that root samples be dried to 65 °C. Root sample mass was then corrected

using mass loss percentages determined by Phiri (2013) for *Eucalyptus grandis x E. camaldulensis* log discs. Phiri (2013) measured sample mass loss between 60 and 105 °C and found that the discs decreased to 93% of their initial mass. They also showed that most of the mass loss for this species was occurring between 65 and 80 °C (Phiri, 2013).

3.3.5 Measuring sample C and nitrogen (N)

Root total C and N content was determined for all samples using a LECO-company CHN elemental analyser. This LECO CHN elemental analyser uses a very pure source of oxygen to fuel a dry-combustion based reaction. McGeehan and Naylor (1988) compared the use of these automated instrumental analysers with the conventional Walkley-Black and Kjeldahl methods for determining organic C and N respectively. Four organic compounds and twenty-nine plant materials were tested. Their results showed that CHN analysers can be used to rapidly and accurately determine C and N simultaneously in plant samples (McGeehan and Naylor, 1988).

3.3.6 Measuring sample lignin content

Lignin has been identified as an important controlling factor of decomposition. Determining lignin content was not a main objective for this study since budget and material restraints did not allow for the measuring of lignin content for all the samples. Lignin concentration was determined from fresh bulked root samples of the different root sizes (2-10 mm, 10-50 mm and >50 mm) in order to serve as a general measurement for the root material.

Samples were milled using a ZM 100 Retsch mill, from 8 mm to 4 mm and finally 2 mm. Samples were then sieved using a Vibratory Sieve Shaker AS basic (600/425 micron). Thereafter followed the conditioning phase at 20 °C and 65 % relative humidity for 48 hours. After conditioning followed: 1) determining sample moisture content, 2) determining sample ash content, 3) determining extractives content (water and ethanol). Thereafter root lignin concentration could finally be determined.

3.3.7 Sample exclusion

There were many samples that had to be excluded from the potential samples that would be used for measuring density, C and nutrient concentrations. This was due to structural damage which was present in most samples and varied with respect to the degree of the damage. In cases where it was not possible to accurately determine sample volume, samples had to be excluded. Accurate measurements of sample volume are critical for accurate estimates of sample density (Harmon and Sexton, 1996) which would be used to determine the rate of root decomposition. Most of the damage can be attributed to difficulty

of sampling decomposing material below-ground. Roots also became increasingly more fragile as the time had passed after felling, which further increased the difficulty of sampling without damage.

Samples were excluded when the amount of structural damage would prevent an accurate volume measurement (Table 3.5). This amount of structural damage differed between the volume measurement techniques:

1. CT-method: If sample total volume could not be accurately reconstructed using the CT software, the sample would be excluded.
2. Displacement method: If the sample was not a representative of the total volume of that same sample (*e.g.* a chunk of material missing), then that sample density would not be included in the data set.
3. Direct measurement technique: The sample volume of all the samples, was accurately measured using the direct measurement technique, hence no samples were excluded. This volume measurement technique was only used to measure stump disc volume.

Table 3.5: Number of samples excluded due to structural damage.

Root size (mm)	Number of samples collected during sampling:	Number of samples that were excluded due to excessive damage:
2-10	36	10
10-50	36	12
>50	36	19
Stump	32	0
Total	140	41

3.4 Statistical analysis

Software from the R Foundation for Statistical Computing was used to analyse and interpret the data (R Core Team, 2017). During the first analysis the displacement method for determining sample was compared to the CT-scanner method. A paired samples *t*-test was performed to test whether the mean volume measurements of the two method were significantly similar for the same samples. The paired samples t-test was used since the two methods were tested on the same samples meaning the tests weren't independent of each other.

The goal of the second analysis was to model coarse root decay and produce decay constants. The relationship between several independent variables and density loss (decomposition) was assessed. The independent variables included tree size (TS), root size (RS), site quality (SQ) and changes in root carbon to nitrogen ratios (C.N) and the dependent variable was density (D) (Table 3.6). A scatterplot matrix ({car} package) was used to visually illustrate the individual relationships between the independent variables and changes in density. Multiple linear regression ({stats} package) was used to determine which of the independent variables had a statistically significant effect on changes in root density (R Core Team, 2017). Stepwise ({RcmdrMisc} package) and AllSubsets regression were used to determine which variables can be used to model density loss (R Core Team, 2017). Thereafter followed the modelling of root decomposition where a negative exponential, linear and semi log transformed linear models were fitted to the data (Section 2.3.3). Spiess and Neumeyer, (2010) showed that conventional R-squared values cannot be used for non-linear models. Therefore, the best model was identified using the residual standard error (RSE) (Spiess and Neumeyer, 2010). The best model was then used to produce decay constants for coarse root decay.

Root carbon concentration was measured and the first analysis was to see if root carbon would vary with RS, TS or SQ (Table 3.6). The same analysis used to investigate the relationships between the independent variables and density loss was used for carbon. A boxplot was used to identify potential outliers in the data. Lignin content of the roots were also measured for fresh root material from both high and low-productivity sites. A two independent samples *t*-test was performed to see whether the lignin concentrations of the roots were significantly different.

Table 3.6: Information of the measured variables

Variable	Description	Measurement	Data type in the R software
D	Density (response variable)	g cm^{-3}	Numerical
C	C concentration	%	Numerical
Time	Time that had passed since clear fell	Years	Numerical
SQ	Site quality	MAI (m^3 ha^{-1} $year^{-1}$)	Numerical
TS	Tree size	Stump diameter (class)	Categorical with three levels (divided into Small, Medium and Large)
RS	Root size	Root diameter (mm)	Categorical with four levels (divided into 2-10 mm, 10-50 mm, >50 mm and a stump cut)
C.N	Carbon to Nitrogen ratio	Fraction	Numerical

CHAPTER 4
RESULTS

4.1 Comparing methods for measuring coarse root volume

The displacement method for determining sample volume was compared to that of the CT-scanner method. To test the hypothesis that the means of the displacement and CT-scanner methods for determining sample volume were statistically significantly similar, a paired samples *t*-test was performed (Table 4.1). The paired t-test showed that there was not a statistically significant difference in the mean volume measurements of the two different volume measurement methods t(16) = -0.78211, and p-value = 0.4456 (Table 4.1). However, the paired t-test operates under the assumption that the differences between the volume measurements need to be sufficiently normally distributed. The Shapiro-Wilk test showed that the differences were not sufficiently normally distributed with a p-value of 0.0138. The non-parametric equivalent of the paired t-test was used to determine whether the means of the displacement and CT-scanner methods for determining sample volume were statistically significantly similar. The Wilcoxon Rank Sum Test showed that there was not a statistically significant difference in the mean volume measurements of the two different volume measurement methods, p-value = 0.7819.

The linear relationship between the displacement and CT-scanner method for determining sample volume is illustrated in Figure 4.1. Comparing the CT-scanner and displacement methods for determining sample volume produced an R-squared of 0.977 (Table 4.2). The linear model that was fitted to the dataset was highly significant yielding a p-value of <0.0001 (Table 4.2).

Table 4.1: Paired sample t-test for the equality of means of the Displacement and CT scanner volume measurements.

	t-test for equality of means				
	t	**df**	**p-value**	**95 % Confidence interval of the difference**	
				Lower	**Upper**
Volume measurements	-0.78211	16	0.4456	-4.2758	1.9711

Table 4.2: Linear regression goodness of fit parameters

Line	**Formula**	**p-value**	**R^2**
Linear (method comparison series)	$y = 0.9342x + 2.33$	<0.0001	0.9770

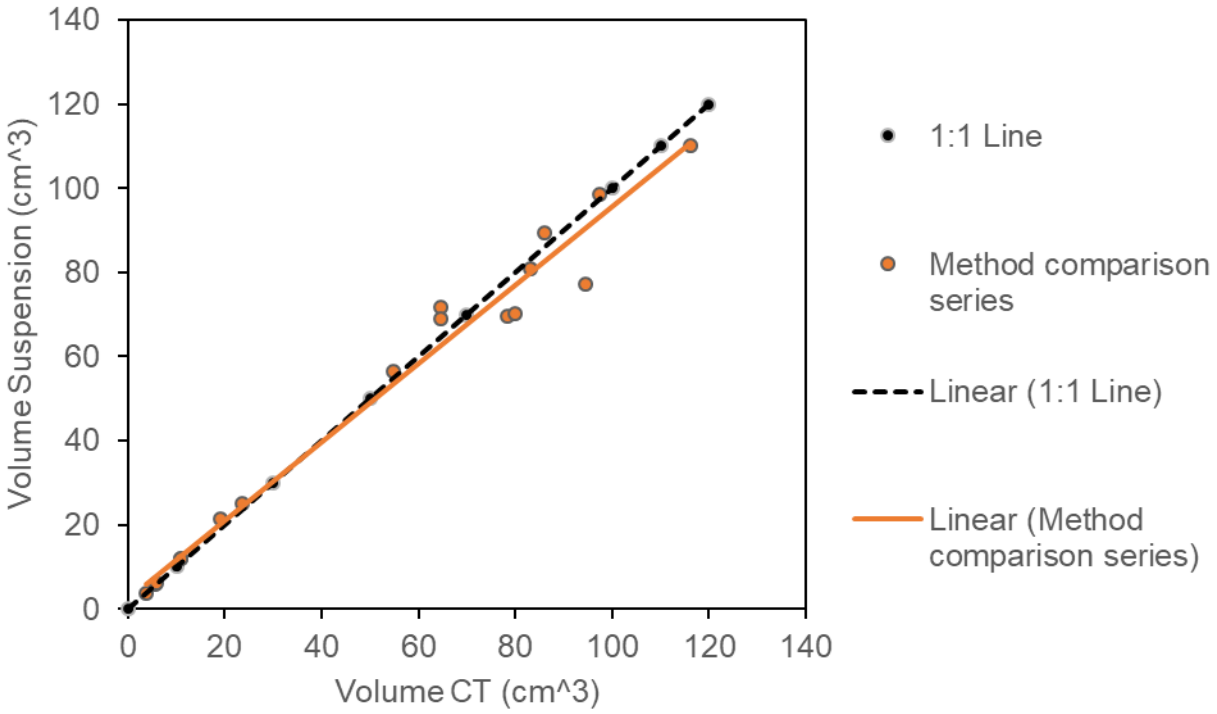

Figure 4.1: Comparison of the CT-scanner and Displacement methods for determining sample volume.

4.2 Modelling changes in density

4.2.1 Distribution of density data

Wood density decreased consistently from a mean density at time zero of 0.4082 g cm^{-3} to 0.1314 g cm^{-3} after 10 years at the last sampling interval of the chronosequence (Table 4.3). Preliminary linear regression analysis showed that there was a significant negative relationship between Density (D) and Time (p-value: <0.0001 and R^2 0.755). The degree of variation of the different time intervals was fairly similar, except at zero years after harvesting where the degree of variation was very low, CV = 6.57 % (Table 4.3).

Table 4.3: Mean coarse root density and coefficients of variation for each age within the chronosequence.

Time (year)	Mean coarse root density (g cm^{-3})	Coefficient of variation (CV) (%)
0	0.4082	6.57
2.5	0.3040	22.17
3.83	0.2516	18.12
5.58	0.2442	20.55
6.17	0.2174	28.84
8.17	0.1851	26.90
10.17	0.1314	28.20

The four points falling beyond the whiskers of the boxplot at time 8.17 and 10.17 years can be regarded as outliers (Figure 4.2). The boxplot defines an outlier as being an observation falling outside the interquartile range (below the 25th or above the 75th percentiles). The outliers outlined by the boxplots at sampling intervals 8.17 and 10.17 years after felling (Figure 4.2) were not removed from the data set since further investigation identified that the samples were in good condition and therefore a measurement error due to a damaged sample was unlikely.

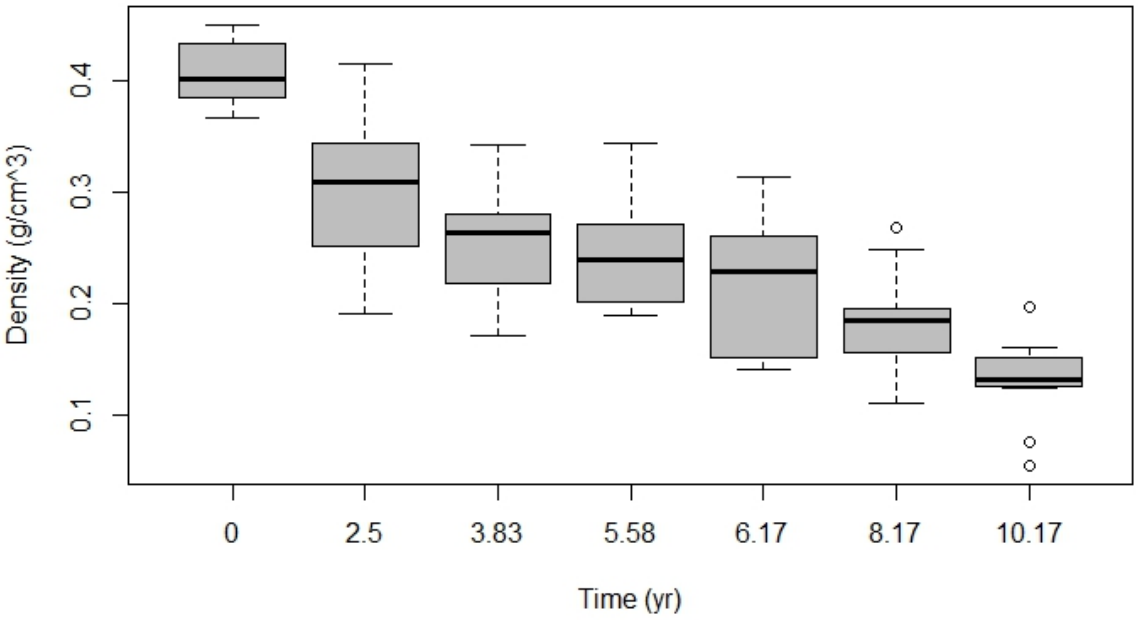

Figure 4.2: Graph showing boxplots of density measurements at each time interval (sampling interval). The boxplots depict possible outliers as bullet points.

4.2.2 Determining significant independent variables

A scatterplot matrix was used to visually illustrate the individual relationship between all the variables in the model by using linear regression (Figure 4.3). A relationship between two variables is shown by the presence of a gradient. If no gradient exists and the linear line is flat then there is no relationship between the applicable variables. The scatterplot is only used as a visual tool to assess potential relationships between variables, but in order to determine whether these relationships have statistical significance we relied on the regression analyses that follow.

Root C to nitrogen ratio (C:N) seemed to have linear relationships with time after felling (time) and the dependent variable density (D) (Figure 4.3). The C:N ratio of the roots also

seemed to have a relationship with root size (RS) Tree size (TS) did not seem to have relationships with any of the other independent variables or the dependent variable density (D). Site quality or productivity (SQ) had a relationship with both time and the dependent variable D. The independent variable time had a linear relationship with the dependent variable, density (Figure 4.3).

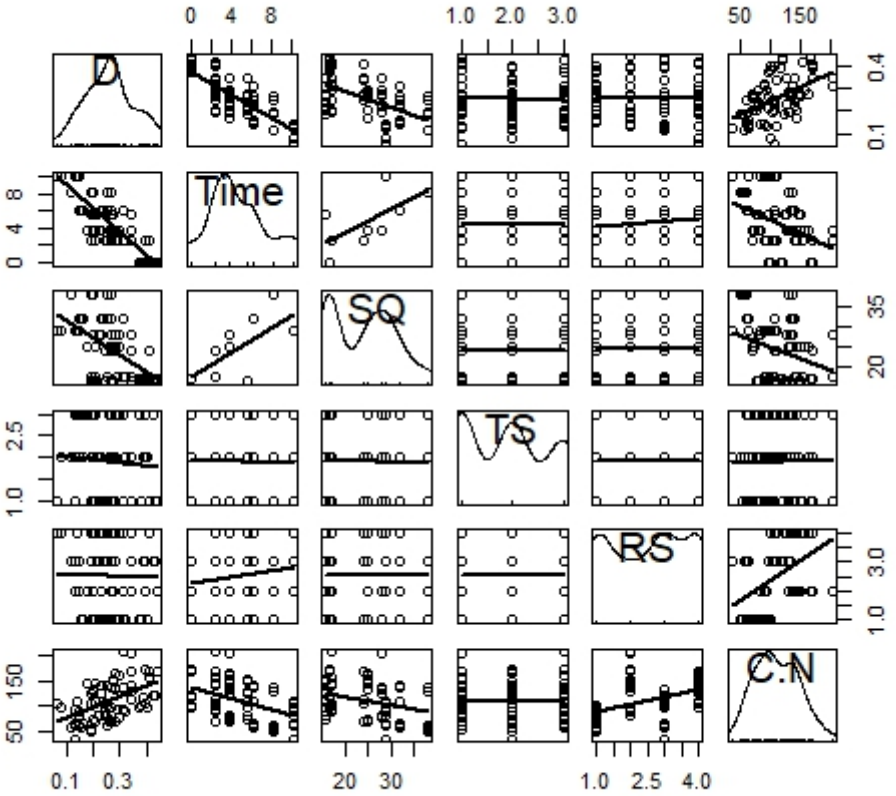

Figure 4.3: Scatter plot matrix showing the individual linear relationships between all variables: density (D), time after felling (Time), site quality (SQ), tree size (TS), root size (RS) and C to nitrogen ratio (C:N).

Multiple linear regression

Multiple linear regression was used to determine which of the independent variables had a statistically significant effect on root density. Both time after felling and C:N ratio had significant relationships with the dependent variable, density (p-values of <0.0001 and 0.0199 respectively) (Table 4.4). Density decreased as the time after harvesting increased, therefore the two variables are negatively correlated. The C:N ratio was positively correlated with density loss, as density decreased so too would the C:N ratio. The multiple linear regression model was globally significant with a p-value = <0.0001 and R-squared = 0.7557.

Table 4.4: Results from the ANOVA of the multiple linear regression model (significant if p-value is smaller than <0.05).

Variable	p-value
Time	<0.0001
SQ	0.9190
TS	0.1843
RS	0.0718
C:N	0.0303

4.2.3 Determining the which variables should be used to model changes in density (decomposition)

Both the *Stepwise* and *Allsubsets* tools were used to see which variables should be used to model changes in density (decomposition).

Stepwise regression

Stepwise regression identifies the best model by adding variables consecutively to a base model. The function can be run in two sequences, forward/backward or backward/forward. The former will start by adding a single variable and then another etc. whereas the latter will start with all of the possible variables included in the model and then removing variables consecutively.

Stepwise regression was run in both the forward/backward and backward/forward sequences (Table 4.5 and Table 4.6). The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) of all the possible models were generated and compared. Density modelled as a function of time (D ~ Time) was the best fitting model for both sequences and for both parameters; AIC = -575.00 and BIC = -570.42 (Table 4.5 and Table 4.6). In these results, changes in density were best modelled using only the time (time after felling) variable.

Table 4.5: Stepwise regression (forward/backward) results that show which model was the best model using the AIC criterion.

Model	AIC
Forward/backward	
D ~ 1 (Starting point)	-440.56
D ~ Time	-575.00
Backward/forward	
D ~ Time + SQ + TS + RS + C:N (Starting point)	-430.76
D ~ Time + SQ + TS + C:N	-434.78
D ~ Time + TS + C:N	-436.76

Table 4.6: Stepwise regression (forward/backward) results that shows which model was the best model using the BIC criterion.

Model	BIC
Forward/backward	
D ~ 1 (Starting point)	-438.27
D ~ Time	-570.42
Backward/forward	
D ~ Time + SQ + TS + RS + C:N (Starting point)	-410.15
D ~ Time + SQ + TS + C:N	-421.03
D ~ Time + SQ + C:N	-425.30
D ~ Time + C:N	-429.40

All possible subsets regression

The *Allsubsets* function tests all the possible combinations of the variables that the function is supplied with. The *Allsubsets* regression analysis confirmed the results from the stepwise regression analysis. Adding additional variables other than Time does not lead to a substantial increase in goodness of fit parameters (Table 4.7).

Table 4.7: The five best models for describing changes in density according to the all possible subsets regression analysis.

Model	Adjusted R^2	AIC
D ~ Time + TS	0.7602	-298.88
D ~ Time + TS + RS	0.7647	-297.88
D ~ Time	0.7525	-297.73

4.2.4 Modelling density change

Several functions were considered for modelling the relationship between density loss and time after felling. The negative exponential model (Model 4.1) gave a slightly better fit or just as good a fit as the semi-log transformed model (Model 4.3) (Table 4.8). The residual standard errors (RSE) and mean standard errors (MSE) for Model 4.1 was 0.04817 and 0.00232 respectively (Table 4.8). The semi-transformed linear model had an RSE and MSE of 0.04824 and 0.00233 respectively (Table 4.8).

Table 4.8: Goodness of fit of models fitted to the data. The variable Y is defined as the proportion of the initial density (Y0), and the density at time t (Yt). Thus, Y = Y0/Yt is defined on the interval 0 ≤ Y ≤ 1. Model parameters: k's (decay constants) and C's.

Model	Model Type	Formula	Rate loss constant k ($year^{-1}$)	C or starting value	RSE	MSE
4.1	Type I Negative exponential	$Y = e^{-k_n t}$	k_n= 0.1058 ± 0.0064	0.40334 ± 0.0089	0.04817	0.00232
4.2	Linear	$Y = c_l - k_l t$	k_l = 0.0405 ± 0.0039	0.3858 ± 0.0083	0.05109	0.00261
4.3	Linear (with natural log of Time)	$Y = c_{l2} - k_{l2}(lnt)$	k_{l2} = 0.0722 ± 0.0128	0.4084 ± 0.0087	0.04824	0.00233

The negative exponential model was used to model decomposition and produce the decay constant (Figure 4.4). The model formula and decay rate constant are shown in Table 4.9. The value of 0.4037 represents the starting value or initial density before decay starts (at age zero), and k represents the decay rate constant of -0.1058 (Table 4.9).

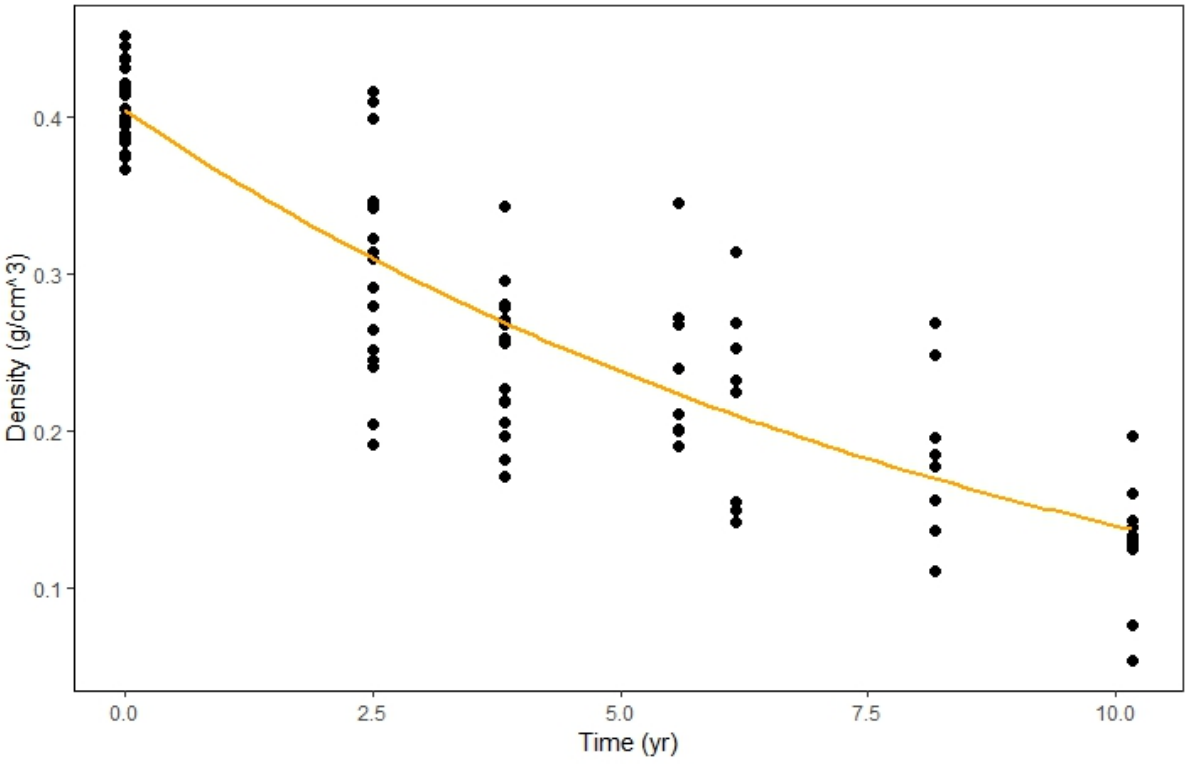

Figure 4.4: Graph showing the changes in coarse root density over time with the *Type I* negative exponential model fitted to the data.

Table 4.9: Negative exponential model formula and model parameters.

Line	Formula	Starting value	Rate loss constant k (year-1)
Type I negative exponential	$Y = 0.4037e^{-0.1058t}$	0.4037± 0.0089	0.1058 ± 0.0064

4.2.5 Time to decay

The time to lose 50%, 75% and 95% of the density was estimated by using the decay rate from the negative exponential formula (Table 4.10). The coarse roots would take on average 6.6 years to lose 50% density, 13.1 years to lose 75% of this density and 28.3 years to lose 95% of its density (Table 4.10).

Table 4.10: Estimated time taken to decay coarse roots from *E. grandis x E. urophylla* hybrids in a subtropical area.

Density loss (%)	Formula	Time taken to decompose (years)
50	$t_{0.5} = -\frac{\ln(0.5)}{k}$	6.6
75	$t_{0.75} = -\frac{\ln(0.25)}{k}$	13.1
95	$t_{0.95} = -\frac{\ln(0.05)}{k}$	28.3

It was important to compare the generated decay rate constant from the current study to that of other similar studies. A range of decay constants from different species, climates and locations were considered for comparison and are listed in Appendix A. Figure 4.5 visually illustrates how the root decay rate from the current study would compare to that of the slowest and fastest decay rates identified in Appendix A.

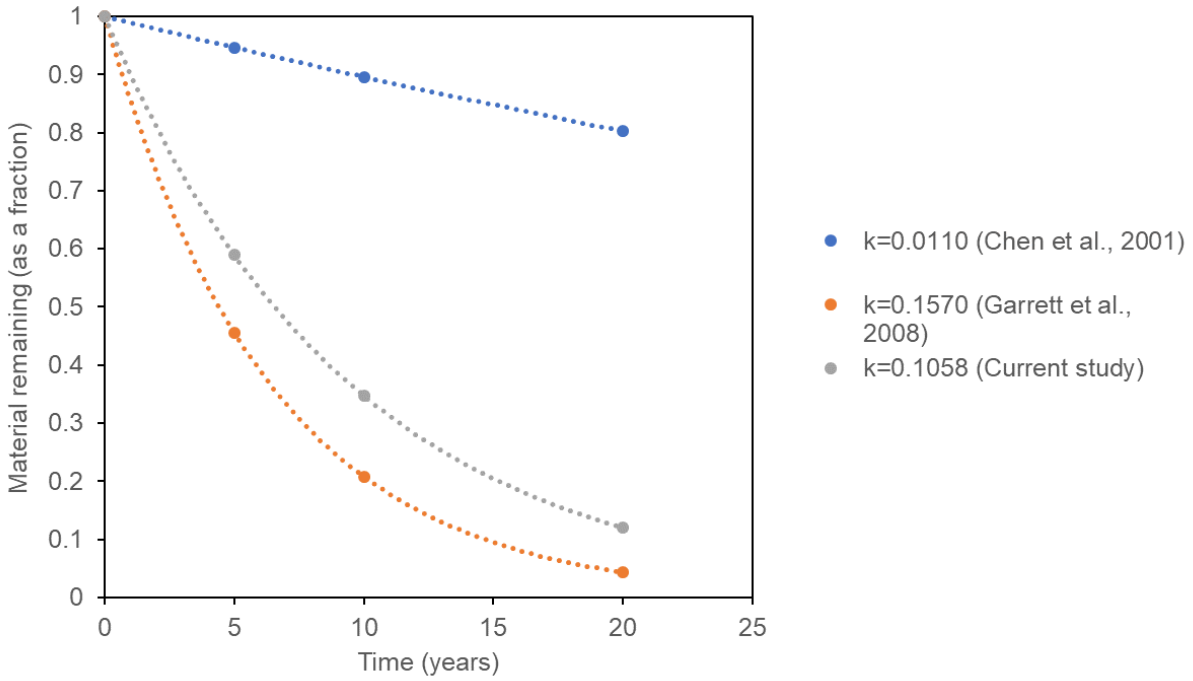

Figure 4.5: Comparing the k-value from the current study to the k-values from other similar studies (Appendix A). The blue regression line represents the slowest decay rate, orange the fastest decay rate and grey the decay rate from the current study.

4.3 Changes in C concentration

4.3.1 C concentration distribution and potential outliers

In the process of analysing the C concentration data a number of outliers were identified (Table 4.11). Outliers were identified as points falling outside the interquartile range of the boxplots (Figure 4.6b). All observations, identified as outliers, were investigated to determine their legitimacy. During the investigation, there seemed to be no indication that any of the samples were measured incorrectly except for one outlier; 97. The investigation brought to light that the stumps from site A56 (which outlier 97 and observations 101 and 105) were mulched after felling whereas the stumps from other sites were left intact. Mulching compromises the integrity of the stump which could increase the difficulty of accurately measuring stump density and C concentration. Although observations 101 and 105 were not identified as outliers by the boxplot (Figure 4.6b), there was strong evidence that these samples were measured incorrectly. Therefore, observations 97,101 and 105 (the C concentration measurements of the stumps from site A56) were removed from the dataset.

A linear regression analysis was performed on the data before the outliers where removed. The ANOVA results from the linear regression model showed that there was a significant linear relationship between the independent variables time after felling and SQ and the dependent variable coarse root C concentration. The fact that the ANOVA results showed a

significant relationship between the two variables time and SQ, and root C concentration was troubling considering the increased leverage that observation 75 and 115 (identified as outliers) would have in influencing this outcome (Figure 4.6a). Therefore outliers 75 and 115 were also removed from the dataset. The effect that all outliers had on the data was illustrated during the second regression analysis where it was found that only root size had a significant effect on coarse root C concentration (Table 4.13). Figure 4.7 shows the carbon content data after all suspicious outliers and observations had been removed.

Table 4.11: Observations identified as outliers

Time interval (year)	Observation number
6.17	75
5.58	90, 93
8.17	97
10	115

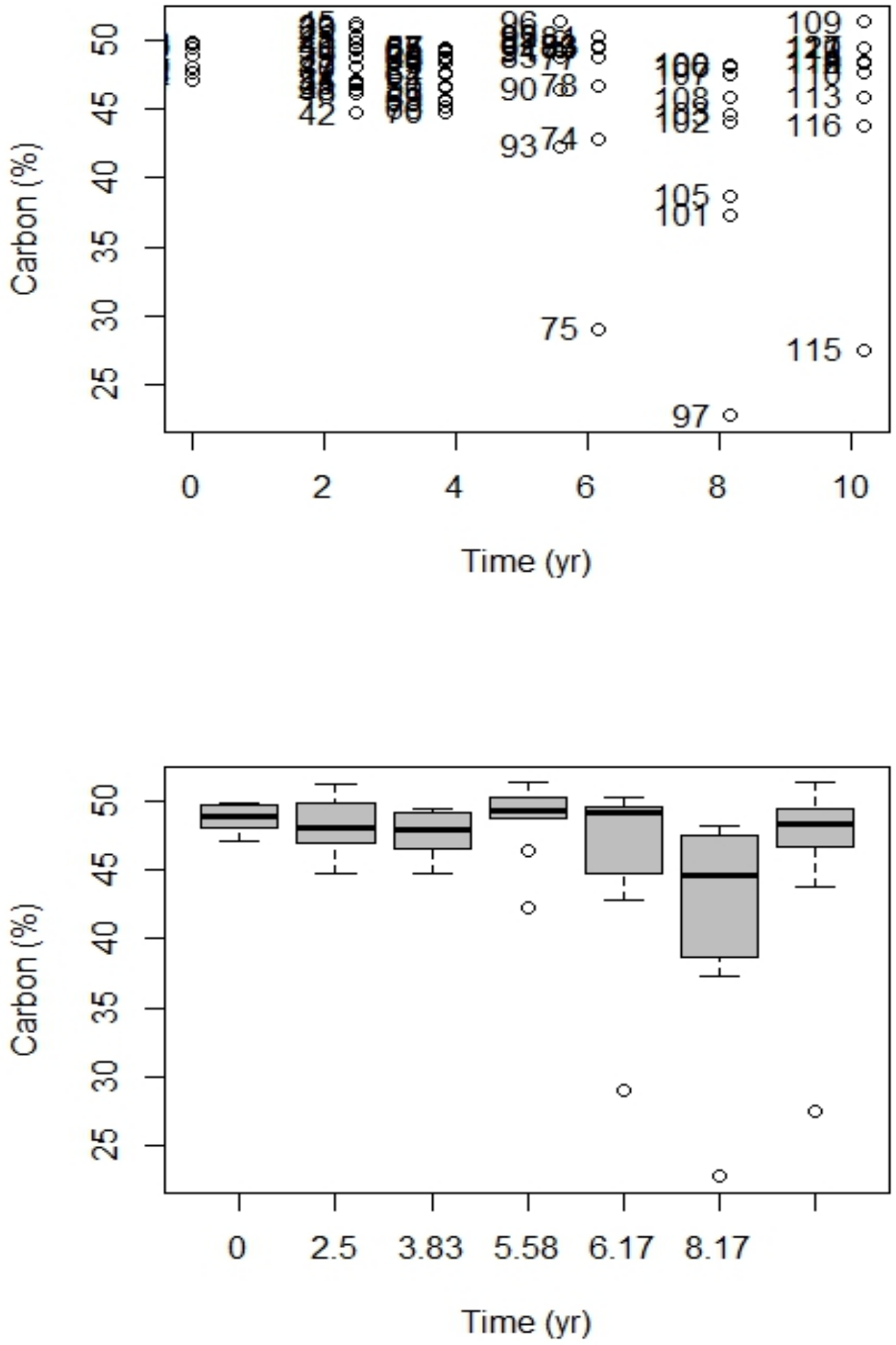

Figure 4.6: Data distribution with observation numbers (4.6a) and boxplot (4.6b) showing outliers as points falling outside the interquartile range (*i.e.* beyond the whiskers of the boxplots). The following observations were identified as outliers: 75, 90, 93, 97, 101, 105, 115.

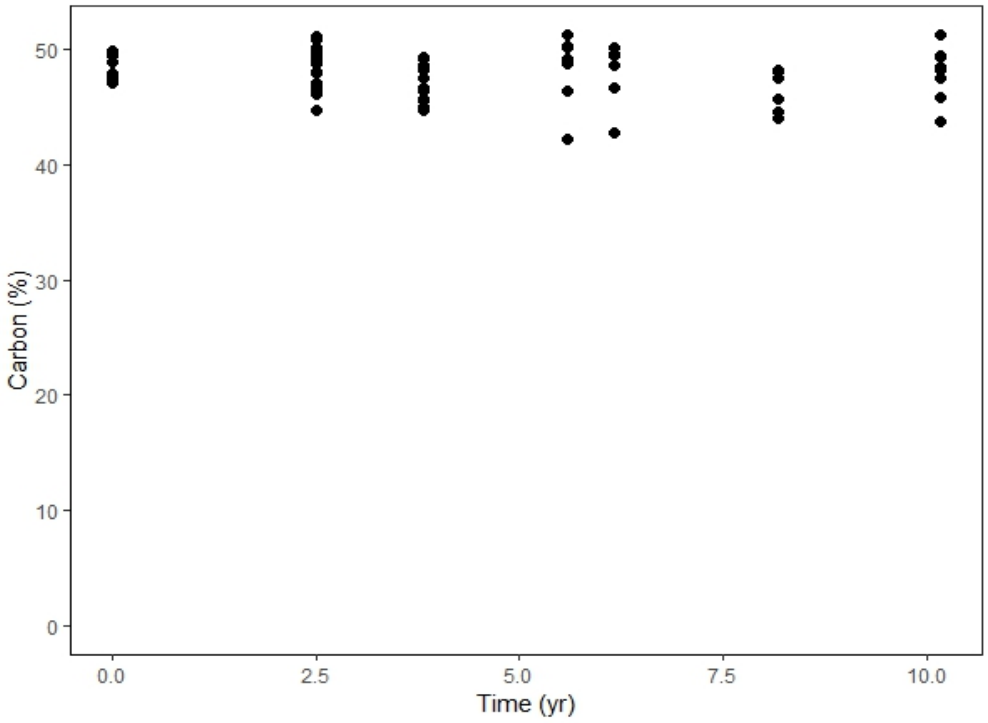

Figure 4.7: C concentration distribution over time after the observations 75 and 115 were removed.

4.3.2 Determining significant independent variables (Outliers included)

It was important to determine whether C concentration would vary amongst different time intervals (within the chronosequence), sites of differing productivity or quality, different tree sizes and different root sizes. Furthermore, it was necessary to determine what the effect of the outliers would be on these relationships. This section will run through the regression analysis of the C concentration dataset with the outliers included whereas the next section will look at the results once the outliers were removed.

The scatterplot matrix was once again used to visually illustrate the individual relationships between all the variables (Figure 4.8). Thereafter regression analysis was used to test the statistical significance of these relationships. TS and RS did not affect any of the other variables, neither did they affect each other. There seemed to be a linear relationship between Time and C concentration (C). SQ also seemed to have a relationship with C (Figure 4.8).

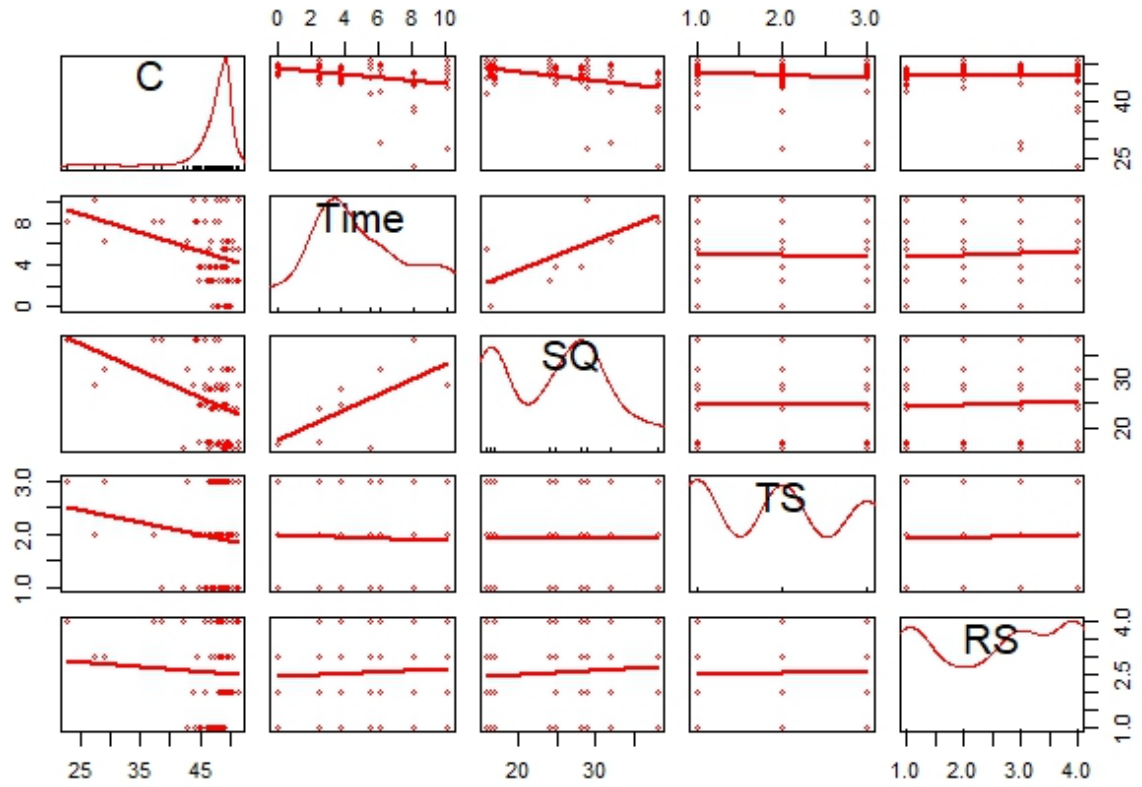

Figure 4.8**:** Scatterplot matrix (including all outliers and suspicious observations) showing the individual relationships between all variables: carbon (C), time after felling (Time), site quality (SQ), tree size (TS), root size (RS).

Multiple linear regression analysis

An analysis of variance (ANOVA) of the multivariate linear model showed that both time and SQ had significant linear relationships with C concentration (C), with a p-values of 0.0103 and 0.0210 respectively (Table 4.12). The fact that the ANOVA results showed a significant relationship between the two variables time and SQ, and root C concentration was troubling considering that all of the suspicious observations that were identified in section 4.3.1, could be exercising above average leverage.

Table 4.12: Results from the ANOVA of the multiple linear regression model (significant if p-value <0.05).

Variable	p-value
Time	0.0103
SQ	0.0210
TS	0.3094
RS	0.5429

4.3.3 Determining significant independent variables after removing outliers and suspicious observations

The new scatterplot (after removal of outliers) showed that both Time and RS seemed to have a relationship with C concentration (Figure 4.9). SQ seemed to have a relationship with time and TS did seem to have a relationship with any of the variables (Figure 4.9).

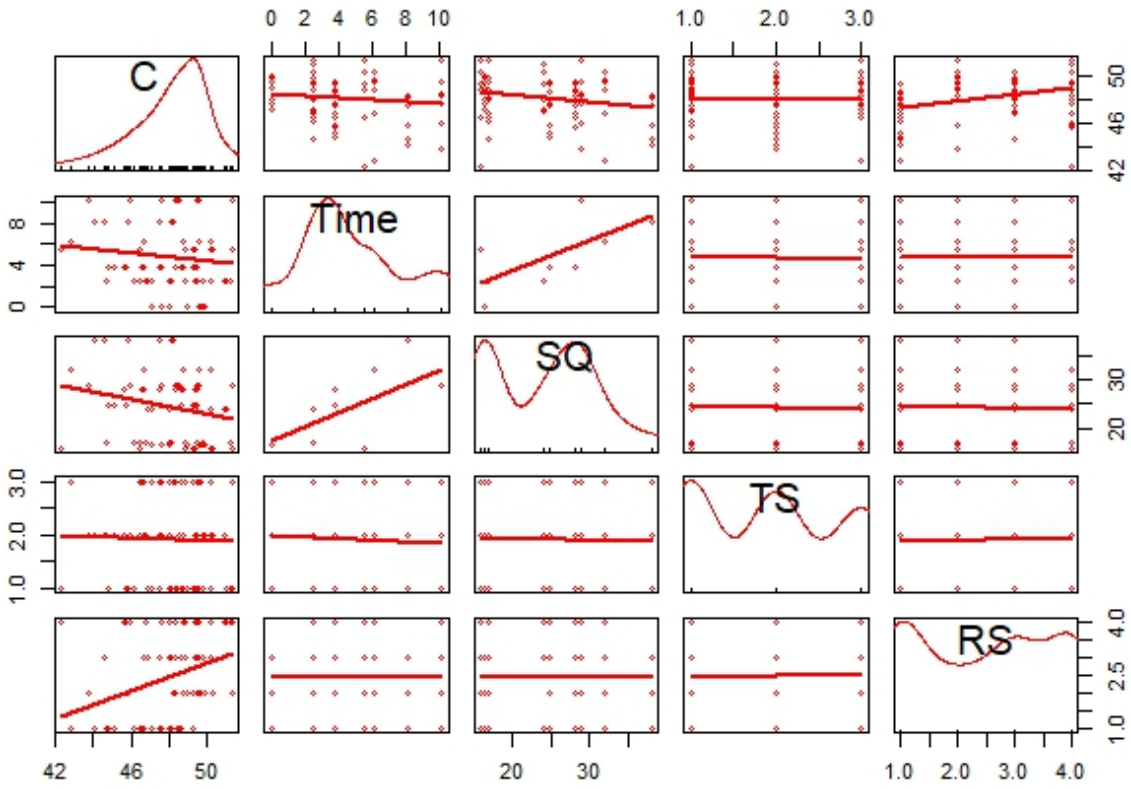

Figure 4.9: Scatterplot matrix (all outliers and suspicious observations removed) showing the individual relationships between all variables: carbon (C), time after felling (Time), site quality (SQ), tree size (TS), root size (RS).

Multiple linear regression analysis

The results from the ANOVA of the multiple linear regression model showed that RS had a significant relationship with C concentration; p-value = 0.0033 (Table 4.13). None of the other independent variables had a significant relationship with C (Table 4.13).

Table 4.13: Results from the ANOVA of the multiple linear regression model after removing outliers 75 and 115 (significant if p-value <0.05).

Variable	p-value
Time	0.2685
SQ	0.0924
TS	0.3237
RS	0.0033

4.3.4 Predicting coarse root C concentration

After removing all suspicious observations and outliers, the results from the ANOVA of the multiple linear regression model showed that root size (RS) had a significant relationship with C concentration. None of the other independent variables had significant relationships with C concentration. Therefore, the final results indicate that C concentration varies with root size alone. The mean C concentration for each root size class appear in Table 4.14.

Table 4.14: C concentration for each root size threshold

Root size (mm)	C concentration (%)
2-10	46.8 ± 1.6
10-50	48.6 ± 1.9
>50	48.8 ± 1.4
stump	48.6 ± 2.3

4.4 Additional controlling factors of root decomposition – Lignin

Lignin concentration was determined from fresh bulked root samples of the different root sizes (2-10 mm, 10-50 mm and >50 mm) in order to have an estimate of the lignin content of the roots. Three bulked samples were from a high productivity site (B7) and three samples from a low productivity site (K3d) (Figure 4.10).

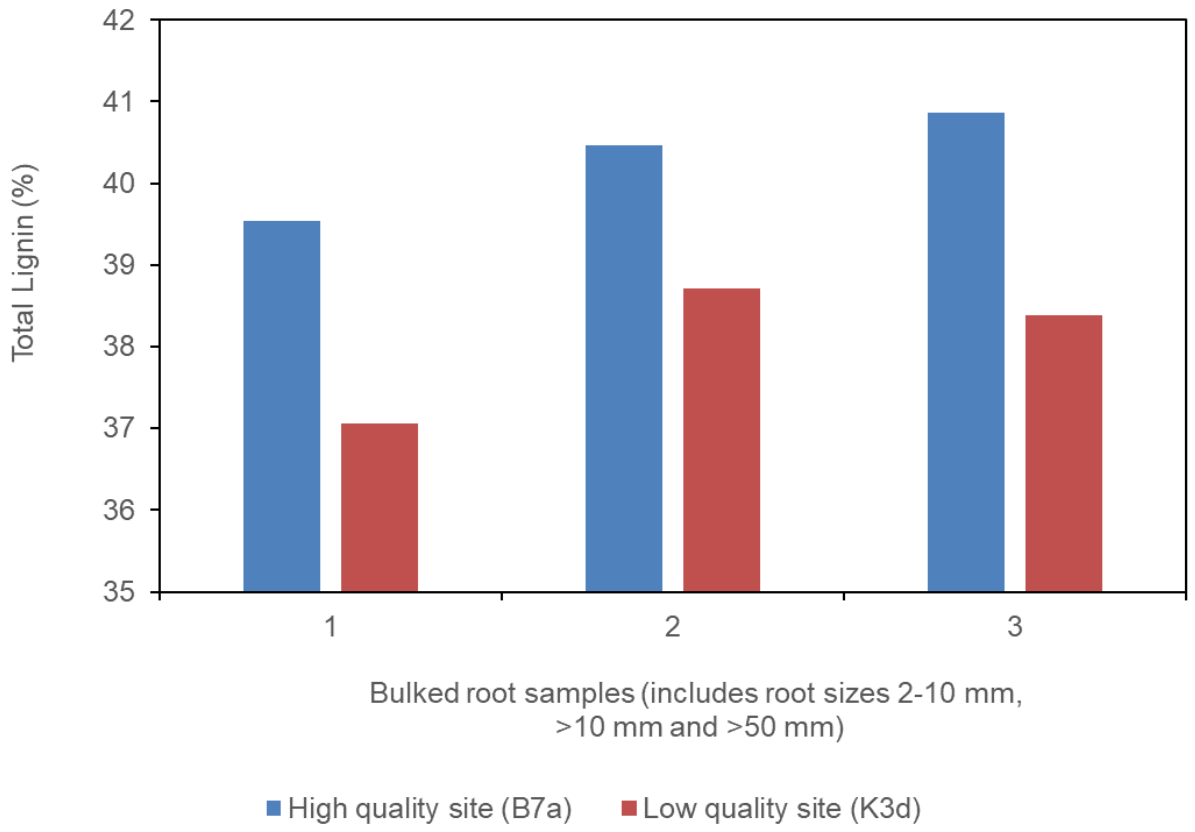

Figure 4.10: Lignin content of freshly felled wood. Each number on the x-axis of the graph below represents one bulked sample. All six samples are independent from each other. MAI for site B7 was 22 and K3d 17 m3 ha-1 annum-1.

To test the hypothesis that the means of the high- and low-quality lignin concentrations were significantly different, a two independent samples *t*-test was performed (Table 4.15). The Shapiro-Wilk test showed that the lignin concentrations were sufficiently normally distributed with a p-value of 0.557 for high quality site and 0.3631 for Low quality site (Table 4.15). Additionally, the assumption of homoscedasticity was tested and satisfied using the F-test(4) = 0.6033, p = 0.7526 (Table 4.15). The independent samples t-test showed that there was a statistically significant difference in the mean lignin concentration between the two different quality sites with t(4) = 3.5094, and p-value = 0.0247. The mean lignin concentration of the high-quality site B7b (40.3 %), was higher than that of the low-quality site K3d (38.1 %).

Table 4.14: F-test for homoscedasticity and t-test results for the effect of site quality (SQ) on lignin concentrations.

	F-test for homoscedasticity		t-test for equality of means				
	F	p-value	t	df	p-value	95 % Confidence interval of the difference	
						Lower	Upper
Lignin concentration	0.6033	0.7526	3.5094	4	0.0247	0.4679	4.0121

CHAPTER 5
DISCUSSIONS AND CONCLUSION

5.1 Comparing methods for measuring sample volume

Initially, two methods were used to measure root sample volume namely the displacement method and the micro-computed tomography (micro-CT) method. Micro-CT was incorporated as an additional method for measuring the volume of highly decomposed root samples. Micro-CT is becoming more popular in many scientific fields mainly because it allows for easy non-destructive imaging of a wide range of morphological structure (including fragile materials such as decomposed roots) (Du Plessis *et al*., 2017).

The goal was to use the displacement method for measuring the less decomposed material and then the micro-CT method for the more decomposed material. The reason being that the displacement method, which is based on the Archimedes principle, can cause an error when samples are highly decomposed (when samples have cavities) due to the methods dependence on the displacement of a fluid. It was not feasible to use the micro-CT method to measure all of the root samples from all of the sampling intervals due to budget restraints. The micro-CT method is more expensive compared to the displacement method mainly due to the expensive specialized equipment and expertise that are needed.

The paired samples t-test showed that the means of the two methods did not differ significantly with t(16) = -0.78211, and *p*-value = 0.4456 (Table 4.1). The goodness of fit of the These results confirm the claims of Du Plessis *et al.*, (2017), that the micro-CT method can be used to accurately measure wood sample volume. However, the paired t-test operates under the assumption that the differences between the volume measurements need to be sufficiently normally distributed. The Shapiro-Wilk test showed that the differences were not sufficiently normally distributed with a p-value of 0.0138. The non-parametric equivalent of the paired t-test was used to determine whether the means of the displacement and CT-scanner methods for determining sample volume were statistically significantly similar. The Wilcoxon Rank Sum Test showed that there was not a statistically significant difference in the mean volume measurements of the two different volume measurement methods, p-value = 0.7819.

An additional third method was used to measure stump sample volume, the direct measurement method (Section 2.3.1). A dedicated experiment to compare the accuracy of this method to the other two volume methods was not conducted since it did not fall within the budget of the study. Harmon and Sexton (1996) explains that the direct measuring of

regularly shaped objects does not differ significantly from that of the displacement method. Unfortunately, the direct measurement method could not be compared to the other two methods during the statistical analysis since only the stump discs were measured using this method.

5.2 Decomposition (density loss)

5.2.1 Relationships between independent variables and density loss (decomposition)

The ANOVA results of the multiple linear regression model showed that the independent variables time after felling (time) and changes in carbon to nitrogen (C:N) ratio were significantly correlated with coarse root decomposition (changes in coarse root density), with p-values <0.0001 and 0.0303 respectively (Table 4.4). Decomposition increases (material becomes more decomposed) as time after felling increases (positively correlated). Coarse root decomposition increased as the C:N ratio decreased (negative correlation). The variables site quality (SQ), root size (RS) and tree size (TS) did not have a statically significant relationship with coarse root density.

C:N ratio

Coarse root C:N ratio was significantly negatively correlated with changes in coarse root density (decomposition) which is consistent with the results from Olajuyigbe *et al.*, (2011) for coarse roots and Silver and Miya, (2001) for total root decomposition (Table 4.4) (Section 2.4). The C:N ratio decreased as the state of decay of the roots increased. This might be due to a relative increase in nitrogen concentration or a decrease in the carbon concentration as decomposition progresses as seen by Olajuyigbe *et al.*, (2011). The current study was not designed to determine the effects of initial root C: N ratio (and other initial root chemistry variables) on *Eucalyptus* hybrid coarse root decomposition. The inclusion of C: N ratio was an additional objective of the current study, which was only considered once it became apparent that root N data was available from the carbon analysis (through the use of the CHN elemental analyser). Root C: N ratio was determined for each age in the chronosequence. The regression analysis showed that C: N ratio can be used as an indication of the state of decay of coarse roots (Table 4.4). However, it would have been impractical to use both C: N and time after felling to model density, since C: N ratio and time explained some of the same variation in coarse root density. It would have been inefficient to have to also measure the C: N ratio of roots each time a prediction of density needs to be made. Therefore, coarse root decay was modelled only as a function of time.

Site quality

Climate has been identified as one of the main factors governing organic matter decay in terrestrial ecosystems (Laiho and Prescott, 2004; Thomas and Packham, 2007). Mean annual temperature (MAT) and mean annual precipitation (MAP) fall within this category of variables, and have been identified as important variables for explaining variation in coarse root decomposition (Silver and Miya, 2001; Garrett *et al.*, 2012; Zhang and Wang, 2015) (Section 2.4).C:N

The Zululand study area did not have extreme variation in climate within the region itself. An additional region with significantly different climatic conditions would have to be measured in order to use MAP or MAT to identify differences in root decay, which would have fallen outside the budget and time frame of the study. Therefore, a measure of site productivity or site quality (in the form of mean annual increment), was used instead of MAP and MAT to potentially identify variation in coarse root decay that might have been explained by MAP or MAT. This hypothesis was based on the fact that warmer and wetter sites usually have higher productivities in the Zululand region. Accurate site productivity data could easily be obtained through the extensive harvesting records provided by Sappi and Mondi.

As illustrated in the regression analysis, site quality or site productivity (SQ) did not have a statistically significant effect on coarse root decomposition (p-value = 0.9190) (Table 4.4). One reason why SQ might not have had a significant effect on coarse root decomposition in this study, could be the fact that the sites did not differ enough in terms of SQ. The initial goal was to select two sites at each sampling interval that had substantial differences in site index. But due to the extensive selection criteria for potential sites (Section 3.1.5) it was not possible to have substantially contrasting sites (in terms of their productivity) at each sampling interval of the chronosequence.

Root size

The results from this study showed that root size (2-10 mm, 10-50 mm, >50 mm and the stump) did not have a significant relationship with decomposition (density loss). Most root studies separate roots into coarse and fine roots, and several other studies sub-divide the coarse root fraction (*e.g.* Silver and Miya, 2001; Zhang and Wang, 2015) (Section 2.1.4 and 2.1.5).

Zhang and Wang, (2015) in their meta-analysis of global patterns in root decomposition, showed that fine roots (≤ 2mm) decomposed significantly faster than coarse roots (>2mm) in middle latitude areas (defined as >30 °N or S), but their decomposition in low latitude

regions (defined as ≤30 °N or S) was not significantly different from that of coarse roots. Zhang and Wang, (2015) did however conclude that the lack of difference in decomposition rate might have been due to species differences, since different species were used to determine fine root decay constants compared to that of coarse roots. Never the less, it is interesting that the results from this study, which also falls within the low latitude area, correlate with that of Zhang and Wang. The results showed no significant difference between the decay rate of small (2-10 mm), medium (10-50 mm) and large roots (≥50 mm). It's also plausible that the root size thresholds used in the current study were unable to identify differences in decay rate.

It was evident from the samples that root decay was also taking place from within the root. Many of the old root samples had live fine roots growing throughout the sample (Figure 3.9). Van Lear *et al*., (2000) also showed that roots would grow into the decomposing loblolly pine (*Pinus taeda* L.) roots. They showed that the growth of new roots establishes root channels within the dead roots. Therefore, the decrease in root surface to volume ratio with increasing root size (diameter) could potentially start to become less of a hindrance to decay for larger roots, since the newly formed root channels effectively increase surface area available for microbes to colonize.

Numerous larger root samples also had portions of their heartwood (and sometimes sapwood) almost completely hollowed out and filled by a sandy organic material. This is again illustrating how root decay could proceed not just from the outside surface but from the inside of the root. This could have been a result of larger soil fauna which could produce small channels to allow the colonization of the inner areas of the root by microbes.

5.2.2 Modelling root decomposition

The stepwise regression results showed that changes in coarse root density can be accurately predicted using only the time variable (AIC = -575.00 and BIC = -570.42) (Table 4.5 and Table 4.6). The all subsets regression analysis confirmed the results from the stepwise regression analysis in that only time is needed to model root decomposition (Table 4.7). These results were not surprising when considering that multiple root decay and wood decay studies recommend modelling woody decay using a single component negative exponential model using only time after death as an independent variable (Olson, 1963; Yavitt and Fahey, 1982; Chen *et al.*, 2001; Silver and Miya, 2001; Shorohova *et al.*, 2008; Melin *et al.*, 2009; Garrett *et al.*, 2012; Zhang and Wang, 2015) (Section 2.3.3). Therefore, even though both time and changes in C:N correlated significantly with root decay, only time was used to model root decay and produce decay constants.

Producing decay constants

Two types of models were considered for modelling changes in coarse root density and producing decay constants (Section 2.3.3). Both a single component negative exponential model (Olson, 1963; Yavitt and Fahey, 1982; Chen *et al.*, 2001; Silver and Miya, 2001; Shorohova *et al.*, 2008; Melin *et al.*, 2009; Garrett *et al.*, 2012; Zhang and Wang, 2015) and a linear model (Lambert *et al.*, 1980; Wieder and Lang, 1982) was fitted to the data (Table 4.8). Of course, the negative exponential model is the most sensible from a theoretical point of view (Section 2.3.3), but studies such as Lambert *et al.*, (1980) have found the linear model to be just as effective at describing bole decay according to R^2 values. Additionally, a second linear model was fitted to the same dataset but with the time scale being log transformed using the natural logarithm (Table 4.8). All of these models operate under the assumption that decomposition is proportional to the density remaining after a certain period of time (Olson, 1963) (Section 2.3.3).

The negative exponential model (Model 4.1) gave a slightly better fit or just as good a fit as the semi-log transformed model (Model 4.3) (Table 4.8). The residual standard errors (RSE) and mean standard errors (MSE) for Model 4.1 was 0.04817 and 0.00232 respectively. The semi-transformed linear model had an RSE and MSE of 0.04824 and 0.00233 respectively. There were only very small differences between the three models, especially between Model 4.1 and Model 4.3 (Table 4.8).

In the current study, non-linear models (Model 4.1 and Model 4.3) were slightly better at describing coarse root decay than a linear model (Table 4.8). The most commonly used equation or model for producing decay constants is the single component negative exponential model (Olson 1963). In support of the current study, many other studies have used the negative exponential model for describing root and CWD decomposition and producing decay constants (Olson, 1963; Yavitt and Fahey, 1982; Chen *et al.*, 2001; Silver and Miya, 2001; Shorohova *et al.*, 2008; Melin *et al.*, 2009; Garrett *et al.*, 2012; Zhang and Wang, 2015) (Section 2.3.3).

Linear models have prevailed in only a few cases. (Lambert *et al.*, 1980) studied the decay of balsam fir (*Abies balsamea*) boles in an upper subalpine forest of the White Mountains, New Hampshire, USA. They showed that the linear and exponential models were equally efficient for modelling decomposition, according to their R^2 values. They decided to use the negative exponential model based on theoretical preference and visual inspection of the data (Section 2.3.3). Silver and Miya, (2001) in their meta-analysis of global root decay fitted

both a linear ad exponential decay function to calculate root decay rates for the studies that reported only mass loss or C loss over time without a decay constant (Silver and Miya, 2001). They found that the exponential equation provided a fit as good or better than the linear model in all cases (Silver and Miya, 2001). In both of the studies above, the best-case scenario for the linear model was that it was equally as good at describing decay as the non-linear (exponential) model, but in no circumstances better. This is consistent with the results for coarse root decay found in the present study (Section 2.3.3).

The negative exponential model (Model 4.1) was used to produce a decay constant; k = 0.1048 year^{-1} (Table 4.8). Only a few studies have produced coarse root decay constants by using the single component negative exponential model and through the chronosequence approach (*e.g.* Chen *et al.*, 2001; Ludovici *et al.*, 2002; Garrett *et al.*, 2008; Palviainen and Finr, 2015) (Appendix A). At the decay rate determined in the current study, it would take on average 6.6 years,13.1 years and 28.3 years for the root system to lose 50%, 75% and 95% of its density respectively (Table 4.10).

The k-value produced in the current study (0.1048 year^{-1}) falls well within the range of k-values from the above-mentioned studies which ranged from 0.011 year^{-1} to 0.157 year^{-1} (Appendix A) (Chen *et al.*, 2001; Garrett *et al.*, 2008). Figure 4.5 visually illustrates how the root decay rate from the current study would compare to that of the slowest and fastest decay rates identified in Appendix A. With a decay constant of 0.011 year^{-1}, half of the density (50%) would be lost after 63 years. With a decay constant of 0.157 year^{-1} it would take 4.4 years and with the constant from the current study (0.1058), 6.6 years for roots to lose half of their density. Unfortunately, no published literature on decay constants for *Eucalyptus* coarse roots (≥2mm) could be found for purposes of direct comparison. However, a decay rate constant has been determined for old *Eucalyptus grandis x E. urophylla* stumps in Brazil by Stape *et al*. (2008) (k = 0.190 year^{-1}).

5.3 Root carbon concentration

Determining root carbon content was necessary to further improve carbon accounting accuracy. The boxplot (Figure 4.6b) identified multiple outliers within the carbon dataset which can be found in Table 4.11. These outliers were investigated to identify if any measurements were made incorrectly. The investigation brought to light that the stumps from site A56 (which included sample 97 (an outlier) and samples 101 and 105 (non-outliers)) were mulched after felling. This was a problem considering that the other sampled stumps were not mulched. The other outliers identified by the boxplot in Figure 4.6b, were investigated but there was no evidence that these observations were measured incorrectly.

None of the outliers or faulty samples could be measured again due to time and budget constraints. Therefore, observations 97, 101 and 105 were removed from the data set because they experienced atypical management interventions (mulching) and could affect the outcome of the analysis.

Observations 75 and 115 as seen in Figure 4.7a showed much lower carbon concentrations than the rest of the data. They were previously identified as outliers (Table 4.11) but were not removed since there was no evidence to suggest measurement error. Although there was no evidence to explain why these observations had such low carbon concentrations, it can clearly be seen in Figure 4.7a that these observations would have a greater leverage on effecting the mean carbon concentration in comparison to the other observations. It is highly likely that something went wrong during the collection, preparation or measurement of these samples. Therefore, observations 75 and 115 were also removed from the dataset.

Initially, before any observations or outliers were removed, the results from the ANOVA of the multiple linear regression model showed that both time and site quality had significant linear relationships with C concentration (C), with a p-values of 0.0103 and 0.0210 respectively (Table 4.12). After removing suspicious observations and outliers (75, 97, 101, 105 and 115), the results from the ANOVA of the multiple linear regression model showed that root size (RS) was the only variable that had a significant relationship with root carbon content, p-value = 0.0033 (Table 4.13). Therefore, the mean root carbon concentration was determined for each root size class.

The mean carbon concentration for each root size class was 46.8 ± 1.6% (< 10 mm), 48.6 ± 1.9% (10-50 mm), 48.8 ± 1.4% (>50 mm) and 48.6 ± 2.3% (stump) (Table 4.14). The default carbon content set by the IPCC (2006) for dead wood or litter was 37% (IPCC, 2006). Small (<10 mm) roots measured the lowest carbon content of all the root sizes, and was about 9.8% higher than the IPCC (2006) default value for dead wood or litter. Medium (10-50 mm) and large (>50 mm) roots and stumps measured carbon content values approximately 11% higher than the IPCC (2006) default value. Although these values are higher than the IPCC (2006) default value, it must be pointed out that the default value is not determined for roots exclusively but rather for deadwood and litter. Therefore, the values for dead root carbon determined in the current study can potentially be a more accurate value for root carbon than the IPCC (2006) default value.

Gifford, (2000), found no trend in the value of carbon content with root diameter between 10mm and 300mm. They did however find that fine roots (<2mm diameter) from poplar box

communities (*Eucalyptus populnea*) and *Pinus radiata* had a carbon content about three percentage points lower than that of coarse woody roots, although for the poplar box community about half the fine root was of grass species (Gifford, 2000). Although the diameter classes are again not directly comparable to the current study, it is still interesting that in both studies, the smallest root size class had the lowest carbon content. Gifford, (2000) determined the average carbon content of woody roots for 23 species to be 49% with a standard deviation of 1% which is similar, although slightly higher than carbon content of roots from the current study.

5.4 Lignin content

Increased concentrations of polyphenols of higher molecular weight such as lignin, have been identified to have strong influence in slowing decomposition (Thomas and Packham, 2007). Lignin is very difficult to decompose due to its structure which can be characterized as being highly heterogenous and unordered (Thomas and Packham, 2007). This structure requires microbes to produce a wide variety of enzymes in order to break down or decompose the compound. For most of the microbial organisms this comes at too high of a price in terms of energy required, making lignin very decay resistant (Thomas and Packham, 2007).

Lignin has not yet been identified as a substantial factor controlling coarse root decomposition, but has been identified as an important factor explaining variation in fine root decomposition (Zhang and Wang, 2015; Bachega *et al.*, 2016; Luo *et al.*, 2017). Zhang and Wang (2015) also found that lignin: N ratio had a significant effect on coarse root decomposition. But there is no reason why lignin content shouldn't also limit coarse root decay. Therefore, lignin content was measured for fresh root samples for the current study in order to investigate the relationship between root decay and lignin. Measuring root lignin did not fall within the scope of the current study from the start and was secondary objective. Root lignin was measured for freshly felled root material from six bulked samples, as a measure of initial root lignin (Figure 4.10). Unfortunately, it was not feasible to measure lignin for each age in the chronosequence due to budget constraints. These lignin values, in relation to the decay rates generated in this study, can then serve as a reference to compare with other studies.

Three of the six bulked samples were sampled from a high productivity site (B07), and three samples from a low productivity site (K3d). The MAI for two sites were 22 m^3 ha^{-1} a^{-1} (B7) and 17 m^3 ha^{-1} a^{-1} (K3d), which is not a large difference. The results from the independent samples t-test showed that there was a statistically significant difference in the mean lignin

concentration between the two different quality sites with t(4) = 3.5094, and p-value = 0.0247 (Table 4.15). The mean lignin concentration of the high-quality site B7b (40.3%), was thus significantly higher than that of the low-quality site K3d (38.1%). Although, no published research could be found regarding the variation in wood lignin between sites of different productivity, there have been studies that have focussed on wood lignin. Clarke *et al*. (1999) found that the wood from the poorer (Helvetia) site had lower lignin concentrations that the wood from the higher quality site (Shafton). However, the majority of the research correlated higher growth rates to lower wood lignin levels (Kirst, 2004; Klash *et al.*, 2010; Novaes *et al.*, 2010).

The results from this study showed that there was no relationship between site productivity (site quality) and decay. Therefore, no proof could be found for a link between lignin concentration, productivity of the site and rate of coarse root decay. Since only bulked samples were used and due to time and budget constrains the effects of lignin on decomposition could not be investigated further. Furthermore, it could be worthwhile for future studies to investigate the relationship between wood lignin content and site productivity. Future studies should perhaps also include investigation of the potential relationship between root initial lignin: N ratio and root decay rate.

5.5 Study limitations

5.5.1 Uncertainties related to the chronosequence approach

Conducting a woody debris decomposition study by using a chronosequence holds a certain degree of uncertainty (Section 2). The risk is due to several unknowns or uncertainties which include differences between sites, fragmentation and uncertainty regarding the age of the material. These differences between sites are a great cause of error in chronosequence data (Harmon and Sexton, 1996).

In this study the additional error that can go along with abovementioned site differences were kept to a minimum by selecting sites that are close to each other and similar in soil type, elevation and topography. Only sites which were burnt after felling were selected for sampling (By using harvesting records from Sappi and Mondi). Losses due to fragmentation was not a problem in this study since the goal was to use density measurements to convert stand level volume measurements into mass (Harmon and Sexton, 1996). If the gaol was to measure mass at each of the sampling intervals of the chronosequence then one would have to account for pieces of the material that might have gone missing (fragmentation) (Harmon and Sexton, 1996). Uncertainties regarding the age of the material was solved by making use of accurate harvesting records of Sappi and Mondi.

Since the study time frame was limited to two years it was simply not possible to follow any approach other than substituting space for time (chronosequence approach). The results obtained from the chronosequence are still insightful, adds to the current body of knowledge and strengthens several results published on coarse root decay for other sites, species and climatic conditions.

5.5.2 Fine root study vs coarse root study

Another area in which this study could be improved, is if decay rates were also generated for fine roots (<2mm). Studies like Zhang and Whang (2015) have shown that separating coarse and fine roots when determining decay, is important. But fine root decay is often studied using shorter intervals and different techniques (Garrett, Davis and Olivee, 2007). The additional effort and time needed for developing the appropriate methods to account for fine root decay did not fall within the budget and time frame of this study.

5.6 Conclusion and recommendations

5.6.1 Conclusion

The main purpose of this study was to determine the longevity of decomposing *Eucalyptus* hybrid roots in managed pulpwood plantations in order to understand their potential for storing C. This goal was achieved by determining the decay rate constant (k = 0.1058) for the roots through the use of a negative exponential model. At this rate there will surely be a degree of accumulation in the below ground carbon content since the average rotation age for pulpwood plantations in the Zululand area is about seven years. However, there was still a large proportion of the variation within the density or decay data that is not accounted for. Therefore, this work provides a good baseline for rate of *Eucalyptus* hybrid coarse root decay within South Africa, but shows that further refinement is required.

Furthermore, the experimental design and sampling methodology succeeded in producing results that were in many instances comparable to the results from other coarse root decay studies thereby strengthening the existing knowledge on root decay. In addition, these methods were able to produce results within a limited two-year time frame, which is an extremely short amount of time to conduct a long-term decay study.

The results from this study showed that neither root size nor site productivity were drivers of *Eucalyptus* hybrid root decomposition in the sub-tropical study area. It was found that root carbon concentration remained constant over time but varied with root size which has been found by other studies as well. Root carbon concentration was measured as 46.8 ± 1.6%, 48.6 ± 1.9%, 48.8 ± 1.4% and 48.6 ± 2.3% for <10 mm, 10-50 mm, >50 mm and stumps

respectively. Lignin content was also determined for fresh root material, 40.3% for high productivity site and 38.1% for low-productivity site, but the relationship between lignin content and measures of site productivity or quality needs to be investigated further.

Finally, the classical displacement method was compared to the micro-CT method for measuring root sample volume. It was found that the micro-CT method was just as accurate at measuring sample volume as the displacement method.

This research was driven mainly by a need for improved Tier 2 carbon accounting systems within the South African pulpwood industry. To date, no decay constants have been produced for *Eucalyptus* hybrid coarse roots in South Africa, although there have been studies that have quantified fresh root biomass. Knowing the decomposition rate of roots in plantations is an essential step to accurately accounting for the biomass and carbon stored within these forests. The decay constants will help industry translate root volume measurements (which have been produced in other recent research), into biomass values for the root systems. The carbon concentration values can then be used to translate this mass into carbon content. Therefore, the decay rates determined for *Eucalyptus* hybrid coarse roots can be used to further improve carbon accounting systems in South African pulpwood plantations in sub-tropical regions. It was also evident from the results that *Eucalyptus* hybrid coarse roots should be regarded as an important long-term biomass pool.

5.6.2 Recommendations

Future studies should consider further investigating the effect of climate on coarse root decay by studying two contrasting climatic regions. This could potentially help to explain some of the variance within coarse root decay identified in the current study. It would also be beneficial to investigate the potential effects of initial root chemistry or substrate such as initial root lignin concentration, lignin: N ratio, C: N ratio and calcium (Ca) concentration on *Eucalyptus* hybrid coarse root decay.

Finally, future studies focussing on coarse root decay should be cautious to use site productivity as a variable for predicting root decay. This is because site productivity might encompass too many other factors which prevents differences in decay being identified.

CHAPTER 6
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APPENDIX A

Root decay constants calculated from negative exponential models for course roots that were sampled in a chronosequence.

Site	Species	MAT (°C)	MAP (mm)	Elevation above sea level (m)	Root size thresholds (cm)	k-value (year^{-1})	Coefficient of determination (R^2) of K	Source
Duke Forest, Durham County, North Carolina	*Pinus taeda*	16	1230	-	Lateral roots	0.068	0.75	(Ludovici *et al.*, 2002)
					Taproot	0.059	0.95	
					Root bark	0.036	0.71	
					Total root system	0.060	0.95	
Tarawera forest, Bay of Plenty region, North Island, New Zealand	*Pinus radiata*	14	1820	90	Root	0.157	0.17	(Garrett *et al.*, 2008)
					Stump	0.110	0.56	
Evo- Vesijako experimental forests, Southern Finland	*Picea abies*	4.2	645	135	5 -10	0.021	0.48	(Palviainen and Finér, 2015)
					> 10	0.017	0.54	
					Stump	0.034	0.79	
Cascade Head Experimental Forest, Pacific coast, Oregon.	*Picea sitchensis* (Sitka Spruce)	10	3420	-	1-5	0.021	0.95	(Chen *et al.*, 2001)
					5-12	0.016	0.84	
	Tsuga heterophylla (Western Hemlock)				1-5	0.040	0.92	
					5-15	0.049	0.97	
H.J. Andrews Experimental Forest, Eugene, Oregon.	*Pseudotsuga menziessii* (Douglas Fir)	8.5	2300	1300	1-5	0.011	0.82	
					5-15	0.013	0.90	
	Tsuga heterophylla (Western Hemlock)				1-5	0.034	0.97	
					5-15	0.033	0.89	
Pringle Falls Experimental Forest, City of Bend, Oregon.	*Pinus contorta* (Lodgepole Pine)	5.7	525	-	1-5	0.025	0.64	
					5-11	0.030	0.62	
	Pinus ponderosa (Ponderosa Pine)				1-5	0.077	0.63	
					5-12	0.073	0.97	