

Above and Below Ground Assessment of *Pinus Radiata*

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ABSTRACT

A comparison of above ground forest metrics with below ground soil CO₂ respiration was carried out in an attempt to reveal if any correlations exist. Above ground measurements of 2720 clonally propagated trees were taken assessing the silvicultural treatments of stocking, herbicide and fertiliser. These were compared to 480 below ground soil CO₂ respiration measurements. Using measurements of mean height, mean dbh and basal area the data was analysed and returned significant results for mean dbh and the interactions of herbicide and clones, and stocking and herbicide. Mean height returned a significant result for the interaction of stocking and herbicide. Below ground measurements showed an interaction between ripping and stocking; however these results were not ratified by the above ground results. Overall the results were encouraging and should aid in future experiments that seek to understand what effect above ground treatments have on below ground CO₂ activity.

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INTRODUCTION

Trees in a forest can be separated into two separate sections, above ground (stems, canopy, branches and foliage) and the roots below ground. All too often the above ground elements receive the majority of attention with little regard for the below ground processes. Above ground, common measures for plantation forest growth are mean top height, diameter at breast height and basal area; however, what has been lacking in this picture is information on what happens underneath the soil. This study aims to bring together above ground and below ground data to give a more complete picture of forest state.

Silvicultural treatments can enhance profits, produce desired wood qualities and shorten forest rotation lengths (Burger, 1994; Lasserre, Mason, & Watt, 2008; Mason & Milne, 1999). To better understand what effects fertiliser, stocking rates, herbicide and clonal genetics has on growth and below ground metrics, this dissertation undertakes an analysis to compare and contrast data from both.

Second only to photosynthesis, soil CO₂ respiration is an important component of global CO₂ cycles and has been shown to account for over sixty percent of total ecosystem respiration in some trials (Davidson, Savage, Verchot, & Navarro, 2002; Kuzyakov, 2006). In plantation forestry knowledge about CO₂ levels with regards to clone type, fertilisation, herbicide and cultivation treatments is a relatively new field.

This study aims to assess CO₂ levels with regards to five different clones, three different initial stocking levels, three different treatments and two cultivation practises. The experiment took place at Rolleston plantation (Christchurch, New Zealand) during the month of July 2013. Above ground data containing tree metrics for a select group of trees was used in conjunction with CO₂ measurements. Over a period of six days, measurements were conducted for each plot in the plantation (a total of 480 measurements) with the total CO₂ and CO₂ efflux rate (ppm and (g (CO₂) m⁻² Hour⁻¹) respectively) being recorded to compare with the above ground site factors.

PROBLEM STATEMENT

Initial stocking, weed control, fertilisation and clonally propagated growing stock are important factors when establishing a *Pinus radiata* (D. Don) (*P. radiata*) plantation, but what effect do these choices have below the ground? Do the interactions observed from above ground silviculture treatments (e.g. herbicidal effects

on clones) correspond to CO₂ levels below ground? This dissertation attempts to compare and contrast data from above ground with CO₂ soil activity below.

RESEARCH QUESTION

H_A: Above ground silvicultural treatments affect below ground CO₂ levels.

H₀: Above ground silvicultural treatments do not affect below ground CO₂ levels.

LITERATURE REVIEW

Above ground measurements of height and diameter at breast height (dbh) which convert to mean top height, mean dbh and basal area at the forest level, are useful benchmarks of how a forest is performing, this review takes account of current research for both above and below ground measurements. Under consideration are the effects of herbicide, fertiliser, stocking densities and differences between five clones with respect to the above ground variables; mean dbh, mean height and basal area. Alongside these above ground measurements below ground CO₂ respiration is also examined, with the aim of integrating above and below components to give a more complete picture of the forest. This review consolidates the literature available for both above-ground effects of silvicultural treatments and current understandings of soil CO₂ respiration measurements.

Soil Respiration

Soil respiration is caused by activity below the surface from roots, soil microbes, and soil fauna within soil and litter layers (Yiqi & Zhou, 2010). Soil CO₂ respiration is the second largest carbon flux on the planet (photosynthesis being first) and contributes a large amount to total ecosystem respiration (Kucera & Kirkham, 1971).

By measuring what levels of total CO₂ are present on the surface level an indication of soil health can be gained, the more activity under the surface the more gaseous CO₂ molecules are being released to the surface. There are three basic pools of carbon that are produced from soil; soil organic matter, above and below ground plant residues and organic matter from living roots like root cells, secretions and exudates (Kuzyakov, 2006). The contribution of soil respiration to total ecosystem respiration is between 40% and 90% (Davidson et al., 2002) and levels of soil CO₂ efflux usually follow seasonal variations (Carlyle & Than, 1988). Levels of CO₂ flux from these

pools of carbon was measured to give a value for total soil ecosystem flux, this was then compared with above ground silvicultural treatments.

The main factors that influence soil respiration are soil and air temperature (Edwards, 1975; Reth, Reichstein, & Falge, 2005), soil water levels (Laine, Byrne, Kiely, & Tuittila, 2007; Reth et al., 2005) and the amount of root material present (Kucera & Kirkham, 1971; Reth et al., 2005; Yiqi & Zhou, 2010). Other documented factors that influence soil CO₂ efflux rates are rates of vegetation growth (M Reichstein et al., 2007), soil substrate amount (Zak, Pregitzer, King, & Holmes, 2000) and pH levels of the soil (Paterson et al., 1997). Total soil CO₂ respiration is the sum of root respiration plus heterotrophic respiration (Carlyle & Than, 1988); all of these above factors give a sum total of how healthy the soil content is, which in turn should be reflected in above ground growth rates.

The Tricarboxylic Acid Cycle:

The production of CO₂ can occur through different pathways, however the most common is the tricarboxylic acid cycle (TCA), also called the citric cycle (a form of tricarboxylic acid). The TCA cycle occurs in aerobic conditions (which predominate in soils), therefore, when soils are waterlogged different processes occur (i.e. fermentation of glucose to organic acids) (Yiqi & Zhou, 2010). The main contributors to soil CO₂ production are root respiration, microbial respiration in the rhizosphere, decomposition of litter and soil organic matter oxidation (Yiqi & Zhou, 2010).

CO₂ respiration is often separated into two different categories, growth respiration and maintenance respiration. Growth respiration provides the energy for biosynthesis of the compounds used to grow plant structures and maintenance respiration provides the energy necessary for day to day functioning of a plant.

Carbon Pools:

When measuring CO₂ flux levels on the soil surface there are five main identified pools or carbon contributing to the total level, these are; root respiration, rhizomicrobial respiration, decomposition from plant residues, root exudates or the addition of plant residues and basal respiration by microbial decomposition of soil organic matter (Kuzyakov, 2006). It is an important to note that all these factors contribute to the total level being emitted at the surface. Reports of attempts to separate the various carbon pools suggest that no completely accurate method is yet

available (Davidson et al., 2002). The study undertaken at Rolleston measured total CO₂ flux from the forest floor without removal of litter.

A closer look at each carbon pool is necessary to better understand the true composition of soil CO₂ measurements. The five pools in detail are;

1. Microbial decomposition of soil organic matter in soil free of roots with no plant remains
2. Microbial decomposition of soil organic matter in root affected or plant residue affected soil
3. Microbial decomposition of dead plant remains
4. Microbial decomposition of rhizodeposits (from living roots)
5. Root respiration

Methods that allow the separation of carbon pools to be estimated include (but are not limited to) root exclusion techniques, excised roots and *in situ* respiration, radio carbon dating of soil and bomb ¹⁴C (Kuzyakov, 2006). These techniques are time intensive, prohibitively expensive and beyond the scope of this dissertation.

Root Respiration:

The proportion of CO₂ production attributable to root respiration is approximately half of the total soil respiration, however this can vary between 10 and 90 percent (Yiqi & Zhou, 2010). The amount and diameter of roots impact on efflux rates considerably, with positive correlations having been found between root mass and CO₂ flux rates (Reth et al., 2005). Root respiration consumes around 10 to 50% of the total assimilated carbon in photosynthesis (Yiqi & Zhou, 2010), hence depending on species and soil type the carbon consumption from root respiration can be considerable. Mycorrhizae are organisms associated with roots from nearly all families of flowering plants and play an important role in carbon cycling (Yiqi & Zhou, 2010), however, attempting to quantify the amount of mycorrhizae populating a given area is problematic and time consuming. Differing environmental conditions make measuring root respiration less accurate, although if the data can be obtained a good representation of site specific conditions can result (Bouma, Nielsen, Eissenstat, & Lynch, 1997).

Temperature effect:

The relationship of soil temperature on CO₂ efflux from soil has been well documented and many studies have established that they are closely correlated

(Edwards, 1975; Wiant, 1967; Yiqi & Zhou, 2010). As soil temperature increases below ground soil respiration also increases; temperature has been reported as the most influential factor affecting soil CO₂ respiration (Fang, Moncrieff, Gholz, & Clark, 1998). Efflux activity below the ground slows down as soil temperatures approach zero, eventually coming to a standstill when soils freeze over (Kucera & Kirkham, 1971). This relationship has been observed in many similar studies (Edwards, 1975; Fang et al., 1998; Wiant, 1967; Yiqi & Zhou, 2010).

Soil Water effect:

Often associated with temperature as the other major influence on soil CO₂ respiration rates, water has a large effect on soil efflux rates (Bouma et al., 1997; Edwards, 1975; Goulden, Munger, Fan, Daube, & Wofsy, 1996; Laine et al., 2007; Linn & Doran, 1984; Markus Reichstein et al., 2002; Reth et al., 2005; Wiant, 1967). Initially, with an increase of water present in the soil, aerobic microbial activity increases up to a point. As soil pores continue to fill up with water, diffusion rates and availability of oxygen are restricted which leads to lower CO₂ efflux rates due to decreased aerobic microbial activity (Kucera & Kirkham, 1971). Water table levels also affect CO₂ efflux rates, as water tables rise there is a corresponding drop in CO₂ respiration rates largely due to the decreased levels of oxygen (Kim & Verma, 1992). The negative effect on CO₂ respiration when soil pores fill up with water has been reported (Reth et al., 2005).

Measuring Techniques

There are several options available to measure soil CO₂ levels. These range from *in situ* options (such as mini-rhizotrons) and semi-permanent measuring stations usually in place for a long period of time (e.g. a year or more), to various chamber methods such as infra-red gas analysers (IRGA) or using alkali to trap CO₂ (Kuzakov, 2006). The latter two options are usually portable whereas the former are normally fixed in place. A complete account of currently available measuring systems is shown in Figure 1. Minirhizotrons are tubes that are usually buried in the ground and house chemical and optical sensors along with other scientific measuring equipment. Root growth can be observed through the clear plastic tube and/or CO₂ efflux can be recorded via sensors. The machine made available for this study was an EGM-4 (or closed dynamic chamber) IRGA and so the majority of literature reviewed is focused around this measuring technique.

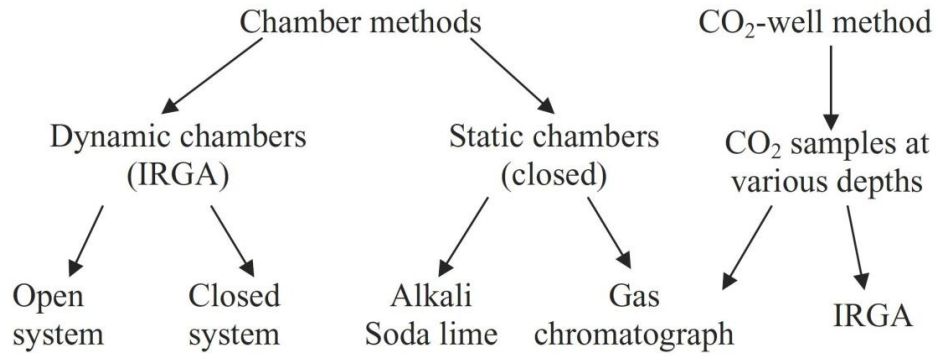


Figure 1: Different methods for measuring soil CO₂ respiration (Source: (Yiqi & Zhou, 2010))

Infrared gas analysers consist of a central unit that houses the analyser which is attached by small diameter hoses to a chamber which is placed atop the soil (or part way in). In closed dynamic chambers the air circulates in a loop between the chamber and a CO₂ detecting sensor (IRGA) shown in the schematic below (Figure 2). Two measurements are usually given;

1. The increase in chamber CO₂ concentration over time
2. A start point and end point are used to calculate the incremental CO₂ efflux over time using the formula;

(1)

$$F = (c_f - c_i)V/\Delta tA$$

Where F = soil efflux, c_i = initial CO₂ concentration, c_f is the final concentration and V is the total volume of the chamber and connecting tubes, Δt is the change in time between the two measurements and A is the soil surface area the chamber covers.

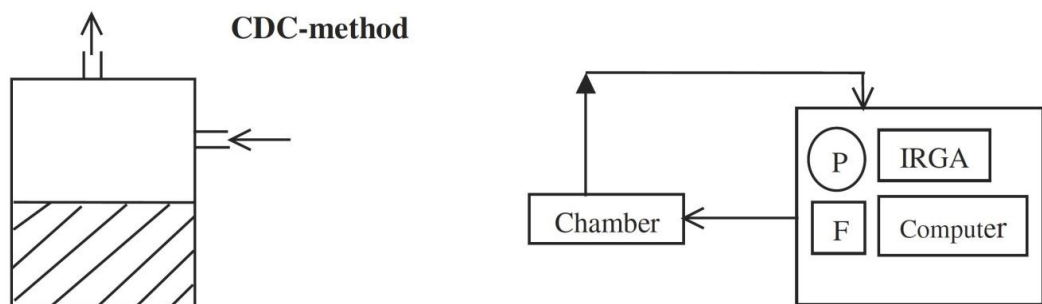


Figure 2: Closed dynamic chamber method (P: air pump, F: flow-meter, IRGA: infrared gas analyser), (Source: Yiqi & Zhou, 2010).

To allow more accurate measurements and to facilitate exact re-measurement in the future it is common practise to prepare experiment sites with plastic collars (100 cm diameter by 50 cm depth) inserted in the ground to a depth of approximately 25cm (Norman et al., 1997; Yiqi & Zhou, 2010). The plastic collars also help contain the lateral diffusion of CO₂ gases at the soil surface which can cause a loss in accuracy of measurements; this positive effect however is balanced by the unavoidable change in soil structure from the insertion of the collars (e.g. roots severed, soil structure changed) (Davidson et al., 2002). Once the collars have been placed in the soil an equilibrating period is observed, usually around thirty days (Yiqi & Zhou, 2010).

Analysis of the EGM-4 Closed Dynamic Chamber System

The operating principle of the EGM-4 is the use of a temporal gradient by building up CO₂ in the closed chamber. Once the chamber is over the soil surface the concentration of CO₂ in the chamber increases due to the release of CO₂ from the soil beneath (Yiqi & Zhou, 2010). The rate at which CO₂ levels increase is directly proportional to the soil CO₂ effluxes. The build-up of CO₂ in the chamber is quantified by the IRGA which measures absorption (and can therefore estimate the number of CO₂ molecules) and by using a precise wavelength can measure the amount of CO₂ molecules in a known volume (Lamouroux, 2008). The EGM-4 works on the principle that gases with di-atomic molecules such as CO₂ strongly absorb photons in the infra-red range; as CO₂ is passed down the sample cell it absorbs part of the infra-red and the sensor reading decreases (Yiqi & Zhou, 2010).

Soil CO₂ measuring systems that use IRGAs are commonly used with notable advantages and disadvantages. Advantages include; the machine is readily available and easy to use; there is not the calibration issues faced with other systems; the measurement time is relatively short and the system is very portable. Disadvantages noted are: the build-up of CO₂ concentration in the chamber can have a distorting effect on the diffusion gradient (which the measurements are calculated from); the measurements can take a long time and require large amounts of labour to complete adequate samples (Yiqi & Zhou, 2010).

Known Issues

Due to the many studies undertaken with an EGM-4 IRGA there have also been numerous studies comparing different machines and examining measurement issues (Davidson et al., 2002; Hutchinson & Livingston, 2001; Janssens, Kowalski, Longdoz, & Ceulemans, 2000; Lamouroux, 2008; Norman et al., 1997; Pumpanen et al., 2004).

Some of the known issues are the effect of wind on chamber measurements (Davidson et al., 2002), possible under-estimation due to chamber artefacts (Davidson et al., 2002) and placement of the chamber itself on top of the soil (Yiqi & Zhou, 2010). Chamber artefacts and biases can cause serious errors; some of the recommendations to reduce these are:

1. Limit measurement to non-windy conditions
2. Set up plastic measuring collars at least a month in advance
3. Use the same machine for all measurements
4. Use the same time period for all measurements

Other findings included improved readings using a vent attached to the chamber in conjunction with a properly designed seal (Hutchinson & Livingston, 2001) and the plastic collar inserted to an appropriate depth thereby reducing lateral diffusion. Reported under-estimation of CO₂ flux levels between -35% to +6% have been published, with highly variable results even with the same IRGA unit (Pumpanen et al., 2004). As the air is sampled by an IRGA it is mixed inside the chamber and this can cause CO₂ levels to rise giving an incorrect reading due to turbulent air (Pumpanen et al., 2004). The EGM-4 has been found to be more accurate without collars (Pumpanen et al., 2004), however, due to the often debris littered nature of forest floors plastic collars are required for this study.

Stone Density Considerations

The amount of stones present in a soil substrate influence CO₂ measurements dramatically and if possible stones (and roots) should be accounted for to allow accurate accounting of soil CO₂ levels (Rodeghiero & Cescatti, 2005). To quantify the amount of matter in soil at plot locations requires exact soil samples using volume tins. Volume tins are cylindrical tins with a known volume, from which a sample is taken and all material removed to a lab for further processing. Once all the roots, stones and any other matter are separated, their volume is calculated through water displacement and reliable estimates of carbon content per unit area can be estimated (Rodeghiero & Cescatti, 2005). A problem with this method is that the plot site is usually damaged through the destructive nature of the sampling technique (Stendahl, Lundin, & Nilsson, 2009).

Another method of determining stone densities and stone dispersion throughout an area has been developed using an auger. While not as accurate as using displacement and intensive bulk density measurements, it has been used with success in certain trials

(Laffan, 2000; Stendahl et al., 2009). This method involves drilling down with an auger until a stone is encountered, then the auger is removed and the depth recorded. Once sufficient samples have been obtained a profile of soil stone depths can be built up across the site, while this method is not as exact as other methods it allows a reasonable understanding of stone frequency underground. In the examples studied the measurements from the auger (or sometimes a rod) could be correlated with volumetric content of stones using site specific models (Stendahl et al., 2009).

ABOVE GROUND LITERATURE:

The main considerations of this review with regards to above ground measurements are the following silvicultural treatments; stocking densities, fertiliser, weed control, and use of clones. Literature researched primarily addresses trees of a younger age, as the trees measured in this study were eight years old. Points of interest include type 1 and type 2 responses evident in tree responses, stocking density interactions and growth differences between clones.

Type 1 responses result from treatments such as weed control and the application of fertiliser; in essence they can advance stand development and do not change site productivity but provide an initial gain in productivity (Mason, 1992). Type 2 responses exhibit longer term changes in site properties, such as correction of a lack of nutrients through application of nitrogen, boron, magnesium etc. (Lasserre et al., 2008; Mason, 1992; Snowdon & Benson, 1992). However, there is the possibility of generating type 2 effects through the early control of a competitive weed species, which could have impacted on mid-rotation growth rates (Mason & Milne, 1999). Attributing which effect has occurred (type 1 or type 2) can be difficult due to tree growth following a sigmoidal curve, which for certain cases makes assigning any increase or decrease to a specific time period complicated. Another option proposed instead of measuring volume based gains is the use of “time” gained to quantify treatment effects, thus, a plantation might gain five years of time and can be harvested earlier due to successful silvicultural treatments (Mason, 1992). Other suggestions in determining which effect is being observed are using a set of testable assumptions that need to be met which would allow better comparisons between experiments (Mason, 1992).

Using these definitions the silvicultural treatments applied at the Rolleston site can be labelled thus:

1. Type 1 responses – Herbicide control, fertiliser application
2. Type 2 responses – Stocking densities

Of all decisions made in the establishment of a forestry plantations, the choice of initial stocking density is one of the most important (Waghorn, Mason, & Watt, 2007). Experiments examining initial stockings and related effects have long been of historical interest to foresters, although to date there have been few studies that compare different clones at different initial stockings (Raison, Myers, & Benson, 1992) although some studies been conducted concerning the above factors and core wood stiffness (Lasserre et al., 2008).

Stocking densities have a large effect on growth rates throughout the rotation length of a plantation (Lasserre et al., 2008; Sjolte-Jorgensen, 1967), the stocking densities found at the experiment reported here were 625, 1250 and 2500 stems/ha, which will be labelled low, medium and high density respectively for the duration of this review. The different effects of stocking densities have been reported in numerous studies and there is now a large amount of knowledge on the subject (Carson, Kimberley, Hayes, & Carson, 1999; Lasserre, Mason, & Watt, 2004; Sjolte-Jorgensen, 1967; Waghorn et al., 2007).

As stocking densities are increased it is usual for stem diameter to decrease, with green crown height, slenderness and per hectare basal area substantially increasing (Carson et al., 1999); the influence of stocking densities is even more important in the current era of shorter rotation lengths (Lasserre et al., 2008). Studies of the effect of stocking on height have yielded conflicting reports, with some experiments reporting no increase in height with increased stocking (Siemon *et al.*, 1976; Hocker, 1979; Cremer *et al.*, 1982; Lanner, 1985) while others have reported an increase in mean top height (Carson et al., 1999; Maclaren, Grace, Kimberley, Knowles, & West, 1995; Mason, 1992; Sjolte-Jorgensen, 1967). The relationship between stocking intensity and tree diameter has been studied numerous times and it is well established that as stocking numbers increase, diameter growth is reduced (Waghorn et al., 2007).

Competition for resources has a considerable impact on growing trees, especially in the early establishment phase of the tree before canopy closure occurs (Lowell, 1988). Large volume gains up to eighty percent have been reported due to effective weed control as the trees have less competition for resources; in one study the main benefit

of weed control was increased solar radiation reaching the tree crown (Watt, Whitehead, Richardson, Mason, & Leckie, 2003). The majority of weed control results to date suggest weed control effects are only of a type 1 variety, however, type 2 responses have been reported in the case of weeds being controlled early that could affect stands later in the rotation (e.g. mid rotation) (Mason & Milne, 1999).

Weed control is an important component of any silvicultural regime and is very influential on young crops, when major gains over weed infested plantations are often reported (Watt et al., 2003). When weed control is not used the reductions in growth are likely to be significant; large reductions in basal area (magnitude 10 fold) where the weed species broom (*Carmichaelia*) was not controlled have been reported (Watt et al., 2003). In dry regions root zone water storage levels have been shown to be closely correlated to the presence or absence of weed competition, with water storage in weed controlled areas remaining higher much later into the summer season than areas with no weed control (Watt et al., 2003).

Fertiliser is sometimes considered to be a type 1 effect (i.e. it does not affect the plantation site productivity in the long term), however type 2 effects have also been observed for fertiliser (Mason & Milne, 1999). The best analogy describing the effect of fertiliser is similar to an acceleration through time resulting in a shorter rotation length. Once fertiliser has been applied it is rapidly taken up by the site system through the tree stand, forest floor, soil, ground vegetation and whatever else is lost through the leaching process (Miller, 1981). The nutritional stages of the tree can be separated into 3 distinct stages;

1. (Prior to canopy closure) High dependency on soil nutrients and a large response to nutrients is usually observed.
2. Responses are less likely unless there has been a thinning event and foliage growth needs to be encouraged.
3. On low nitrogen sites deficiencies can appear over a long period of time and may eventually disappear with the reduced tree demands that occur with age.

METHODS AND MATERIALS

Rolleston Experiment

The experiment used for the study reported here is located 25km southwest (E 172.345184°, N -43.617534°) of Christchurch, New Zealand, was planted in 2005 and is 7.5 ha in extent. The layout of the plantation comprises 48 plots made up of 4 blocks which are laid out in a randomised complete block with a split-split plot arrangement of factors within these blocks. Factors that are included for the current experiment are;

1. Differences in stocking (625, 1250 and 2500 stems/ha) (main plots)
2. Weed Competition (yes/no) (mid-level subplots)
3. Fertilisation (NPKS + trace elements, yes/no) (mid-level subplots)
4. 5 clones (which were randomly allocated) (lowest-level subplots)

Weed control application has been applied in two phases; initially the entire experiment was subjected to weed control with 1 m swaths of herbicide applied down planted rows. Prior to the third growing season some plots were subjected to broadcast herbicide applications to completely control weeds, while in others, weeds were left to reinvade plots. The layout of the experiment is shown in Figure 3. The different sized plot areas (small, medium and large), correspond to 2500, 1250 and 625 stems/ha respectively and the corresponding treatments (Fertiliser (F), Herbicide (H), Fertiliser and Herbicide (FH) or no weed control or fertiliser treatments are indicated in each subplot . Clone numbers are shown in these subplots, with each number corresponding to two rows of a clone (one cultivated, one not cultivated).

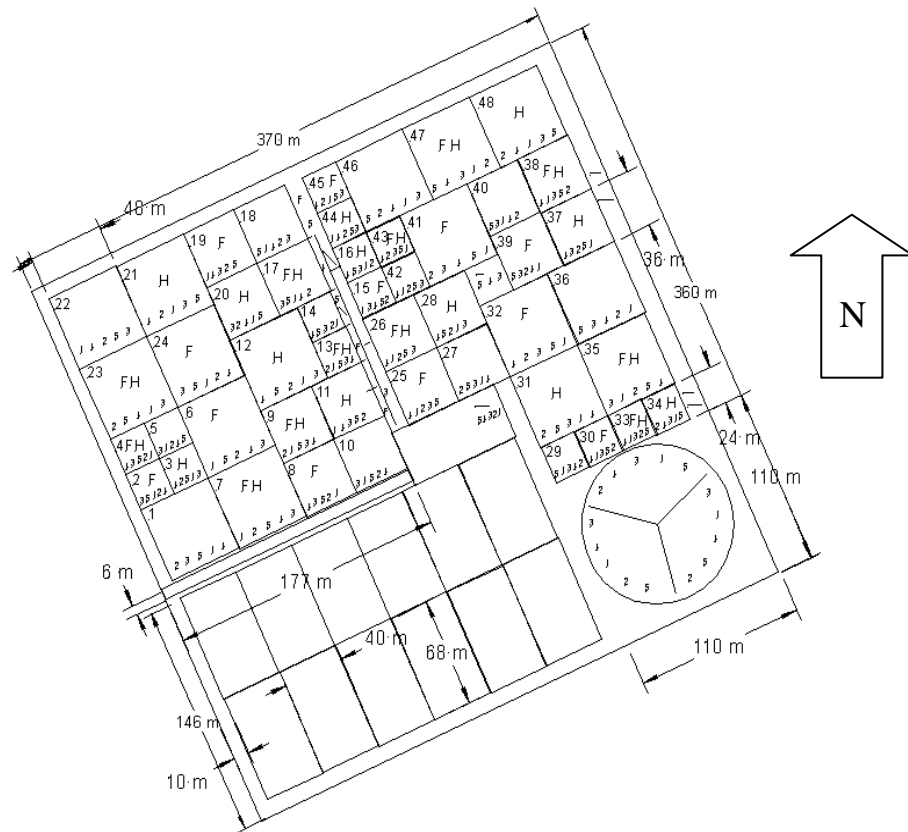


Figure 3: Overview of Rolleston Forestry Trial showing plots (1 – 48) and treatments (F=fertiliser, H=Herbicide).

Within each plot there are two different tree layouts (Figure 4), with both the 625 stems/ha and the 2500 stems/ha stocked plots having a vertical orientation and the 1250 stems/ha plot a diagonal layout. Inside of each plot is a marked square/rectangle that contains the trees measured for both the above ground and below ground measurements. In each measured area there are five clones (which have two rows apiece); all measurements for both above and below ground data come from within these areas.

The following above ground results were derived from data measured in 2012, both height and dbh of 2720 trees were recorded (ideally the 2013 tree measurements would have been used, however they were still in progress). Tree height was measured using a Haglöf Vertex IV Ultrasonic Hypsometer, which takes two measurements (the top of the tree and usually 130cm off the ground) and calculates height using the difference in angle. Diameter was measured using a diameter tape which is specially marked to allow the measurement of cylinder shaped objects.

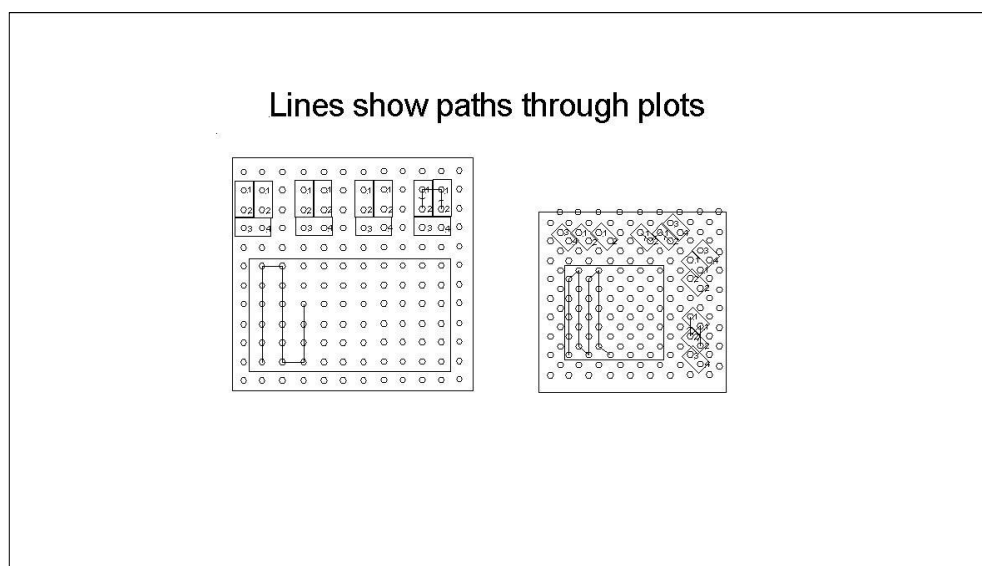


Figure 4: Schematic of Rolleston Plots (Left hand image = 625, 2500 stems/ha, Right hand image = 1250 stems/ha).

Soil Composition:

The soil at the Rolleston site is split into two classifications; seventy five percent of the soil is classified as pallic firm brown soil from the Lismoref family, with a silty loam texture, which is also well drained. The soil is classified as moderately stony with a topsoil clay range between 15 – 25 percent and has a shallow stony layer class. The depth to hard rock is classified shallow with moderate to low soil moisture. The other 25 percent is classed as Balmoralf also consisting of a silty loam texture with very stony topsoil which drains very well. The ‘S-map Online’ database from Landcare Research rates the soil types shown above with a medium confidence level and the site area is classed as a double symbol map, which relates to a confidence level of ± 20 percent of estimates.

Stone Consideration:

An obvious influence on soil CO₂ respiration is the unknown amount of stones contained below each plot location, this consideration was identified early in the experiment. In an attempt to solve the issue various experts and science reports were consulted. To accurately measure stone and/or root volumes in soil, exact volumes must be known (i.e. volume tins) and water displacement methods used. For a suitable statistical sample to be collected, samples from all forty eight plots would have been necessary. Given the time constraints faced by a short term study it was decided to use a less time consuming method for determining stone content than more time intensive methods (such as exact soil volume measures using water displacement and separating all objects in the soil).

The method chosen for the assessment of stone density uses an auger and involves drilling down until a stone is struck and then the depth is recorded. Samples were taken a distance of 30cm from a randomly selected clone in each of the 48 plots; there were four samples per collar (two in a rip line and two outside of a rip line). This gave enough information to allow a decision to be made on the influence of stone densities across the Rolleston experiment.

Water

There is well known correlation between soil water levels and CO₂ respiration levels (Edwards, 1975; Linn & Doran, 1984; Reth et al., 2005), as the soil holds more water the level of CO₂ efflux decreases. For this reason it was important to determine the water levels across the whole plantation to determine if there were any localised effects that might distort the results. To determine soil water levels the gravimetric approach was used in which the mass of the water in the soil is divided by the mass of dry soil (McLaren & Cameron, 1996). The resulting number is expressed as a percentage (i.e. soil content was 83 percent, water content was 17 percent).

(2)

$$\theta m = \frac{\text{mass of water}}{\text{mass of dry soil}}$$

Above Ground Data

To assess above ground growth rates measurements were taken at the Rolleston forest, the measurements analysed and compared were mean dbh, mean height, and basal area/ha. Measurements were of the trees located in the outlined rectangles which contain the clones and therefore are the same measurement locations for the below ground measurements (Figure 4). Other factors recorded were stocking, weed competition, fertilisation and the location of the five randomly allocated clones. The results reported here used the data from 2012 to assess the different effects of various silvicultural treatments.

The measurements for the above ground factors are taken by Marcel van Leeuwen annually and entered into data sheets which are then entered into the “R” statistical package. The means of these variables were then used for comparison purposes along

with various mixed effect linear models to determine the significance of interactions between the variables analysed. The level of significance used was $\alpha = 0.05$.

Comparison

To facilitate more timely experiments a comparison of two EGM-4's was conducted to determine if they could be used in conjunction. Using a series of eight separate collars (placed next to each other) located in similar soils and no further than 30 cm from each other recordings from the two machines were then compared. Although the readings were very similar there was enough difference in the results to abandon this option and it was decided to use one machine only. An expert on the operation of EGM-4's was also consulted (Horacio Bown) and his suggestion was to use one machine due to accuracy issues that have been reported (Norman et al., 1997).

Preparation

In order to measure CO₂ efflux the EGM-4 has a chamber that plugs into the main unit via a power socket and two gas tubes. Each measurement is taken by inserting the cylinder directly into the soil or atop of tight fitting plastic collars; for our purposes the plastic collars were chosen for three reasons;

1. To protect the cylinder from stones and general debris.
2. To allow accurate re-measurement for trials in the future. CO₂
3. To reduce CO₂ leakage

Once the collars had been placed in the soil a waiting period of one month was observed to allow the soil in and around the collar to recover from any disturbance. Any disturbance of the soil will result in a large release of soil CO₂ efflux (Yiqi & Zhou, 2010). Leaf litter or other woody debris sitting atop the collars is cut with a pair of scissors, so the amount that is over top of the collar falls inside leaving the other amount to fall outside. This is to simulate what would have landed on that particular spot had the collar not been in place (Yiqi & Zhou, 2010), this is common to most soil CO₂ studies using plastic collars although sometimes leaf litter is excluded (Kucera & Kirkham, 1971). If there happened to be live vascular flora growing in the location (most often grass at the Rolleston site), this was removed when the collars were installed as the flora would have likely influenced the soil CO₂ reading (Kim & Verma, 1992).

The location of the collars was pre-determined from the layout of the original plantings. Each clone had two collars placed at regular intervals; one collar in a rip-line and the other outside of a rip-line (Figure 4). Each collar was inserted to a depth of

around 30 mm which left a lip of 20 mm protruding from the soil allowing the SRC-1 chamber to easily be placed on top. A foam gasket was added around the SRC-1 chamber to stop any leakage from the collar-chamber seal; use of a gasket is common in most types of these experiments (Davidson et al., 2002; Hutchinson & Livingston, 2001; Yiqi & Zhou, 2010).

Measuring Soil CO₂ Efflux

The device used in this study to measure CO₂ soil respiration is the environmental gas monitor (EGM-4) made by PP Systems. In this study an environmental sensor was attached to measure temperature at each measurement location and the device was used as a closed dynamic system.

The CO₂ exchange rate is calculated by plotting the rate of change in chamber CO₂ concentration. A quadratic fit was chosen as this is recommended by the manufacturer's specifications; if the rate of CO₂ change was less than 0.2 ppm/second then a linear fit is automatically used.

At the site each collar was inserted into the soil either in a rip or outside of a rip; the exact position of this was determined by the stocking density of each plot (i.e. the position was such so that each clone was represented the same). In each of the 48 plots there are ten collars placed (two for each clone); each clone has a collar in a rip-line and outside of a rip-line.

Measurements below Ground

Over a period of five days 480 measurements were taken with the EGM-4; each measurement takes around five minutes. Every time a measurement is recorded the chamber flushes for a period of ten seconds so that it is equilibrated to the ambient atmosphere (Yiqi & Zhou, 2010). Each measurement would result in a total CO₂ and a CO₂ exchange rate recording; temperature was also recorded at each location.

Soil samples were taking using a core extractor (Diameter = 25mm) at each of the forty eight different plots (four for each plot). Each sample was bagged on the day and contained at a low temperature; at the end of each day they would be taken to the lab to be weighed (wet weight). The samples where then dried at 110°C for a period of 72 hours and weighed again (dry weight), the gravimetric water content was then calculated for each sample (Figure 12).

Due to the soil at Rolleston being classified as “stony Lismore” (“Landcare Research” 2013), it was necessary to determine if the soil stone content was homogeneous over the whole site (as the stone content will effect CO₂ respiration). By

using an auger with a diameter of 25mm and drilling into the ground at five random locations for each plot an assessment of stone ratios could be made (Figure 13).

EGM-4 Infrared Gas Analyser

The company PP Systems manufactures the EGM-4 which was used for measuring CO₂ soil respiration in this experiment. Using infra-red gas analysing techniques the EGM-4 is capable of measuring CO₂ to within a few ppm. The EGM-4 consists of a measuring unit which has ports for attaching various implements depending on the type of measurements required. For this study a chamber (Model name "SRC-1") which is placed on the soil to measure CO₂ and a temperature probe (Model name STP-1) were used. The EGM-4 gives an output that includes total CO₂ (measured in (ppm) parts per million and the CO₂ exchange or rate (measured in (g (CO₂) m² Hour)). With regards to soil CO₂ flux levels two measurements were taken;

1. The total soil CO₂ level at the time of the measurement (usually around 3 minutes) in units of parts per million (ppm).
2. Soil CO₂ assimilation over the time length of the measurement which is measure in grams of CO₂ per meter squared per minute (g/CO₂/m²/minute).

ANALYSIS

Upon receiving the raw Rolleston 2012 above ground data a quick check was made for any suspicious outliers; these were double checked with the original sheet recordings and either corrected (i.e. 2.17 → 21.7) or removed (in the case of dead trees).

Analysis was conducted using a series of non-linear mixed effect models due to the models used containing mixed and random effects. Non-linear mixed effects also allow for the specification of correlation structure amongst the explanatory variables and auto-correlation of the response variable. The response variable (dbh, height, basal area and CO₂ exchange rate) were modelled as a function of the explanatory variables and any interactions (including - stocking, fertiliser, herbicide, clone and block). Following this an analysis of variance table was used to assess any significant

interactions; the significant findings were then graphed using the `tapply` command in R.

RESULTS

The results comprise two sets of main measurements: above ground measurements of height, diameter at breast height (dbh), basal area (G) and below ground soil CO₂ efflux levels.

Above Ground Results

The analysis of mean dbh as the response variable showed a significant interaction of herbicide and clones, with the clones that received herbicide treatment recording a larger mean dbh (Figure 5). The clones that had herbicide applied showed more variance than the non-herbicide clones. The results for the interaction between herbicide and clones on the response variable dbh was significant ($p < 0.001$).

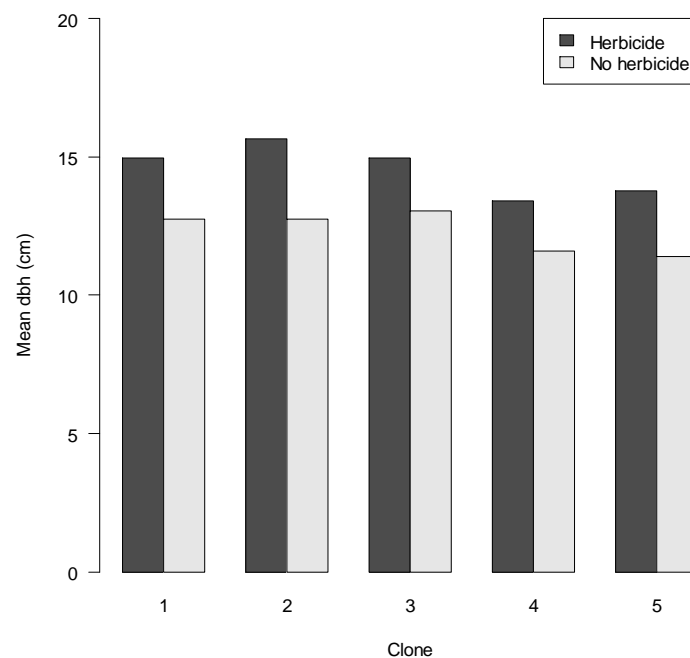


Figure 5: Comparison of clones with and without herbicide versus mean dbh.

The interaction between stems/ha and herbicide on mean dbh is shown below (Figure 6). Of interest is that stocking density was starting to impact on mean dbh with the lower stocked plots reporting larger diameters than the higher stocked areas. The effect of stocking on mean dbh shows a clear reduction in the highest stocked plots compared to the medium and low stocked plots. The interaction between stocking and herbicide recorded a significant value of $p < 0.001$.

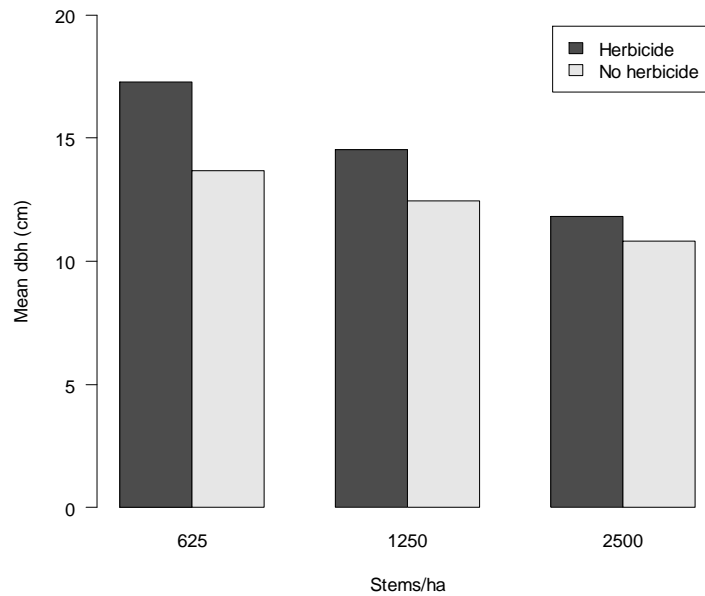


Figure 6: Stems/ha with & without herbicide versus mean dbh.

The interaction between stocking and herbicide on mean height is shown below in Figure 7. Although noticeably different, there is a lesser effect of the interaction on the higher stocked plots. Both the 625 stem/ha and 1250 stem/ha plots show nearly identical mean heights. The no-herbicide plots recorded similar mean heights across all stocking densities. The interaction of stocking and herbicide on mean height was significant ($p < 0.001$), and just as for dbh, heights were more influenced by pasture competition at lower stockings.

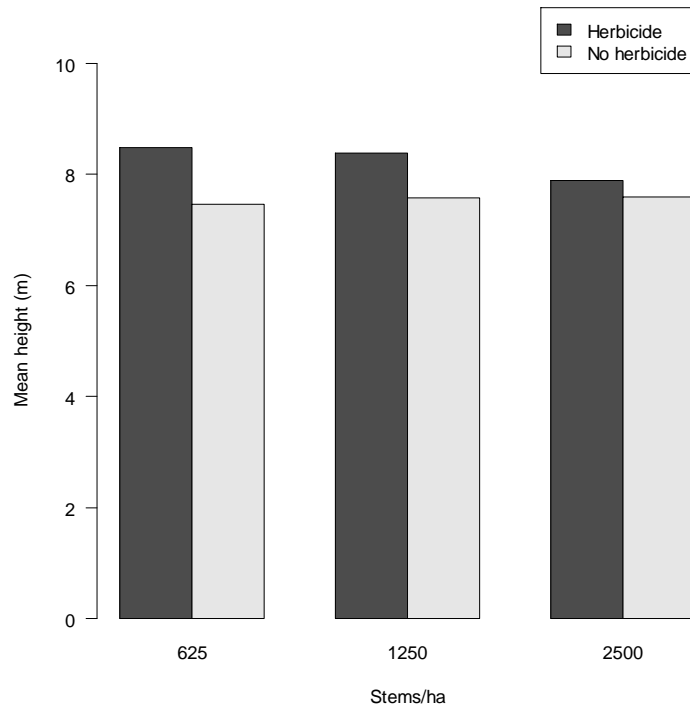


Figure 7: Interaction between stocking, herbicide and mean height.

All other results for both mean height and mean dbh reported insignificant p values. This included all interactions other than those reported above, between herbicide, fertiliser, stocking, clone and cultivation.

Basal Area Analysis

A surrogate for above-ground biomass was calculated using the formula for basal area per hectare (known as 'G') shown below. The basal area for each individual tree was calculated then scaled up using a plot specific factor (initial stocking divided by number of trees per plot); this resulted in basal area ($\text{m}^2 \text{ha}^{-1}$) for each area containing the clones.

(3)

$$G = \pi \cdot \frac{dbh^2}{40000}$$

Results of basal area as the dependent variable showed that stocking was significant ($p < 0.001$) and also herbicide was significant ($p < 0.001$). Fertiliser and all other interactions did not return significant results.

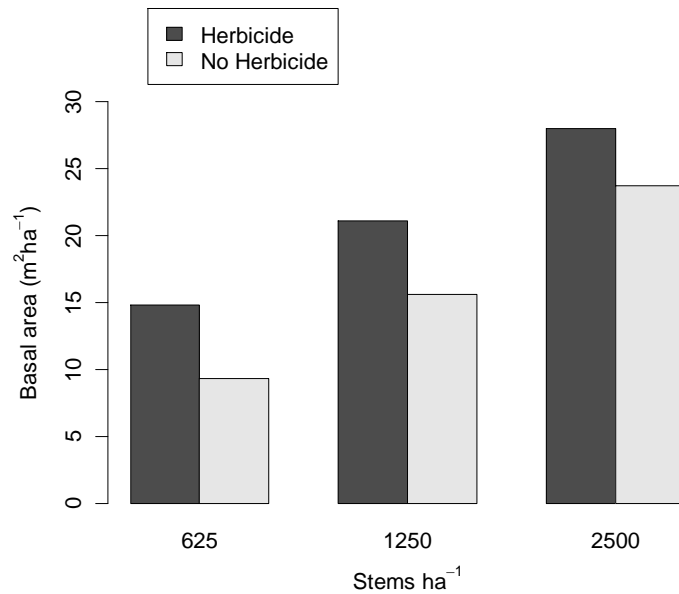


Figure 8: Above ground biomass indicator for all stocking (with and without herbicide).

The calculation of basal area was used in an attempt to connect the above ground findings with the below ground CO₂ efflux values (Figure 14). The two higher stocked plots resembled the significant findings observed in Figure 14 (i.e. as the bio-mass increases due to higher stocking the CO₂ readings increase accordingly for the ripped category that should show higher biomass due to easier root penetration). The low stocked plots did not follow this trend; the low stocked plots reported the lowest biomass (Figure 8), with the highest CO₂ flux reading for the un-ripped plot and second highest CO₂ reading in the ripped plots (Figure 14).

Below ground results

The next series of results concern the measurements taken with the EGM-4 IFGA; there are also accompanying measurements for soil temperature, soil water content (gravimetric) and soil stone content.

CO₂ readings

The first results for below ground measurements show total CO₂ across the entire Rolleston forest (Figure 9). There is a high level of heteroscedasticity for the higher values of CO₂. The overall mean was 501.46 ppm.

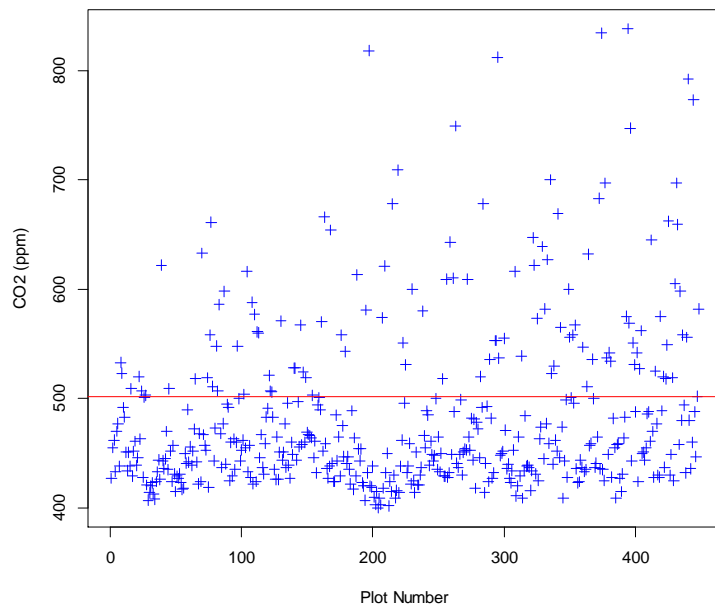


Figure 9: Total CO₂ measurements for all plots.

Carbon Soil Efflux

The assimilation of carbon flux rates for all 480 plots is shown below (Figure 10). There are some outliers however the majority of the spread is around the mean (mean = 0.21 g/CO₂/m²/sec).

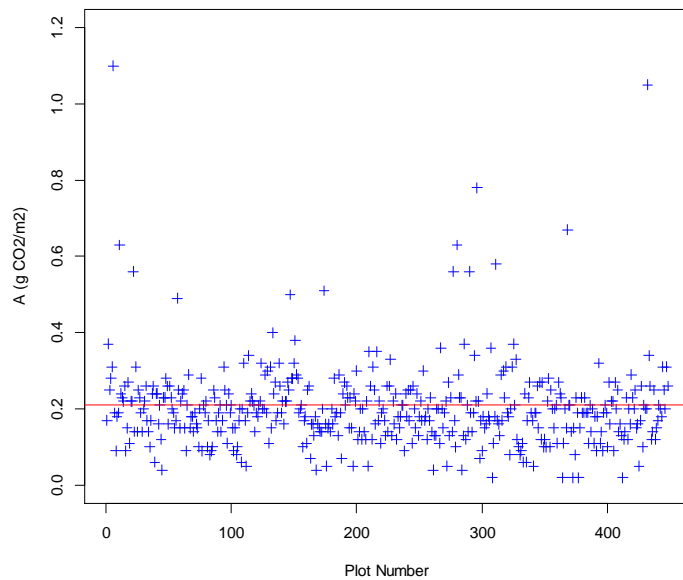


Figure 10: Assimilation of Carbon for all plots.

Temperature

A temperature probe was fitted to the EGM-4 and used to measure soil temperature at the 480 plot locations (within 10cm of a plastic collar). The results ranged between

3° and 7° Celsius (Figure 11), with measurements generally stable over the course of a day.

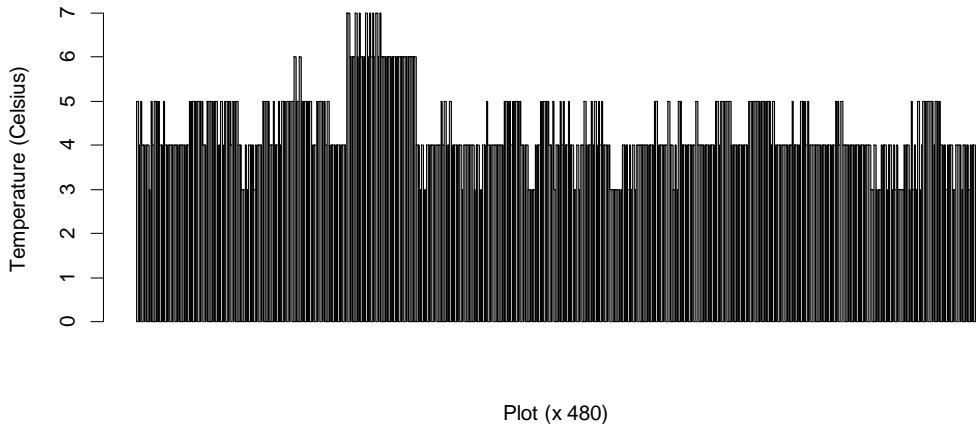


Figure 11: Soil Temperature over duration of measurements (Total plots = 480).

Soil Water Levels

Results for the soil water gravimetric content samples showed a strong homogeneity across all of the plots (Figure 12). There was an average of 15.2 percent water content across the samples with a standard deviation of 1.6 percent. The low variance across samples resulted in soil water content being treated as homogeneous across the sampled area.

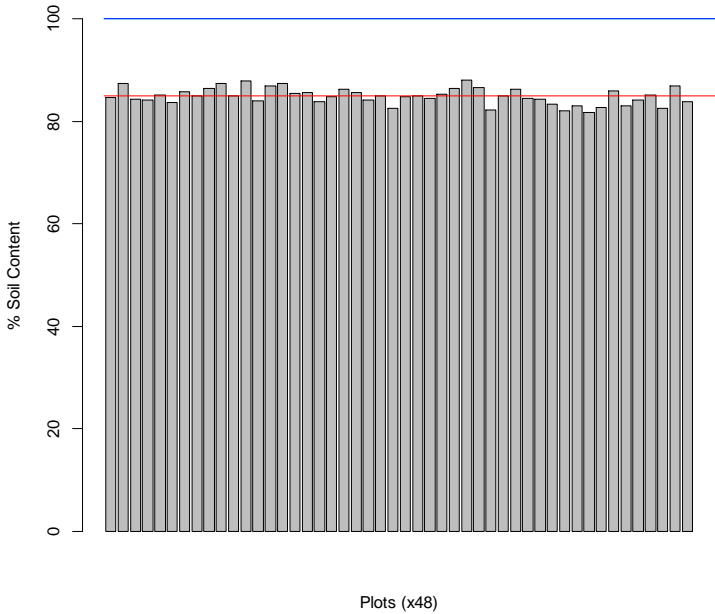


Figure 12: Soil water levels (bars show soil content after water removed, blue line = 100%, red line = average soil content).

Stone Density

Stone depth was measured four times in each plot; two times around a collar positioned in a rip and two times around a collar positioned outside of a rip. The results returned a mean of 11.79cm and a standard deviation of 5.54cm. Each measurement was 30cm from a collar; the collar was predetermined to be the third clone from the west side of each plot (This allowed a random allocation as the clones were originally allocated randomly). Stone depths were found to be highly variable across the plantation (Figure 13). Due to the high heterogeneity and variance within the sampled plots; it was necessary to factor in the effect of this finding.

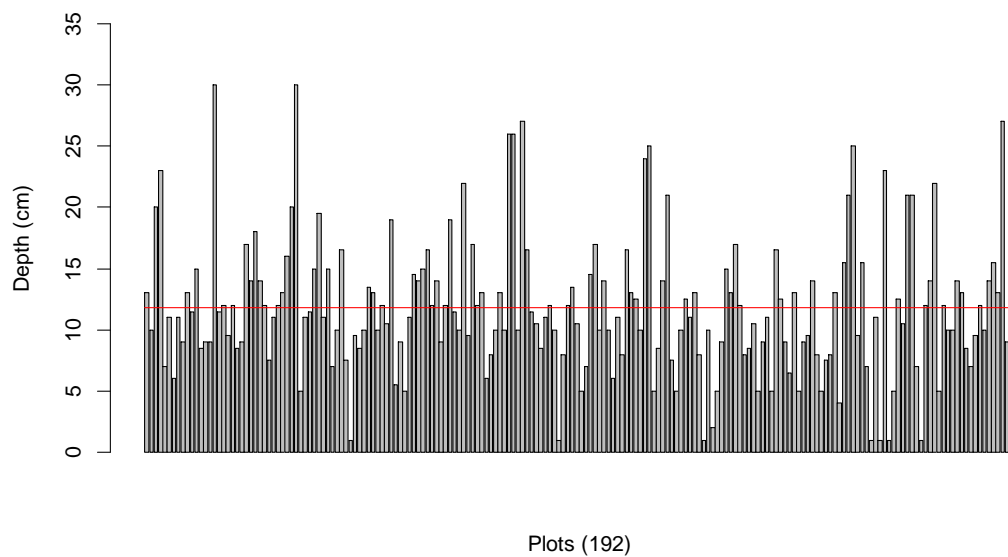


Figure 13: Stone depth measurements (192 plots- red line represents the mean of 11.86 cm).

Soil CO₂ Exchange Rate

The main finding from the below ground CO₂ measurements was the significant interaction between stocking and ripping on CO₂ flux levels (Figure 14). For both the 1250 and 2500 stocked plots the mean flux level was higher in the ripped rows. In contrast to this, the lower stocked plots (625) showed the reverse trend. The significant value reported for this interaction was $p < 0.001$.

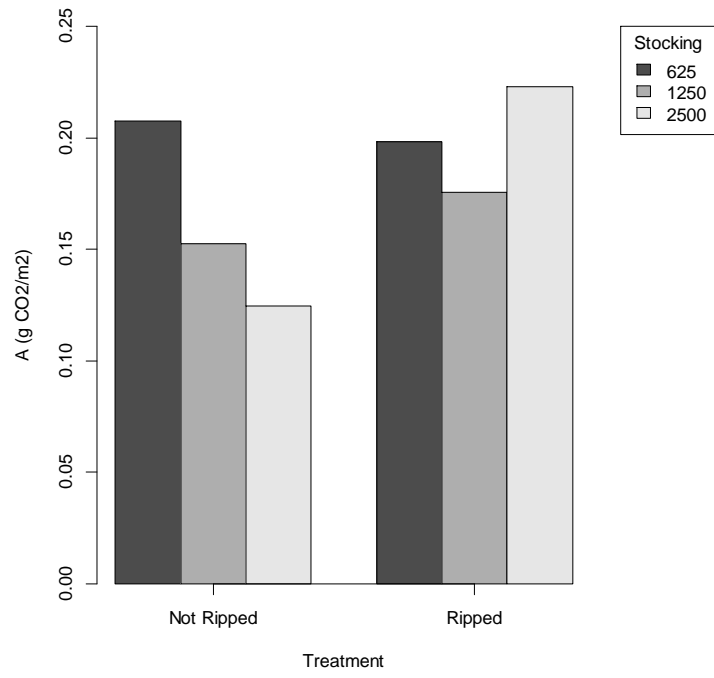


Figure 14: Interaction between ripping and stocking on CO₂ exchange rate "A".

DISCUSSION

There were significant findings for both the above and below ground measurements; however there are various issues that need addressing for future experiments of this nature. The silvicultural interactions reported are an on-going dynamic process that will change as the trees transition from juvenile to mature. Below ground measurements should prove useful if variables such as soil stone and root quantities can be quantified more accurately.

A noticeable effect was the significant herbicide interaction on clone mean dbh (Figure 5) which showed a large difference in mean dbh between the clones (~ 4cm for clone 2). With such a difference in mean dbh it was assumed that soil respiration levels might also yield similar differences between clones. It is fair to assume that if a tree is growing faster (increasing volume) than other trees then it is likely to be producing more CO₂ due to the increased growth rate, however, the results showed mixed findings with regard to this. Considering the interaction of herbicides on clones it will be of interest to keep studying this as the plantation matures.

Somewhat surprising was the small effect of stocking on mean dbh in the plots without herbicide (Figure 5). The effect of herbicide significantly increased mean dbh for the low stocked sites whereas in the high stocking sites the difference was less substantial. As the plantation matures the impact of the different initial stockings on mean dbh is likely to become more apparent in the non-herbicide treatments (Maclaren et al., 1995). Stocking effects on below ground CO₂ soil respiration are hard to quantify due to the difficulty in determining root volumes for each particular measurement plot (Kuznyakov, 2006). If an accurate assessment of underground root volumes was carried out and then modelled for the different stocking densities then a useful correlation between root mass and CO₂ efflux might eventuate.

The interaction between stocking and herbicide on mean height also appears to build on the mixed results being reported from other studies, with the height differences being very similar between the three different stocking levels (Figure 7). There have been cases studies showing no effect (Cremer, Borough, McKinnell, & Carter, 1982; Lanner, 1985; Siemon, Wood, & Forrest, 1976) but also experiments which have found significant effects of stocking densities on mean height (Maclaren et al., 1995; Mason & Milne, 1999).

Soil CO₂ respiration levels were predictably low due to measurements taking place in the middle of winter. There are many reports on the strong correlation between air

and ground temperature with soil respiration levels (Baldocchi, Vogel, & Hall, 1997; Bouma et al., 1997; Edwards, 1975; Pypker & Fredeen, 2002; Reth et al., 2005; Wiant, 1967; Yiqi & Zhou, 2010). As ground temperatures decrease, soil CO₂ levels also decrease (Bouma et al., 1997; Edwards, 1975; Reth et al., 2005). Fluctuations of soil temperature over a day's measurements are commonly recorded (Reth et al., 2005; Wiant, 1967; Yiqi & Zhou, 2010). While this relationship was known before the trial, due to time constraints, there was no opportunity to take measurements at any other time (CO₂ measurements were taken in July when ground temperatures are approaching freezing).

Even with the low soil temperatures an adequate result was obtained which showed a significant interaction between ripping and stocking on CO₂ efflux levels (Figure 14). It is likely the low soil temperatures (Figure 11) limited the range of results that might be reported when soil temperatures are warmer; ideally a yearlong set of soil CO₂ measurements would be recorded (or at least one set for each season).

A major consideration in interpreting soil CO₂ levels at the Rolleston plantation was the variability of stone density throughout the area. While the stone depth results were not comprehensive they do illustrate the high variability across the entire site. Any future studies of soil efflux levels at the Rolleston site would benefit from an accurate assessment of stone densities, this would require multiple soil samples quantifying root volume (dry and wet), stone volume and any other debris present. With enough samples, a more descriptive account of stone and root densities should allow for more accurate measurements of soil CO₂ levels as the actual soil content would be more accurately known. To assess directly underneath the sample locations would entail the destruction of each measurement site allowing for only one measurement and potentially creating damage to root systems. This is not an option at this site.

Another interesting point is the significant finding of cultivation (ripped or not ripped) on soil CO₂ levels. This was only reported for below ground results and did not feature in the above ground analysis. As the below ground measurements took place in winter it is feasible that a study conducted in the summer might report more below/above ground significant correlations. A series of measurements for the summer period has been propositioned which can then be compared to the 2013 above ground data. Ideally both above and below ground measurements would be taken in the same year because of differences in rainfall, sunshine hours and other climatic inputs that differ from year to year.

An attempt at connecting the significant above ground data with the below ground data was made using a calculation of basal area per hectare. A strong relationship between above and below ground findings was not forthcoming for this study. This is likely due to the miss-alignment of the timing of measurements. There is more likelihood of a correlation when soil temperatures and corresponding CO₂ efflux levels are similar (e.g. mid-summer).

There is increasing interest in the amount of CO₂ flux coming from different species in different environments; however, this is the first study to look at differences between CO₂ efflux levels between *P.radiata* clones. As well as five different clones, the effects of fertiliser (with and without), herbicide (with and without), are examined for their effect on CO₂ fluxes. The effect of cultivation on CO₂ flux levels is also considered; for each clone a measurement inside and outside of a cultivation line were analysed.

CONCLUSION

The analysis of mean dbh as the response variable showed a significant interaction of herbicide and clones, with all clones responding strongly to the herbicide treatment. The interaction between stocking and herbicide was significant; the lower stocked plots reporting large gains from herbicide treatment while the lowest stocked plots showed minimal gains. The effect of herbicide and stocking on mean height was significant; both the medium and low stocked plots showing higher gains than the high stocked plots.

Herbicide and stocking were the only significant factors in the basal area analysis, with significant gains apparent across all three stocking densities. Higher stocking produced smaller dbh measurements but higher basal area/ha. From the below ground CO₂ results the only significant finding was the interaction between stocking and ripping. The medium and high stocked plots showed greater CO₂ efflux levels with cultivation, while the low stocked plots did not. Overall the results did not support a connection between above ground silvicultural treatments and below ground CO₂ efflux levels.

With increased below ground efflux levels and the corresponding larger efflux levels respired from clones, any relationships that might exist are more likely to be exposed. Combining descriptive stone density surveys, high soil temperature measurements, continuing dbh and height measurements will eventually provide an answer as to whether a correlation can be made.

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