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# Biochar application during reforestation alters species present and soil chemistry

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## Abstract

Reforestation of landscapes is being used as a method for tackling climate change through carbon sequestration and land restoration, as well as increasing biodiversity and improving the provision of ecosystem services. The success of reforestation activities can be reduced by adverse field conditions, including those that reduce germination and survival of plants. One method for improving success is biochar addition to soil, which is not only known to improve soil carbon sequestration, but is also known to improve growth, health, germination and survival of plants. In this study, biochar was applied to soil at rates of 0, 1, 3 and 6 t ha$^{-1}$ along with a direct-seed forest species mix at three sites in western Victoria, Australia. Changes in soil chemistry, including total carbon, and germination and survival of species were measured over an 18 month period. Biochar was found to significantly increase total carbon by up to 15.6 % on soils low in carbon, as well as alter electrical conductivity, Colwell phosphorous and nitrate- and ammonium-nitrogen. Biochar also increased the number of species present, and stem counts of *Eucalyptus* species whilst decreasing stem counts of *Acacia* species. Biochar has the potential to positively benefit reforestation activities, but site specific and plant-soil-biochar responses require targeted research.

Keywords: afforestation, climate change mitigation, nitrogen, phosphorus, revegetation, soil carbon, species diversity

## 1 Introduction

Reforestation plays an important role in mitigating climate change and global biodiversity loss in agro-ecosystems (George et al., 2012; Cunningham et al., 2014). It can help reclaim unproductive land (Bartle et al., 2007), and improve conservation values (Bartle et al., 2007; Lal, 2008; Smith, 2008). Afforestation of agricultural land not only acts as a carbon sink, reducing global atmospheric $CO_2$ concentrations (Johnson et al., 2007; Lal, 2008), it can also generate new income to land through carbon credits and offset schemes (Lal, 2008; Baumber et al., 2011; Schirmer and Bull, 2014).

Worldwide, reforestation is essential to tackle the negative effects of extensive vegetation clearing for agriculture. In 2000, global deforestation was estimated at being 7.3 million ha annually and is due to a complex interplay between socio-cultural, economic, political values, with the prime reason being agricultural expansion (Ghazoul, 2013). This loss of vegetation has resulted in the decline of biodiversity worldwide (Freudenberger and Brooker, 2004; Ghazoul, 2013). This is in combination with reduced and lost ecosystem services, negative impacts on climate change, and economic losses (Ghazoul, 2013). Protected vegetation reserves and preserving remnant vegetation has been used extensively to tackle vegetation loss, but this alone is not sufficient (Freudenberger and Brooker, 2004; Cunningham et al., 2008). Reforestation, along with vegetation conservation methods, has been determined to be key in the conservation and recovery of biodiversity and ecosystem functions in agroecosystems (Freudenberger and Brooker, 2004; Cunningham et al., 2014), with reforestation methods planned for particular cases to consider environmental and socio-economic needs (Bennett and Mac Nally, 2004).

One common method used in large-scale reforestation is direct-seeding (Schirmer and Field, 2002; Greening Australia, 2003), although the success of this method for large-scale reforestation can be influenced by soil conditions. On degraded agricultural lands, salinity, lack of water-holding capacity, and lack of nutrient availability all affect the success of direct-seeding methods (Bell, 1999). Soil amendments, such as biochar, composts and others, can be used to improve physiochemical properties of soils (Quilty and Cattle, 2011; Barrow, 2012), and may help overcome these limitations for reforestation success.

Biochar is effective in improving degraded soils, and is a vehicle for soil carbon sequestration (Lehmann, 2007). Biochar is produced by the pyrolysis of a traditional organic amendment, such as manure and/or wood chip, to form a high-carbon product that can be applied to soil (Lehmann and Joseph, 2009). There are overwhelming benefits of biochar application, a few of which include improved plant-available nutrients and micronutrients, increased soil carbon (C) and soil-C sequestration potential, slow release of nitrogen (N) and phosphorus (P), increased soil microbial biomass, and improved soil physical properties (Atkinson et al., 2010; Joseph et al., 2010; Lehmann et al., 2011; McHenry, 2011; Quilty and Cattle, 2011; Barrow, 2012). The effects of biochar, however, are specific to soil type, soil biota, plant species and biochar type and/or feedstock specific (Joseph et al., 2010; van Zweiten et al., 2010; Lehmann et al., 2011) and have focused on changes to soil in agronomic conditions (e.g. van Zweiten et al., 2010; McHenry, 2011; Xu et al., 2012). To the author’s knowledge, biochar has not been assessed for its effectiveness in ameliorating soils for reforestation.

In addition to soil responses, biochar can have a positive influence on plant productivity, growth, yield, and survival. Research on biochars has thus far focused on biochar and crop species (e.g. van Zweiten et al., 2010; McHenry, 2011), naturally derived charcoal in plantation and natural forest soils (DeLuca et al., 2006; Atkinson et al., 2010; Stavi, 2013), and application of wood ash derived from biomass burning in power plants to plantation soils (Stavi, 2013). This includes findings of improved productivity and performance of crop plants (van Zweiten et al., 2010; McHenry, 2011), and greater yields of agricultural crops and trees (DeLuca et al., 2006; Atkinson et al., 2010; McHenry, 2011). Biochar also has had a mixed effect on plant germination and survival. For example, both the rate and type of biochar can influence the germination of a range of agricultural species, including an increase or decrease dependant on the exact combination of char, species and soil type (van Zweiten et al., 2010; Solaiman et al., 2012; Buss and Masek, 2014). The survival of plant species, including *Abutilon theophrasti* (Chinese Lantern) and *Prunella vulgaris* (Self Heal), in human-induced saline soils significantly improved with 50 t $ha^{-1}$ biochar addition to the topsoil (Thomas et al., 2013). The noted benefits of biochar and natural charcoal for a wide variety of crop and plantation species give credence to the postulated benefits to direct-seeded tree species. A review into the potential use of biochar in afforestation activities supports the notion that biochar has potential to benefit reforested systems, yet also highlights the need for targeted research on biochar application in reforested environments (Stavi, 2013). To our knowledge there have been no field investigations on the role of purpose-made biochar in germination and survival of common species used in reforestation, in addition to soil improvements.

Given the lack of research on biochar use in reforestation, our study examines the potential for an enriched form of biochar to have a benefit in direct-seeded plant systems, both in improving a range of soil properties, including increasing soil carbon, whilst concomitantly improving plant germination, species diversity and survival. An enriched biochar is a biochar mixed with manures, minerals and/or clays, which is then reprocessed at a low temperature (Chia et al., 2014). Field trials were established in three different locations in Victoria, Australia, with the aims to determine if:

1. Biochar improves overall total soil carbon and other key soil physiochemical properties; and
2. Number of germinants, species present, and survival of direct-seeded mixed woody native Australian species were improved with biochar additions.

This research will improve understanding of biochar as not only an ameliorant for improving soil carbon and nutritional properties, but also its potential to improve the germination and survival of key species important in reforestation.

# 2 Methods and Materials

## 2.1 Site locations

Field trial sites were established at three locations across western Victoria, Australia, covering a range of soil types, rainfall gradients and vegetation communities. The field trials were located near Milltown (38.05 S. 141.75 E), Minimay (36.691 S. 141.27 E) and Nhill (36.26 S. 141.56 E). The characteristics of each site are available in Table 1. The temperature and rainfall data was obtained from the Bureau of Meteorology (Hamilton and Nhill, Bureau of Meteorology). The soils present at the sites were classified using the Australian Soil Classification (Isbell, 1996), and this was used to determine the Great Soil Group. These sites fall within priority zones for landscape restoration activities, within the Habitat 141° project area (http://habitat141.org.au/; last accessed October 2014).

## 2.2 Site layout and preparation

Each field site comprised a 1 ha area, which was fenced to prevent livestock and kangaroo access. Each of these fenced areas contained 36 plots, all of which were 100 m long and 0.3 m wide. The width of the plots was determined by the furrow used to create the rip-line, as part of the standard approach to the direct-seeding method (Greening Australia, 2003), and nine plots were used for each treatment to reduce variation that may be associated with a narrow plot. The plots were arranged in a grid of 12 rows x 3 columns, with 3 m spacing between the plots. Treatments were randomised within blocks, with a total of nine blocks as divided by rows and columns, with four rows and one column making a block. Within each block, each plot row then received a treatment of either: control (no biochar), low (1 t $ha^{-1}$), medium (3 t $ha^{-1}$) and high (6 t $ha^{-1}$) biochar rates, completely randomised by row and column blocks.

Prior to biochar application, each plot was ripped to a depth of half a metre. This was done approximately six months prior to biochar application and direct-seeding. Ripping is performed on Australian soils to prevent fracturing of root structures when soils dry out in summer, and also allows moisture penetration to greater depth (Greening Australia, 2003). The sites were sprayed with RoundUp ® (active constituent: 360g/L present as the isopropylamine salt) approximately one month prior to spreading the biochar, and again before seeding. These chemicals were used to kill any weeds on the side, and reduce competition between weed and the direct-seeded species (Greening Australia, 2003).

## 2.3 Biochar requirements, characteristics and application

The feedstock used for the enriched biochar was a combination of Southern Blue Gum fines (*Eucalyptus globulus*) mixed with small amounts of chicken manure (10%), and follows the method of Chia et al. (2014). Using this blend, a low and high temperature biochar was produced, as well as a high temperature biochar made just from the Southern Blue Gum fines. This made a total of three biochars that were then used to make one blend used in this research. The low temperature biochar was produced at 350 – 400 °C and the high temperature char was produced at 500 °C. Phosphoric acid (10%, 1:1 solution to biochar) was added to the biochars to oxidise the surface of the biochar whilst concomitantly stabilising carbonyl groups and improve loss of H from the biochar surface (Chia et al., 2014). A final biochar blend, using the three biochars, was produced which possessed an Hydrogen to Carbon (H/C) ratio of 0.697, which is consistent with International Biochar Initiative guidelines of an H/C ratio <0.7 (International Biochar Initiative, 2013). Quantities used to make the final biochar blend used in this trial were: 90 kg of high temperature biochar with no added chicken manure, 457 kg high temperature biochar with added chicken manure, and 966 kg of low temperature biochar with added chicken manure. Biochar was chosen as an amendment due to its known value in C-sequestration (Lehmann et al., 2011), its reappropriation of locally produced waste products, and its potential to stimulate germination (van Zweiten et al., 2010; Solaiman et al., 2012; Buss and Masek, 2014).

Three samples of the one blended biochar were sent for full analysis as per Routine Agricultural Soil Analysis - Australian Reams/Albrecht Testing at the Environmental Analysis Laboratory, Lismore, New South Wales, Australia (Table 2). Analysis or calculations included: pH and electrical conductivity (EC) (1:5 water) (Rayment and

Higginson, 1992); exchangeable sodium (ESP) (Rayment and Higginson, 1992); cation exchange capacity (CEC) (Rayment and Higginson, 1992); ammonium- ($NH_4^+$-N) and nitrate-N ($NO_3^-$ -N) (Wolf and Beegle, 2009); Colwell phosphorus (Colwell-P) (Rayment and Higginson, 1992); total carbon (% TC) and total nitrogen (% TN) on a LECO CNS Analyser; and calculated TC:TN.

The biochar was applied to the field plots using a purpose-built mechanical spreader attached to a tractor that dispensed a calibrated amount of biochar in a 30 cm wide band. This band was matched with the width of the rip-line prepared for direct seeding. The biochar was then incorporated into the surface soil (top 10 cm) using a pasture harrow. Plots designated as a control were ripped and harrowed in the same manner as the biochar plots to ensure uniformity of soil disturbance. The biochar was applied to the sites under favourable calm and dry weather conditions in September/October 2012.

### 2.4 Plant selection, seeding and monitoring

Species seed mixes varied according to site, and were made up of a mixture of indigenous trees and shrubs representative of the vegetation assemblages associated with these landscapes. The full species selection for Milltown and Minimay is listed in Table 3. Nhill has not been included, as there was no germination at the site throughout the 18 months of this research, due to localised extreme drought conditions. Following application of the biochar to the soil, a suite of indigenous species (Table 3) was direct-seeded into each plot line that had been previously ripped and received one of four biochar treatments. The equilibration period between applying the biochar and direct-seeding was 7 – 20 days. Seeding was undertaken using a proprietary single-row seeding machine (Burford Seeder, Rod Burford, Australia), which is designed to dispense a measured amount of seeds along each plot’s existing furrow line using two seed boxes – one for larger and one for smaller seeds. The amount of seeds used (Table 3) was based on recommended application rates for this region (Greening Australia, 2003).

The monitoring of germinants, species diversity and survival was undertaken using a transect method along each of the sites’ 36 plots. A transect aligned along each plot was marked at 10 m intervals. The first 10 m and last 10 m of each plot transect was omitted from survey. Three surveys were taken along each 100 m transect at 10 m, 50 m and 80 m respectively from the start of the plot. The survey strategy is based on obtaining an adequate number of seedling measurements across each plot, and each site, and is determined by the expected number of germinants as a function of the rate of seed dispensed per linear kilometre of seed line (Table 3). The number of seedlings that appear early in a direct-seeding site can be in the order of 5000 to 10000 stems per hectare (Heydenrych and Ten Seldam, 2011). Germinants in each plot were divided by the length of the survey transect to standardise the number as germinants per metre (m). Milltown and Minimay were monitored every two months for the first six months, starting four weeks after seeding, then quarterly to measure germination and survival of seedlings. The number of germinants, and their classification (to genus or species level), were also recorded.

### 2.5 Soil sampling and analysis

Prior to biochar spreading, three bulk density (BD) cores (0-10 cm) were taken at all sites. The BD was 1.03, 1.18 and 0.97 g $cm^{-3}$ at Nhill, Minimay and Milltown respectively. Soil sampling was undertaken at time zero (T0), to provide baseline soil carbon analysis of biochar-incorporated soil and controls (no biochar) prior to seeding, followed by soil sampling undertaken at ~6 months (T6) and ~18 months (T18) into the trial. Soil was sampled from the 0-10 cm horizon. Ten samples were taken from each plot and combined into one composite sample, giving a total of 36 composite samples for each site.

Samples were submitted for full analysis as per Routine Agricultural Soil Analysis - Australian Reams/Albrecht Testing at the Environmental Analysis Laboratory, Lismore, NSW, Australia, as per the above methods. The TC was also standardised as g-C $m^{-3}$ using the TC (%), BD (as above), and a depth of 10cm. This same formula was also used to: a) predict the contribution of TC with biochar addition, b) predict the TC of soil with biochar addition, as the control soil TC plus predicted contribution from biochar (a), c) the predicted increase in TC relative to the control (%), d) the actual increase in TC with biochar addition (using TC in Table 4), and e) the actual increase in TC compared to the control (%).

## 2.6 Data analysis

Due to the sites having different soil and climatic characteristics, all three of the sites were analysed separately. A Repeated Measures Restricted Maximum Likelihood (RM REML) were used to understand: a) if biochar application affected total germination density (germinants per m), number of species present, or germinant density per m of an individual species for Milltown and Minimay, and b) if biochar application affected key soil physiochemical properties (pH, EC, ESP, ECEC, $NH_4^+$-

N, $NO_3^-$ -N, Colwell-P, %TC, %TN, and TC:TN) of all three sites. All replicates (36 on each farm) were given unique identifying numbers and these were used as the subjects, with repeated measures undertaken using time as a covariate. For soil analysis, Minimay and Nhill have only two time points, 0 and 18 months. Milltown has three time points, 0, 6 and 18 months. Milltown and Minimay were the only two sites with germination, with seven sampling times for Milltown and six for Minimay recorded. Nhill was not analysed for germination, as none occurred. For the model, the fixed factors were rates of biochar (0, 1, 3, and 6 t $ha^{-1}$) and random factors were designated as the interaction between row and column location of the samples. Where P-values were significant, F-values were checked against the appropriate orthogonal contrast, and main effects pairwise comparisons using Least Significant Difference (LSD), and confidence intervals of 95%.

Survival was not recorded on individual trees at each time point. Instead, percentage loss of germinants was calculated. This was calculated as: the final survey of germinants (stems per m) - the first survey of germinants (stems per m) divided by the first survey of germinants (stems per m) and multiplied by 100 as % stem loss. To determine if there was a decrease in % stem loss with biochar, the loss was analysed via Analysis of Variance (ANOVA) with fixed factors being biochar rate, and random factors being the interaction between the block design (row by column). Where P-values were significant, F-values were checked against the appropriate orthogonal contrast, and main effects pairwise comparisons LSDs, and confidence intervals of 95%. All data analysis was undertaken using SPSS Version 21 (IBM, USA).

# 3 Results

## 3.1 Soil characteristics

### 3.1.1 Nhill

Plant available (Colwell) P, TC and TN increased significantly ($P < 0.05$) with biochar addition (Table 4). In contract, the soil pH, EC, ESP, ECEC, $NO_3^-$ -N, $NH_4^+$-N and C:N did not change in response to biochar addition ($P>0.05$) (Table 4). Specifically, where 6 t ha$^{-1}$ of biochar were added to the soil, there was higher Colwell-P compared to 0 t ha$^{-1}$. With pairwise comparisons, there were significant increases in TC and TN with 6 t ha$^{-1}$ of biochar relative to those with 0 and 1 t ha$^{-1}$. Further, the predicted increase in TC relative to the control (Table 5), and the actual increases with each rate of biochar addition are all very similar. The exception is 6 t ha$^{-1}$ of biochar, where the actual increase was slightly lower at 15.6% compared to the predicted of 18% increase in TC.

### 3.1.2 Minimay

There was a significant difference ($P < 0.05$) associated with biochar application for $NH_4^+$-N, EC and $NO_3^-$ -N (Table 4). In contrast, here was no difference in soil pH, ESP, ECEC, Colwell-P, TC, TN and C:N associated with biochar addition (Table 4). Specifically, with pairwise comparisons, EC was higher in plots with 6t ha$^{-1}$ of biochar relative to those with 0 and 1 t ha$^{-1}$, and higher $NO_3^-$-N with 6 t ha$^{-1}$ biochar compared to 0, 1 and 3 t ha$^{-1}$. There was a decreasing trend in $NH_4^+$-N with greater use of biochar, with 3 and 6 t ha$^{-1}$ having similar values of $NH_4^+$-N compared to each other, and less with 0 and 1t ha$^{-1}$ (Table 4). The comparisons of predicted and actual increases in TC with biochar addition (Table 5) were inconsistent. The 1 t ha$^{-1}$

application rate actually showed a decrease of -4.9 % in TC relative to the control when there should have been a 1.6 % increase. The 3 and 6 t ha$^{-1}$ application rates did result in actual increases in TC, close to the predicted values (Table 5).

### 3.1.3 Milltown

With biochar addition, there was a significant difference ($P < 0.05$) in EC and Colwell-P (Table 4). However, there was no difference in soil pH, ESP, ECEC, $NO_3^-$-N, $NH_4^+$-N, TC, TN and C:N associated with biochar addition (Table 4). In particular, there was higher EC and Colwell-P in plots with 3 and 6 t ha$^{-1}$ of biochar compared to those with no biochar. The predicted and actual increase in TC with biochar addition (Table 5) was also inconsistent across biochar application rates. Both 1 and 3 t ha$^{-1}$ had actual increases in TC that surpassed the predicted increase, but 6 t ha$^{-1}$ had a lower increase of 4.3 % relative to the predicted value of 5.8 %.

## 3.2 Number of germinants, diversity and survival

There were significant differences ($P < 0.05$) between biochar application rates for the number of species present, and the number of germinants per metre of *Acacia melanoxylon, A. paradoxa, Eucalyptus viminalis* and *E. ovata* at Milltown (Table 6), and *Dodonea viscosa* at Minimay (Table 7). With 6 t ha$^{-1}$ of biochar, the number of species present was higher, relative to all other rates of biochar. There were fewer germinants per m of *A. paradoxa* with 3 t ha$^{-1}$ of biochar compared to the control, and fewer germinants per meter of *A. melanoxylon* with 3 and 6 t ha$^{-1}$ compared to no biochar treatment. *Eucalyptus viminalis*, however, had significantly greater number of germinants per m with 3 t $ha^{-1}$ compared to 0 and 6 t $ha^{-1}$, and *E. ovata* had more germinants per m with 6 t $ha^{-1}$ than all other treatments. At Minimay, for *D. viscosa*, the model revealed a significant difference between germination for particular rates of biochar application. This, however, is most likely a Type I error, due to high skew in the data from absence of germinants in some transects, and so should be treated with due caution. Biochar had no effect on decreasing the percentage loss of germinants at either site (Table 8).

# 4 Discussion

## 4.1 Soil carbon and chemical properties

The significant increase in TC and TN with increasing application rate of biochar at Nhill, may be related to the low initial TC and TN content of the soil. Biochar is well known to increase soil TC and TN (e.g. Chan et al., 2008; Barrow, 2012), with increasing rates of application concomitantly increasing soil TC (Chan et al., 2008). Carbonised or pyrolised chicken manure and poultry litter wastes present in the biochar are known to increase soil TN (Chan et al., 2008; Tagoe et al., 2008). At Nhill, the largest increase in TC was 15.6% with 6 t $ha^{-1}$ of biochar, which was just under the predicted increase of 18 % (Table 5). Due to the higher TC content of the soils at Minimay and Milltown, the predicted and actual increases in TC with biochar were lower.

It is interesting that there was no detected significant increase in TC at Milltown and Minimay, despite an expected contribution of 29.1 and 35.4 g-C $m^{-3}$, similar to the 30.9 g-C $m^{-3}$ at Nhill. The differences in TC increases between sites may have been related to differences in plant growth and biological activity within sites (Joseph et al., 2010; Lehmann et al., 2011), such as positive microbial priming, where the degree and nature of priming is soil-char specific, and this may vary the C-loss as $CO_2$ within the sites (Schulz and Glaser, 2012). Considering measurements of microbial respiration and dissolved organic carbon in future biochar studies will help determine C balance in these environments. Other variation in TC may be due to erosive loss of biochar, over or underestimate due to application and sampling methods, or differences in TC that are within analytical error range.

Furthermore, application rates of biochar for Minimay and Milltown were possibly too low to see a significant increase outside of natural variation, as the predicted rates were only 9 and 6 %. To measure a significant increase in TC, an application rate of biochar that increases the soil TC by a predicted amount of 18 % or more is likely required for the Minimay and Milltown sites, as demonstrated at Nhill. Therefore, application rates of 12.1 t $ha^{-1}$ and 18.6 t $ha^{-1}$ would be necessary to produce a statistically significant 18 % increase.

Plant available (Colwell) P and EC was altered with biochar application, but this change was site dependent. The biochar had a very high Colwell-P and a moderate EC, which is likely to be due to the chicken manure added into the feedstock, and phosphoric acid used in the charring process to produce an enriched biochar (Chia et al., 2014). Chicken manure is known to have a high P content, and a high EC depending on feed given to the chickens (Cameron et al., 1997). Poultry litter biochar is known to have a high available P content, and is known to increase available P and EC in soil (Chan et al., 2008). The relative differences in Colwell-P between the sites and by rate of biochar may be due a range of different conditions, including P sorption properties of the soil (McDowell and Condron, 2001); binding potential of the biochar (Barrow, 2012); and enhanced soil biological processes with biochar application (Steiner et al., 2008a). Similar to Chan et al. (2008), our research also found increasing Colwell-P with increasing rates of biochar applied. Although the EC increased, it was no more than 0.02 dS $m^{-1}$, and unlikely to have a negative biological impact.

Interestingly, $NO_3^-$-N and $NH_4^+$-N at Minimay was influenced by biochar addition, and may be the result of several different mechanisms. Poultry and chicken manure char known to increase soil N (Chan et al., 2008; Tagoe et al., 2008), and this biochar had a very high $NO_3^-$-N content and low $NH_4^+$-N. Therefore, it is unsurprising that $NO_3^-$-N increased relative to the control. This effect, however, only occurred at one site out of the three. At Minimay, the increase in $NO_3^-$-N also came with a decrease in $NH_4^+$-N in the presence of biochar. This indicates a potential site-specific biological interaction between soil and biochar, and a greater net conversion of $NO_3^-$ -N with the biochar addition at a rate of 6 t $ha^{-1}$. Improved N cycling efficiency and N fixation has also been found to occur in other biochar studies (DeLuca et al., 2006; Steiner et al., 2008b) and in this case the contribution and influence of N cycling and N fixation may be related to a site-specific biochar-biological interaction. These interactions have been noted as inconsistent across sites and biochar types (Berglund et al., 2004). The biological fixation of N and cycling of N in soil with biochar addition is an area that is widely regarded as requiring further research (Berglund et al., 2004; Atkinson et al., 2010) and this work further supports that more research is needed.

## 4.2 Plant germination, survival and species present

Although there was germination at Minimay and Milltown sites, there was also a decline in survival over time. The loss of germinants was due to unusually dry climatic conditions during establishment in the summers (December – March) of 2012-2013 and 2013-2014, with a rainfall of 56.5 mm and 88.6 mm compared to a normal average of 137.7mm (Hamilton Airport, Bureau of Meteorology). Despite these adverse conditions, the findings in this research highlight the positive influence of the addition of biochar on direct-seeded species.

The changes in the number of species present and their individual density at Milltown may be the result of competition between species with increased available nutrients. There was a greater number of species present with the highest biochar rate (6 t ha$^{-1}$). When comparisons were made for individual species, some exhibited significant different increases and decreases in density dependant on the rate of biochar application. Unique plant-soil responses with biochar additions are not uncommon and many authors have demonstrated distinct and different trends for different biochars across a range of sites and plant species (e.g. Chan et al., 2008; van Zweiten et al., 2010; McHenry, 2011). The increased availability Colwell-P at Milltown, however, likely increased *E. ovata* and *E. viminalis* density, as P fertilisation is known to improve *Eucalyptus* growth in several species (Hunter, 2001; Graciano et al., 2006). In mixed species plots, the water and nutrient availability is known to alter competitiveness of individual species (Forrester et al., 2005), and the higher Colwell-P with biochar addition may have altered the competiveness of *E. ovata* and *E. viminalis* relative to the *Acacia* species.

As the effect of biochar is rate and species-specific, biochar is likely to be creating conditions where particular species have optimum growth and survival parameters as matched with biochar application. Therefore, understanding the requirements of each individual species in the seed mix and their response to biochar, as well as competitive interactions, is essential in the prediction of changes to the number of species present with biochar addition on a site-by-site basis. Despite this, if the biochar is being added to the site at a rate of 1 - 6 t ha$^{-1}$ as to increase C-sequestration, it is unlikely to have a negative effect on overall seedling survival. This application rate may positively influence the number of species present, reducing density of some species and increasing others.

## 5 Conclusion

This is the first study to find that biochar, as an enriched form, positively influences success of direct seeded reforestation. This includes an increase in the number of species present as germinants from a direct-seeded mix, as well as soil carbon, available nitrogen and phosphorus, and EC. Responses to biochar were site and species-specific. The apparent influence of biochar application rate on the number of species present may have a flow on effect to final site biodiversity and ultimately upon the overall C-sequestration of agroforestry plots. Biochar addition to sites should always consider the soil chemistry of the site, especially the TC content, and the required rate of biochar needed to significantly increase soil C and other nutrients. Furthermore, targeted research on common reforestation seed mixes and N cycling with biochar application is required to understand precise influences of biochar on plant diversity and nutrient availability in direct-seeded plots. The outcome of this study shows that biochar does have a positive benefit in enhancing outcomes for reforestation, and can help to improve both biodiversity and soil carbon sequestration targets.

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