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Changes in δ^{15}N in a soil – plant system under different biochar feedstocks and application rates

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Abstract

The application of biochar in soils has been hypothesised to improve soil quality while enhancing carbon (C) sequestration. However, its effect on nitrogen (N) dynamics in the soil – plant system is still not fully understood. In the present work, N isotope composition ($\delta^{15}N$) was used to facilitate the understanding of the processes involved in the N cycling when biochar is applied. We evaluated, through a wheat pot trial, the effect of different application rates of two types of biochar produced from jarrah and pine woodchips on the wheat biomass at harvest, and on the soil and plant C and N contents and $\delta^{15}N$. In addition, the potential benefit of using nutrient-saturated biochar for the soil – plant system was also investigated. Whilst biochars produced from different feedstocks had similar effects on soil and plant nutrient contents, they induced differences in wheat grain biomass and plant $\delta^{15}N$. The effect of the biochar application rate was more pronounced and at rates higher than 29 t ha^{-1}, the application of biochar decreased grain biomass by up to 39% and potentially increased N losses. Isotopic analyses indicated that this acceleration of N dynamics had probably occurred before the stage of wheat grain formation. The application of nutrient-enriched biochar resulted in an improved wheat grain production, most likely due to the enhanced nutrient availability, and in reduced N cycling rates in the plant-soil system, which could offset the competition between biochar and plants for nutrients and decrease adverse environmental impacts due to N losses.

Keywords: Jarrah biochar; pine biochar; nutrient enrichment; nitrogen cycling; isotopic composition

Introduction

Biochar is a carbon (C) rich substance that is created by heating organic matter under low oxygen conditions and is applied to soils to improve its fertility (De Pasquale et al. 2012). Biochar can be made from a wide range of feedstocks (Dai et al. 2013), and has been recently proposed as a means to condition soil while reducing the global C footprint through soil C sequestration (Lehmann 2007; Barrow 2012). Adding biochar to the soil decreases soil bulk density whilst increasing its cation exchange capacity, nutrient cycling, and its ability to retain plant available water (Laird et al. 2010; Anderson et al. 2011). This observation had led to an expectation that biochar addition would enhance plant nutrient uptake and water use, and ultimately stimulate plant productivity while reducing dependence of agro-systems on fertilisers (Glaser et al. 2002; Lehmann 2007). These high expectations have been mitigated by previous reports showing that the effects of biochar on soil fertility and crop yields depend on the biochar feedstock and production process (Jeffery et al. 2011). Different biochar feedstocks have different effects on soil pH, nitrogen (N) mineralisation and water holding capacity (Struebel et al. 2011), which are further influenced by biochar application rates (Glaser et al. 2002). High rates of biochar application can disturb the biological balance of nutrients by increasingly modifying soil pH, enhancing N immobilisation or stimulating the loss of native soil organic matter (Wardle et al. 2008; Van Zwieten et al. 2010; Ippolito et al. 2012), thereby negatively impacting crop yields. The total nutrient content of biochar and the proportion of it made readily available to the plants are largely dependent upon the type of biochar and pyrolysis conditions (Mukherjee and Zimmerman 2013), which explains the contrasting results that have been obtained for enhancement of crop productivity by biochar addition. These results range from negative or neutral effect on crop yield (Wang et al. 2012; Güereña et al. 2013), unless combined with fertiliser application, to increases in plant biomass (Zhang et al. 2011; Rajkovich et al. 2012).

The influence of biochar application on N cycling is especially complex (Struebel et al. 2011). Biochar addition has been reported to increase N mineralisation (Berglund et al. 2004), to decrease it (Deenik et al. 2010), or to have no effect on N availability for plants (DeLuca et al. 2006). Soil amendment with biochar can also lead to N immobilisation (Bruun et al. 2012), can modify nitrification rates (DeLuca et al. 2006; Wang et al. 2011) and potentially adsorb N compounds (Lehmann et al. 2003; Liang et al. 2006), thereby reducing NH_4^+ leaching (Glaser et al. 2002). On the other hand, negative yield responses with biochar application in some experiments have been attributed to a decline in available NH_4^+ (Deenik et al. 2010). The mechanisms through which biochar acts on N availability, and hence plant productivity, remain relatively unclear, although they seem to be principally mediated through soil microflora (Anderson et al. 2011; Güereña et al. 2013).

The use of N isotope composition ($\delta^{15}N$) to examine more closely the interactions between biochar and its environment would allow researchers to gain insights into N dynamics (Taghizadeh-Toosi et al. 2012). Isotopic analyses have been already used to unravel the mechanisms governing N cycling in plant – soil systems but they have rarely been applied to biochar studies (Spokas et al. 2012). Soil $\delta^{15}N$ provides an indication of N transformations in non-leguminous plants, since discrimination against the heavier ^{15}N isotope during microbial assimilation, soil N mineralisation and nitrification, leaves the residual NH_4^+-N in soils enriched in ^{15}N (Högberg 1997). Denitrification and volatilisation can also lead to soil $\delta^{15}N$ enrichment (Pu et al. 2001). Ultimately, the mechanisms influencing soil $\delta^{15}N$ also influence plant $\delta^{15}N$ via their N uptake (Ibell et al. 2013), which makes $\delta^{15}N$ a good tracer of N transformations in the environment. A better understanding of the effect of biochar application on N cycling rates and $\delta^{15}N$ is thus clearly needed. The objective of this study was therefore to investigate the changes in soil and plant $\delta^{15}N$ under controlled experimental conditions when different biochar feedstocks and application rates were used. We hypothesised that biochar feedstock and application rates would influence N transformations and availability, and hence soil and plant N content and $\delta^{15}N$.

Since the use of biochar is often recommended in conjunction with the application of a fertiliser (Steiner et al. 2007), we also tested if saturating biochar in a nutrient-rich solution would affect $\delta^{15}N$ and enhance N availability, thereby improving plant growth.

Methods

Description of the pot experiment

The experiment was designed to investigate the effect of different application rates of two types of biochar on wheat (*Triticum aestivum* L.) and on soil N content. Plants were grown in a Vertosol soil collected at Bindi Bindi, in the Wheatbelt region of Western Australia (coordinates 30°37'S, 116°28'E). The sampling soil depth was 10 cm, and its texture was 50%, 22% and 27% of clay, silt and sand respectively, with 1% of organic matter. Its bulk density was 1.13 g cm^{-3}.

We used a hardwood biochar produced from jarrah (*Eucalyptus marginata* Donn ex. Sm.) and a softwood biochar produced from pine (*Pinus radiata* D. Don) to amend the soil. The woodchips were produced from a pine plantation near Bunbury, about 200 km south of Perth, Western Australia. The biochars were produced by ANSAC (www.ansac.com.au) using ANSAC HK indirectly fired kiln, at a maximum pyrolysis temperature of 700 °C and a residence time of 20 minutes under depleted oxygen environment. Biochars were then cooled indirectly to below 90 °C before being discharged in air. Biochar pH measured in water (1:5 ratio) was 9.22 for jarrah biochar and 8.92 for pine biochar. Different quantities of either jarrah or pine biochar (0, 50, 100 and 200 g per pot) were placed in 4 L pots and mixed with soil in order to reach a total volume of 3.5 L, with four replicates per treatment. Resulting rates of biochar application were 0, 1.3, 2.6 and 5.3% (w/w), and corresponded to rates of 0, 14, 29, and 60 t ha^{-1} respectively. Furthermore, we included two additional treatments consisting of applying 100 g (2.6% or 29 t ha^{-1}) of both types of biochar saturated in a nutrient-rich solution (Hoagland's No. 2 Basal Salt Mixture, Sigma) to investigate its effect on soil fertility.

Wheat seeds were placed in Petri dishes and soaked in water for two days prior to sowing in order to accelerate germination. One seed was sown per pot. Plants were watered during the whole period of the experiment and pots were kept at 80% field capacity. Plastic bags were used to seal the pots to ensure that nutrients were not leached out of the soil during watering. Plants were harvested after reaching full maturity, 105 days after planting.

Sample analyses

Soil samples were collected from each pot after harvest. Fifty grams of each soil sample were air-dried and ground to a fine powder using a Rocklabs ring grinder. Total C, total N, and N isotope composition (^{15}N) were determined for each soil sample by mass spectrometry (spectrometer GV Isoprime, Manchester, UK), following the procedure reported by He et al. (2008). Soil pH was measured in water (1:5 ratio), and hot water extractable organic C and total N were measured using a Shimadzu TOC-V$_{CSH/CSN}$ TOC/N analyser. Ammonium–N and NO_3^-–N were determined following hot water extraction using a SmartChem 200 Discrete Chemistry Analyser as described in Hosseini Bai et al. (2012). Original non-modified biochar samples were also analysed by mass spectrometry to measure their total C and N contents. The BET (Brunauer–Emmett–Teller) surface area, micropore volume and average pore diameter of biochars were determined by N_2 adsorption using a TriStar 3000 analyser (Micromeritics).

Plant samples were dried at 65°C to a constant weight and the dry weights of different plant components, including grains, tillers and roots, were measured. Plant samples were then ground finely to measure total C, total N and $\delta^{15}N$ using mass spectrometry.

Statistical analyses

Variable normality was verified using Shapiro-Wilk test and homogeneity of variance was tested with Levene's test. Variables that did not meet the latter assumption were log transformed. Multiple univariate ANOVA and Tukey *post-hoc* test were used to investigate significant differences in plant biomass and in soil and plant nutrient contents among the treatments. Regression curve estimations were performed where ANOVA results were significant to predict the relationships between the rates of added biochar and the measured soil and plants variables ($n = 16$ measurements). All analyses were considered significant at $p < 0.05$. Statistics were computed in SPSS (version 19, SPSS Inc.).

Results

Biochar characterisation

BET surface area and micropore analyses showed that pine biochar presented a more porous structure than jarrah biochar. The BET surface area of pine biochar was larger than jarrah biochar (337 against 252 $m^2 g^{-1}$ respectively) and its micropore area was also greater (296 against 228 $m^2 g^{-1}$). The average pore diameter of pine biochar was larger than jarrah biochar (2.14 against 2.24 nm respectively). Moreover, pine biochar presented a higher total C content than jarrah biochar (81.5% against 74.7% C respectively). The total N content was also slightly higher in pine biochar than in jarrah biochar (0.14% against 0.11% N respectively).

Effect of biochar on soil properties

Soil analyses were carried out after harvesting, and the results are presented in Table 1. The application of non-treated biochar resulted in differences in soil pH, soil C/N ratio and soil $\delta^{15}N$. Soil pH was significantly lower in the control treatment (no biochar addition) than in the soil amended with the highest rate of jarrah biochar. Regression analyses showed that pH increased linearly with the rate of biochar addition for both types of biochar (Table 2). No significant differences were found in soil total N content among the treatments. Consequently, soil C/N, similarly to soil total C content, increased with the rate of biochar addition for both types of biochar (Table 2). No significant differences were found among the treatments for labile C and N fractions and for NH_4^+–N and NO_3^-–N (Supplementary Table 1). Soil $\delta^{15}N$ was significantly higher when 14 t ha^{-1} jarrah biochar were applied than when 60 t ha^{-1} pine biochar were added to the soil (Table 1). However, no significant relationship was detected between soil $\delta^{15}N$ and the rate of biochar addition (Table 2).

Enriching biochar in a nutrient solution prior to its application had no significant effect on the measured soil variables (Table 1).

Effect of biochar on plant biomass

Adding non-treated biochar induced differences in wheat biomass. Grain dry weight was significantly larger when 14 t ha^{-1} of pine biochar were applied than under the same application rate of jarrah biochar (Fig. 1a). Grain biomass was also significantly reduced by 36% and 39% compared to the control when 29 and 60 t ha^{-1} of jarrah biochar respectively were added to the soil. Regression analyses indicated that adding biochar decreased wheat grain dry weight linearly for both types of biochar (Table 2). On the other hand, no significant differences were found in wheat tiller and root biomass among treatments (Fig. 1b and 1c).

Saturating biochar in a nutrient solution prior to application had a positive effect on wheat grain biomass, particularly for jarrah biochar, when compared with the same application rate of non-saturated biochar (Fig. 1a). However, it did not influence wheat tiller or root biomass (Fig. 1b and 1c).

Effect of biochar on plant nutrient contents and $\delta^{15}N$

The C and N contents of the harvested biomass were analysed and the results are presented in Table 3. Generally, no significant differences were observed in the plant C content amongst treatments, with the exception of grain C content being higher when 60 t ha^{-1} pine biochar was applied than when 14 t ha^{-1} of the same biochar was added to the soil. Regression analyses showed that soil amendment with pine biochar linearly increased wheat grain C content (Table 2).

Similar results were found for wheat N contents. Whilst no significant differences were observed in grain N content following soil amendment with biochar, tiller N content was lower when 14 and 29 t ha^{-1} pine biochar were applied than under the 60 t ha^{-1} jarrah biochar treatment. Regression analyses showed that increasing rates of jarrah biochar increased tiller N content. No relationship was detected between rates of biochar application and root N content (Table 2).

The different treatments had distinct effects on plant $\delta^{15}N$. Grain $\delta^{15}N$ was higher when 29 t ha^{-1} pine biochar was applied than when the same rate of jarrah biochar was added to the soil (Fig. 2a). Similarly, the highest tiller and root $\delta^{15}N$ were found when 29 t ha^{-1} pine biochar were applied and were significantly larger than when no biochar or 14 t ha^{-1} pine biochar were added to soil (Fig. 2b and 2c). Regression analyses indicated that the application of jarrah biochar linearly decreased grain $\delta^{15}N$, but that a quadratic regression model was best fitted for grain $\delta^{15}N$ values under jarrah biochar amendment ($R^2 = 0.753$, $F = 19.775$, $p < 0.01$). Tiller $\delta^{15}N$ values under pine biochar application were also best explained by a quadratic regression model ($R^2 = 0.502$, $F = 6.551$, $p < 0.05$) whilst soil amendment with increasing rates of pine biochar enhanced root $\delta^{15}N$ linearly (Table 2).

Nutrient-enriched biochar did not have any effect on plant C and N contents, but decreased $\delta^{15}N$ in tillers and roots in the case of pine biochar (Fig. 2b and 2c).

Discussion

Biochar application, regardless of feedstock, did not produce significant differences in soil C and N pools nor in plant nutrient content. However, wheat grain dry weight was affected by the different treatments and the observed differences in plant $\delta^{15}N$ following biochar amendment showed that N dynamics were modified as well.

Our results demonstrated that the rates of biochar application used in this study had little influence on soil C and N contents or soil $\delta^{15}N$, and that the two biochars had similar effects on the measured soil parameters. The effect of biochar application rate on soil pH, total soil C content and labile C fraction found in this study are consistent with other reported findings. The addition of biochar led to an increase in soil pH, as described in Jones et al. (2012) and Nelissen et al. (2012), who showed that the higher pH of fresh biochar (9.2 and 8.9 in this study for jarrah and pine biochar respectively) increased the pH of the more acidic soil. As expected, biochar also linearly enhanced soil total C content but did not have any effect on the labile C fraction of the soil, probably due to the fact that the C provided by biochar is more chemically and biologically stable (Cross and Sohi 2011). Furthermore, the similar effects of jarrah and pine biochars on soil fertility could be explained by the fact that both biochars were produced under the same pyrolysis conditions. As Atkinson et al. (2010) summarised in their review, pyrolysis temperature influences biochar physical and chemical properties, such as functional group composition or N and P concentration, which in turn influence soil and plant nutrient contents.

Whilst significant effects of biochar application rates and feedstock on soil were scarcely observed, our results demonstrated that wheat grain biomass was influenced by the different treatments. Biochar feedstock induced differences in grain biomass, with pine biochar being more beneficial than jarrah biochar at the same rates of application, but not when compared to the control treatment. Rajkovich et al. (2012) also reported a large influence of biochar feedstock on crop yield in a pot experiment and concluded that biochar made from plant residues such as oak or pine failed to enhance plant growth significantly. Taghizadeh-Toosi et al. (2012) found that biochars with higher pH had less impact on plant growth, due to a lower adsorption of NH_3. Additionally, the high pH of biochar can further inhibit plant development and decrease plant N use efficiency (Xie et al. 2013). Jarrah biochar had a higher pH than biochar made from pine, which may explain that it reduced grain biomass when compared to the control at rates higher than 29 t ha^{-1}. Linear regressions also showed that grain biomass decreased with increasing rates of biochar application, and previous findings have attributed the negative impact of biochar on crop yields to microbial N immobilisation in the short term (Novak et al. 2010; Nelissen et al. 2012). However, N immobilisation and the subsequent decrease in N availability are usually limited to the period of initial mineralisation of the more labile fraction of biochars (Rajkovich et al. 2012). Bruun et al. (2012) reported that this initial N immobilisation did not influence the levels of soil mineral N at the end of their 65-day experiment, which concurs with the lack of effect of biochar on soil labile N pools or plant N content at the end of our 105-day study. The adsorption of soil N by biochar might also have temporarily reduced the available N for plant growth, thus causing the lower grain dry weight, although recent findings have shown that at least some of the N compounds adsorbed to biochar are bioavailable (de la Rosa and Knicker 2011; Taghizadeh-Toosi et al. 2012).

The influence of biochar on N dynamics was further confirmed by the differences observed in plant $\delta^{15}N$. For all plant components (grains, tillers and roots), plant $\delta^{15}N$ was the highest when 29 t ha^{-1}pine biochar were applied. Additional differences were observed between biochar feedstocks, as regression analyses showed that jarrah biochar decreased grain $\delta^{15}N$ whilst pine biochar increased tiller and root

$\delta^{15}N$ when more than 29 t ha^{-1} were applied to the soil. Plant $\delta^{15}N$ reflects the conditions of N nutrition where and when the plants were grown and as such relates to soil $\delta^{15}N$ status at the time of growth (Xu et al. 2003; Ibell et al. 2013). An increase in $\delta^{15}N$ therefore demonstrates an acceleration of N dynamics (Ibell et al. 2010) and could indicate an enhancement of soil microbial activity at the higher rates of pine biochar addition, which would, in turn, enhance microbial N immobilisation. Pine biochar is more porous than biochar made from jarrah wood, and these micropores provide microhabitat for soil microorganisms involved in nutrient cycling and organic matter decomposition (Barrow et al. 2012). However, the lack of impact of biochar addition on plant N content makes unlikely that N immobilisation was the main mechanism causing the observed differences in grain biomass. The differences between plant components, due to a discrimination of the lighter N isotope during N transformation and translocation, may also reveal different conditions at the later stage of grain formation. Plant $\delta^{15}N$ enrichment has been attributed to soil N losses via NH_3 volatilisation, denitrification or NO_3^- leaching, as these processes tend to increase soil $\delta^{15}N$ due to isotopic fractionation (Choi et al. 2006; Rui et al. 2011). At application rates higher than 29 t ha^{-1}, pine biochar may therefore favor N losses from the soil-plant system when compared to jarrah biochar, though most likely during plant early growth as grain $\delta^{15}N$ was not affected by pine biochar addition. Biochar amendment has been reported to enhance NH_3 volatilisation and N_2O emissions in already alkaline soils (pH = 7.9 in the control treatment) (DeLuca et al. 2009; Xie et al. 2013). By further increasing soil pH, biochar could favor the activity of denitrifying microorganisms and promote N gaseous losses (Xu et al. 2013). Coupling stable isotope studies with measurement of gaseous N emissions following the addition of biochar would therefore permit to better predict the amount of N losses from the system and to gain insights in the long-term effect of biochar addition on plant and soil productivity.

The need for experimental data that provide realistic evidence of biochar effect on soil has prompted the implementation of greenhouse experiments where the concentration of biochar in each pot can be accurately measured. However, the negative impact of biochar at high application rates on wheat productivity needs to be considered in light of the relatively short-term nature of the pot trials and more beneficial effects may be visible after a longer period of time or under field conditions. The negative effects for wheat biomass described in this study when the highest rates of biochar were applied may also be due to the smaller amounts of soil used for these treatments. In this pot experiment, we used different amounts of soil depending on the amount of applied biochar, which may have provided less nutrients to the plant and thus reduce plant growth. However, significantly lower grain biomass under high biochar application rates when compared to the control was only observed with jarrah biochar. It is therefore unlikely that a drastic nutrient reduction occurred due to the slight difference in the amount of soil and influenced biomass production. The negative effect of biochar on plant productivity shown by regression analyses may therefore be due to the reduction of the bioavailability of other macro and micronutrients that are necessary for plant growth. Phosphorus for instance is a crucial element for crop yield, and the availability of P can be influenced by biochar amendment (Cui et al. 2011). Parvage et al. (2013) showed that different rates of biochar application had different effect on soil solution P concentration and therefore on plant yield. Further work is therefore required to investigate if P deficiency could be an additional factor reducing grain weight when increasing rates of biochar are added to the soil, and in that case, if treating biochar with a nutrient-rich solution could also offset this effect.

Nutrient-enrichment of biochar prior to its application had a positive effect on grain dry weight, especially for pine biochar. Pine biochar had a more porous structure as assessed by adsorption of N_2, and may have adsorbed larger quantities of the nutrient-rich solution, although the adsorption of this solution was not directly quantified. The adsorption of soil nutrients by biochar and the following decrease in bioavailability could be offset by nutrient-enrichment of biochar, which would no longer compete with plants for nutrients. Hoagland’s solution is a fertiliser widely used in the horticulture industry to provide soils with essential nutrients such as ammonium phosphate, magnesium sulphate and potassium nitrate. Although nutrients other than N were not measured in this study, we can hypothesise that nutrient-enriched biochar helped counteract the negative effect of biochar on plant productivity by enhancing nutrient availability in the plant-soil system. An increase in nutrient availability may also indirectly improve plant N uptake, by enhancing root growth and stimulating the production of root exudates and hence the activity of soil microorganisms responsible for N mineralisation (Cornish and Raison 1977; Cesco et al. 2012). Furthermore, nutrient-enriched pine biochar decreased tiller and root $\delta^{15}N$, indicating a decrease in N cycling rates and in potential N losses from the system. Treating biochar before its application could therefore decrease denitrification and its associated risk of environmental pollution. Although it is still necessary to assess whether the positive effect of nutrient-enriched biochar remains after repeated growth cycles, this treatment constitutes an innovative agricultural management practice such as those that Spokas et al. (2012) recommend in order to fulfill the increasing demand for food while minimising negative environmental impacts.

Conclusions

The different biochar feedstocks and the rate of biochar application to soil in a series of wheat pot trials did not produce significant differences in the measured soil and plant nutrient contents. However, isotopic analyses showed that biochar amendment had an influence on the N dynamics of the plant – soil system at the time of growth and increased N losses, probably through ammonia volatilisation or denitrification, under the two highest rates of biochar application. The increase in the soil pH combined with the acceleration of N losses from the system following biochar application were likely to be responsible for the adverse effect of biochar addition on the wheat grain biomass. A decrease in the availability of other nutrients may further reinforce the negative effect of high rates of biochar application on plant productivity. Nutrient-enriched biochar offset the entrapment of N and other nutrients by biochar and should be tested both in the long-term and under field conditions to verify its potential reduction of gas emissions.

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Figure captions

Fig. 1 Effects of biochar feedstocks and application rates on wheat dry weight of a) grains; b) tillers; and c) roots. The asterisk in the biochar rates axis represents biochar nutrient-enrichment. Different letters represent significant differences (Tukey post-hoc test, $p < 0.05$)

Fig. 2 Effects of biochar feedstocks and application rates on N isotopic composition ($\delta^{15}N$) of a) wheat grains; b) wheat tillers; and c) wheat roots. The asterisk in the biochar rates axis represents biochar nutrient-enrichment. Different letters represent significant differences (Tukey post-hoc test, $p < 0.05$)

Table 1 Influence of biochar feedstocks and application rates on soil characteristics. Values represent means ± standard errors. Different letters within columns represent significant differences (Tukey post-hoc test, $p < 0.05$)

Application rate (t ha^{-1})	pH-H_20	Total N (mg kg^{-1})	C/N	$\delta^{15}N$ (‰)
0	7.9 ± 0.16 b	1.16 ± 0.02 abc	17.5 ± 0.3 b	3.23 ± 0.12 ab
Jarrah biochar:				
14	8.1 ± 0.02 ab	1.09 ± 0.01 abc	24.6 ± 1.7 ab	3.97 ± 0.27 a
29	8.2 ± 0.05 ab	1.11 ± 0.04 abc	27.4 ± 3.7 ab	3.51 ± 0.18 ab
60	8.3 ± 0.07 a	1.04 ± 0.03 bc	39.0 ± 5.5 a	3.15 ± 0.13 ab
Pine biochar:				
14	8.1 ± 0.04 ab	1.02 ± 0.04 c	21.6 ± 0.6 b	3.38 ± 0.30 ab
29	8.1 ± 0.04 ab	1.09 ± 0.03 abc	26.9 ± 1.5 ab	3.55 ± 0.22 ab
60	8.2 ± 0.04 ab	1.05 ± 0.01 bc	38.5 ± 5.1 a	2.95 ± 0.10 b
Nutrient-enriched biochar:				
29 (jarrah)	8.0 ± 0.07 ab	1.17 ± 0.02 ab	23.2 ± 3.0 b	3.16 ± 0.13 ab
29 (pine)	8.1 ± 0.02 ab	1.22 ± 0.03 a	21.2 ± 1.4 b	3.21 ± 0.07 ab

Table 2 Linear correlation coefficients (R2) for relationships between rates of biochar application and measured soil and wheat variables (n = 16). One asterisk means significance at $p < 0.05$ while two asterisks mean significance at $p < 0.01$

	Soil						Plant dry weight		
	pH	Total C [a]	Total N [a]	Soil C/N	δ^{15}N [b]		Grains [c]	Tillers [c]	
Jarrah biochar	0.39**	0.61**	0.34*	0.63**	0.07		0.60**	0.39**	
Pine biochar	0.31*	0.73**	0.13	0.74**	0.08		0.45**	0.17	
	Plant nutrients								
	Grain C [d]	Grain N [d]	Grain δ^{15}N [b]	Tiller C [d]	Tiller N [d]	Tiller δ^{15}N [b]	Root C [d]	Root N [d]	Root δ^{15}N [b]
Jarrah biochar	0.04	0.004	0.45**	0.29*	0.43**	0.15	0.14	0.005	0.01
Pine biochar	0.46**	0.01	0.02	0.12	0.01	0.23	0.21	0.11	0.51**

[a] in g kg^{-1}
[b] in ‰
[c] in g plant^{-1}
[d] in mg g^{-1}

Table 3 Effects of biochar feedstocks and application rates on wheat carbon (C) and nitrogen (N) contents. Values represent means ± standard errors. Different letters within columns represent significant differences (Tukey *post-hoc* test, $p < 0.05$)

Application rate	Grains		Tillers		Roots	
(t ha^{-1})	Total C (mg g^{-1})	Total N (mg g^{-1})	Total C (mg g^{-1})	Total N (mg g^{-1})	Total C (mg g^{-1})	Total N (mg g^{-1})
0	416.9 ± 3.8 ab	24.3 ± 2.3 a	391.5 ± 4.0 a	3.60 ± 0.36 ab	367.3 ± 10.4 a	4.36 ± 0.38 a
Jarrah biochar:						
14	416.3 ± 1.3 ab	23.7 ± 0.4 a	395.4 ± 4.6 a	3.24 ± 0.19 ab	365.6 ± 11.4 a	4.38 ± 0.32 a
29	423.4 ± 3.6 ab	22.8 ± 1.7 a	386.3 ± 4.8 a	4.32 ± 0.36 ab	335.6 ± 11.2 a	4.47 ± 0.86 a
60	419.8 ± 3.9 ab	24.7 ± 1.7 a	380.6 ± 2.6 a	5.35 ± 0.74 a	346.0 ± 13.7 a	4.53 ± 0.48 a
Pine biochar:						
14	415.9 ± 2.1 b	21.5 ± 1.1 a	384.8 ± 5.2a	2.81 ± 0.33 b	356.9 ± 9.4 a	3.80 ± 0.22 a
29	424.1 ± 2.4 ab	21.4 ± 1.0 a	383.7 ± 4.3 a	2.53 ± 0.36 b	319.5 ± 18.7 a	4.18 ± 0.84 a
60	436.4 ± 8.2 a	24.6 ± 2.1 a	382.1 ± 5.0 a	3.72 ± 0.88 ab	323.1 ± 27.9 a	5.69 ± 1.58 a
Nutrient-enriched biochar:						
29 (jarrah)	428.9 ± 5.3 ab	24.6 ± 2.3 a	399.9 ± 9.1 a	3.33 ± 0.20 ab	328.6 ± 12.3 a	3.82 ± 0.42 a
29 (pine)	416.7 ± 2.5 ab	22.6 ± 0.9 a	390.7 ± 2.3 a	3.88 ± 0.32 ab	337.8 ± 14.5 a	4.19 ± 0.33 a

Fig 1

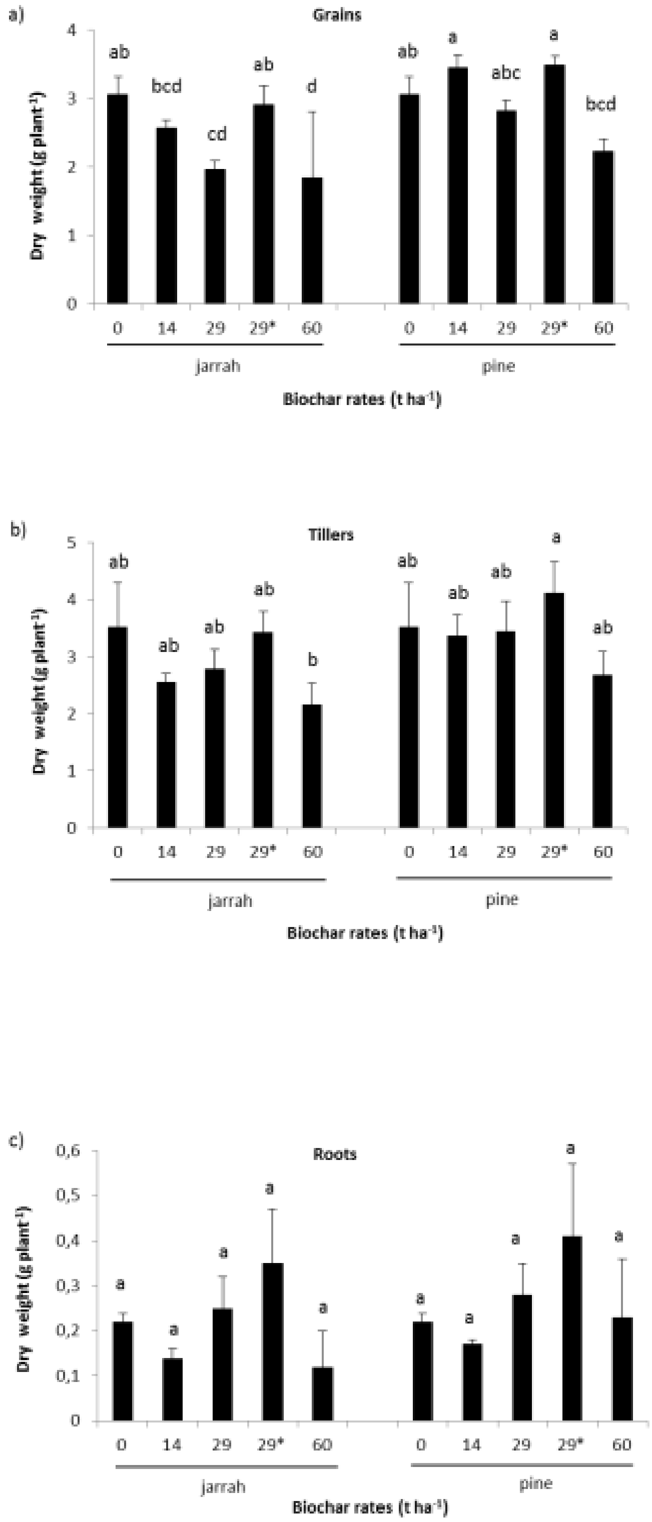
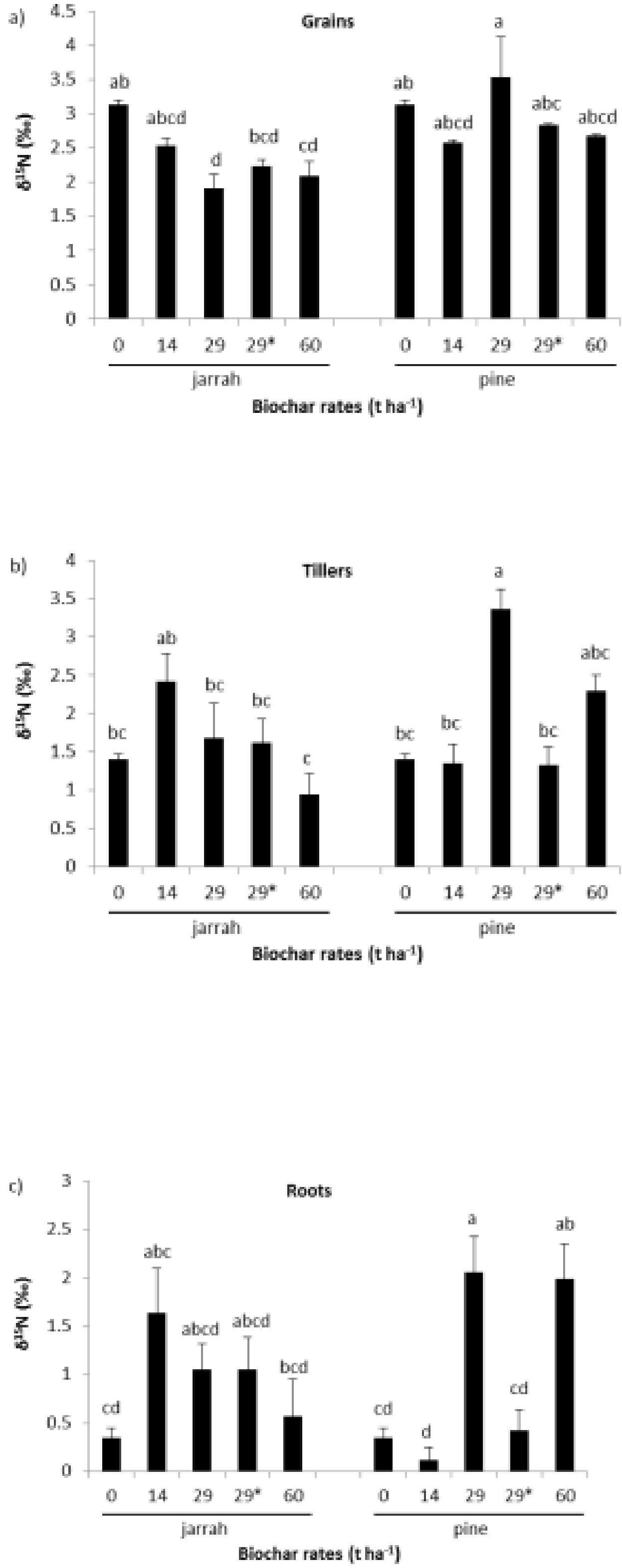

a)
Grains
Dry weight (g plant⁻¹)
4
3
2
1
0
ab
bcd
cd
ab
d
ab
a
abc
a
bcd
0
14
29
29*
60
0
14
29
29*
60
jarrah
pine
Biochar rates (t ha⁻¹)
b)
Tillers
Dry weight (g plant⁻¹)
5
4
3
2
1
0
ab
ab
ab
ab
b
ab
ab
ab
a
ab
0
14
29
29*
60
0
14
29
29*
60
jarrah
pine
Biochar rates (t ha⁻¹)
c)
Roots
Dry weight (g plant⁻¹)
0,6
0,5
0,4
0,3
0,2
0,1
0
a
a
a
a
a
a
a
a
a
a
0
14
29
29*
60
0
14
29
29*
60
jarrah
pine
Biochar rates (t ha⁻¹)

Fig 2


a)
Grains
δ15N (‰)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
ab
abcd
d
bcd
cd
ab
abcd
a
abc
abcd
0
14
29
29*
60
0
14
29
29*
60
jarrah
pine
Biochar rates (t ha-1)
b)
Tillers
δ15N (‰)
4
3.5
3
2.5
2
1.5
1
0.5
0
bc
ab
bc
bc
c
bc
bc
a
bc
abc
0
14
29
29*
60
0
14
29
29*
60
jarrah
pine
Biochar rates (t ha-1)
c)
Roots
δ15N (‰)
3
2.5
2
1.5
1
0.5
0
cd
abc
abcd
abcd
bcd
cd
d
a
cd
ab
0
14
29
29*
60
0
14
29
29*
60
jarrah
pine
Biochar rates (t ha-1)