Tarin MWK, Fan L, Cai Y, Tayyab M, Chen L, He T, Rong J, Zheng Y (2020).
Biochar amendment regulated growth, physiological, and biochemical responses of conifer in red soil
iForest – Biogeosciences and Forestry – doi: 10.3832/ifor3416-013

Supplementary Material

Appendix 1 - Equations used for calculation of leaf biochemical traits and photosynthetic pigments.

$$Amino\ acids(\mu g/g) = \frac{(Measure - Blank)}{(Standard - Blank)} * 50\,\mu\frac{g}{ml} * Soluble\ protein\ content \quad (1)$$

$$Total\ Soluble\ Protein(g/lit) = \frac{(Measure - Blank)}{(Standard - Blank)} * 0.563\,g\ Pro/lit \quad (2)$$

$$Ascorbic\ Acid\left(\mu\frac{g}{g}\ wet\ weight\right) = \frac{(Measure - Blank)}{(Standard - Blank)} * 6\,\mu\frac{g}{ml} * 4 * Soluble\ protein\ content \quad (3)$$

$$\text{Soluble Sugar Content (μg/g wet weight)} = \frac{(Measure - Blank)}{(Standard - Blank)} * 1000\ \mu g/ml/sample\ weight/(10 * volume\ of\ distilled\ water) * Multiple\ of\ sample\ Dilution \quad (4)$$

Chlorophyll A content ($mg \cdot g^{-1}$) = (12.21*D663-2.81*D645*V/ (1000*W) (5)

Chlorophyll B content ($mg \cdot g^{-1}$) = (20.13*D645-5.03*D663*V/ (1000*W) (6)

Total chlorophyll content ($mg \cdot g^{-1}$) = (20.2*D645+8.02*D663*V/ (1000*W) (7)

Carotenoids ($mg \cdot g^{-1}$) = (1000*D470-3.27*Ca-104*Cb)/229*V/ (1000*W) (8)

Appendix 2- Light-response curves of photosynthesis fitting**.**

The light-response curves of photosynthesis were fitted following the modified model of a rectangular Hyperbola as follows;

$$Pn=\frac{(1-\beta PPFD)}{(1+\Upsilon PPFD)}(\alpha PPFD+Rd) \quad (9)$$

where Pn is net photosynthetic rate at the light (PPFD), Rd is the rate of dark respiration, and α is initial slope and also α, β and ϒ are the coefficients which are independent of PPFD (Light). At low PPFD, α also shows the increasing rate of Pn.

Light use efficiency (LUE)

$$LUE=\alpha\frac{1-\beta PPFD}{1+\Upsilon PPFD}-\frac{Rd}{PPFD} \quad (10)$$

According to eqn. 9 and eqn. 10, light use efficiency = Pn/PPFD can be calculated, so maximum light use efficiency is equal to α. Hence, α also indicates the maximum LUE of leaves at very low PPFD. Light compensation point (LCP), light saturation point (LSP) and maximum photosynthetic rate (Pn-Max) were calculated as follows:

$$LCP=\frac{-Rd}{\alpha} \quad (11)$$

$$LSP=\frac{\sqrt{\frac{\beta+\gamma}{\beta}}}{\Upsilon}-1 \quad (12)$$

$$Pn-max=\alpha\frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\Upsilon}-Rd \quad (13)$$

Tab. S1 - Physiochemical properties of soil and hardwood biochar. (EC): electrical conductivity; (TN): total nitrogen; (TC): total carbon; (TP): total phosphorous; (AP): available phosphorous; (OM): organic matter; (AK): available potassium; (C/N): carbon nitrogen ratio.

Characteristics	Hardwood Biochar	Soil
pH	5.70	4.69
EC ($mScm^{-1}$)	6.34	106.67
TN ($g\ kg^{-1}$)	8.29	0.49
TC ($g\ kg^{-1}$)	330.53	3.36
TP($g\ kg^{-1}$)	0.30	0.25
AP ($mg\ kg^{-1}$)	24.31	10.24
OM ($g\ kg^{-1}$)	569.83	5.79
AK ($g\ kg^{-1}$)	0.32	0.36
C/N Ratio	39.846	6.864

Fig. S1 - Seasonal comparison in conductance to H_2O (µmol H_2O $m^{-2}s^{-1}$) of seedling at different photosynthetic photon flux density levels (PPFD) under different hardwood biochar (BH) levels. B0: control soil without hardwood biochar amendment; BH5: hardwood biochar-amended soil at 5 $g.kg^{-1}$; BH20: hardwood biochar-amended soil at 20 $g.kg^{-1}$; and BH80: hardwood biochar-amended soil at 80$g.kg^{-1}$, respectively. June 2017: 1st season; September 17: 2nd season; December 17: 3rd season; and March-18: 4th season, respectively indicate four different seasons. Error bars represent the standard error of the mean (n=3)

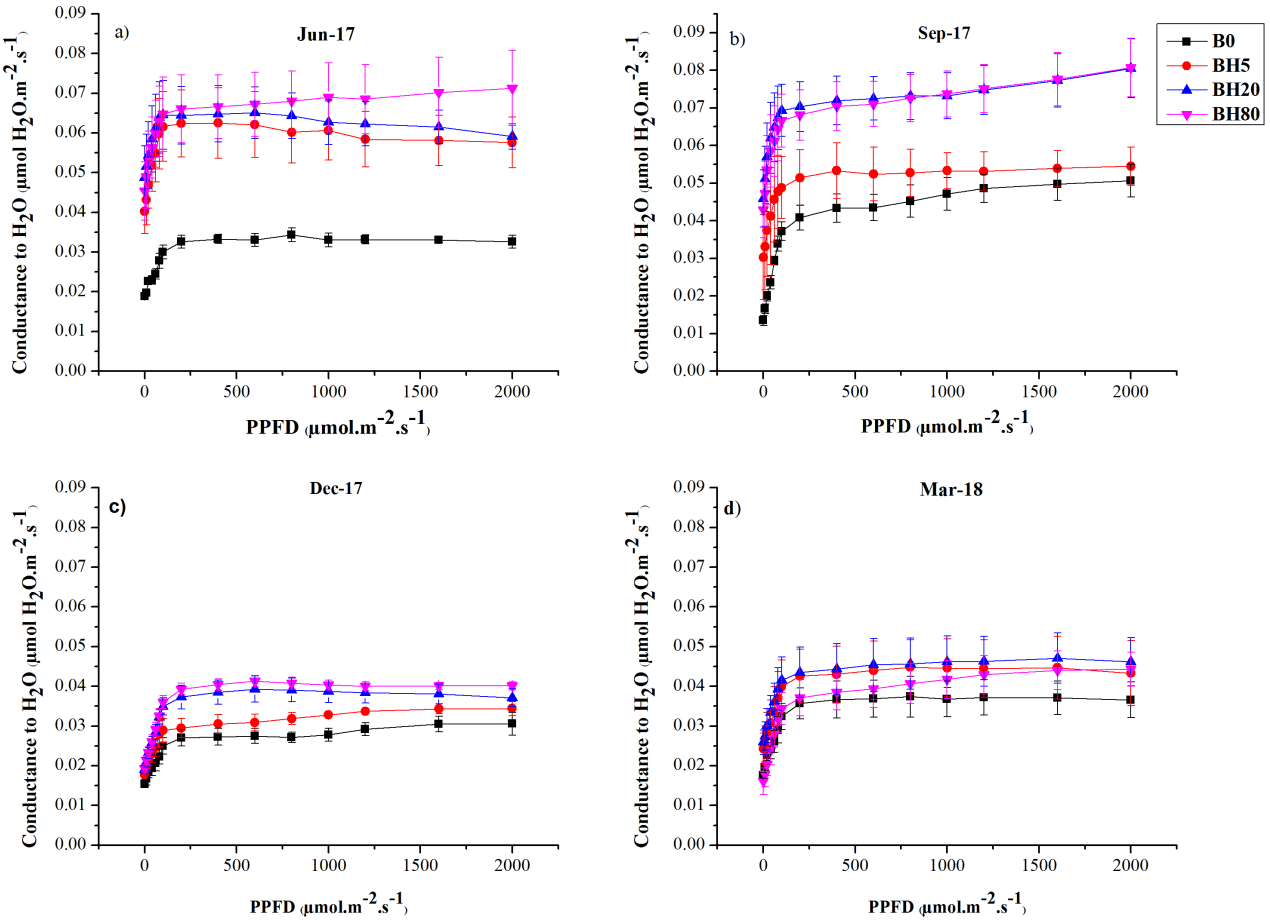

Fig. S2 - Seasonal comparison in intercellular CO_2 concentration (µmol CO_2 mol^{-1}) of seedling at different photosynthetic photon flux density levels (PPFD) under different hardwood biochar (BH) levels. B0: control soil without hardwood biochar amendment; BH5: hardwood biochar-amended soil at 5 $g.kg^{-1}$; BH20: hardwood biochar-amended soil at 20 $g.kg^{-1}$; and BH80: hardwood biochar-amended soil at 80$g.kg^{-1}$, respectively. June 2017: 1st season; September 17: 2nd season; December 17: 3rd season; and March-18: 4th season, respectively indicate four different seasons. Error bars represent the standard error of the mean (n=3).

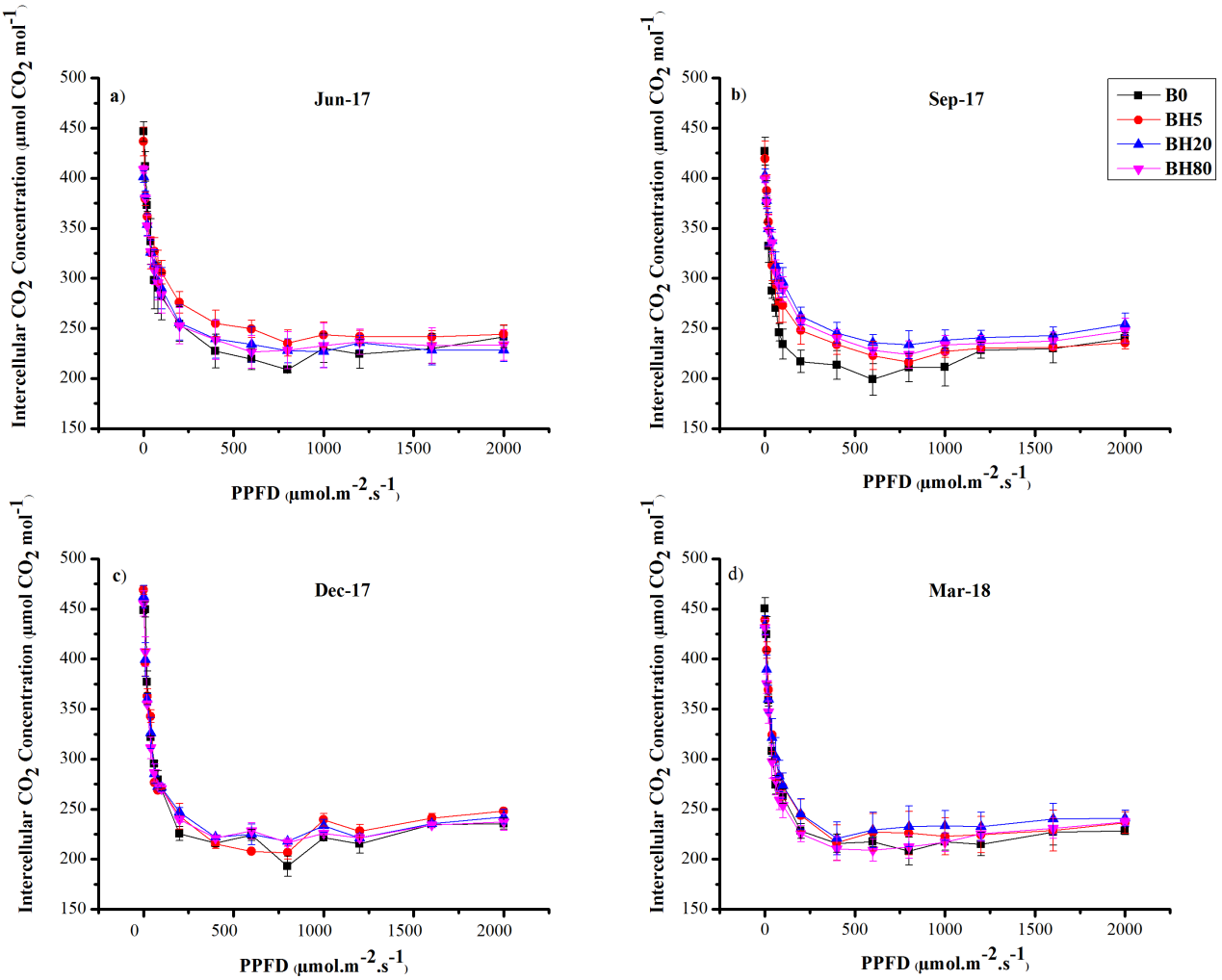

Fig. S3 - Seasonal comparison in transpiration rate (µmol $H_2O.m^{-2}.s^{-1}$) of seedling at different photosynthetic photon flux density levels (PPFD) treated with different hardwood biochar (BH) levels. B0: control soil without hardwood biochar amendment; BH5: hardwood biochar-amended soil at 5 $g.kg^{-1}$; BH20: hardwood biochar-amended soil at 20 $g.kg^{-1}$; and BH80: hardwood biochar-amended soil at 80 $g.kg^{-1}$, respectively. June 2017: 1st season; September 17: 2nd season; December 17: 3rd season; and March-18: 4th season, respectively indicate four different seasons. Error bars represent the standard error of the mean (n=3).

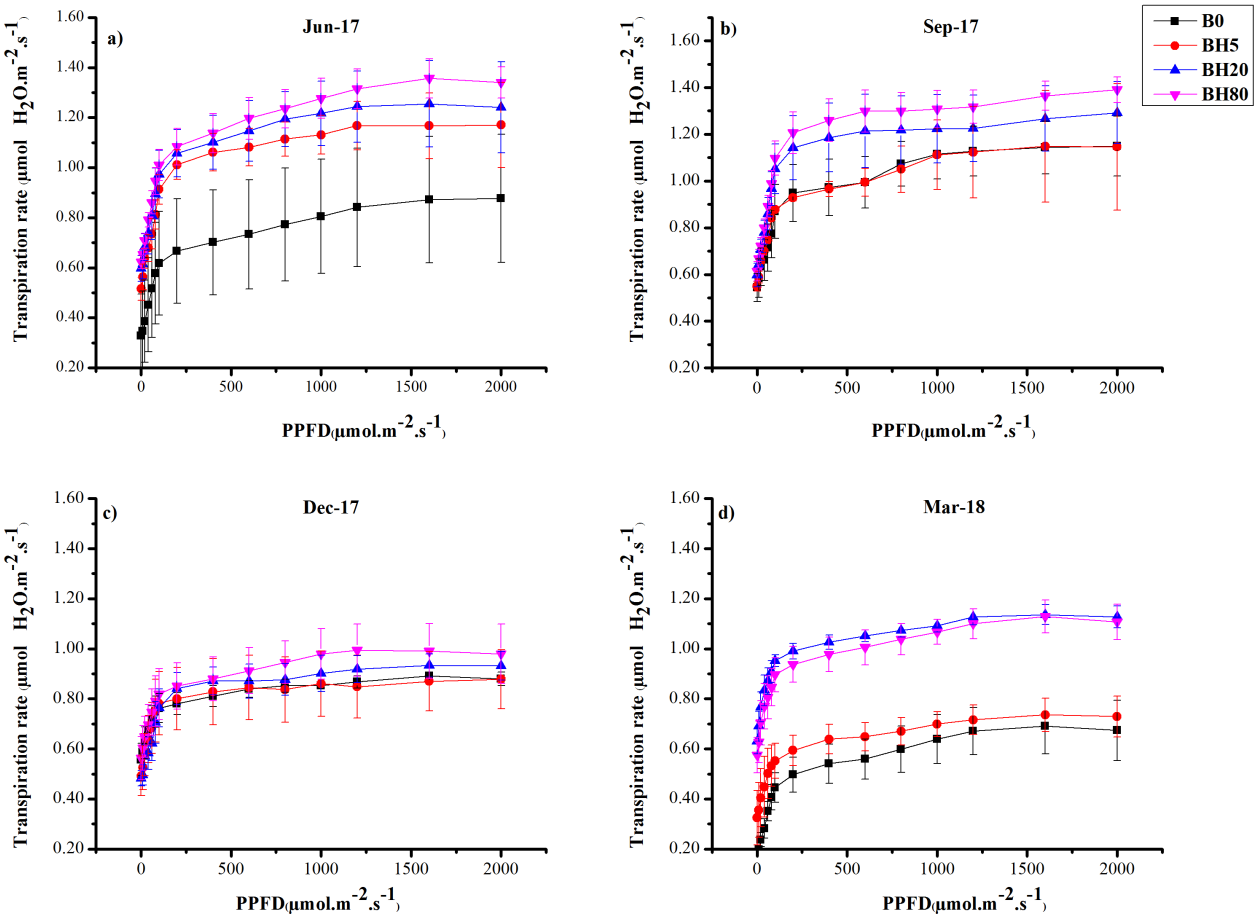

Fig. S4 – Metrological information of study area, during the entire experiment period (source: https://www.worldweatheronline.com)