Journal of Soil Science and Plant Nutrition (2021) 21:2815–2825
https://doi.org/10.1007/s42729-021-00568-z

ORIGINAL PAPER

Effects of Biochar on Pulse C and N Cycling After a Short-term Drought: a Laboratory Study

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Received: 4 February 2021 / Accepted: 3 August 2021 / Published online: 17 August 2021

Abstract

This study aimed to explore the effects of biochar on pulse CO_2 and N_2O emissions and N cycling microbial functional genes after a short-term drought through a soil incubation experiment. Soil samples were collected in a macadamia orchard where biochar was applied 5 years prior to the incubation. Samples were wetted after being subjected to short-term (2-month) drought conditions. Samples were analysed for gas emissions (N_2O and CO_2), available NH_4^+-N, and NO_3^--N, water soluble organic carbon (WSOC), water soluble total N (WSTN), and N cycling microbial gene abundance for a period of 21 days post-drought. Soil CO_2 emissions were significantly higher in the drought-affected soil with no biochar than in the control soil with no biochar. No effect of biochar was detected on CO_2 emissions for drought-affected soil. Available labile C (WSOC) in drought-affected soil was higher than in soils not subjected to drought, regardless of the presence of biochar. Therefore, C loss after adding water could be explained by the release of labile C accumulated during drought. Drought-affected soil with biochar did not influence N_2O emissions compared with control soil subject to drought. In soils not subjected to drought, biochar had higher NO_3^--N than the soil without biochar at day 7 post-drought, which could partly be explained by increased soil ammonia-oxidising bacteria (AOB) gene abundance. Our study suggested that a pulse C loss was more likely to occur post-drought whereas pulse N loss through N_2O emission was not evident regardless of biochar application particularly within first day after being rewetted. Our study highlights the pulse effects of drought on GHG emissions from the soil after being wetted.

Keywords Climate change · Microbial functional genes · Carbon sequestration · Wood biochar

1 Introduction

Some climate models forecast an increase in the frequency of drought spells and alteration of seasonal rainfall during the twenty-first century (Dai 2013). Drought spells influence carbon (C) and nitrogen (N) fluxes (Canarini et al. 2017; Homyak et al. 2017). Alteration of C and N fluxes after drought may have important feedback effects on climate change (Bai et al. 2015a; Zhou et al. 2016, 2017). Generally, drought spells lead to a reduction in C and N loss through different gaseous forms (Canarini et al. 2017; Homyak et al. 2017; Leitner et al. 2017). However, drought-affected soils

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have the potential to lose C when wetted after drought, due to the release of labile C pools which can be consumed by soil microbes (Canarini et al. 2017). Drought-affected soils may also lose N through mobilisation of the accumulated N during the drought period and stimulation of microbial N transformations (Homyak et al. 2017; Leitner et al. 2017). Mobilised/mineralised N can then be quickly converted to N_2O or leached (de Vries et al. 2012; Zhou et al. 2016). Soil management practices, including the addition of organic amendments, such as biochar, may further interact with C and N cycling in soils subjected to drought. Therefore, it is important to understand the extent to which particular soil organic amendments affect soil C and N cycling in a drought-affected soil.

Biochar is a C-rich material produced through pyrolysis of organic material when oxygen is limited under high temperatures. Biochar is widely used in management practices to amend soils and is usually claimed to retain soil C and N (Choudhary et al. 2021; Hannet et al. 2021; Rodrigues et al. 2021; Saffeullah et al. 2021). Recent evidence showed that biochar could increase long-term soil C retention through a stabilization of soil organic C and root-derived C (Weng et al. 2017). On the other hand, soil N retention after biochar application is driven by decreased N leaching and N_2O emission (Van Zwieten et al. 2010b, 2014; Bai et al. 2016; Darby et al. 2016; Nguyen et al. 2017a). Biological processes such as nitrification and denitrification are also affected by biochar application and further control N retention (Clough and Condron 2010; Bai et al. 2015b,c; Aamer et al. 2020). However, contradictory results have been reported regarding the effect of biochar application on soil inorganic N, N_2O emissions, abundance of soil N cycling microbes and soil microbial activity (assessed through CO_2 emissions) (Hardie et al. 2014; Griffin et al. 2017; He et al. 2017; Nguyen et al. 2017a,b). Considering the expected increase in drought events associated with global climate change, the influence of biochar amendment on C and N retention and on soil microbes needs to be thoroughly investigated to understand the mechanisms behind potential C and N retention by biochar in drought-affected soils.

Soil N cycling microbes may be influenced by drought events (Reverchon et al. 2015; Kaurin et al. 2018; Hammerl et al. 2019). Although some reports indicate that denitrifiers, ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) may be resilient to drought (Sher et al. 2012; Hartmann et al. 2013; Kaurin et al. 2018), other findings suggest soil denitrifying and nitrifying microbial communities may not be able to recover, or may have a prolonged recovery time after a drought spell (de Vries et al. 2012; Liang et al. 2014). As these functional groups have been recently recommended as indicators of microbial response to climate change (Gschwendtner et al. 2014; Thion and Prosser 2014), investigating the abundances and activities of soil N-cycling microbes after drought is necessary to understand how microbially mediated soil processes are altered by drought (Fuchslueger et al. 2014).

The objective of this study was to investigate the effects of biochar on soil C and N cycling and on N cycling microbial communities in soil subjected to a short-term drought, through a laboratory experiment. As drought spells are expected to increase under climate change, the long-term C and N fluxes and greenhouse gas (GHG) emissions from drought-affected soils have been predicted or evaluated in a wide range of soils (Jia et al. 2018; Aronson et al. 2019; Leitner et al. 2020). However, short-term fluxes known as 'pulses' have been overlooked and most studies fail to integrate the contribution of N cycling microbes, which are likely to be involved in the immediate mobilisation of labile C and N pools. Since organic amendments are increasingly applied to improve soil quality and resilience, it is critical to assess the impact of biochar amendment on short-term C and N fluxes in drought-affected soils to inform sustainable management practices under climate change scenarios. We hypothesised that biochar application in soils subjected to drought may help mitigate C and N losses through enhanced soil C and N retention.

2 Materials and Methods

2.1 Site Description

The experimental site was located in Beerwah, Australia (26°50′14.6″ S; 152°56′49.96″ E). In 2012, a biochar experiment was established in an orchard that was planted in 2003 with macadamia HAES variety 741 (*Macadamia integrifolia*, Proteaceae). Soil in the orchard is a Kurosol with an acidic pH of 5.0 (Bai et al. 2015b). Details of the biochar experiment establishment have been provided in Bai et al. (2015b). In brief, pine wood chips were used to produce biochar at the highest treatment temperature of 550 °C with residence time of 45 min. Randomised blocks were set up in the orchard with six replicates per treatment. Biochar was applied to the soil surface at 30 t ha^{-1} dry weight. Soil and biochar (1.5:1 ratio) were mixed prior to application. In plots that received no biochar, an equal quantity of soil (with no biochar) was applied. The orchard management was not altered after biochar application.

2.2 Incubation Experiment

Soil samples were collected with cores (60 mm inner diameter) from two points at each replicate plot. In total, 12 cores in non-biochar plots and 12 cores in biochar treated plots were collected in March 2017, 65 months after biochar application. Soil samples were collected within a 50 cm

radius directly from the base of the tree stems at a depth of 0–10 cm. The collected soils were sieved using a 2 mm sieve. After sieving, non-biochar soils and biochar treated soils were homogenised separately to constitute one biochar soil and one no-biochar soil to decrease heterogeneity. A total of 48 vials (70 ml) were prepared with approximately 50 g of soil; 24 vials received non-biochar soil and 24 vials received soil treated with biochar. Initial soil moisture was 8.44% in the soil without biochar and 10.15% in the soil with biochar. Half of the vials in each treatment were kept at 20% water holding capacity (WHC) (drought soils) and the other half (control soils) were kept at 60% WHC. All samples were incubated for 60 days at 27 °C. After 60 days of incubation, all samples were kept at 60% WHC for a further 21 days at 27 °C (wetted period). Soil WHC was maintained throughout the incubation period by weighing the samples every 2–3 days and the water loss was replaced with DI water. Four vials were randomly selected from each one of the four treatments (drought and control biochar and non-biochar soils) at days 1, 7 and 21 post-drought. These soils were then used for biochemical analyses.

2.3 Gas Collection and Soil Chemical Analyses

All collected vials were processed for gas collection. Gases were collected by placing the 70 ml vials containing the soil sample into individual 1 L glass jars for 1 h incubation. A 25 ml syringe was used to pierce the rubber septum to extract gas and inject it into separate 12 ml (Exetainer, Labco Ltd, High Wycombe, UK) vacuum-sealed vials (Darby et al. 2016). Gases were analysed using gas chromatography (Shimadzu GC-2010 Plus) to detect N_2O and CO_2 emissions.

Further biochemical analyses were undertaken immediately after gas collection, on the same day of sample collections. From each vial, a 5 g subsample was added to 40 ml 2 M KCl and then shaken for 1 h, followed by centrifuging at 4000 rpm for 10 min to measure inorganic N. The solutions were used to measure soil available NO_3^-—N and NH_4^+- N, after being filtered through a Whatman 42 filter paper, using a SmartChem 200, Discrete Chemistry Analyser (DCA).

Another subsample soil was used to measure water soluble organic C (WSOC) and water soluble total N (WSTN) by adding 5 g soil to 25 ml of DI water. The soil–water mixture was then shaken for 10 min followed by centrifuging for 10 min at 10,000 rpm. The samples were filtered through a 33-mm Millex syringe-driven 0.45-µm filter. The concentrations of WSOC and WTSN were measured using a Shimadzu TOC-VCSH/CSN TOC/N.

2.4 Abundance of Microbial N Functional Genes

DNA was extracted from 0.25 g of soil using a MoBio PowerSoil® DNA isolation kit (MO BIO, Carlsbad, CA, USA), following manufacturer's instructions. Abundances of 16S rRNA gene for total bacteria, *nifH*, AOA and AOB *amoA*, *nifH, nxrA*, *nxrB*, *narG, nirK, nosZ* and *ureC* genes were assessed using the primer sets presented in Table S1 in the supplementary material. The reaction volume for all the genes was 10 µl, including 5 µl of SYBR SensiMix (Bioline), 0.35 µl of each primer (10 µM), and 2 µl of a tenfold diluted DNA. Standard curves were generated using tenfold serial dilutions of plasmids containing correct inserts of the target genes. All qPCR analyses were undertaken on a Bio-Rad CFX96 optical real-time PCR detection system (Bio-Rad, Laboratories Inc., Hercules, CA, USA) at the University of Melbourne.

2.5 Statistical Analysis

Three-way analyses of variance (ANOVA) were performed with time post-drought, biochar and drought as main effects on CO_2, N_2O, WSOC, WSTN, available NH_4^+-N and NO_3^--N and microbial gene abundances. However, significant 2-way and 3-way interactions were detected. All data were then analysed using one-way ANOVA at each sampling time to detect differences among the four treatments including drought no-biochar, drought biochar, control no-biochar and control biochar at each sampling day. CO_2 data were analysed using generalized linear model followed by Sequential Sidak. Pearson's correlations were run to determine the relationships between soil chemical properties and the abundances of N cycling genes at each sampling day. The statistical program used for analysis was SPSS version 21 (SPSS Inc. Chicago, IL, USA).

3 Results

3.1 Short-term Drought and Biochar Effects on GHG Emissions

Differences in CO_2 and N_2O emissions between treatments were only observed at day 1 post-drought. At day 1 post-drought, CO_2 emissions were higher in drought-subjected soils than in control soils, whilst biochar significantly decreased CO_2 emissions in control soils only. The highest CO_2 emissions at that sampling time were from the drought-affected soil with no biochar which did not significantly differ from soil with biochar subject to drought (Fig. 1a). No significant differences in soil CO_2 emissions were observed among all treatments at day 7 and day 21 post-drought (Fig. 1a). Furthermore, at day 1 post-drought, N_2O emissions from the drought-affected soil with biochar were significantly lower than the control soil where no biochar and no drought was applied (Fig. 1b). However, within the drought-affected soil, biochar did not influence N_2O

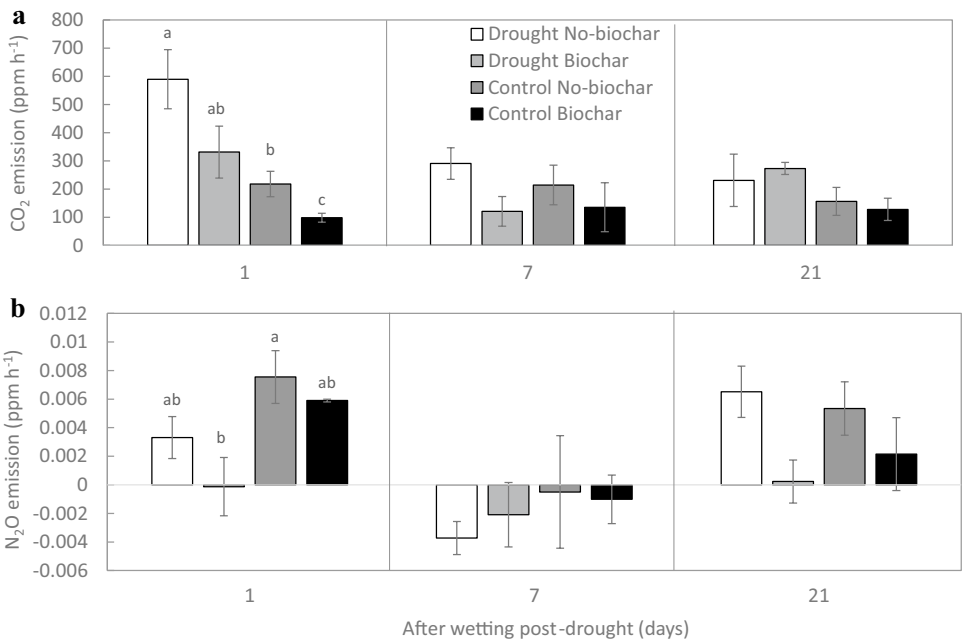

Fig. 1 Soil CO_2 fluxes at days 1 (**a**), 7 (**b**) and 21 (**c**) and N_2O fluxes at days 1 (**d**), 7 (**e**) and 21 (**f**) post-drought. Different lowercase letters indicate significant differences among treatments at $p < 0.05$. Error bars represent mean standard errors

emissions. No significant difference in N_2O emissions was observed among treatments at days 7 and 21 post-drought (Fig. 1b).

3.2 Short-term Drought and Biochar Effects on Soil Physicochemical Parameters

The WSOC, a measurement of the labile soil organic fraction, was significantly influenced by drought. At all sampling dates, WSOC was significantly higher in the drought-subjected soils than in the control soils, except at day 7 when WSOC from drought-subjected soils did not differ from that of the control soil with biochar (Fig. 2a). Although the presence of biochar did not influence WSOC at days 1 and 21 post-drought, control soils with biochar had lower WSOC than control soils with no biochar at day 7 (Fig. 2a). At day 21 post-drought, drought-affected soil with biochar had significantly higher WSOC than that of control soil with and without biochar (Fig. 2a). Regarding the soil labile N, drought-subjected soils had significantly lower WSTN, a measure of the labile soil N, compared with the control treatments, regardless of the presence of biochar, at days 1 and 7 post-drought (Fig. 2b). At day 21, lower WSTN was recorded in drought-subjected soils relative to the control soil with biochar. Within the drought treatments, biochar did not have any influence on soil WSTN.

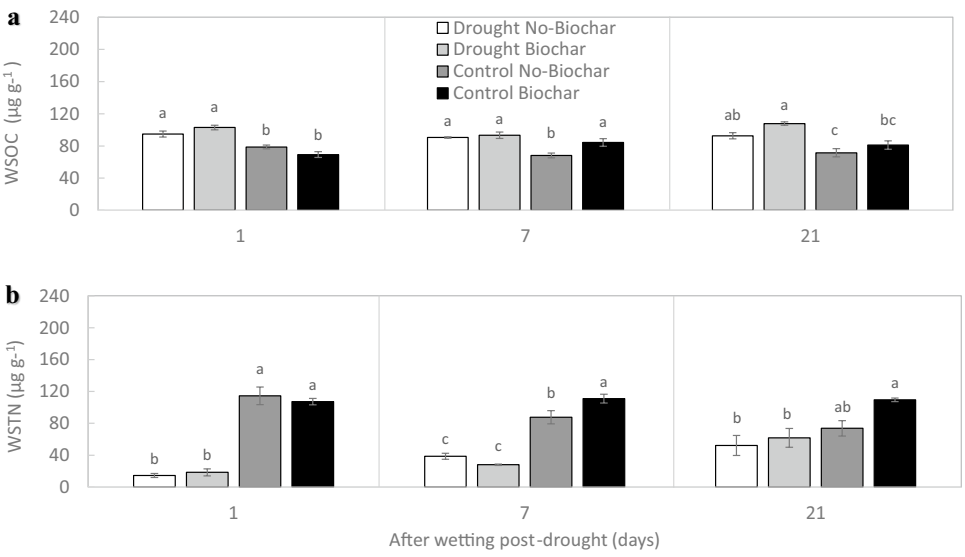

Fig. 2 Soil water soluble organic carbon (WSOC) (**a**) and water soluble total nitrogen (WSTN) (**b**) at days 1, 7 and 21 post-drought. Different lowercase letters indicate significant differences among treatments at $p < 0.05$ at that sampling day. Error bars represent mean standard errors

Drought-affected soils with no biochar treatment had significantly higher available NH_4^+-N than control no-biochar soils at days 1 and 7 post-drought (Fig. 3a). In the presence of biochar, soil available NH_4^+-N did not differ between drought-affected and control soil at days 1 and 21 post-drought (Fig. 3a). However, at day 7 post-drought, the drought-affected treatment had higher soil available NH_4^+-N, even in the presence of biochar. Wetting soil after drought did not alter available NO_3^-- N at days 1 and 21 post-drought, as no differences were observed between drought-affected soils and control soils, regardless of the presence of biochar (Fig. 3b). However, at day 7 post-drought, available NO_3^-- N in control soils was higher in the presence of biochar than in the no-biochar treatment (Fig. 3b).

3.3 Short-term Drought and Biochar Effects on N Cycling Microbial Genes

Differences in microbial gene abundances were observed among treatments at days 1 and 21 post-drought. At day 1 post-drought, the abundance of AOB *amoA* and *nirK* was highest in the control soil with biochar (Table 1). On the other hand, at that sampling time, the drought-affected soil with no biochar treatment had significantly higher *nxrA*, *nosZ1* and *nosZ2* than the drought-affected soil with biochar treatment (Table 1). At day 21 post-drought, the drought-affected soil with no biochar treatment had significantly lower AOA *amoA* than the drought-affected soil with biochar (Table 1). The abundance of the 16S rDNA gene was significantly higher in control biochar soil than in soils subjected to drought by day 21 post-drought (Table 1). Conversely, the control soil with biochar had the lowest abundance of *nxrA* and *nosZ1* at day 21 post-drought. Furthermore, drought treatment resulted in a higher abundance of *ureC* in the absence of biochar at day 21 post-drought (Table 1).

A positive but weak relationship between WSOC and available N was detected (Supplementary Fig. 2b; $X^2 = 37.553$, $p = 0.051$). Significant correlations were found between the abundance of N cycling genes and the evaluated soil parameters, at the different sampling dates. At day 1 post-drought, soil AOA *amoA* gene abundance was positively correlated with N_2O and was negatively correlated with CO_2 ($r = 0.576$ and -0.728, respectively; Table 2). At that sampling time, the abundance of AOB *amoA* was negatively correlated with WSOC ($r = -0.660$; Table 2). Soil pH was positively correlated with denitrification genes *nirK* and *nosZ1*, but negatively correlated with 16S gene abundance. The abundance of *nosZ1* was also positively correlated to soil NH_4^+-N. At day 7 post-drought, the abundances of *nxrA, nosZ1* and *nosZ2* genes were negatively correlated with WSOC, a pattern which did not perdure to the last sampling date (Table 2). At day 21, the AOA *amoA*, *nosZ2* and 16S gene abundances were negatively correlated with available NH_4^+-N (Table 2). However, available NH_4^+-N was positively correlated with *ureC*. Finally, the abundance of the denitrification gene *nosZ2* was negatively correlated with soil pH (Table 2).

4 Discussion

Soil CO_2 emissions from drought-subjected soils were not influenced by the application of biochar in this short-term study. However, regardless of the presence of biochar

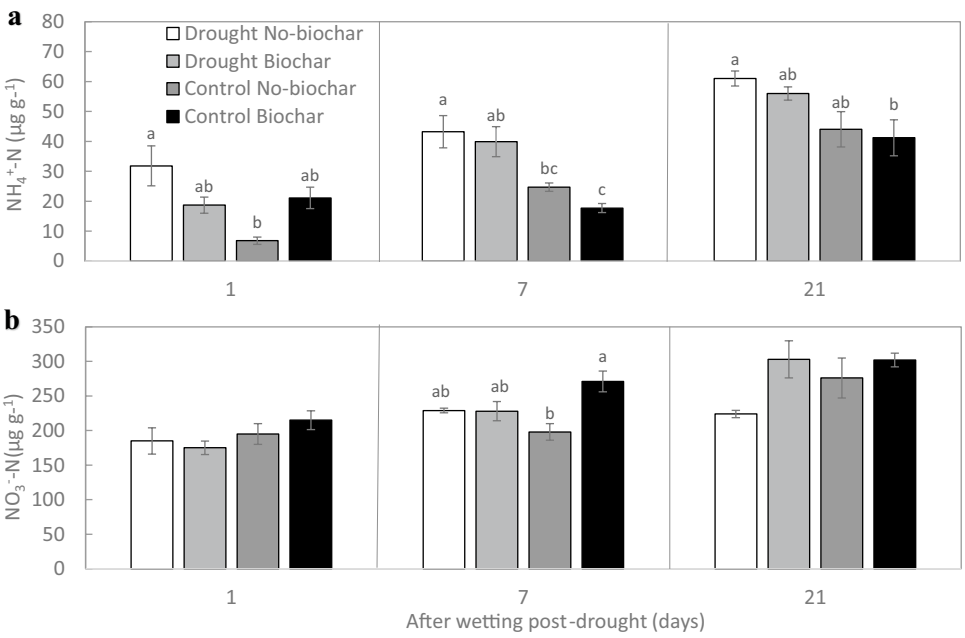

Fig. 3 Soil available NH_4^+-N (**a**) and available NO_3^--N (**b**) at days 1, 7 and 21 post-drought. Different lowercase letters indicate significant differences among treatments at $p < 0.05$ at that sampling day. Error bars represent mean standard errors

Table 1 Abundances of N-cycling genes (number of gene copies g^{-1} soil ± standard error) resulting from treatments of biochar application and rewetting after drought. Lower-case letters in the columns indicate differences among treatments at the same sampling day at $p < 0.05$

	Day 1			Day 7		Day 21		
AOA *amoA*								
Drought No-biochar	3.49×10^5	$\pm9.08\times10^4$		2.35×10^6	$\pm1.30\times10^5$	1.82×10^5	$\pm9.79\times10^4$	b
Drought Biochar	9.40×10^5	$\pm3.37\times10^5$		1.89×10^6	$\pm2.78\times10^5$	1.58×10^6	$\pm3.02\times10^5$	a
Control No-biochar	9.32×10^5	$\pm7.30\times10^4$		1.51×10^5	$\pm9.63\times10^4$	1.56×10^6	$\pm2.96\times10^5$	a
Control Biochar	1.56×10^6	$\pm5.28\times10^5$		5.08×10^5	$\pm1.18\times10^5$	1.22×10^6	$\pm2.20\times10^5$	ab
AOB *amoA*								
Drought No-biochar	2.61×10^6	$\pm4.14\times10^5$	b	2.40×10^6	$\pm1.32\times10^6$	1.73×10^6	$\pm1.21\times10^5$	
Drought Biochar	1.46×10^6	$\pm6.67\times10^4$	b	2.46×10^6	$\pm3.47\times10^5$	3.28×10^6	$\pm6.76\times10^5$	
Control No-biochar	1.15×10^6	$\pm6.49\times10^5$	b	3.43×10^6	$\pm1.74\times10^6$	2.38×10^6	$\pm4.39\times10^5$	
Control Biochar	7.39×10^6	$\pm1.37\times10^6$	a	2.21×10^6	$\pm7.68\times10^5$	1.92×10^6	$\pm3.14\times10^5$	
nifH								
Drought No-biochar	1.61×10^6	$\pm2.98\times10^4$		1.60×10^6	$\pm2.38\times10^5$	1.74×10^6	$\pm2.75\times10^4$	
Drought Biochar	1.65×10^6	$\pm1.17\times10^5$		1.47×10^6	$\pm1.08\times10^5$	1.86×10^6	$\pm1.43\times10^5$	
Control No-biochar	1.70×10^6	$\pm8.97\times10^4$		1.32×10^6	$\pm2.15\times10^4$	1.86×10^6	$\pm1.75\times10^5$	
Control Biochar	1.61×10^6	$\pm1.60\times10^5$		1.75×10^6	$\pm1.79\times10^5$	1.49×10^6	$\pm1.32\times10^5$	
nxrA								
Drought No-biochar	3.58×10^5	$\pm3.37\times10^4$	a	2.34×10^5	$\pm1.16\times10^5$	4.10×10^5	$\pm5.77\times10^4$	a
Drought Biochar	1.68×10^5	$\pm2.57\times10^4$	b	1.16×10^5	$\pm1.53\times10^4$	2.13×10^5	$\pm5.04\times10^4$	ab
Control No-biochar	2.02×10^5	$\pm8.51\times10^3$	ab	4.30×10^5	$\pm6.23\times10^4$	2.40×10^5	$\pm3.80\times10^4$	ab
Control Biochar	2.73×10^5	$\pm4.06\times10^4$	ab	2.32×10^5	$\pm5.75\times10^4$	9.76×10^4	$\pm5.29\times10^3$	b
nxrB								
Drought No-biochar	1.75×10^4	$\pm3.31\times10^3$		2.16×10^4	$\pm1.41\times10^3$	1.90×10^4	$\pm5.46\times10^2$	
Drought Biochar	2.13×10^4	$\pm1.52\times10^3$		1.62×10^4	$\pm3.10\times10^3$	1.92×10^4	$\pm2.31\times10^3$	
Control No-biochar	1.57×10^4	$\pm2.50\times10^3$		1.62×10^4	$\pm2.56\times10^3$	2.26×10^4	$\pm4.03\times10^3$	
Control Biochar	2.17×10^4	$\pm4.68\times10^2$		2.26×10^4	$\pm3.32\times10^3$	1.65×10^4	$\pm2.89\times10^3$	
narG								
Drought No-biochar	4.21×10^6	$\pm6.34\times10^5$		3.82×10^6	$\pm1.89\times10^6$	4.71×10^6	$\pm9.39\times10^5$	
Drought Biochar	3.87×10^6	$\pm3.44\times10^5$		2.64×10^6	$\pm1.35\times10^5$	3.66×10^6	$\pm7.96\times10^5$	
Control No-biochar	1.98×10^6	$\pm9.25\times10^5$		4.19×10^6	$\pm1.04\times10^5$	2.64×10^6	$\pm1.46\times10^5$	
Control Biochar	4.31×10^6	$\pm2.75\times10^5$		2.82×10^6	$\pm3.85\times10^5$	3.01×10^6	$\pm6.52\times10^4$	
nirK								
Drought No-biochar	1.23×10^7	$\pm1.76\times10^6$	ab	1.61×10^7	$\pm8.39\times10^6$	1.08×10^7	$\pm4.53\times10^6$	
Drought Biochar	1.14×10^7	$\pm2.50\times10^6$	ab	9.06×10^6	$\pm1.82\times10^6$	1.19×10^7	$\pm2.46\times10^6$	
Control No-biochar	4.74×10^6	$\pm1.81\times10^6$	b	9.01×10^6	$\pm4.55\times10^6$	1.09×10^7	$\pm3.18\times10^6$	
Control Biochar	1.61×10^7	$\pm2.86\times10^6$	a	9.20×10^6	$\pm1.77\times10^6$	9.62×10^6	$\pm4.93\times10^5$	
nosZ1								
Drought No-biochar	5.03×10^6	$\pm2.66\times10^5$	a	3.56×10^6	$\pm2.00\times10^5$	6.80×10^6	$\pm1.48\times10^5$	a
Drought Biochar	4.31×10^6	$\pm3.70\times10^5$	a	2.51×10^6	$\pm1.74\times10^5$	3.95×10^6	$\pm9.14\times10^5$	ab
Control No-biochar	1.98×10^6	$\pm5.65\times10^5$	b	4.94×10^6	$\pm6.66\times10^5$	4.31×10^6	$\pm4.88\times10^5$	ab
Control Biochar	4.40×10^6	$\pm6.18\times10^5$	a	3.69×10^6	$\pm7.31\times10^5$	3.05×10^6	$\pm2.80\times10^5$	b
nosZ2								
Drought No-biochar	7.92×10^6	$\pm5.55\times10^5$	a	4.37×10^6	$\pm3.26\times10^5$	4.89×10^6	$\pm7.67\times10^5$	
Drought Biochar	2.68×10^6	$\pm2.35\times10^5$	b	5.10×10^6	$\pm5.66\times10^5$	3.47×10^6	$\pm2.95\times10^5$	
Control No-biochar	1.98×10^6	$\pm5.81\times10^5$	b	7.84×10^6	$\pm1.11\times10^5$	8.39×10^6	$\pm1.74\times10^6$	
Control Biochar	3.30×10^6	$\pm3.44\times10^5$	b	5.28×10^6	$\pm1.37\times10^5$	5.84×10^6	$\pm1.47\times10^6$	
ureC								
Drought No-biochar	5.13×10^6	$\pm5.10\times10^5$		7.91×10^6	$\pm4.12\times10^5$	6.08×10^6	$\pm2.46\times10^5$	a
Drought Biochar	4.38×10^6	$\pm9.32\times10^4$		4.25×10^6	$\pm3.13\times10^5$	4.95×10^6	$\pm3.72\times10^5$	ab
Control No-biochar	2.94×10^6	$\pm1.54\times10^6$		4.61×10^6	$\pm4.35\times10^5$	3.84×10^6	$\pm3.63\times10^5$	b

Table 1 (continued)

	Day 1			Day 7		Day 21		
Control Biochar	4.66×10^{6}	$\pm2.83\times10^{5}$		3.32×10^{6}	$\pm2.09\times10^{5}$	4.40×10^{6}	$\pm1.90\times10^{5}$	ab
16S rRNA								
Drought No-biochar	2.09×10^{9}	$\pm5.19\times10^{7}$	ab	2.65×10^{9}	$\pm8.68\times10^{7}$	3.19×10^{9}	$\pm1.60\times10^{8}$	b
Drought Biochar	1.18×10^{9}	$\pm4.18\times10^{8}$	b	2.91×10^{9}	$\pm7.20\times10^{7}$	3.50×10^{9}	$\pm2.28\times10^{8}$	b
Control No-biochar	2.41×10^{9}	$\pm1.58\times10^{8}$	a	3.12×10^{9}	$\pm1.31\times10^{8}$	4.72×10^{9}	$\pm1.43\times10^{8}$	ab
Control Biochar	2.32×10^{9}	$\pm4.74\times10^{7}$	ab	3.04×10^{9}	$\pm1.51\times10^{8}$	5.91×10^{9}	$\pm6.22\times10^{8}$	a

Table 2 Correlations between chemical variables (CO_2, N_2O, pH, WSOC, NO_3^--N and NH_4^+-N) and gene abundance (AOA, AOB, *nifH, nxrA, nxrB, narG, nirK, nosZ1, nosZ2, ureC* and 16S rRNA) at days 1, 7 and 21 post-drought. * Correlation is significant at the 0.05 level (2-tailed) ** Correlation is significant at the 0.01 level (2-tailed)

	CO_2	NO_2	pH	WSOC	NO_3^--N	NH_4^+-N
Day 1						
AOA *amoA*	**−0.728****	**0.576***	−0.075	−0.522	0.179	−0.309
AOB *amoA*	−0.454	0.102	0.162	**−0.660***	0.442	0.338
nifH	−0.155	0.389	0.092	0.041	−0.290	0.016
nxrA	0.193	0.138	−0.017	−0.047	−0.076	0.488
nxrB	**−0.613***	0.194	0.441	−0.079	0.060	−0.082
narG	−0.114	−0.002	0.510	0.052	−0.244	0.325
nirK	−0.166	−0.260	**0.615***	−0.083	0.113	0.572
nosZ1	0.297	−0.367	**0.672***	0.266	−0.290	**0.671***
nosZ2	0.554	−0.176	0.117	0.356	−0.402	0.508
ureC	−0.066	0.149	0.234	0.039	−0.423	0.167
16S rRNA	−0.426	**0.594***	**−0.653***	−0.513	0.370	−0.246
Day 7						
AOA *amoA*	0.243	−0.010	0.101	0.426	0.063	0.350
AOB *amoA*	0.546	−0.126	−0.221	−0.447	−0.213	−0.224
nifH	0.227	0.026	0.272	0.016	0.193	−0.369
nxrA	0.442	−0.053	−0.364	**−0.777****	−0.499	−0.527
nxrB	0.548	0.008	−0.057	−0.177	0.112	−0.347
narG	0.436	0.115	−0.268	−0.314	−0.328	−0.213
nirK	0.531	0.001	−0.151	0.00	−0.035	−0.032
nosZ1	0.497	−0.065	−0.281	**−0.716***	−0.376	−0.545
nosZ2	0.363	−0.013	−0.067	**−0.753****	−0.475	−0.499
ureC	0.345	−0.027	−0.207	0.065	−0.149	0.025
16S rRNA	−0.286	0.048	0.046	−0.339	0.169	−0.469
Day 21						
AOA *amoA*	−0.043	−0.361	−0.023	0.214	0.396	**−0.605***
AOB *amoA*	0.209	−0.167	−0.26	0.272	−0.003	0.076
nifH	0.143	−0.309	−0.1	0.28	−0.394	−0.156
nxrA	0.129	0.134	−0.256	0.062	−0.571	0.475
nxrB	0.078	−0.001	−0.347	0.084	−0.256	−0.297
narG	0.254	−0.133	−0.092	0.298	−0.179	0.501
nirK	−0.060	0.118	−0.433	0.181	−0.096	−0.097
nosZ1	0.134	0.092	−0.146	0.135	−0.528	0.486
nosZ2	−0.456	0.270	**−0.712****	−0.425	−0.419	**−0.747****
ureC	0.260	−0.033	0.089	0.524	−0.248	**0.660***
16S rRNA	−0.575	0.151	−0.498	−0.323	0.193	**−0.663***

in soils, adding water to soil after drought significantly increased CO_2 emissions at day 1 post-drought. Our results are consistent with other studies reporting a sharp release of CO_2 within the first 24 h after soil irrigation (Casals et al.

2011; Maucieri et al. 2017). The lack of biochar influence on soil CO_2 emissions in drought-affected soils was corroborated by our WSOC results. Available labile C in soil usually stimulates microbial activity, leading to increased microbial respiration rates (Zhou et al. 2016; Bongiorno et al. 2020). In our study, WSOC was not affected by biochar application at day 1 post-drought, although soils subjected to drought (with or without biochar) presented higher WSOC concentrations than their respective controls. Adding water may have released the immobilised labile C accumulated throughout the drought period. Therefore, our experiment indicated that short-term drought led to greater C loss and that drought-subjected soil without biochar had higher potential to lose C when compared with control soils. We also observed that biochar soil that was not subjected to drought had significantly lower CO_2 emissions compared with all treatments. There are contradictory reports regarding CO_2 emissions after biochar application (He et al. 2017), although recent findings show that biochar application may decrease CO_2 emissions, most likely through a binding of available labile C to the biochar surface (Zhang et al. 2015; Darby et al. 2016; Fan et al. 2020). As demonstrated by Weng et al. (2017), biochar-induced soil C retention may occur in the long-term, which may explain the lack of biochar effect on C cycling in drought-affected soils in our short-term incubation study.

In our study, the application of biochar to drought-affected soils did not influence soil N loss through N_2O emissions over the 21-day sampling period. In general, biochar mitigates N_2O emissions through several mechanisms (Harter et al. 2014; Darby et al. 2016; He et al. 2017; Liao et al. 2021). By altering soil physical, chemical and biological properties, biochar may lead to sorption of NO_3^--N and NH_4^+-N (Van Zwieten et al. 2010a,b, 2015; Bai et al. 2015b). Consequently, substrate availability is altered, affecting N_2O emissions. Decreased microbial activity is another mechanism to mitigate N_2O emissions (Cayuela et al. 2013; Darby et al. 2016; Wu et al. 2017), since microbial transformations of NH_4^+-N and NO_3^--N are considered some of the main processes responsible for gaseous N_2 and N_2O emissions from soil (Xu et al. 2014). Our results showed that biochar did not influence soil N substrate availability, measured through WSTN, NH_4^+-N and NO_3^--N, with the exception of WSTN and NO_3^--N at day 7 post-drought in control soils (not subjected to drought). The fact that biochar had no effect on soil N substrate availability for microbial N transformations in drought-subjected soils is likely to be the underlying cause of the lack of influence of biochar on soil N_2O emissions.

Higher available WSTN and NO_3^--N were observed in the control biochar soil (not subjected to drought) than in the control no-biochar soil at day 7. Biochar is known to increase soil NO_3^--N through different mechanisms. Biochar application has been reported to decrease NO_3^--N leaching and increase NO_3^--N retention time in several studies (Bai et al. 2015b; Asadyar et al. 2020). Furthermore, biochar application may increase microbial N transformations, for example by accelerating nitrification rates, which would also lead to an increase in soil NO_3^--N (Nguyen et al. 2017a, 2018). Overall, microbial communities appeared resilient to the impacts of drought over the period of our study. Biochar soil that was not subjected to drought had significantly higher AOB *amoA* gene abundance than the control no-biochar soil at day 1, which may have contributed to the observed increase in available NO_3^--N detected at day 7. Nitrification in agricultural soils has been mainly associated with AOB *amoA* gene abundance (Jia and Conrad 2009). Increased NO_3^--N availability in the control soil with biochar, however, did not lead to increased N_2O emissions. Biochar has been shown to activate soil microbes to convert N_2O to N_2 (Van Zwieten et al. 2014; Liao et al. 2021), evidenced through increases in *nosZ* gene abundance. In soil not subjected to drought, we also detected higher abundance of the *nosZ1* gene at day 1 in biochar soil compared with no-biochar soil. However, we did not find any evidence of microbial stimulation by biochar, as presented by our CO_2 emission results.

Our study indicated that soil rewetting after a short-term drought may not lead to a significant pulse N loss but may increase soil C loss and these effects were not driven by biochar. Our finding is inconsistent with another short-term study under laboratory conditions which has shown a ~ 10% decrease of GHG emissions in the presence of biochar (Maucieri et al. 2017). It should be noted that in our soil, biochar was added to the soil over five years prior to our incubation study. Biochar properties may change over time. For example, a decrease of approximately 50% in biochar effect size on N_2O emissions has been observed over three years following biochar application (Fungo et al. 2019). We did not add fresh biochar to soil in our experiment due to the fact that the biochar at high rates is applied only once and no reapplication is considered. Inconsistency between our study and Maucieri et al. (2017) is likely to be related to changing biochar properties over time. Nonetheless, our study emphasised the importance of understanding the pulse effects of drought and the resulting increase in GHG emissions from the soil after drought; these short-term consequences of drought spells should be taken into consideration in future studies.

5 Conclusions

This study investigated the effectiveness of biochar in decreasing soil pulse C and N loss in a soil subjected to a short drought spell. CO_2 emissions were increased

post-drought and the presence of biochar had a negligible effect on mitigating CO_2 emissions. Adding water to the drought-affected soil led to increased labile C which could explain the observed increase in microbial respiration. Similarly, biochar did not influence N loss through N_2O emissions in drought-affected soils. However, biochar increased NO_3^--N in the soil not subjected to drought, most likely through an increase in nitrification rates, as evidenced by microbial functional genes. The importance of understanding the pulse effects of drought on GHG emissions from the soil after being wetted was highlighted in this study suggesting these short-term consequences of drought spells should be taken into consideration in future studies.

Acknowledgements The authors acknowledge Dr T. Smith, D. Bottrell, Dr I. Tahmasbian, I. Darby, and G. Lambert for their assistance in soil collection and laboratory analyses. This study was funded by the University of the Sunshine Coast (Faculty of Science, Health, Education and Engineering) to fulfil N. Citerne's Honours study. We also thank Griffith University and University of Melbourne for assisting us with sample processing.

Author Contribution Conceptualisation, experimental design (NC, HW, TL, SHB), data collection, sample analysis (NC, HW, TL, MF, NO, XS, HH, FR, MR, SHB), statistical analysis (NC, HW, TL, MF, SHB), manuscript writing (NC, FR, SHB), manuscript revision (NC, HW, TL, MF, NO, XZ, GZ, HH, FR, MR, SHB), supervision (HW, TL, SHB).

Data and Materials Availability Data will be made available upon the requests.

Code Availability Not applicable.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethics Approval and Consent to Participate All authors have consented to the submission of this manuscript to the journal.

Consent for Publication All authors have read and commented on this manuscript and given their consent for this publication.

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