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Combination of Biochar-Based Fertilisers and Reactive Barriers Improved Soil Carbon Storage, Soil Moisture Retention, and Crop Yield in Short Term

Negar Omidvar¹  | Stephen Joseph^{1,2,3}  | Lakmini Dissanayake¹  | Michael B. Farrar¹  | Frédérique Reverchon⁴  | Russell Burnett⁵  | Mehran Rezaei Rashti⁶  | Apsara Amarasinghe⁶  | Sara Tahery²  | Zhihong Xu¹  | Wendy Timms³  | Brittany Elliott⁷  | Hongdou Liu¹  | Shahla Hosseini Bai¹ 

¹Centre for Planetary Health and Food Security, School of Environment and Science, Griffith University, Brisbane, Queensland, Australia | ²School of Materials Science and Engineering, University of New South Wales, Sydney, New South Wales, Australia | ³School of Engineering, Deakin University, Geelong, Victoria, Australia | ⁴Red de Diversidad Biológica del Occidente Mexicano, Centro Regional del Bajío, Instituto de Ecología, A.C., Pátzcuaro, Michoacán, Mexico | ⁵Rainbow Bee Eater Pty Ltd, Berrigan, New South Wales, Australia | ⁶Australian Rivers Institute and School of Environment and Science, Griffith University, Brisbane, Queensland, Australia | ⁷School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Queensland, Australia

Correspondence: Shahla Hosseini Bai (s.hosseini-bai@griffith.edu.au)

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ABSTRACT

Climate change threatens long-term soil health because of increased severity and frequency of drought periods. Applying biochar to soils before a drought can increase non-biochar soil carbon (C) and water storage over the long term and sustain crop yield. However, the on-farm benefit of buried solid biochar and applied liquid biochar at low rates remains uncertain. This study examined the effects of two novel biochar-based soil amendments on soil C, water storage and crop yield. The biochar-based amendments included a biochar reactive barrier (RB) made by layering wood-based biochar, straw mulch and cow manure into a series of open surface trenches, and a liquid biochar mineral complex (BMC) applied twice, at low rate (200 kg ha⁻¹) to one side of RB (fertilised area), while the other side of RB received no treatments (non-fertilised area). Moisture concentration within the RB ranged from 6.76% up to 56.68% after large rainfall, more than double the surrounding soils and gradually started migrating from the RB outwards. Soil within 50 cm distance of the RB showed a 24.5% increase in non-biochar soil C compared with soil at 600 cm distance of the RB, 2.54% versus 2.04%, respectively, in the non-fertilised area, which was supported with lowering soil microbial activity. Pasture yield increase was associated with liquid BMC fertiliser rather than proximity to the RB. Pasture yield was 44% higher in the fertilised area compared with the non-fertilised area 27.89 t ha⁻¹ versus 19.31 t ha⁻¹. Approximately 158 kg CO₂e was removed from the atmosphere for each cubic meter of RB and an annual removal of 150 kg CO₂e ha⁻¹ was estimated by liquid BMC application. Income earned by increased yield was still profitable even though applied liquid BMC could cost between USD 400–520 ha⁻¹ including shipping costs. Overall, our study suggested biochar-based RB and BMC fertilisers can effectively increase soil moisture retention while building non-biochar soil C storage in the surrounding soil. The adoption of biochar-based techniques has the potential to improve drought resilience while increasing soil C in wide range of non-irrigated cropping systems.

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1 | Introduction

Climate change is exacerbating the frequency, duration and severity of drought periods that decrease soil moisture and pose a significant threat to soil health and agricultural productivity (Grillakis 2019; Liu et al. 2023; Wang and Wang 2023). Soil biochemistry is directly moderated by soil moisture, which in turn affects carbon (C) cycling, because C decomposition rates slow down under drought conditions leading to reduced soil fertility (Singh et al. 2021). Moreover, the combination of rising atmospheric carbon dioxide (CO₂) concentrations and elevated temperatures is expected to exacerbate the impact of drought by limiting soil C through effects on plant respiration and photosynthesis (Zhou et al. 2023). Soil C losses may be greater than anticipated with fluctuating moisture level, even with the potential of microbes to adapt to these conditions (Allison 2023). Collectively, soil C models show that innovative C-based amendments are needed to mitigate drought impacts and increase soil C storage, while improving soil fertility (Nepal et al. 2023; Paustian et al. 2019).

Biochar is a C-rich solid produced through the pyrolysis of organic wastes and is an effective approach for atmospheric CO₂ drawdown (IPCC 2019; Almaraz et al. 2023). Biochar application as a soil amendment has been shown to increase soil moisture, C and nutrient retention, and plant yield (Bai et al. 2022; Gupta, Gupta, and Mondal 2019; Lehmann et al. 2021; Razzaghi, Obour, and Arthur 2020). Therefore, incorporating biochar into the soil brings various co-benefits to farming systems, which may be critical in the context of climate change. However, large field-scale biochar application where biochar is traditionally spread broadly on the soil surface and then incorporated may not be economically viable (Williams and Arnott 2010). As a result, biochar application practices are continually evolving to minimise cost, while achieving specific purpose-driven requirements of sites, soils and crops (Gabhane et al. 2020; Joseph et al. 2017). Trench burial in soil has been an effective strategy to store large quantities of C in the soil while capturing soil moisture (Ogawa and Okimori 2010; Sierra et al. 2024). The buried biochar in trenches is usually covered by a layer of soil or sand (Ogawa and Okimori 2010). However, creating trenches filled with biochar without a top-soil cover could further enhance the trapping of air humidity and rainfall. The cost of biochar burial in trenches can vary between 64 USD ha⁻¹ for an application rate of 12 Mg ha⁻¹ to 3160 USD ha⁻¹ at 185 Mg ha⁻¹, depending on the trench depth, the feedstock type and application rate (Campion et al. 2023; Williams and Arnott 2010). The cost of trenching may be reduced by activating biochar with organic matter, which enhances the composting process of the organic material in the mixture and could create an optimal niche for microorganisms, fostering nutrient availability for plants while enhancing soil C storage, particularly when moisture levels are high (Filiberto and Gaunt 2013; Joseph and Taylor 2024; Öztürk et al. 2012). Trenches created using biochar-activated organic matter were referred to as reactive barriers (RB) in the current study, given the reactive biogeochemical and hydrological processes that are created in the trench barriers within the soil. A 13-years old study has shown that biochar-based trenches in tree crops increases soil C and

water storage (Mahajan et al. 2021). However, the extent in which the beneficial properties of RB would expand laterally to surrounding soils, particularly in the short term to justify the initial cost of RB establishment, remains uncertain.

Another way to reduce the cost of biochar application is to enhance the biochar before application to soil (Joseph et al. 2013, 2021; Rasse et al. 2022). Enhanced biochar is usually combined with chemical or organic fertilisers to produce biochar compound fertilisers, added to compost, or enhanced with clay, minerals, and nutrients prior to application to create biochar mineral complex (BMC) (Bai et al. 2024; Joseph et al. 2013, 2021). Nutrient-rich BMCs are innovative approaches to applying biochar at low rates whilst maximising its benefits (Darby et al. 2016; Farrar et al. 2019). For example, the application of BMC has been shown to increase yield in different crops, including ginger, pepper, peanut, wheat, and maize (Farrar et al. 2019; Glaser et al. 2015; Yao et al. 2015; Zheng et al. 2017), most likely due to improved nutrient use efficiency (Rasse et al. 2022). The BMCs are usually developed as solid formulations, but sometimes there is a need to apply liquid nutrients to expedite nutrient inputs and plant uptakes. To date, only a limited number of trials have been conducted to show the potential of BMCs to increase crop yield (Kilowasid et al. 2022; Kumar et al. 2021; Lou et al. 2016); however, no trials have yet explored the co-benefits of bespoke liquid fertilisers on soil moisture, C storage, and nutrient availability in addition to crop yield.

This study used a pasture model system because: (1) livestock production industry has a huge C footprint with 18% contributions to greenhouse gas emissions (Chiarello et al. 2021; Franzluebbers 2020; Mogensen et al. 2014), and (2) pasture soils are often depleted in soil C and nutrients, and therefore require frequent fertiliser applications to sustain yield (Silveira and Kohmann 2020). This study examined the effects of combined use of a novel open surface RB filled with biochar and liquid BMC on soil C storage, moisture retention, plant nutrient uptake and yield (Figure 1), and assessed the cost effectiveness of this innovative approach in the short term. Since biochar is known to promote microbial growth due to its porous structure, the presence of micro and nanosized mineral nutrients and labile C (Dai et al. 2021), we expected that the RB would start storing moisture, thereby affecting soil microbial community within the RB. However, it was uncertain to what extent soil and grass near the RB would benefit from moisture accumulation and microbial growth within the RB. We hypothesized that (1) the stored moisture within RB would migrate and diffuse into the surrounding soils, enhancing microbial growth and activity. This, in turn, would result in high pasture yields on the top of the RB; (2) a few months after RB establishment, there would be a build-up of moisture, nutrients, C and microorganisms in the soil adjacent to the RB, as the positive effect of the RB would expand laterally; and (3) BMC application would increase soil moisture and C storage, and an improvement of pasture yield would be observed next to the RB, as a result of the increases in soil moisture, nutrients and microbial activity in the RB proximity. Altogether, our study provides evidence on how RB and BMC can be effectively utilized to retain soil moisture while building C storage, making this method applicable to a broad range of planting systems.

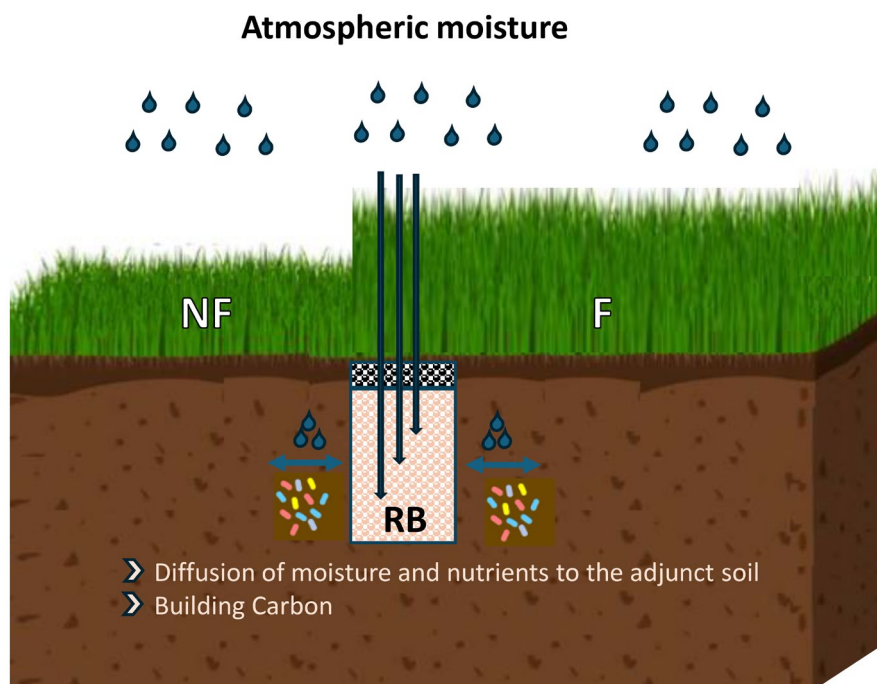


FIGURE 1 | Schematic of a reactive barrier (RB), fertilised (F) and non-fertilised (NF) areas, illustrating the drawdown of atmospheric moisture and rainfall and its release into the soil, as well as the accumulation of soil carbon and microbes in the adjunct soil.

2 | Methods

2.1 | Site Description, Treatment Preparation, Establishment, and Experimental Design

The experimental site was located in a pastureland at Berrigan, New South Wales (NSW) (S35.677594 E145.804731. 35°67' S 145°80' E). The soil is classified as a chromosol and is a red-brown duplex soil with sandy-loam texture (Isbell 2016). The climate is characterised by hot summers and cool winters with low annual precipitation of 438 mm. Daily average temperatures range between 13.1°C and 31.0°C and overnight between 4.0°C and 17°C. The site was used to grow pasture for 10 years with minimal diammonium phosphate (DAP) fertiliser applied for 7 years prior to establishment. The experimental site was not grazed for 3 months prior to establishment and sown with grass mix 3 days prior to site establishment in May 2023. The grass contained a mixture of Italian ryegrass (*Lolium multiflorum*) at a rate of 18 kg ha⁻¹ and three clover varieties of balansa clover (*Trifolium michelianum*), Crismin clover (*Trifolium incarnatum*) and Berseem clover (*Trifolium alexandrinum*) sown at a rate of 4 kg ha⁻¹ each.

Wood-biochar used in RB was prepared onsite at 600°C using a commercial type of kiln. Then, the biochar was mixed with cow manure, straw, basalt dust, diatomite, and gypsum in a ratio of 31:30:30:5:2:2 (dry w/w), with 5% wood vinegar added before being placed in the RB. Biochar used in the production of liquid BMC was made at 450°C from straw and poultry litter (50:50) feedstocks combined with minerals, basalt, diatomite, rock phosphate, bentonite, kaolinite, and ferrous sulfate. The liquid BMC was produced by micronizing the BMC solid (200 kg ha⁻¹ equivalent in dilution) and diluting with water, a water-based dispersion agent, and a commercial organic fertiliser rich in N (% 10.1) (Charlie Carp, NSW, Australia). The solution was then adjusted to pH 6.5 by adding 5%

wood vinegar. The chemical composition of solid biochar incorporated into the RB and liquid BMC has been summarised in Table 1.

Biochar used in the RB was characterised by scanning electron microscope (SEM) analyser (Nano SEM 350 FEI Netherlands) equipped with a Bruker X-ray energy dispersive spectroscope (EDS). The chromium coating was used to make the sample electrically conductive. The biochar used in the RB and liquid BMC were further characterised by PerkinElmer Fourier transform infrared (FTIR) spectroscopy using the wavenumber range (450–4000 cm⁻¹) with a 16 cm⁻¹ spectral resolution and 64 accumulations for each collection by the array at the Chemical Analytical Facility Griffith University.

Six replicated plots with an area of 26 m × 10 m were established in May 2023 and plots were 2 m apart. Each plot contained two RBs spaced 10 m apart (Figure 2a). Each RB was excavated to dimensions of ≈10 m length, 0.5 m width and 0.8 m depth using a mechanical trencher. Each reactive barrier was filled with a 0.6 m layer of the wood-biochar mixed with straw and manure mixture, and 0.2 m layer of wood-biochar (Figure 2a). The fertilised areas between two RBs received liquid BMC on two occasions at week 0 (May 2023) and week 9 (June 2023) at a rate of 200 kg dry BMC ha⁻¹, for a total application rate of 400 kg ha⁻¹. These areas were also top-dressed with DAP at week 0 and urea at week 9, each applied at the rate of 100 kg ha⁻¹. The other sides of RB received no fertiliser (Figure 2a).

2.2 | Soil Sample Collection and Bio-Chemical Analyses

Soil biochemical data were collected from the topsoil, which is the major hotspot for microbes involved in nutrient cycling and

TABLE 1 | Characterization of soil, biochar mixture used inside the reactive barriers (RB), pure biochar used on top of the RB, and liquid biochar mineral complex (BMC) used in the field trial.

	Units	Soil	Mixture used in the RB	Pure biochar	Liquid BMC
pH _{H₂O}		6.18	6.58	7.50	3.28
EC	(mS/cm)	0.06	0.91	0.40	283.00
Total C	%	1.86	11.7	70.74	12.1
Total N	%	0.14	0.5	1.26	4.09
δ ¹³ C	‰	−27.14	−27.40	−26.9	−25.9
δ ¹⁵ N	‰	4.36	4.2	3.2	−2.70
P	mg 100 g ^{−1}	25.56	64.72	47.2	4719.00
K	mg 100 g ^{−1}	209.94	316.6	162.2	1037.50
Al	mg 100 g ^{−1}	1397.10	752.78	295.2	349.80
As	mg 100 g ^{−1}	BDL	BDL	5.30	BDL
B	mg 100 g ^{−1}	0.51	0.53	19.1	4.60
Ca	mg 100 g ^{−1}	107.55	312.13	1296.7	121.60
Cd	mg 100 g ^{−1}	BDL	BDL	0.10	BDL
Cr	mg 100 g ^{−1}	1.62	9.03	—	0.80
Co	mg 100 g ^{−1}	0.31	BDL	BDL	0.20
Cu	mg 100 g ^{−1}	0.63	1.53	8.70	1.00
Fe	mg 100 g ^{−1}	865.74	488.98	588.8	502.00
Hg	mg 100 g ^{−1}	BDL	1.36	0.10	BDL
Mg	mg 100 g ^{−1}	77.60	116.86	279.8	95.80
Mn	mg 100 g ^{−1}	18.29	15.63	19.50	8.10
Mo	mg 100 g ^{−1}	BDL	0.29	BDL	0.10
Na	mg 100 g ^{−1}	9.23	39.56	187.4	246.90
Pb	mg 100 g ^{−1}	1.97	1.61	—	0.20
S	mg 100 g ^{−1}	14.79	41.43	76.00	221.60
Se	mg 100 g ^{−1}	BDL	BDL	4.90	BDL
Si	mg 100 g ^{−1}	7.36	3.51	BDL	29.10
Zn	mg 100 g ^{−1}	1.42	2.77	23.4	3.50

Abbreviations: Al, aluminium; As, arsenic; B, boron; BDL, below detectable level; Ca, calcium; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; EC, electrical conductance; Fe, iron; Hg, Mercury; K, potassium; Mg, magnesium; Mn, manganese; Mo, molybdenum; Na, sodium; P, phosphorus; Pb, lead; S, sulphur; Se, selenium; Si, silicon; Total C, total carbon concentration; Total N, total nitrogen concentration; Zn, zinc; δ¹³C, C isotopic composition; δ¹⁵N, N isotopic composition.

where microbial communities are influenced by land use (Bai et al. 2023; Xue et al. 2023). Volumetric moisture concentration (VMC %) was recorded throughout the summer and at a depth of 0–10 cm at weeks 16, 22, 28, 34, 36 and 39 following the RB establishment (Field Scout TDR 300, Spectrum Technologies Ltd, UK). The soil samples were collected at the 0–10 cm depth using an auger with inner diameter of 60 mm at week 16. The soil samples were collected at 10 cm, 50 cm and 600 cm distances away from the RB in both fertilised and non-fertilised areas. Biochar from the centre of the RB was also collected (Figure 2b).

A sub-sample of the collected soil from each point and biochar from the top of the RB was immediately stored at −20°C to be

used for soil respiration and microbial community composition analyses. The remaining of the soil and biochar samples were air-dried and sieved to 2 mm prior to chemical analysis.

A sub-sample (≈20 mg) of the air-dried and sieved soil and biochar was transferred to 8 × 5 mm tin capsules to measure total C, total N, and C and N isotope composition (δ¹³C and δ¹⁵N) using an Isotope Ratio Mass Spectrometer connected to a CN Eurovector Elemental Analyser, Italy (Ibell, Xu, and Blumfield 2013). Concentrations of NH₄⁺–N and NO₃[−]–N in soil samples were determined using 2 M KCl extraction and analysed with a SmartChem 200 Discrete Chemistry Analyser (Unity Scientific, Brookfield, CT, USA) (Rayment and Lyons 2011). Macro- and

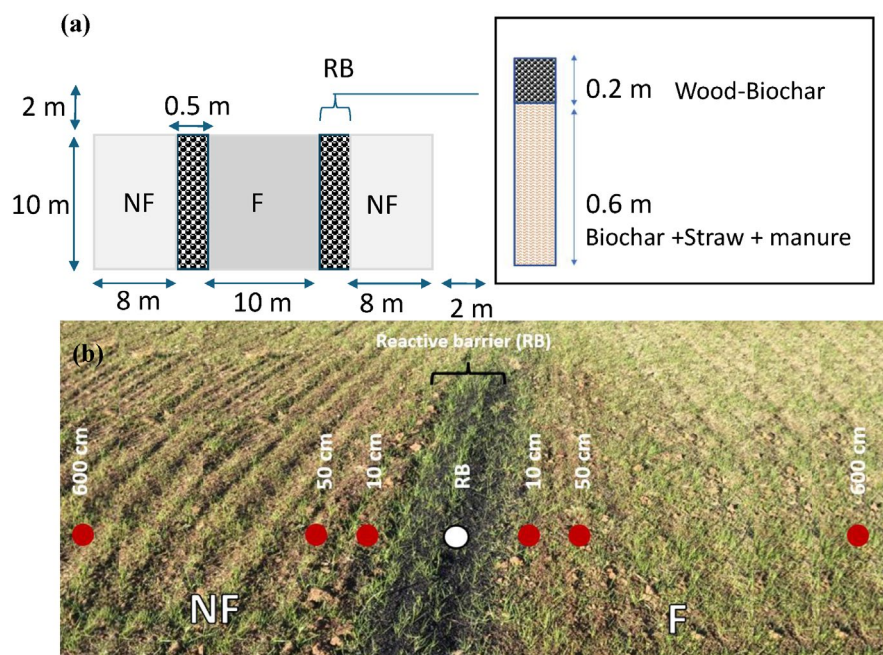


FIGURE 2 | (a) Experimental design and profile view (from top to bottom) of a reactive barrier (RB); areas with dark solid grey received biochar mineral complex with fertiliser top dressing (F) and areas with light solid grey received no fertiliser (NF); (b) Schematic of samples collected in the middle of the RB, 10, 50 and 600cm away from the RB in both fertilised (F) and non-fertilised (NF) areas.

micro-nutrient concentrations including phosphorus (P), potassium (K), aluminium (Al), arsenic (As), boron (B), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), lead (Pb), sulphur (S), silicon (Si), and zinc (Zn) concentrations were determined using ICP-OES (Optima-5300V, Perkinelmer, Waltham, WA, USA) (Bai et al. 2017).

2.2.1 | DNA Extraction, Next-Generation Sequencing (NGS) Library Preparation, and Illumina MiSeq Sequencing

DNA extractions were carried out in triplicate from collected soil in designed sampling points and biochar on top of RB using the DNeasy PowerSoil DNA Isolation Kit (QIAGEN) following the company protocol. The extracted DNA were sent to GENEWIZ of Azenta Life Sciences company (Suzhou, China) for the preparation of amplicon libraries and Illumina sequencing. The sequencing libraries were constructed using a MetaVX Library Preparation Kit. Briefly, 20–50ng of DNA was used to generate amplicons that cover V3 and V4 hypervariable regions of the 16s rRNA gene of bacteria and ITS rRNA gene of fungi. The forward and reverse primers were 338F (5'-CCTACGGRBGCASCAGKVRVGAAT-3') and 806R (5'-GGACTACNVGGGTWTCTAATCC-3') for 16s rRNA and 'GTGAATCATCGARTC' and 'TCCTCCGCTTATTGAT' for ITS rRNA respectively. Amplicon sequencing (paired-end, 2×300bp) was conducted on an Illumina MiSeq/Novaseq Platform (Illumina, San Diego, USA) by GENEWIZ from Azenta Life Sciences. The effective sequences after quality filtration were grouped into operational taxonomic units (OTUs) by using the clustering program VSEARCH (1.9.6) against the Silva 132 database pre-clustered at 97% sequence identity.

2.2.2 | Soil Microbial Respiration and Substrate-Induced Respiration

Microbial respiration and functional profile (substrate-induced respiration) were determined using the MicroResp technique (Campbell et al. 2003). Soil sub-samples (~0.4g each) were carefully placed into 96 deep-well plates using a filling device and the moisture content adjusted to 30% their maximum water holding capacity (WHC) by adding distilled water. Prior to conducting the MicroResp method, the deep-well plates with the moisture-adjusted soils were incubated at 25°C for 14 days in a sealed container with self-indicating soda lime and a damp paper towels (Amarasinghe et al. 2021). The CO₂ detection plates were also prepared by mixing the indicator dye, cresol red (12.5 ppm, wt/wt), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM) into 150 µL of 1% Noble agar for each well (Campbell et al. 2003). Eight nutrient substrates including C sources using fructose, glucose, maltose, sucrose, and amino acid sources using alanine, leucine, tryptophan, were used to determine the functional diversity of the microbes. Basal respiration was also measured by adding sterilized water. The agar and indicator solutions were prepared separately and combined with 1:2 ratio (agar: indicator) prior to use. These plates were stored in sealed plastic boxes with wet paper towels and a beaker containing water and soda lime to ensure that they do not desiccate or react with atmospheric CO₂. Carbohydrates and amino acids substrate solutions were prepared based on their solubility in water. Once the required volume was prepared, the solution was filter-sterilized and stored in sterile tubes at 4°C. Thirty microliters (30 µL) from each prepared substrates and filter-sterilized distilled water (to measure the basal respiration) were added to the pre-incubated soil (14 days) in the deep-well plates. In order to calculate C utilization, the detection plate colour was measured as absorbance at 570 nm immediately before and after

the 6 h incubation using a microplate reader (Burton et al. 2010; Campbell et al. 2003).

2.3 | Grass Yield Collection and Analysis

We harvested the grass just before conventional harvest time on two occasions of weeks 16 and 22 following the site establishment. Dry season commenced after the final harvest and grass stops growing. Grass yield, height and chlorophyll concentration were measured within a 1 m² quadrant placed randomly adjacent to the RB and at a distance meant to include the corresponding soil samples for 10 and 50 cm distances. A second sample using the quadrant was taken at a distance of 550 cm to include soil taken at 600 cm in the centre of the quadrant. Height and chlorophyll concentration were measured at the centre of the quadrant. Chlorophyll concentration of the grass was measured using a Soil Plant Analysis Diagnostic (SPAD) index, determined by a SPAD chlorophyll meter (atLEAF CHL PLUS). The grass of each quadrant was then manually harvested using grass shears at about 5 cm above the ground to determine yield in each quadrant. After harvesting, the fresh weight of the grass sample from each sampling point were immediately recorded using a digital balance (Salter Heston Blumenthal Precision Dual Platform Digital Kitchen Scale) and yield data were calculated and reported as t ha⁻¹. We also collected grass yield, height and chlorophyll concentration on top of the RB in an area of 1 m².

A sub-sample of approximately 100 g fresh grass was weighed separately for moisture determination and oven-dried at 60°C for 72 h to constant weight to estimate dry biomass. Dry biomass samples were then ground to a fine powder using a Rocklabs ring grinder and used to measure total C, total N, and C and N isotope composition, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, and nutrient concentrations. Micro- and micro-nutrient concentrations including P, K, Al, As, B, Ca, Cr Co, Cu, Fe, Mg, Mn, Na, Pb, S, Si, and Zn concentrations were analysed using an ICP-OES (Optima-5300V, Perkinelmer, Waltham, MA, USA) at the Chemical Analytical Facility at Griffith University (Bai et al. 2017).

2.4 | Data Calculations and Statistical Analyses

Potential CO₂ removed (kg) per kg biochar applied in each cubic meter of RB was calculated using the VERA method as described in Adhikari et al. (2024). In brief, we calculated CO₂ removal using the following equation:

$$\begin{aligned} &\text{Potential CO}_2 \text{ removed (kg) per kg biochar} \\ &= \text{Total C of biochar (\%)} \times 3.67 \times \text{PR}_{\text{de}} \end{aligned} \quad (1)$$

Where total C content of biochar was obtained from elemental analysis of pure biochar collected from 0.2 m layer of RB, and from biochar mixed with straw and cow manure collected from 0.6 m layer of RB. The C content for 0.6 m layer of the RB was based on the sum of measurement from samples taken at depths of 0.3–0.4 m, 0.4–0.6 m and 0.6–0.8 m in each RB. The 3.67 is a multiplying factor to convert quantity of elemental C to its equivalent quantity of CO₂ derived from the molecular weight ratio of CO₂ to C (44/12). The PR_{de} is permanence adjustment

factor accounts for stability of C in biochar. For hydrogen to C ratio less than 0.4, the factor is 0.74 indicating the higher stability (used for 0.2 m layer of the RB). While, for hydrogen to C ratio greater than 0.4, the factor is 0.56 indicating lower stability (used for 0.6 m layer of the RB).

Total N, P and K uptake were separately estimated as the grass yield (kg ha⁻¹) and nutrient concentrations in grass tissues following the equation below (Baptistella et al. 2020):

$$\begin{aligned} &\text{Nutrient uptake (Kg NPK ha}^{-1}\text{)} \\ &= \frac{\text{Grass dry biomass content (Kg DM ha}^{-1}\text{)} \times \text{nutrients (\%)}}{100} \end{aligned} \quad (2)$$

The nutrient use efficiency (NUE) was calculated as the differences in nutrient uptake in the fertilised area and the control of each plot, divided by the nutrient (N, P and K) applied using the following equation (Baptistella et al. 2020; Bastidas et al. 2024):

$$\text{NUE (\%)} = \frac{\text{Kg Ni} - \text{Kg N0}}{\text{nutrient rate of fertilised treatment}} \times 100 \quad (3)$$

Where N_i is the nutrient uptake in fertilised area (kg ha⁻¹), N₀ is the nutrient uptake in non-fertilised area (kg ha⁻¹) and rate of nutrient applied in fertilised area (kg ha⁻¹).

Partial nutrient (N, P and K) balances were calculated as the differences between the nutrient input and nutrient output representing the nutrient uptake over the period of trial using the following equation (Touhami et al. 2022).

$$\begin{aligned} &\text{Partial nutrient balance (kg NPK ha}^{-1}\text{)} \\ &= \text{nutrient input} - \text{nutrient output} \end{aligned} \quad (4)$$

A simple economic and environmental estimation of RB was calculated (Table S1). This analysis was mainly focused on costs, including construction of RB, excavation and soil removal, as well as the expenses associated with biochar, straw and cow manure used inside of RB. On the benefit side, it emphasized environmental benefits such as the CO₂ removal per each cubic meter of RB, along with the economic benefits from selling the pasture grass.

The estimate of total costs and break-even of BMC are summarised in Table S1. Costs were divided into two categories, fixed and variable. Fixed costs include freight within 100 km radius on the experimental site and cost to apply all fertiliser products. Variable cost was the price to produce BMC fertiliser and has been estimated at USD\$700–1000 t⁻¹ based on the ex-factory sales price of liquid BMC provided by the producer involved in this study. Revenue increase was calculated as the differences in yield between fertilised and non-fertilised area, multiplied by the sale price of the grass using the following equation (Imran, Salim, and Adam 2023):

$$\begin{aligned} &\text{Revenue increase (USD kg}^{-1}\text{)} \\ &= (\text{Yield}_{\text{fertilised area}} - \text{Yield}_{\text{non-fertilised area}}) \times \text{Sale price USD ha}^{-1} \end{aligned} \quad (5)$$

The break-even of BMC was calculated where the sum of the total costs equaled the increase in revenue from the additional yield in the fertilised area using the following equation (Horváth 2017)

$$\text{Total revenues increase} = \text{Total cost of production} \quad (6)$$

This marks the point at which the costs are fully covered, and no profit or loss is incurred.

In all statistical analyses, we excluded data collected from the RB. Three-way analysis of variance (ANOVA) was used to detect the main effects of sampling time, fertilisation and distance from RB, and their interactions for soil moisture concentration followed by Tukey test when the main effects were significant. Two-way ANOVA was used to determine the main effects of fertilisation and distance from RB, and their interactions on soil and grass properties followed by Tukey test when the main effects were significant. The means were compared using Tukey test at 0.05 probability level. Soil total C concentrations in soil samples collected from 10 cm points varied between 1.70% and 5.90% as a result of biochar presence in the soil and hence were excluded from analysis to ensure soil total C changes were not influenced by biochar contamination from the RB.

Differences in basal respiration and substrate-induced respiration between the fertilisation and distance from the RB were analysed using two-way ANOVA and Tukey test. Where, interaction between effects of fertilisation and distance from RB was found to be significant, a series of one-way ANOVA test were performed (Omidvar et al. 2023). All statistical analysis was performed with IBM SPSS 29.0. Differences among patterns of microbial metabolic function profiles were tested with permutational analysis

of variance (PERMANOVA) and visualised using multidimensional scaling (MDS) of bootstrap averages. Monte Carlo P-values ($p < 0.05$) were used to detect significant differences among different treatments (Garcia-Palacios et al. 2016). MDS and PERMANOVA were run in PRIMER v7 with PERMANOVA+.

3 | Results

3.1 | Characteristics of Biochar Used in RB and Liquid BMC

The SEM images of biochar used in RB showed that the pores had been filled with minerals such as K, Ca, O, Si, and Al with high concentrations (Figure 3a–c). The biochar used in the RB showed distinct peaks corresponding to different functional groups, including hydroxyl ($-\text{OH}$), carbonyl ($-\text{C}=\text{C}$), lignin ($-\text{CH}$), aliphatic phosphate ($\text{P}-\text{O}-\text{C}$), cellulose ($\text{C}-\text{O}-\text{C}$) and silicon ($\text{Si}-\text{O}-\text{Si}$) using FTIR analysis (Figure 3d). The liquid BMC showed distinct peaks at 3400, 1660, 1653, 1430, 1150, 1030, and 940 cm^{-1} , corresponding to functional groups including hydroxyl ($-\text{OH}$), amide ($-\text{N}-\text{C}=\text{O}$), carboxyl ($-\text{C}=\text{O}$), carbohydrate ($\text{C}-\text{O}-\text{C}$), silicon ($\text{Si}-\text{O}-\text{Si}$), and phosphate ($-\text{P}-\text{O}$) using FTIR analysis (Figure 3e). Chemical properties of the mixture used in the RB, biochar used in the RB, and liquid BMC are summarized in Table 1.

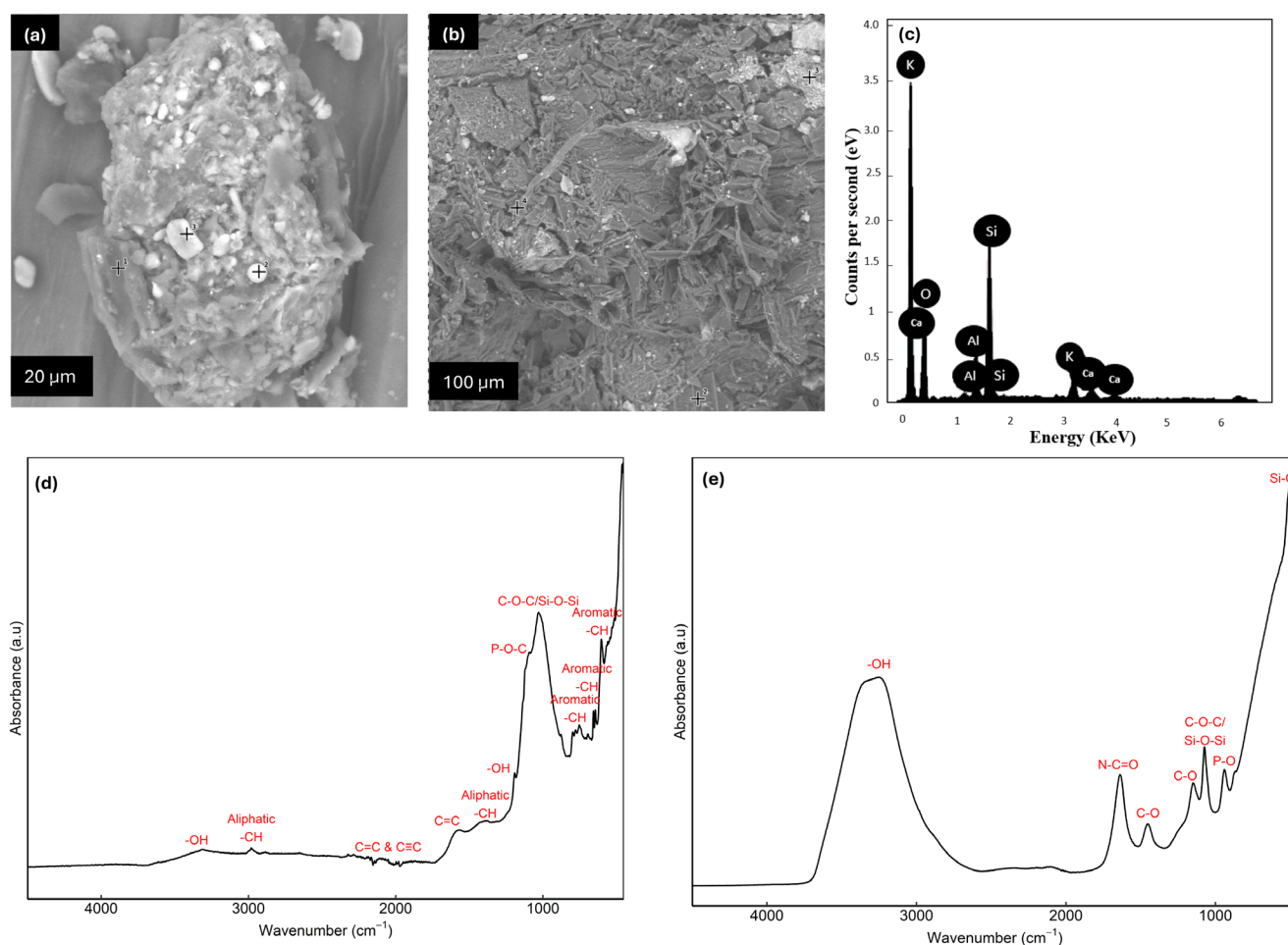


FIGURE 3 | Scanning electron microscope (SEM) of (a, b and c) biochar used in reactive barriers (RB); fourier transform infrared (FTIR) spectroscopy of (d) biochar used in the RB and FTIR of (e) liquid biochar mineral complex (BMC).

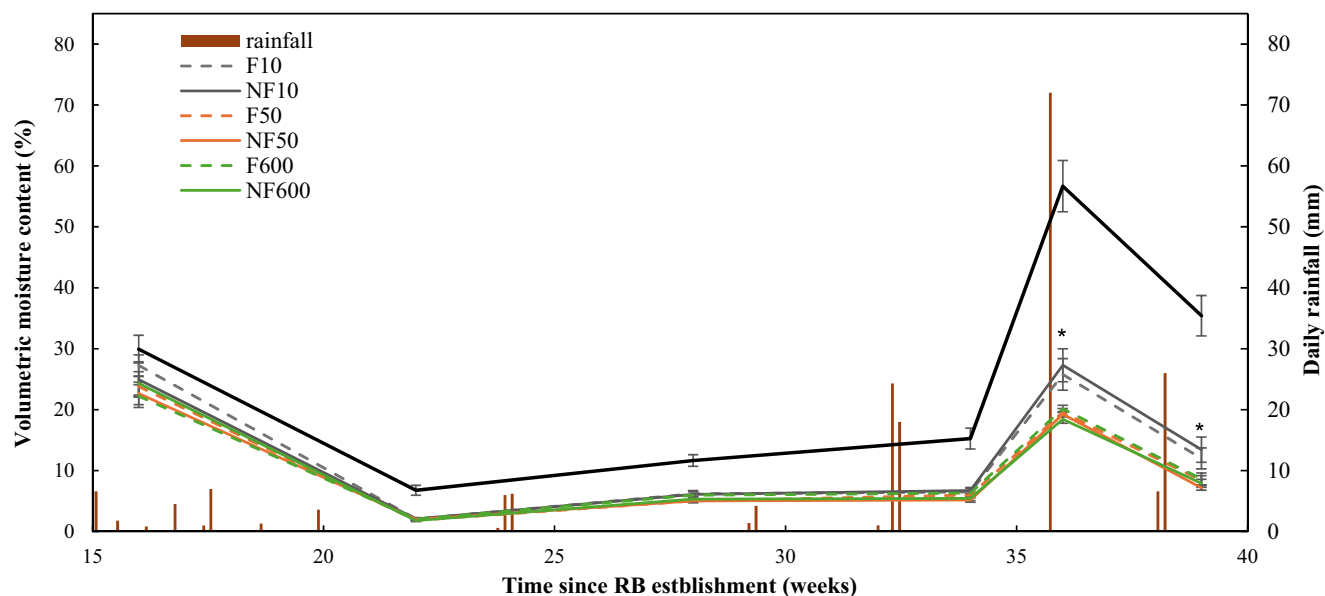


FIGURE 4 | Soil moisture concentration (%) in reactive barriers (RB—Solid black line) and soil at 10, 50 and 600 cm away from the RB in both fertilised (F) and non-fertilised areas (NF) at weeks 16, 22, 28, 34, 36 and 39 since RB establishment. Values shown are mean $\pm$ SE ($n = 6$), * represents soil moisture concentration at 10 cm was significantly higher than that of 50 and 600 cm away from the RBs.

3.2 | Soil Moisture Concentrations and Biochemical Properties of Reactive Barriers and Soil

From week 36 onwards, soil moisture concentration was higher within a 10 cm distance from the RB compared with 50 and 600 cm distances in both fertilised and non-fertilised areas of the RB (Figure 4). The moisture concentration within the RB varied between 6.76% and 56.68% throughout the assessment period. The moisture concentration within the RB varied in response to rainfall (Figure 4).

Distance from the RB was the main cause for significant effect on soil total C, specifically, on the non-fertilised area, soil total C was higher close to the RB (50 cm) compared with 600 cm distance from the RB (Figure 5). Soil total N concentrations and $\delta^{15}\text{N}$ values were similar among treatments regardless of fertiliser application and distance from the RB (Table 2). Soil NH_4^+-N and NO_3^--N concentrations were significantly higher in the fertilised area compared with the non-fertilised area, and with no effect of distance away from the RB (Table 2). Majority of soil nutrient concentrations was not affected by fertilisation and distance from the RB. However, significant distance effects were detected in soil P, B, Na, Pb and Si concentrations, which were the highest at the closest point to the RB (10 cm) (Table 2).

3.2.1 | Soil Bacterial and Fungal Community Composition

A total number of 2,121,461 16S DNA quality-filtered reads were observed with an average of 91,847 sequenced reads and read length of 451 bp per sample. Soil bacterial sequences were clustered into 17,146 OTUs with 97% sequence similarity after quality trimming and sequence filtering. The bacteria sequences were distributed in 44 individual bacterial phyla, 1085 genera,

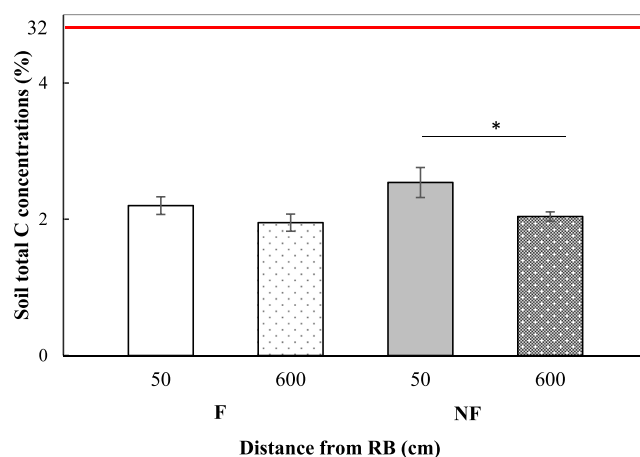


FIGURE 5 | Soil total carbon (C) concentrations in 50 and 600 cm away from reactive barriers (RB—Red solid line) in both fertilised (F) and non-fertilised (NF) areas at week 16 following application of liquid biochar mineral complex (BMC) and RB establishment. Values shown are mean $\pm$ SE ($n = 5$).

and 1370 species. The most dominant bacterial phyla were *Actinobacteria* (24.10%), *Firmicutes* (21.23%), *Proteobacteria* (20.65%), *Acidobacteria* (11.03%) and *Bacteroidota* (7.14%), which accounted for 84.16% of the total number of bacterial OTUs detected (Figure 6). A heatmap of bacterial community composition at the phylum level is presented in Figure S1.

A total number of 2,123,900 ITS DNA quality-filtered reads were observed with an average of 92,049 sequenced reads and a read length of 328 bp per sample. Soil fungal sequences were clustered into 5591 OTUs with 97% sequence similarity. The fungal sequences were distributed in 19 individual bacterial phyla, 657 genera, and 1171 species. The most dominant fungal phyla were *Ascomycota* (69.65%), *Unclassified fungi* (11.90%), *Mortierellomycota* (9.29%), and *Basidiomycota* (4.47%)

TABLE 2 | Nutrient concentration in reactive barriers (RB) and soil sampled at 10, 50 and 600 cm away from the RB in both fertilised (F) and non-fertilised areas (NF) at week 16 following application of liquid biochar mineral complex (BMC). Values shown are mean $\pm$ SE ($n = 5$).

Nutrients	Units	RB	NF10	F10	NF50	F50	NF600	F600	F	D	F $\times$ D
Total N	(%)	0.72 $\pm$ 0.05	0.18 $\pm$ 0.02	0.20 $\pm$ 0.02	0.20 $\pm$ 0.02	0.20 $\pm$ 0.02	0.20 $\pm$ 0.02	0.18 $\pm$ 0.02	ns	ns	ns
$\delta^{15}\text{N}$	(‰)	3.84 $\pm$ 0.77	6.41 $\pm$ 0.52	5.83 $\pm$ 1.12	7.74 $\pm$ 1.29	6.25 $\pm$ 0.70	7.55 $\pm$ 0.77	6.25 $\pm$ 0.58	ns	ns	ns
$\text{NH}_4^+ \text{-N}$	($\mu\text{g g}^{-1}$)	6.14 $\pm$ 1.10	11.63 $\pm$ 1.24	26.90 $\pm$ 8.67	12.84 $\pm$ 0.97	29.56 $\pm$ 5.94	9.67 $\pm$ 1.78	16.03 $\pm$ 2.09	$p < 0.008$	ns	ns
$\text{NO}_3^- \text{-N}$	($\mu\text{g g}^{-1}$)	14.59 $\pm$ 2.89	7.65 $\pm$ 1.57	18.47 $\pm$ 7.55	7.00 $\pm$ 0.96	22.52 $\pm$ 4.95	8.06 $\pm$ 1.85	10.41 $\pm$ 3.38	$p < 0.002$	ns	ns
P	(mg 100 g $^{-1}$)	54.31 $\pm$ 4.08	29.61 $\pm$ 3.04	34.86 $\pm$ 1.81	39.03 $\pm$ 1.09	35.86 $\pm$ 1.48	35.30 $\pm$ 1.62	36.91 $\pm$ 2.89	ns	$p < 0.056$	ns
K	(mg 100 g $^{-1}$)	220.60 $\pm$ 16.58	336.45 $\pm$ 12.67	355.13 $\pm$ 13.67	309.49 $\pm$ 14.55	305.88 $\pm$ 16.85	293.89 $\pm$ 23.11	340.25 $\pm$ 27.17	ns	ns	ns
Al	(mg 100 g $^{-1}$)	585.43 $\pm$ 37.31	1850.02 $\pm$ 108.59	2008.98 $\pm$ 25.06	1965.80 $\pm$ 44.01	1869.70 $\pm$ 79.30	1993.39 $\pm$ 130.94	2058.58 $\pm$ 82.88	ns	ns	ns
B	(mg 100 g $^{-1}$)	9.68 $\pm$ 1.48	4.52 $\pm$ 1.51	5.05 $\pm$ 0.93	1.58 $\pm$ 0.83	1.43 $\pm$ 0.48	0.78 $\pm$ 0.05	1.22 $\pm$ 0.40	ns	$p > 0.001$	ns
Ca	(mg 100 g $^{-1}$)	768.23 $\pm$ 52.32	182.16 $\pm$ 24.37	152.97 $\pm$ 9.72	146.06 $\pm$ 4.36	146.24 $\pm$ 5.02	149.81 $\pm$ 6.57	166.09 $\pm$ 19.06	ns	ns	ns
Cr	(mg 100 g $^{-1}$)	8.69 $\pm$ 2.26	1.96 $\pm$ 0.26	1.92 $\pm$ 0.07	2.24 $\pm$ 0.07	2.17 $\pm$ 0.09	2.29 $\pm$ 0.10	2.24 $\pm$ 0.09	ns	ns	ns
Co	(mg 100 g $^{-1}$)	0.28 $\pm$ 0.02	0.40 $\pm$ 0.05	0.43 $\pm$ 0.02	0.44 $\pm$ 0.01	0.42 $\pm$ 0.01	0.44 $\pm$ 0.02	0.45 $\pm$ 0.01	ns	ns	ns
Cu	(mg 100 g $^{-1}$)	3.72 $\pm$ 0.40	0.87 $\pm$ 0.09	0.72 $\pm$ 0.03	0.87 $\pm$ 0.02	0.84 $\pm$ 0.05	0.87 $\pm$ 0.02	0.85 $\pm$ 0.04	ns	ns	ns
Fe	(mg 100 g $^{-1}$)	638.19 $\pm$ 19.44	1057.32 $\pm$ 95.51	1174.99 $\pm$ 59.89	1179.02 $\pm$ 8.69	1180.89 $\pm$ 27.05	1193.96 $\pm$ 44.39	1220.44 $\pm$ 25.25	ns	ns	ns
Mg	(mg 100 g $^{-1}$)	204.93 $\pm$ 18.93	121.08 $\pm$ 7.49	117.27 $\pm$ 3.93	104.47 $\pm$ 1.93	103.88 $\pm$ 1.07	110.56 $\pm$ 10.39	116.11 $\pm$ 5.44	ns	ns	ns
Mn	(mg 100 g $^{-1}$)	35.62 $\pm$ 8.94	18.79 $\pm$ 1.93	18.82 $\pm$ 0.92	19.72 $\pm$ 0.55	19.36 $\pm$ 0.44	18.46 $\pm$ 0.70	19.56 $\pm$ 0.67	ns	ns	ns
Na	(mg 100 g $^{-1}$)	82.56 $\pm$ 3.59	31.09 $\pm$ 8.34	18.99 $\pm$ 2.61	12.97 $\pm$ 1.04	13.66 $\pm$ 0.59	13.08 $\pm$ 2.40	13.66 $\pm$ 0.68	ns	$p > 0.006$	ns
Pb	(mg 100 g $^{-1}$)	7.44 $\pm$ 0.75	2.13 $\pm$ 0.27	2.05 $\pm$ 0.09	2.60 $\pm$ 0.19	2.55 $\pm$ 0.22	2.78 $\pm$ 0.13	2.64 $\pm$ 0.21	ns	$p < 0.01$	ns
S	(mg 100 g $^{-1}$)	45.58 $\pm$ 3.41	16.72 $\pm$ 2.05	17.46 $\pm$ 1.89	20.07 $\pm$ 0.60	18.84 $\pm$ 0.80	19.77 $\pm$ 0.73	20.08 $\pm$ 1.76	ns	ns	ns
Si	(mg 100 g $^{-1}$)	13.60 $\pm$ 0.82	27.01 $\pm$ 3.07	31.80 $\pm$ 4.10	13.16 $\pm$ 1.04	15.82 $\pm$ 1.14	12.37 $\pm$ 1.52	13.88 $\pm$ 1.47	ns	$p > 0.001$	ns
Zn	(mg 100 g $^{-1}$)	9.51 $\pm$ 0.70	2.52 $\pm$ 0.32	2.16 $\pm$ 0.05	2.16 $\pm$ 0.05	2.13 $\pm$ 0.08	2.11 $\pm$ 0.14	2.14 $\pm$ 0.07	ns	ns	ns

Note: Arsenic (As) was below detectable level (BDL) and excluded. F (fertilised); D (distance from RBs); F $\times$ D (interaction of fertiliser and distance); ns (not significant at $p < 0.05$).
Abbreviations: Al, aluminium; B, boron; Ca, calcium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; $\text{NH}_4^+ \text{-N}$, ammonium; $\text{NO}_3^- \text{-N}$, nitrate; P, phosphorus; Pb, lead; S, sulphur; Si, silicon; Total N, Soil total nitrogen concentration; Zn, zinc; $\delta^{15}\text{N}$, nitrogen isotopic composition.

(Figure 7). These accounted for 95.33% of the total number of fungal OTUs detected. A heatmap of fungal community composition at the phylum level is presented in Figure S2. No significant differences were observed in bacterial and fungal Shannon diversity and Chao1 Indices (Figure S3).

3.2.2 | Microbial Metabolic (Catabolic) Activities

Fertilised area at 10 and 50cm from the RB had higher metabolic activities than the non-fertilised area at the same distances for fructose, maltose, sucrose, alanine, and leucine substrates (Table 3). In general, the rate of carbohydrate utilization was greater than that of amino acids (Table 3). The respiration rate across all the substrates in the non-fertilised area with 600cm distance from the RB was significantly higher than the non-fertilised area with 10 and 50cm distance from the RB (Table 3). The Multidimensional Scaling (MDS) analysis, based on substrate-induced respiration data of soil microbes (i.e., functional capacity of the microbial community to metabolize different substrates, reflecting the metabolic diversity and adaptability), showed the differences in soil microbial functional diversity between the fertilised and non-fertilised areas being also affected by distances from the RB (Figure S4).

3.3 | Changes in Grass Yield, Height, and Chemical Properties

Grass yield was higher in the fertilised than that of non-fertilised area (Figure 8). Grass yield ranged from 16.65 to 19.31 t ha⁻¹ for

the non-fertilised area, and from 23.26 to 27.89 t ha⁻¹ for the fertilised area (Figure 8). Grass yield harvested directly from the RB was 29.61 t ha⁻¹ (Figure 8). Chlorophyll concentration (%) of grass was similar for all treatments (Table 4). Grass height was significantly higher for grass grown in the fertilised area compared with the non-fertilised area regardless of distance from the RB (Table 4). Grass total C concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were not affected by fertilisation nor distance from the RB (Table 4).

Grass N, P, and K concentrations were significantly higher in grass grown in the fertilised area compared with the non-fertilised area, but distance from the RB was not significant (Table 4). Uptake of N, P, and K were significantly higher in grass grown in the fertilised area compared with the non-fertilised area (Table 5). Uptake of P was also higher for grass grown 50cm distance from the RB than those grown at 600cm distance from the RB (Table 5). We observed positive P balance in the fertilised area and negative balances were observed for N and K in both fertilised and non-fertilised areas (Table 5). Majority of grass nutrient concentrations was not affected by fertilisation and distance from the RB (Table 4). However, grass S and Zn concentrations were significantly higher in the fertilised area compared with the non-fertilised area (Table 4). A significant distance effect was only detected in grass Mn and Zn concentrations, where Mn and Zn concentrations were higher at the 50cm than 600cm away from the RB (Table 4).

3.4 | Environmental and Economic Benefits

The RB contributed to approximately 158 kg CO₂e removal per cubic meter of established RB and also applying liquid BMC is

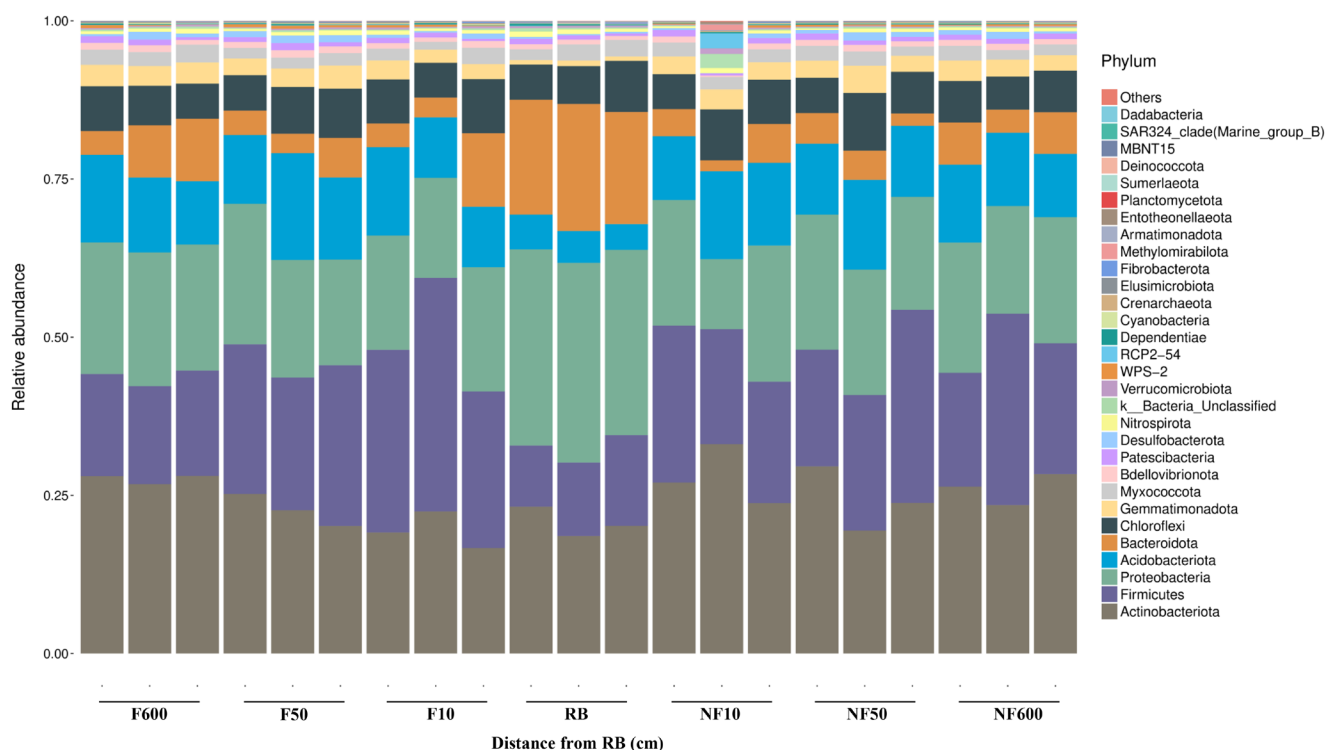


FIGURE 6 | Bacterial community structure at the phylum level in reactive barriers (RB), and in the soil sampled at 10, 50 and 600cm away from the RB in both fertilised (F) and non-fertilised areas (NF) at week 16 following application of liquid biochar mineral complex (BMC). Relative abundance was based on the proportional frequencies of DNA sequences that could be classified.

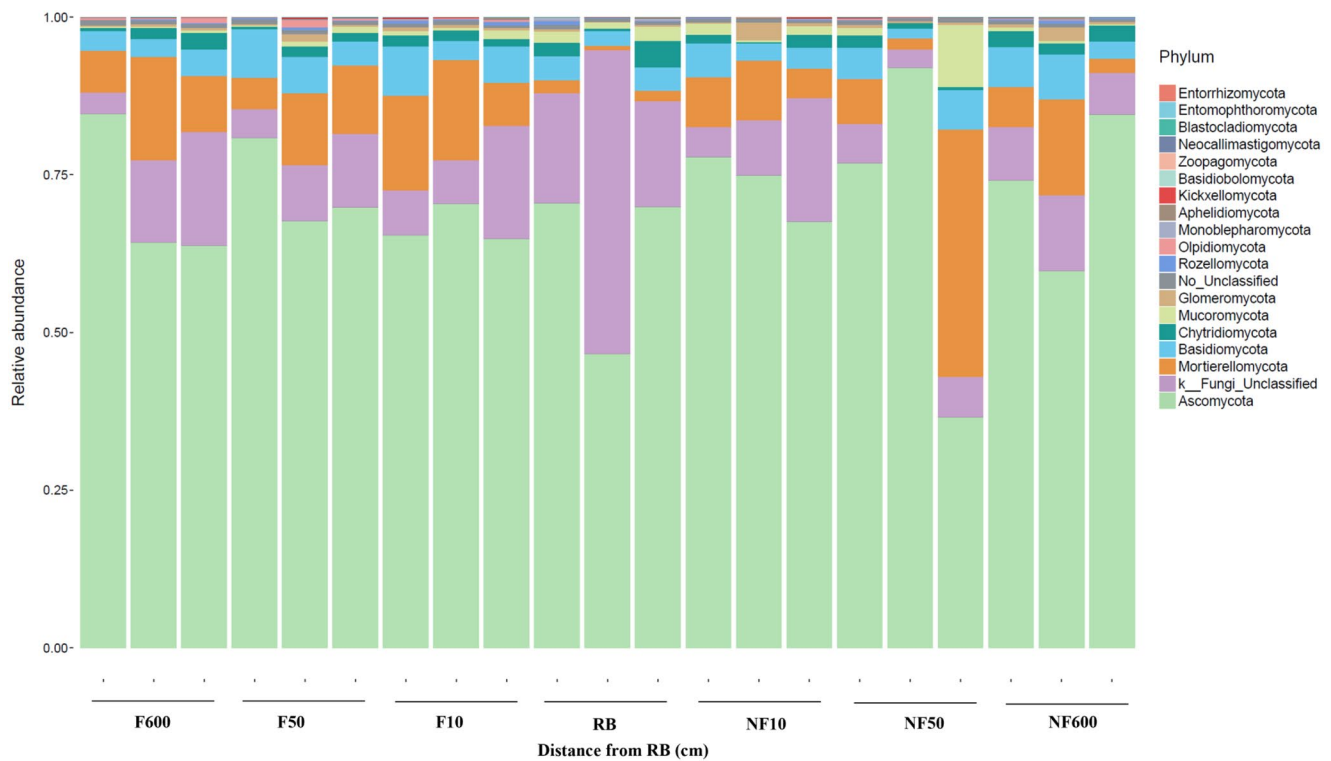


FIGURE 7 | Fungal community structure at the phylum level in reactive barriers (RB), and in the soil sampled at 10, 50 and 600 cm away from the RB in both fertilised (F) and non-fertilised areas (NF) at week 16 following application of liquid biochar mineral complex (BMC). Relative abundance was based on the proportional frequencies of DNA sequences that could be classified.

TABLE 3 | The average rate of nutrient substrate utilization by the microbes in reactive barriers (RB) and soil sampled at 10, 50, and 600 cm away from the RB in both fertilised (F) and non-fertilised (NF) areas at week 16 following application of liquid biochar mineral complex (BMC). Values shown are mean $\pm$ SE ($n=5$). Different letters in a row show significant differences among treatments excluding RB using Tukey's post hoc test following one-way ANOVA at $p < 0.05$.

Substrates	Source	RB	NF10	F10	NF50	F50	NF600	F600
Distilled Water	Distilled Water	2.13 $\pm$ 0.08	0.95 $\pm$ 0.06d	2.04 $\pm$ 0.12a	0.86 $\pm$ 0.02d	1.52 $\pm$ 0.04bc	1.93 $\pm$ 0.14ab	1.51 $\pm$ 0.09c
Fructose	Carbohydrates	2.70 $\pm$ 0.15	1.13 $\pm$ 0.07c	2.34 $\pm$ 0.10ab	1.00 $\pm$ 0.02c	1.99 $\pm$ 0.13b	2.73 $\pm$ 0.25a	1.98 $\pm$ 0.06b
Glucose	Carbohydrates	2.77 $\pm$ 0.20	1.06 $\pm$ 0.06bc	2.52 $\pm$ 0.30a	0.98 $\pm$ 0.03c	1.80 $\pm$ 0.04abc	2.63 $\pm$ 0.37a	1.88 $\pm$ 0.04ab
Maltose	Carbohydrates	2.55 $\pm$ 0.14	1.11 $\pm$ 0.07b	2.10 $\pm$ 0.06a	1.00 $\pm$ 0.02b	2.00 $\pm$ 0.29a	2.70 $\pm$ 0.29a	2.18 $\pm$ 0.17a
Sucrose	Carbohydrates	2.55 $\pm$ 0.16	1.12 $\pm$ 0.07b	2.10 $\pm$ 0.05a	0.99 $\pm$ 0.02b	2.04 $\pm$ 0.24a	2.57 $\pm$ 0.18a	2.02 $\pm$ 0.09a
Alanine	Amino acids	2.50 $\pm$ 0.16	1.08 $\pm$ 0.07c	1.86 $\pm$ 0.05b	0.97 $\pm$ 0.02c	1.64 $\pm$ 0.09b	2.39 $\pm$ 0.12a	1.82 $\pm$ 0.08b
Leucine	Amino acids	2.31 $\pm$ 0.15	1.04 $\pm$ 0.07c	1.63 $\pm$ 0.02b	0.93 $\pm$ 0.01c	1.49 $\pm$ 0.07b	2.29 $\pm$ 0.09a	1.61 $\pm$ 0.04b
Tryptophan	Amino acids	2.12 $\pm$ 0.16	0.99 $\pm$ 0.06c	1.42 $\pm$ 0.02b	1.45 $\pm$ 0.06b	1.35 $\pm$ 0.03bc	2.21 $\pm$ 0.18a	1.44 $\pm$ 0.05b

Note: Distilled water (to determine the basal respiration).

estimated to contribute an additional 150 kg CO₂e ha⁻¹ removal annually (Table 6). Net revenue increase for BMC application varied between USD 767 and USD 887 ha⁻¹ based on low and high BMC costs of USD 280 ha⁻¹ and USD 400 ha⁻¹, respectively (Table S1). Although commercial production of these liquids has not been undertaken, the producers involved in this study estimated the ex-factory sales price of liquid BMC would be between USD 700 and USD 1000 t. Assuming the liquid BMC was produced within a 100-km radius of the farm with a freight cost of USD 300 t (based on current rates), then the cost for an application rate of 0.4 t ha⁻¹ would range between USD 400 and USD

520 ha⁻¹ (Table S1). The costs of DAP and UREA applications were USD 400 and USD 440 t, respectively (Table S1). Therefore, an application rate of 100 kg ha⁻¹ would cost between USD 40 and USD 44 ha⁻¹. The cost of liquid BMC application was estimated at USD 17 ha⁻¹. In this study, a simplified economic assessment estimated that the total establishment cost of 1 m³ of RB was at USD 59, including USD 47.5 for 1 m³ of biochar (at USD 228 wet weight t⁻¹), and USD 11.5 for construction cost per 1 m³ of the RB (Table 6). No additional cost was incurred for manure and straw, as they were farm waste; however, the cost of adding straw and manure can range from USD 0 to 20 per 1 m³,

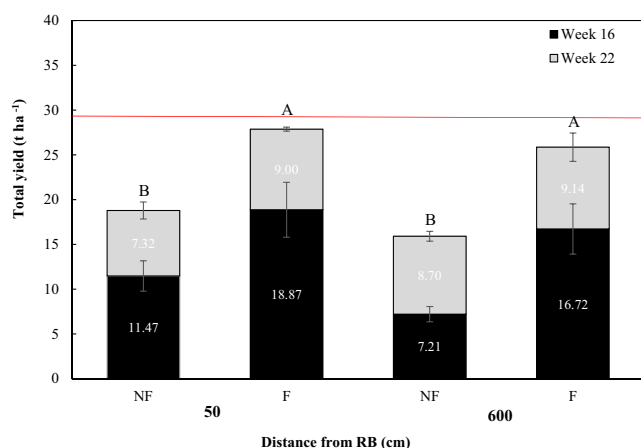


FIGURE 8 | Grass yield in reactive barriers (RB, solid red line), and at 50 and 600 cm away from the RB in both fertilised (F) and non-fertilised areas (NF) at weeks 16 and 22 following the liquid biochar mineral complex (BMC) application. Values shown are mean $\pm$ SE ($n=6$). Letters represent significant differences between NF versus F, using Tukey's post hoc test following two-way ANOVA at $p < 0.05$.

depending on livestock availability on the farm. Our simplified economic analysis showed that the project remained profitable since the increased income from the fertilised area was sufficient to cover the costs of BMC, leading to overall profitability in the short term. The break-even point of BMC was estimated to be USD 1167.7 ha⁻¹ including shipping costs, which would result in a zero net projected return (Table S1).

4 | Discussion

Our results confirmed several of our hypotheses. First, this study highlighted that the RB was able to store moisture with various reactive hydro-biochemical processes over time. Moreover, increased soil C storage was also found in the soil adjacent to the RB (50 cm vs. 600 cm) and in the non-fertilised area only. Our last hypothesis was partially supported because we found that increased yield was driven by BMC addition rather than proximity to the RB, although adding BMC by itself did not increase soil moisture or C storage.

The RB increased moisture retention and provided a high grass yield. The RB stored moisture that over time migrated into the surrounding soil. The moisture movement could be explained by a combination of soil hydrological processes, including diffusion of both water vapour and liquid moisture through soil pores and fractures (Adhikari, Timms, and Mahmud 2022). Accumulated moisture would initially be used to ensure optimal conditions for composting the mixture used in the RB (biochar, mulch, and manure in the lower part of the RB), thus providing food for microbes and plants. The relatively high moisture storage within the RB would facilitate decomposition of labile nutrients from the mulch and manure used in the RB structure, which in turn could enhance the pasture yield and microbial growth. Biochar mixed with straw mulch has shown to increase soil nutrient concentrations and crop yield (Gu et al. 2018; Hu et al. 2021; Khan et al. 2022). The majority of the grass root growth occurs within the top 30 cm

of soil (Guo et al. 2024). Grass roots, grown on top of the RB, could have reached the composted mixture of biochar, straw mulch, and manure zone, allowing plants to assimilate readily available nutrients from the mixture despite the fact that the top 20 cm of the RB were filled with biochar only. Thus, a combination of high moisture and nutrient availability in the RB could partly explain the high pasture yield (29 t ha⁻¹) observed growing on top of the RB.

The RB enhanced soil C storage in areas adjacent to the RB compared with those located away from the RB (50 cm vs. 600 cm) in the non-fertilised area only, supporting our second hypothesis. Direct mixing of biochar and soil is widely reported to increase soil C storage (Li et al. 2023; Li and Tasnady 2023). However, soil C increase detected in the soil collected 50 cm away from the RB was not explained by soil direct mixing with biochar. The increased soil C storage adjacent to the RB could then be associated with various mechanisms. For example, soil C stabilisation can occur due to alterations in soil moisture, temperature, and microbial communities (Kan et al. 2020; Zhang et al. 2022). In our study, we observed increased moisture levels from week 36 onward, whereas the samples for C storage assessment were collected on week 16 following the RB establishment. Furthermore, no significant shift in microbial community composition was detected between areas immediately adjacent to the RB and those at 600 cm distance away. However, differences were detected for microbial respiratory capabilities, as catabolic activities were lower adjacent to the RB than at a 600 cm distance and in the non-fertilised area. Lower catabolic activity could be related to slower decomposition and C mineralisation rates at 50 cm than at 600 cm, which could explain the observed increase in C storage (Damian et al. 2021; Dignac et al. 2017).

There was no evidence of microbes being laterally transferred from the RB to the adjacent soil. However, the RB presented a distinct microbial community and abundance compared with the adjacent soil, with a larger dominance of some microbial taxa. For example, the RB had higher relative abundance of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* than the adjacent soil. It is also likely that microbes migrated from surrounding soil. For example, *Actinobacteria* form mycelium-like networks that are adapted to the structure of biochar (Fu et al. 2022). In this study, a wood-based biochar with 5–10 μ m diameter pores covering ~15% of biochar surface was applied inside the RB and could be categorized as a high porous structure (Leng et al. 2021). High porosity and high moisture storage would provide a suitable environment in the RB for *Actinobacteria* growth originated from soil. *Proteobacteria* are mainly composed of copiotrophic taxa which contribute to high C decomposition rates (Fierer, Bradford, and Jackson 2007). High labile C presented in the RB may have favoured *Proteobacteria* growth in the RB being originated from soil. Interestingly, we found that the relative abundances of *Rhizobiales*, *Cytophagales*, *Micrococcales*, *Chitinophagales* and *Flavobacteriales*, at the order level, were higher in the RB than those of the adjacent soil. Additionally, soil microbial growth and community composition are highly sensitive to pH changes (Fierer and Jackson 2006). For example, the biochar buried in the RB had pH of 7.5, which may enhance the viability of *Actinobacteria* (Fu et al. 2022).

TABLE 4 | Grass nutrient concentration in reactive barriers (RB) and grass sampled at 50 and 600 cm away from the RB in both fertilised (F) and non-fertilised areas (NF) at week 16 following application of liquid biochar mineral complex (BMC). Values shown are mean $\pm$ SE ($n = 5$).

Nutrients	Units	RB	NF50	F50	NF600	F600	F	D	F $\times$ D
Chlorophyll	(mg g ⁻¹)	44.80 $\pm$ 1.60	39.96 $\pm$ 1.60	48.03 $\pm$ 2.47	44.20 $\pm$ 1.55	43.48 $\pm$ 1.35	ns	ns	ns
Height	(cm)	56.50 $\pm$ 1.50	29.00 $\pm$ 2.29	44.20 $\pm$ 3.40	25.33 $\pm$ 3.02	40.50 $\pm$ 3.31	$p < 0.001$	ns	ns
Total C	(%)	39.95 $\pm$ 0.46	38.57 $\pm$ 2.35	40.49 $\pm$ 0.32	42.28 $\pm$ 0.62	51.48 $\pm$ 10.08	ns	ns	ns
$\delta^{13}\text{C}$	(‰)	-30.68 $\pm$ 0.17	-30.24 $\pm$ 0.09	-30.38 $\pm$ 0.10	-30.34 $\pm$ 0.21	-27.98 $\pm$ 2.02	ns	ns	ns
Total N	(%)	4.11 $\pm$ 0.21	2.80 $\pm$ 0.22	3.83 $\pm$ 0.17	3.18 $\pm$ 0.16	3.57 $\pm$ 0.33	$p < 0.012$	ns	ns
$\delta^{15}\text{N}$	(‰)	1.08 $\pm$ 0.28	-0.44 $\pm$ 0.51	-0.71 $\pm$ 0.53	-0.62 $\pm$ 0.14	0.43 $\pm$ 1.16	ns	ns	ns
P	(mg 100 g ⁻¹)	571.34 $\pm$ 10.35	334.99 $\pm$ 49.57	510.85 $\pm$ 13.55	300.71 $\pm$ 19.83	423.01 $\pm$ 15.59	$p < 0.001$	ns	ns
K	(mg 100 g ⁻¹)	5589.26 $\pm$ 162.28	3592.60 $\pm$ 302.42	4556.78 $\pm$ 55.65	3629.58 $\pm$ 249.92	4445.76 $\pm$ 420.95	$p < 0.007$	ns	ns
Al	(mg 100 g ⁻¹)	60.80 $\pm$ 6.34	207.94 $\pm$ 113.53	79.66 $\pm$ 16.22	43.36 $\pm$ 5.30	81.76 $\pm$ 18.01	ns	ns	ns
B	(mg 100 g ⁻¹)	0.50 $\pm$ 0.04	0.63 $\pm$ 0.08	0.67 $\pm$ 0.03	0.67 $\pm$ 0.10	0.75 $\pm$ 0.07	ns	ns	ns
Ca	(mg 100 g ⁻¹)	288.65 $\pm$ 23.26	381.63 $\pm$ 20.47	391.07 $\pm$ 16.11	408.52 $\pm$ 34.28	393.56 $\pm$ 27.31	ns	ns	ns
Fe	(mg 100 g ⁻¹)	41.20 $\pm$ 5.52	111.59 $\pm$ 57.77	48.92 $\pm$ 9.20	27.75 $\pm$ 3.38	48.85 $\pm$ 9.70	ns	ns	ns
Mg	(mg 100 g ⁻¹)	179.53 $\pm$ 2.66	156.08 $\pm$ 4.62	178.05 $\pm$ 3.68	163.77 $\pm$ 6.46	160.65 $\pm$ 5.92	ns	ns	ns
Mn	(mg 100 g ⁻¹)	4.14 $\pm$ 0.36	9.97 $\pm$ 0.57	8.65 $\pm$ 0.79	7.27 $\pm$ 0.50	7.54 $\pm$ 0.86	ns	$p > 0.014$	ns
Na	(mg 100 g ⁻¹)	401.81 $\pm$ 32.17	204.36 $\pm$ 19.23	338.66 $\pm$ 61.37	254.20 $\pm$ 39.41	230.76 $\pm$ 24.35	ns	ns	ns
S	(mg 100 g ⁻¹)	232.19 $\pm$ 5.76	173.94 $\pm$ 13.98	215.70 $\pm$ 9.21	185.21 $\pm$ 9.03	196.53 $\pm$ 9.95	$p < 0.025$	ns	ns
Si	(mg 100 g ⁻¹)	14.29 $\pm$ 3.19	15.33 $\pm$ 3.23	11.88 $\pm$ 2.67	9.51 $\pm$ 1.61	13.86 $\pm$ 2.12	ns	ns	ns
Zn	(mg 100 g ⁻¹)	3.86 $\pm$ 0.33	1.70 $\pm$ 0.09	2.02 $\pm$ 0.05	1.57 $\pm$ 0.04	1.75 $\pm$ 0.07	< 0.001	< 0.006	ns

Note: Arsenic (As), chromium (Cr), cobalt (Co), copper (Cu) and lead (Pb) were below detectable level (BDL) and were excluded. F (fertiliser); D (distance from RBs); F $\times$ D (interaction of fertiliser and distance); ns (not significant at $p < 0.05$).
Abbreviations: Al, aluminium; B, boron; Ca, calcium; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; P, phosphorus; S, sulphur; Si, silicon; Total C, total carbon; Total N, total nitrogen; Zn, zinc; $\delta^{13}\text{C}$, carbon isotopic composition; $\delta^{15}\text{N}$, nitrogen isotopic composition.

TABLE 5 | Nitrogen (N), phosphorus (P), and potassium (K) uptake and balance in 50 and 600 cm away from the reactive barriers (RB) in both fertilised (F) and non-fertilised areas (NF) at week 16 following application of liquid biochar mineral complex (BMC). Values shown are mean $\pm$ SE ($n = 5$).

Treatments	N uptake	P uptake	K uptake	N balance	P balance	K balance
NF50	109.38 $\pm$ 18.10	12.87 $\pm$ 2.35	138.89 $\pm$ 21.04	-109.33 $\pm$ 18.09	-12.86 $\pm$ 2.35	-138.80 $\pm$ 21.04
F50	217.17 $\pm$ 38.24	28.11 $\pm$ 3.35	253.37 $\pm$ 34.31	-63.77 $\pm$ 38.24	42.12 $\pm$ 3.35	-241.07 $\pm$ 34.31
NF600	106.50 $\pm$ 10.52	10.17 $\pm$ 1.22	122.77 $\pm$ 15.43	-106.44 $\pm$ 10.52	-10.16 $\pm$ 1.22	-122.69 $\pm$ 15.43
F600	165.48 $\pm$ 27.58	19.07 $\pm$ 0.94	187.77 $\pm$ 14.41	-12.08 $\pm$ 27.58	51.15 $\pm$ 0.94	-175.47 $\pm$ 14.40
F	$p < 0.006$	$p < 0.001$	$p < 0.002$	$p < 0.016$	$p < 0.001$	$p < 0.005$
D	ns	$p < 0.023$	ns	ns	$p < 0.023$	ns
F $\times$ D	ns	ns	ns	ns	ns	ns

Abbreviations: D, distance from RBs; F $\times$ D, interaction of fertiliser and distance; F, fertiliser; ns, not significant at $p < 0.05$.

TABLE 6 | Environmental and economic benefit analysis of reactive barriers (RB) used in this study.

Costs	USD 1 m ³ of RB	USD 6 RBs (24 m ³)
Construction including excavation and soil removal	\$11.5	\$276
Biochar used inside the RBs (5 t biochar wet weight used)	\$47.5	\$1140
Straw and cow manure	0	0
Total cost of RB	\$59	\$1416
Benefits	kg CO ₂ per 1 m ³ of RB	kg CO ₂ per 6 RBs (24 m ³)
Environmental benefits: CO ₂ removal	158 ^a	3792
Economic benefits: Yield USD t ha ⁻¹		\$231

Note: Biochar price at USD 228 wet weight t⁻¹; Sale price at USD 170 t ha⁻¹.

^aBMC contributed an additional 1 kg CO₂e ha⁻¹ removal annually.

Therefore, a combination of high surface area, elevated pH, high labile C and high moisture retention could have contributed to increased colonization of some microbes in the RB, some of which may have been originated from soil. The lateral expansion of those microbes into the soil might be facilitated by moisture diffusion from the RB to the soil over time, which then will have implications for soil fertility.

Our last hypothesis was partially supported since BMC application increased yield but did not increase soil moisture or C storage in the short term. The BMC used in this study was produced from a mixture of nutrients, biomass and minerals such as clay that contain micronutrients that were pyrolyzed at 450°C. Inclusion of clay with the biomass and presence of various functional groups in organic biomass, such as oxygen, carboxylic, acid, alcohol, phenolic alcohol, and aldehyde enhance soil nutrient retention (Mandi et al. 2021). Increased pasture yield following application of BMC was mainly due to increased nutrient availability from the soil for plants. Improvement in grass yield was specifically explained by an increase in grass N, P, and K uptakes. BMC can specifically enhance K available for plant uptake by adsorbing and retaining K⁺ ions in the biochar pores, leading to decreased K leaching (Farrar et al. 2021). Increased K concentration could also be explained by increased aggregation in biochar-clay particles because the BMC formula was blended with minerals and clays, including bentonite and kaolinite. Hence, our study suggested the importance of using

BMC to increase nutrient availability for plants in the short term while waiting for the RB to affect the surrounding soil over the longer term.

The BMC application led to improved soil P, whereas N and K mining from soil was confirmed with negative values observed for N and K and only partial nutrient balance in the fertilised area. The N added by co-application of BMC and chemical fertiliser was 153 kg N ha⁻¹ year⁻¹, which was 60% less than the recommended high-rate use of 400 kg N ha⁻¹ year⁻¹ but was higher than the recommended strategic use rate of 100 kg N ha⁻¹ year⁻¹ (DPI 2021). The P added by co-application of BMC and chemical fertiliser was 70 kg P ha⁻¹ year⁻¹, which was a sufficient rate to maintain pasture growth (DPI 2021). The K addition by the BMC application was 12 kg K ha⁻¹ year⁻¹ because our initial soil test had a high K, thus it was not required to add extra K (DPI 2021). The optimal pasture growth for clover and ryegrass varies between 6 and 9 t dry mass ha⁻¹ in Australia (Neal, Neal, and Fulkerson 2007). In our experimental site, we had planted a mixture of clover and ryegrass with an average yield of 3.5 and 5.2 t dry mass ha⁻¹ for non-fertilised and fertilised areas, respectively. Therefore, the BMC used would need further N and K adjustments to potentially increase yield without mining nutrients from the soil. Our study confirmed that annual fertilisation was required because N, P, and K mining was found in the non-fertilised area despite the fact that the area was subject to fertilisation

for the past 10 years. Our study further highlighted the importance of nutrient budgeting to develop BMCs to prevent further depletion of soil nutrients.

The presence of RB in combination of annual BMC application had the potential to help our farming systems to decrease their C footprint. We used biochar prepared at temperature 600°C for the RB. The biochar prepared at temperature 600°C is highly persistent and it is estimated that over 80% of C remains in the soil for centuries (Joseph et al. 2021). The BMC application rate was low to ensure it was cost effective for annual application, leading to facilitate the industry adoption. Hence, it was not surprising that BMC did not significantly increase soil C and moisture retention due to low application rate of the BMC. However, it was expected that the BMC application overtime builds up soil resilient and increase soil moisture and C storage.

Liquid BMC application and RB establishment were expensive. Although commercial production of these liquids has not yet been undertaken, the producers involved in this study estimated the ex-factory sales price of liquid BMC would be between USD 700 and USD 1000/t, a cost ranging between USD 400 and USD 520 ha⁻¹ for an application rate of 0.4 t ha⁻¹. In this study, a simplified economic assessment estimated that the total cost of the RB was at USD 59 for 1 m³ of biochar. The RB cost would have the potential to further decrease subject to the on-farm waste availability and livestock availability. Our simplified economic analysis showed that the project remained profitable since the increased income from the fertilised area was sufficient to cover the costs of BMC, leading to an overall profitability in the short term. The highest BMC process of USD 520 ha⁻¹ was still 50% less than the break-even point of BMC, estimated at USD 1167.7 ha⁻¹ to result in a zero net projected return. The cost of RB establishment was a one-off cost, which an in-depth long-term cost benefit analysis would determine the overtime return from the RB establishment.

Environmental benefits of the designed RB and liquid BMC application were also important. For example, approximately 158 kg CO₂e were removed from the atmosphere per each cubic meter of established RB, and liquid BMC application could add an additional removal of 1 kg CO₂e ha⁻¹ annually. Currently, international C credit schemes support projects and farmers that are using new technologies to reduce C footprint by reducing emission or storing C. Depending on the scheme, biochar stored in the RB was eligible to be sold for C credit units on the secondary market under a private commercial agreement, potentially providing an additional income stream for either or both farmers and biochar producers. Biochar burial has been shown to increase soil non-biochar C over time (Mahajan et al. 2021), which will allow farmers to apply for further C credit schemes. Additionally, biochar burial in trenches being established on hills has the capacity to decrease runoff of rainfall and soil loss at approximately of 12.3 t ha⁻¹, 13 years since the establishment (Mahajan et al. 2021). Another benefit includes improving drought resilience. For example, under drought conditions, dry organic matter and dead trees become fire hazard and turning those materials into biochar would also reduce fire risks under climate change (Sahoo et al. 2021). Then, storing biochars produced from dry organic farm wastes in open surface RB designed

in our study would have the potential to capture and store air humidity in addition to rainfall, leading to further increasing farming systems resilience to drought when drought spells struck. Therefore, biochar is not only a tool to increase C sequestration; its multifaceted benefits, including improved drought resilience, reduced fire risks, enhanced soil health, improved moisture retention, and increased income diversification, are invaluable to making farming systems climate-resilient.

5 | Conclusions

Our results highlight that innovative C-negative solutions, such as the combination of open surface RB and liquid BMC application, could collectively enhance soil moisture and C retentions while increasing crop yield in a pasture cropping system, ultimately improving farm profitability. The increased pasture yield was more responsive to the liquid BMC application rather than proximity to the RB due to increased nutrient use efficiency of the plants in the fertilised area. The RB contributed approximately 158 kg CO₂e removal per cubic meter of established RB and BMC application could add an additional removal of 150 kg CO₂e ha⁻¹ annually. Despite the cost of liquid BMC, which ranged between USD 400 and USD 520 ha⁻¹ for an application rate of 0.4 t ha⁻¹, increased income revenue gained by higher yield, made the project profitable even in the short term. The cost of BMC production is expected to decrease when produced at commercial levels. Overall, our results indicate that the combination of the RB and liquid BMC application could be effectively utilized to mitigate C footprint in farming systems. The adoption of biochar-based techniques could be used as an effective strategy to combat the adverse effects of climate change and increase drought residence, thereby promoting sustainable agricultural practices.

Author Contributions

Negar Omidvar: data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing – original draft, writing – review and editing. **Stephen Joseph:** conceptualization, funding acquisition, validation, writing – review and editing. **Lakmini Dissanayake:** data curation, investigation, methodology, writing – review and editing. **Michael B. Farrar:** formal analysis, methodology, validation, writing – review and editing. **Frédérique Reverchon:** validation, writing – review and editing. **Russell Burnett:** data curation, formal analysis, methodology, project administration, writing – review and editing. **Mehran Rezaei Rashti:** data curation, methodology, writing – review and editing. **Apsara Amarasinghe:** data curation, formal analysis, methodology, writing – review and editing. **Sara Tahery:** data curation, methodology, writing – review and editing. **Zhihong Xu:** conceptualization, funding acquisition, writing – review and editing. **Wendy Timms:** data curation, formal analysis, methodology, writing – review and editing. **Brittany Elliott:** methodology, validation, writing – review and editing. **Hongdou Liu:** data curation, methodology, writing – review and editing. **Shahla Hosseini Bai:** conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at <https://zenodo.org/records/14649373>, reference number 10.5281/zenodo.14649372.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.