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Effect of Harvesting Time in Growth Performance and Energy Crops Productivity of Napier (*Pennisetum purpureum* cv. Taiwan) Exposed under CO_2 Elevated Conditions

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ABSTRACT

Napier grass is crucial in reducing greenhouse gas emissions by substituting non-renewable resources. When Napier grass is burned, the carbon dioxide (CO_2) released is roughly equal to the amount absorbed during its growth, making it a potentially carbon-neutral energy source. This study investigates the impact of ratooning (repeated harvesting) on various aspects of Napier grass, including growth, physiology, biomass production, nutrient content, and chemical analysis. It also explored the interaction between elevated CO_2 levels and ratooning. Two experiments were conducted over 12 months. Experiment 1 took place in an open field at the Faculty of Agriculture, Universiti Putra Malaysia (UPM), with two treatments: no ratooning and ratooning at three months after planting (MAP). Experiment 2 was conducted in an open field at UPM and a greenhouse at Tenaga National Berhad Research, Kajang, Selangor. Eight combination treatments were studied: (T1) 1-month elevated CO_2 ($MECO_2$) - no ratooned, (T2) 1 $MECO_2$-R at 3 MAP, (T3) 2 $MECO_2$-noR, (T4) 2 $MECO_2$-R at 3 MAP, (T5) 5 $MECO_2$-noR, (T6) 5 $MECO_2$-R at 3 MAP, (T7) 12 $MECO_2$-noR, and (T8) 12 $MECO_2$-R at 3 MAP. The results indicated that, in Experiment 1, no ratooning was more favourable for all parameters compared to ratooning. In Experiment 2, a 1-month exposure to elevated CO_2 showed better results compared to longer exposure periods. In conclusion, Napier grass performed

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better when not subjected to ratooning and exposed to short-term elevated CO_2 levels. This research highlights the potential of Napier grass as a sustainable and carbon-neutral energy source.

Keywords: Elevated CO_2, green energy, Napier grass, productivity, ratooning, renewable sources

INTRODUCTION

Pennisteum purpureum CV. Taiwan or Napier grass was originally developed and popularised in Taiwan and has great potential as a green energy source due to the conversion efficiency into various forms of renewable energy (Long et al., 2004). Before this, Napier grass was widely known as a forage crop for livestock feeding. However, it received attention as a bioenergy crop due to its high productivity, adaptability, and nutritional value (Ainsworth & Rogers, 2007). Besides that, this cultivar exhibits vigorous growth, and it is characterised by tall, robust stems that can reach heights up to 3–4 m, which can produce a substantial amount of production under favourable conditions (Osborne et al., 2008). *Pennisetum purpureum* cv. Taiwan also has relatively good nutritional content with appreciable crude protein, fibre, and other essential nutrients (Ansah et al., 2010). However, Napier grass quality depends on several factors, such as maturity and growing conditions, based on the purpose of use (Polle et al., 1997). This cultivar demonstrates excellent adaptability to a wide range of environmental conditions (Assuero & Tognetti, 2010). It thrives in warm and humid climates but tolerates moderate drought conditions (Namiki, 1990).

The high biomass yield of Napier grass makes it an excellent option for bioenergy production (Behnke et al., 2010). It can be harvested and processed to produce biofuels like biogas, bioethanol, and bio-oil (Rangnekar & Thorpe, 2001). Biogas can be generated through the anaerobic digestion of Napier grass, providing a renewable source of clean energy for electricity or heating generation (Bendary et al., 2013). Napier grass is rich in organic matter that is easily digestible and is an ideal material as a feedstock for biogas production (Boyer, 2015). Anaerobic digestion of the grass releases methane, a potent greenhouse gas, which can be trapped and fully utilised as a clean and sustainable energy source (Babbar et al., 2015). The dry matter of Napier grass is used as a combustion tool that can produce steam, which drives turbines to generate electricity (Gulfam et al., 2017). This process is considered carbon neutral as the carbon dioxide released during combustion is absorbed by growing Napier grass, making it a renewable energy source (Byers & Guerrero, 1995).

Known as a fast-growing grass with high carbon sequestration, it has enlightened the Napier grass to play a role in mitigating climate change (Caird et al., 2007). During the growing phase, it absorbs significant amounts of carbon dioxide from the atmosphere, helping to offset greenhouse gas emissions (Chan et al., 2008).

Practically, Napier grass is beneficial in developing regions as it can be an accessible and renewable energy resource for rural communities, which reduces dependence on non-renewable energy resources (Chaparro & Sollenberger, 1997). While Napier grass shows a promising outcome as a green source, the large-scale utilisation for energy production may need extra precautionary steps and management to avoid negative impacts on the environment, such as habitat loss and competition with food crops (Chen et al., 2004). Proper management, such as sustainable cultivation practices and integrated land-use planning, is essential to responsibly harnessing its potential as a renewable energy resource (Orodho, 2012). There are four objectives of the study: (1) to study the effect of ratooning on the growth, physiology, and biomass production of Napier grass, (2) to analyse the effect of ratooning on nutrient content, proximate, and ultimate analysis of Napier grass, (3) to identify the effect of elevated CO_2 ratooning on growth, physiology, biomass production, and chemical content of Napier grass, and (4) to analyse the interaction between elevated CO_2 and ratooning on growth, physiology, biomass production, and chemical content of Napier grass.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at two locations: Field 15, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, with the GPS coordinate 2˚59'01.9" N, 101˚44'01.7"E (Figure 2), and a greenhouse at Tenaga National Berhad Research, Kajang, Selangor with the GPS coordinate 2.9683° N, 101.7326° E (Figure 3). The open field for experimentation was cleared using a bulldozer to remove all weeds and unwanted plants in the experiment area. The soil was ploughed 25 cm deep so the soil could turn over the uppermost soil. Ploughing soil needs to be done twice, two weeks after the first plough, to ensure the soil is uniformly ploughed. The soil was left for two weeks before the liming process, and after that, the ploughed soil needed to be tested for pH level. The plot size for the experiment was 23.0 m x 12.0 m. Each treatment has four replicates, which represent four plots. Each plot was raised by 2.0 m x 3.0 m and built with a separation block consisting of 6 crops that have been planted and prepared the cutting from a mature stem of Napier grass within 15 cm to 20 cm, which consisted of three nodes. The cuttings were prepared under two conditions: (1) planting in an open area and (2) exposure under different periods of elevated CO_2 conditions (800 µmol/mol) before being transplanted to an open field. The CO_2 greenhouse was constructed so that the plant can receive 12 hr photoperiod and average photosynthetic photon flux density of 800 µmol/mol. Day and night temperatures and relative humidity were recorded. Vapor pressure deficit ranged from 1.11 to 2.32 kPa. Pure CO_2 at 99.8% purity was supplied from a high-pressure CO_2 cylinder and injected through a pressure regulator into the fully sealed 5 m × 3.67 m growth houses (Figure 1). The

CO_2 concentrations were measured using SenseAir CO_2 Sensors (USA), designated to each chamber during CO_2 exposition (Figure 4a-4e). The CO_2 was elevated slowly to 800 µmol/mol. The Average rainfall documented for the experimented region was 117.06 mm with a minimum and maximum temperature of 21.9°C and 38.0°C, respectively.

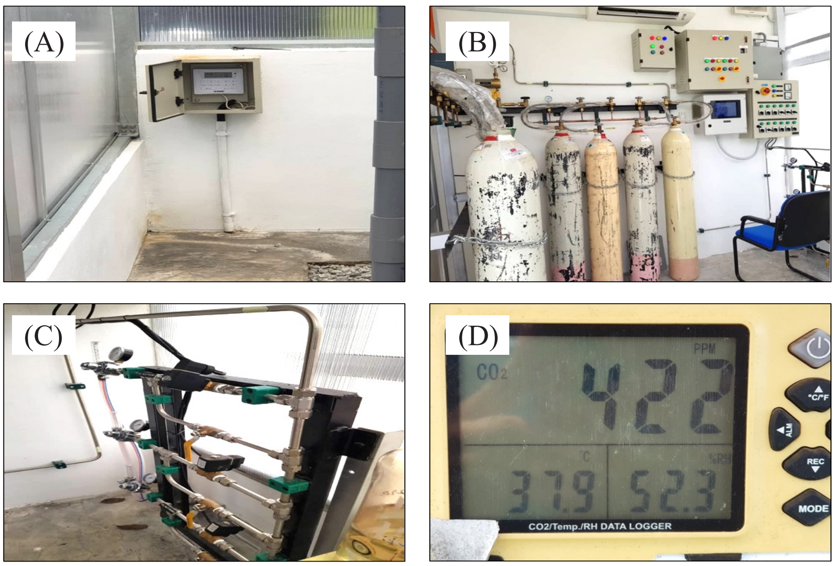

Figure 1. Monitoring of temperature, relative humidity (RH), and carbon dioxide (CO_2) level using a sensor (A); Source of pure CO_2 connected to the CO_2 greenhouse system (B and C); A data logger for CO_2, temperature, and RH was used in the greenhouse at Tenaga Nasional Berhad Research (D)

Figure 2. Open area experimental site

Figure 3. Greenhouse experimental site

Land Preparation and Fertilisation

The cutting propagated in polybag size, 61 cm x 61 cm arranged in the greenhouse and exposed at four different periods of elevated CO_2. At the same time, the ploughed land was applied with 300 kg/ha organic matter before crop transplanting. All cuttings were transplanted at the same time for open field and greenhouse. Sprinkler irrigation was the method of applying water in this experiment at the greenhouse, which was applied at the necessary time. An overhead sprinkler was built in an open area surrounding the experiment area so that the flow rate reaches 60 to 70%. Surface broadcast is a fertiliser application method used in both experimental areas. The fertiliser urea (NPK 46-0-0, YaraTera™, Norway) was applied on the soil surface of an entire experiment plot either in granule or liquid form. The fertiliser was weighed with the amount of 10 kg/ha and applied after one week of planting and every month for up to 1 year to get the maximum yield of Napier grass as a bioenergy crop. Urea fertiliser (nitrogen) was applied as a single fertiliser to provide nutrients for Napier grass's shoot and root development.

Experimental Design and Treatments

Randomised complete block design (RCBD) was used for both experiments, with all assigned treatments randomly placed within each block. This design helps to control variability and ensures that each treatment has an equal chance of being influenced by different factors. Experiment 1 consisted of 2 treatments: T1: open field (OF)—no ratooned and T2: open field—ratooned at 3 MAP. In Experiment 2, the Napier grass was subjected to 2 factorials, i.e., ratooning and period of CO_2 elevation at 800 µmol/mol CO_2, for the ratooning plant was subjected to ratooned (R) and no ratooned (noR). The plant was exposed to a short period at 1-month elevated CO_2 ($MECO_2$) and 2 $MECO_2$ and a long period at 5 $MECO_2$ and 12 $MECO_2$, respectively. Eight combination treatments were studied: (T1) 1 $MECO_2$-no ratooned, (T2) 1 $MECO_2$-R at 3 MAP, (T3) 2 $MECO_2$-noR, (T4) 2 $MECO_2$-R at 3 MAP, (T5) 5 $MECO_2$-noR, (T6) 5 $MECO_2$-R at 3 MAP, (T7) 12 $MECO_2$-noR, and (T8) 12 $MECO_2$-R at 3 MAP. Both experiments were carried out for 12 months, which was a total of 10 treatments.

Data Collection

Plant growth was measured throughout the experimental period, while total biomass and proximate analysis were measured at 12 MAP.

Plant Growth

Field measurement and sampling were done every month for up to one year. At the time of field measurement, a 1 m x 1 m sample size from each plot was cut to measure all the growth parameters. The tiller number was counted in the sample size for each plot, including other growth parameters, plant height (PH), stem diameter (SD), and tiller bunch circumference (TBC).

PH and TBC were measured using measuring tape. The hook is a feature in measuring tape that helps measure one side of Napier grass. The measuring tape was stretched across the height or width of the Napier grass from the tip of the shoot to the root part or around the circumference of the Napier grass as straight as possible when a measurement was being done. The tape was locked, and the reading was written down.

SD was measured using a digital calliper. The device was re-zeroed before use to minimise the instrumental error. If the device is not properly zeroing, the reading would be inaccurate. Technically, callipers have two jaws, i.e., large and small jaws. Large jaws are used to measure the outside of an object, while small jaws are used to measure the inside of an object. The large jaws were used to measure the stem diameter in this experiment. The calliper was unlocking the top lock screw before measurements. The large jaws were adjusted to widen the jaws by sliding the thumbscrew on the bottom to the right. The large jaws were placed around the stem. The slide should be moved to the left until the jaws are clamped around the Napier stem. The screw was locked to ensure the jaws were set to read.

Total Biomass

Biomass production is the net amount of plant-dried matter before and after the drying method at a certain harvesting period. Total biomass was measured on a plant or unit of land basis and closely related to the plant's carbon assimilation capacity. The plot size needed to harvest from each treatment was 3 m x 2 m to calculate the total biomass. All the fresh samples must be cleaned and weighed immediately after being harvested before the samples are brought back to the lab and dried. It is to minimise any errors that could happen during the total biomass calculation. The fresh weight of Napier grass was calculated by using the formula below:

$$\text{Crop yield (t/ha)} = \frac{\text{Fresh weight of tuber (g)}}{1{,}000} \times \frac{10{,}000\ \text{m}^2\ \text{(1 ha)}}{\text{Size of the quadrant (m}^2\text{)}}$$

Ultimate Analysis: Carbon (%), Hydrogen (%), Nitrogen (%), and Sulphur (%)

A sample was prepared by adding concentrated nitric acid to 1.0 g of dried sample and allowed to stand overnight before being digested for 2 hr at 115°C. A concentrated hydrochloric acid (LabChem, USA) was added to the sample mixture, and the digestion was continued until a clear solution was observed. The sample solution was then diluted into 100 ml with deionised water, filtered and left to dry before being burned and analysed in the ultimate analyser. An amount of 0.25 g from the prepared sample is put in a designated sample vial of refractory-grade clay. The designated vial was combusted at a temperature of 1,350°C. This sample was combusted in the ultimate analyser to determine the percentage of weight of carbon, hydrogen, nitrogen, and sulphur produced from the combustion process. All the parameters were determined simultaneously from the same sample in the analyser. Below are the total carbon, hydrogen, nitrogen, and sulphur calculations.

$$\text{Total carbon (\%)} = \frac{\text{Organic carbon + Inorganic carbon produced}}{\text{Total weight of sample}} \times 100\%$$

$$\text{Total hydrogen (\%)} = \text{Total hydrogen (ad)} \times \left[\frac{100\% - \text{TM (\%)}}{100}\right] + [0.1119 \times \text{TM}]$$

$$\text{Total nitrogen (\%)} = \frac{\text{Weight of nitrogen produced}}{\text{Total weight of sample}} \times 100\%$$

$$\text{Total sulphur (\%)} = 100\% - (\text{C\%} + \text{H\%} + \text{N\%})$$

where,
ad = As determined
C% = Carbon in percentage
H% = Hydrogen in percentage
N% = Nitrogen in percentage
TM = Total moisture

Statistical Analysis

All data collected were analysed using a two-way analysis of variance (ANOVA) by Statistical Analysis System (SAS 9.4) for RCBD with factorial and replicated four times to determine the significant differences between treatment means. Difference between means separated using least significant difference (LSD) at P<0.05 level.

RESULTS AND DISCUSSION

Experiment 1

Plant Growth

Figures 4A and 4C prove that the open field-no ratooned (OF-noR) showed significantly higher than open field-ratooned (OF-R) at plant height and tiller bunch circumference reading by increasing 5.03% and 12.09%, respectively. At the same time, stem diameter showed significantly higher at OF-R compared to OF-noR, which increased by 18.92% (Figure 4B). Extending the planting period for both treatments can increase plant growth for OF-R, as the crops have more time to establish and grow new crops without relying on the regrowth of existing plants (Engineer et al., 2016). Previous studies showed that plant age significantly affected leaf area and height by 39% and 53%, respectively (Chun et al., 2003). At the same time, the previous study reported a significant effect on plant height and basal circumference as it increased by 41.02% and 23.84% after cutting interval (Durand & Kawashima, 1980).

Despite these findings, all OF-R treatments will surpass the OF-noR at a certain period as if it grows continuously (Côté et al., 2010). The OF-R start to cross the OF-noR line at 9 MAP for Figure 4A, while 4 MAP for Figure 4B. From Table 1, ratooning does not show a significant effect between OF-noR and OF-R, with only a 0.5% difference. The result was in line with previous reports; the regrowth method has reduced the maise grain yield by 15.9% compared to continued growth in 2019 (De Graaff et al., 2006). Although the yield has significantly reduced the yield, across the years, the grain yield increased by 2.7–10.8% in 2020, better than the continued growth (Durand & Kawashima, 1980).

Table 1
The effect of ratooning on plant height, stem diameter, tiller number, and tiller bunch circumference of Napier grass (Pennisetum purpureum *cv. Taiwan*)

Treatments	Tiller number
Ratooned (R)	
Open field-noR	13.68a
Open field-R	13.61a
LSD P<0.05	NS

Note. Means followed by the same letter within a column are not significantly different at P>0.05 by least significant difference (LSD) test with n = 32; NS = Not significant; noR = No ratooned

Ultimate Analysis: Carbon (%), Hydrogen (%), Nitrogen (%), and Sulphur (%)

The ratooning application significantly (P<0.05) decreased the nitrogen of Napier grass by 15.13% in Figure 5A. However, it showed a significantly increased sulphur of Napier grass by 18.75% (Figure 5B). Meanwhile, carbon and hydrogen showed no significant effect due to ratooning. The reason behind this result was that regrowth

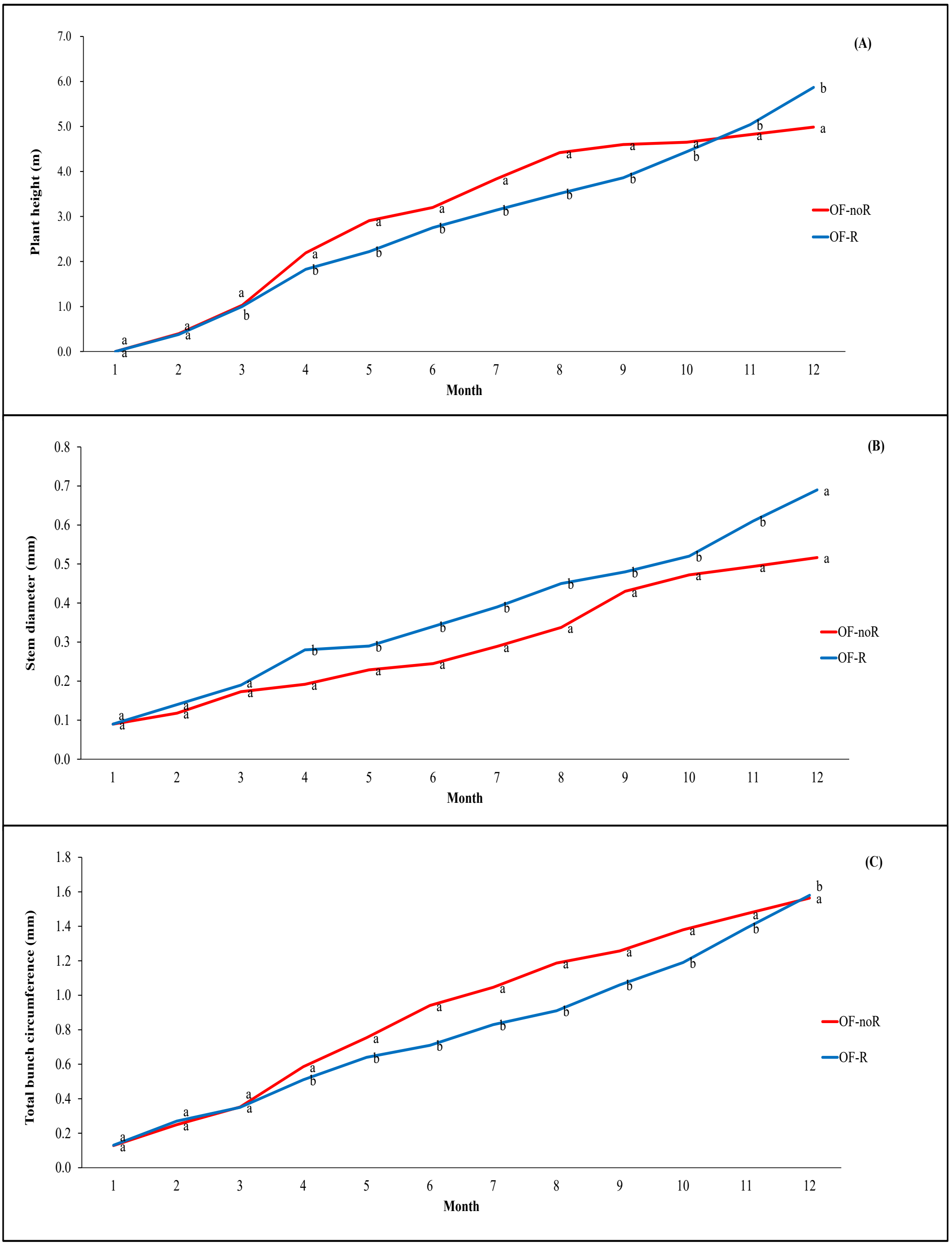

Figure 4. The effect of ratooning on (A) plant height, (B) stem diameter, and (C) tiller bunch circumference of Napier grass (*Pennisetum purpureum* cv. Taiwan). Mean values with the same letter are not significantly different at P>0.05 by the least significant difference (LSD).

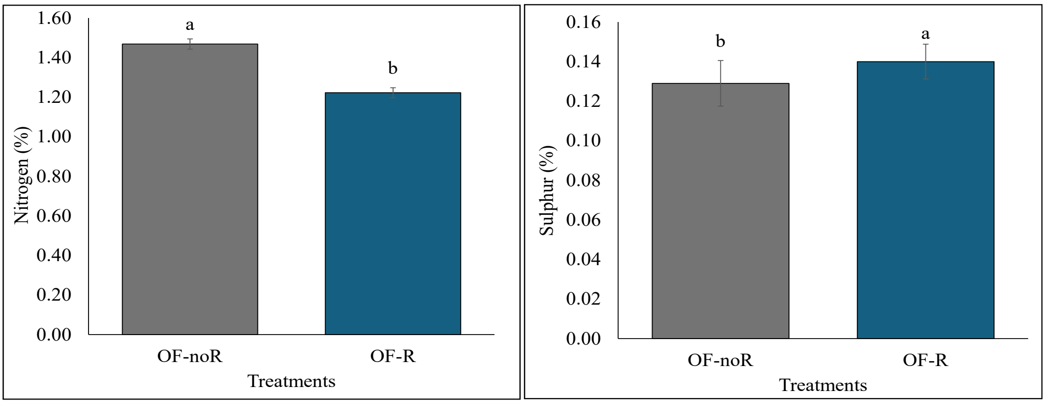

Figure 5. The effect of ratooning on (A) nitrogen and (B) sulphur contents of Napier grass (*Pennisetum purpureum* cv. Taiwan), respectively

Note. OF-noR = Open field-no ratooned; OF-R = Open field-ratooned; a and b indicate significant differences between means using the least significant difference at $p \leq 0.05$.

often consists of young, actively growing plant tissues that contain relatively higher levels of carbon and hydrogen compared to mature plant parts (Elehinafe et al., 2021). Carbon and hydrogen are key components of organic compounds in plant growth and development (Xu et al., 2021). During ratooning, plants need more nitrogen resources for protein synthesis. At the same time, their nitrogen-containing compound has already achieved continuous growth since day one transplant, resulting in mature plants containing high nitrogen (%) compared to young plants (Falster & Westoby, 2003). Ratooning can impact the cycling of sulphur in the soil (Imran et al., 2007). When the ratooning happens, the remaining sulphur content in the soil becomes a critical factor in determining the sulphur uptake by the regrown crop (Feng et al., 2008). Based on the results, the soil has sufficient sulphur levels to support the subsequent growth of Napier grass with adequate sulphur content. Thus, Napier grass must allocate nutrients differently during ratooning compared to the initial growth phase (Geuns, 2003). Nitrogen and sulphur are important for various physical and physiological reasons, including protein synthesis, enzyme activity, and plant growth (Grodzinski et al., 1996).

From the results, Table 2 shows no significant effects of ratooning on carbon (%) and hydrogen (%). These results were relatively due to their proportions in plant tissue, which remains stable (Gupta et al., 2016). Primarily, ratooning will affect only aboveground biomass, while the below-ground root system will remain intact and contribute to nutrient reserves (Haegele et al., 2017). Post-ratooning causes the stored nutrients in the roots to execute to support the regrowth process; thus, carbon and hydrogen content do not experience significant shifts (Hager et al., 2016). Unlike nitrogen and sulphur content, these elements were translocated from older to newer tissues after ratooning, but carbon and hydrogen content do not undergo substantial redistribution within the plants, which reflects no fluctuate significant effect due to ratooning (Halim et al., 2013). Besides, the carbon and hydrogen turnover rate in plant tissues is also generally slower compared to nitrogen, which is involved in more dynamic processes (Pérez-López et al., 2010). Carbon and hydrogen take longer to manifest, making them less likely to show significant shifts within a short period, for example, between harvesting time and ratooning cycles (Hanna & Monson, 1988).

Table 2
The effect of ratooning on carbon and hydrogen of Napier grass (Pennisetum purpureum *cv. Taiwan*)

Treatments	Carbon (%)	Hydrogen (%)
Ratooned (R)		
Open field-noR	45.03a	5.50a
Open field-R	44.23a	5.59a
LSD *P*<0.05	NS	NS

Note. Means followed by the same letter within a column are not significantly different at *P*>0.05 by least significant difference (LSD) test with n = 32; NS = Not significant; noR = No ratooned

Total Biomass

Based on Figure 6, OF-noR showed significantly higher compared to OF-R on total biomass at every harvesting period, which increased by 63.60% (6 MAP), 64.28% (8 MAP), and 28.86% (12 MAP), respectively. This result corresponded with photosynthesis rate (PR), stomatal conductance (SC), transpiration rate (TR), shoot fresh weight (SFW), and shoot dry weight (SDW) (McDonald & Ho, 2002). When an aboveground portion of the crop is removed, the stubble and roots are left behind. As a result, the overall biomass of the plant is temporarily reduced (Pérez-López et al., 2010). The regrowth and biomass recovery rate will depend on crop type, growing conditions, and management practices (Harris, 1992). If ratooning is practised multiple times, the plant's biomass declines with each successive ratoon (Heijnen et al., 2001). This decline can be attributed to factors like nutrient depletion, exhaustion of energy reserves, and reduced plant vigour. As a result, the biomass of each subsequent ratoon is lower than the previous one (Jaafar et al., 2008). A previous study has shared the same result in their findings. The performance of Napier grass before ratooning was 63.16%, while the performance after ratooning was 42.11% and concluded the growing process was interrupted and led to a decrease in plant biomass (Wangchuk et al., 2015)

Normally, the first growth cycle benefits from all essential nutrients in the soil, establishing the root system and leading to robust and vigorous growth, resulting in relatively higher biomass (Polle et al., 1997). However, it is less likely to be available after ratooning (Imran et al., 2007). Due to ratooning practices, it can influence the number of nutrient reserves stored in the root systems, which affects the regrowth potential (Ibrahim et al., 2011). Adequate management with proper fertilisation, good irrigation, and close pest control monitoring can provide a conducive environment for

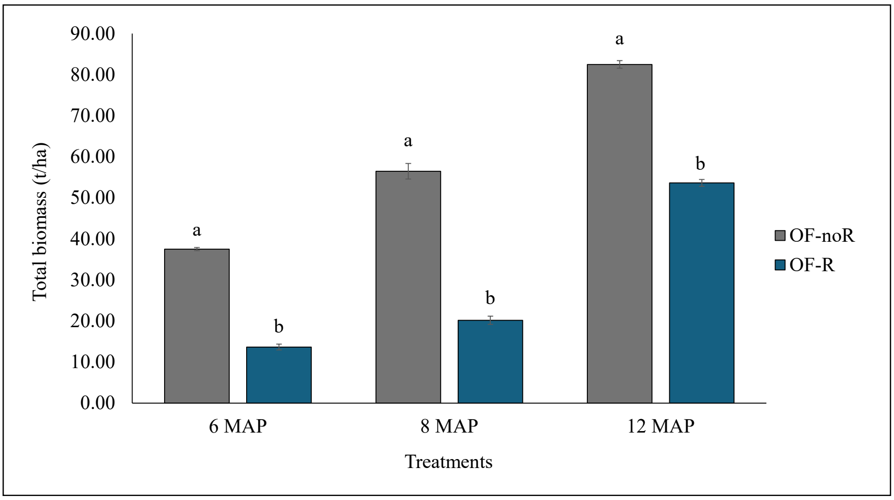

Figure 6. The effect of ratooning on total biomass of Napier grass (*Pennisetum purpureum* cv. Taiwan) at six months of planting (MAP), 8 MAP, and 12 MAP, respectively

Note. OF-noR = Open field-no ratooned; OF-R = Open field-ratooned; a and b indicate significant difference between means among MAP using least significant difference at $p \leq 0.05$

growth (Heijnen et al., 2001). A healthier soil environment positively impacts root growth and overall plant height (Ishii et al., 2015).

Experiment 2

Plant Height

Based on Figure 7A, the 5 $MECO_2$-noR showed a significantly higher plant height compared to another elevated CO_2, which increased by 22.15% (T1), 17.93% (T2), 30.57% (T3), 40.30% (T4), 22.57% (T6), 1.05% (T7), and 23.00% (T8), respectively. CO_2 is an important element during photosynthesis that produces carbohydrates and energy (Jaafar et al., 2008). The concentration of CO_2 stimulated photosynthesis, which affected sugar production and other compounds needed in plant growth (Chen et al., 2004). This potentially helps plants to grow taller in size as they have a sufficient amount of energy (Jørgensen et al., 2010). This result was in line with Jaafar et al. (2008), which proved the statement above based on his findings in the previous experiment. In Figure 7B, the 2 $MECO_2$–noR showed a significantly higher stem diameter compared to another elevated CO_2, which increased by 38.24% (T1), 42.16% (T2), 52.94% (T4), 56.86% (T5), 73.53% (T6), 59.80% (T7), and 72.55% (T8), respectively. Higher carbon dioxide levels can enhance the production of building blocks by growing and allocating more resources for cell division, expansion and elongation, which results in an increase in stem diameter (Jampeetong et al., 2014). These results were in line with the previous, which showed the effect of the tiller number on the C_4 crop by exposure to long CO_2 exposure (Zailan et al., 2016).

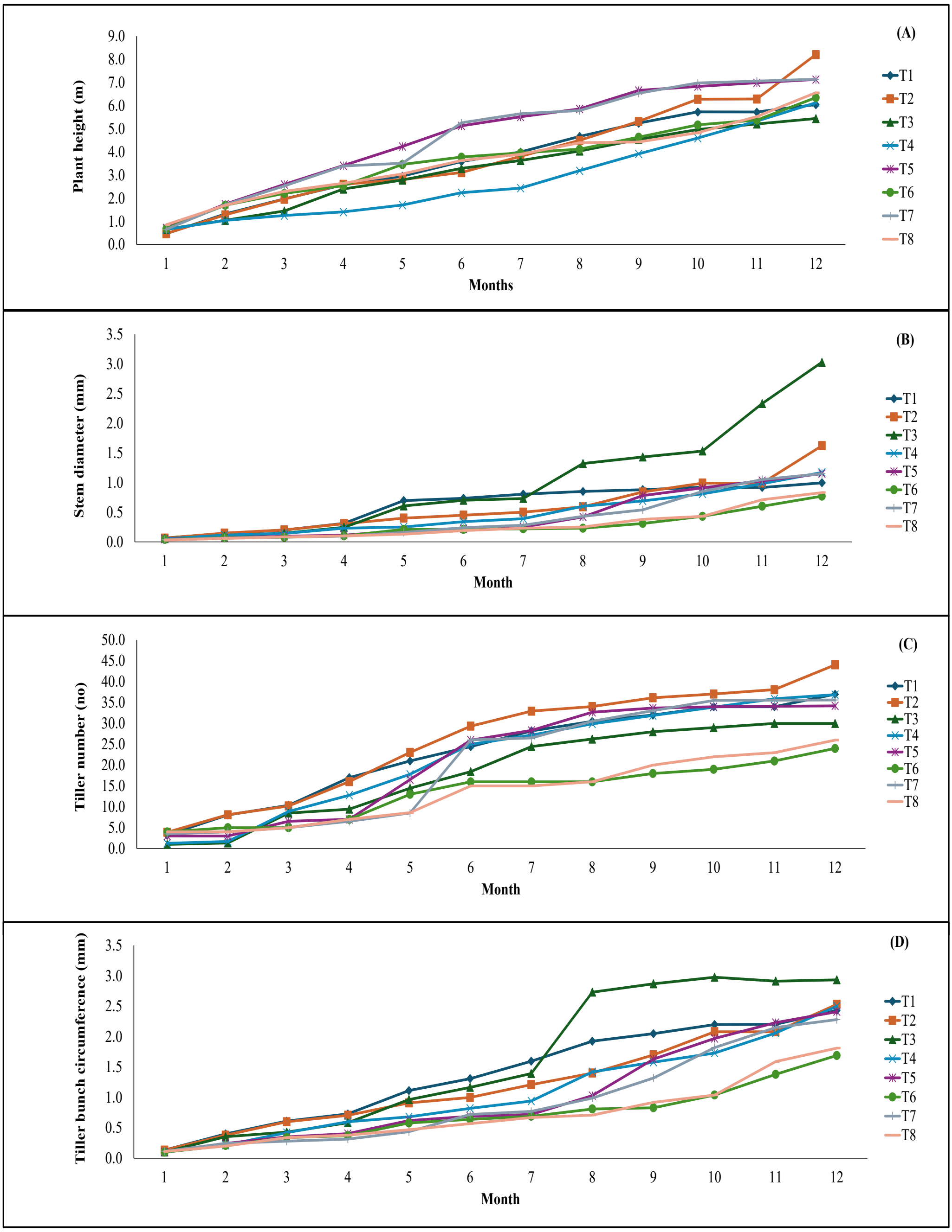

Figure 7. The interaction effect of ratooning and different periods of elevated carbon dioxide on (A) plant height, (B) stem diameter, (C) tiller number, and (D) tiller bunch circumference of Napier grass (*Pennisetum purpureum* cv. Taiwan)

Note. T1 = 1-month elevated CO_2 ($MECO_2$)-no ratooned (noR); T2 = 1 $MECO_2$-ratooned (R) at three months after planting (MAP); T3 = 2 $MECO_2$-noR; T4 = 2 $MECO_2$-R at 3 MAP; T5 = 5 $MECO_2$-noR; T6 = 5 $MECO_2$-R at 3 MAP; T7 = 12 $MECO_2$-noR; T8 = 12 $MECO_2$-R at 3 MAP

Whereas, in Figure 7C, the 1 $MECO_2$-R showed a significantly higher tiller number compared to another related CO_2, which increased by 10.58% (T1), 29.41% (T3), 15.91% (T4), 17.22% (T5), 47.58% (T6), 19.98% (T7), and 47.09% (T8), respectively. Elevated CO_2 and ratooning have been proven to increase aboveground biomass production in either shoot or tiller numbers (Long et al., 2004). The growth rate depends on the proportion of allocations; if the plant allocates essential nutrients and other elements to tiller parts, the tiller number increases biomass production (Poudel & Dunn, 2017). It can result in the initiation of more tiller buds and the subsequent growth of new tillers (Pritchard et al., 1999). On the other hand, the 2 $MECO_2$-noR showed a significantly higher tiller bunch circumference compared to another elevated CO_2, which increased by 14.01% (T1), 24.14% (T2), 32.70% (T4), 36.15% (T5), 55.29% (T6), 41.06% (T7), 54.62% (T8), respectively (Figure 7D). The longer Napier grass is exposed to elevated CO_2, the more significant changes in tiller bunch circumference (Rahman et al., 2019). However, if the impact has reached maximum production, any longer exposure will not help to increase the tiller bunch circumference (Kimball, 2016). The result corresponds with a previous study, which states that a high carbon supply under elevated CO_2 helps accelerate cell division and expansion in tissues and enhance early growth and development in the meristematic tissues of the plant. Kimball (2016) and Wangchuk et al. (2015) have proven that elevated CO_2 significantly affected plant height and tiller number with increasing plant height throughout the experiment.

Ultimate Analysis: Carbon (%), Hydrogen (%), Nitrogen (%), and Sulphur (%)

Table 3 shows a significant interaction between ratooning and different periods of elevated CO_2 in nitrogen and sulphur. While in Figure 8A, the 12 $MECO_2$-R at 3MAP shows the highest reading of total nitrogen (%) compared to other treatments, which are increased by 14.04% (T1), 11.70% (T2), 20.47% (T3), 17.54% (T4), 26.90% (T5), 9.37% (T6), 33.33% (T7), and 35.87% (T8) respectively. Elevated CO_2 levels will have different approaches to photosynthesis, transpiration, and stomatal conductance rates (Zakaria et al., 2019). High CO_2 concentration can theoretically lead to increased photosynthesis rate in plant growth, resource allocation, and tissue composition due to high carbon assimilation (Gulfam et al., 2017). Besides, elevated CO_2 plays an important role in microbial activities, which results in an increase in the decomposition rates of organic matter (Zhou et al., 2022). Thus, nitrogen fixation has increased due to the large amount of nitrogen compound released from organic matter (Long et al., 2006).

Elevated CO_2 concentrations can stimulate photosynthesis and enhance plant carbon uptake (Wangchuk et al., 2015). The increased carbon assimilation results in higher carbon content in organic materials (Lounglawan et al., 2014). Elevated CO_2 can improve plant nitrogen use efficiency,

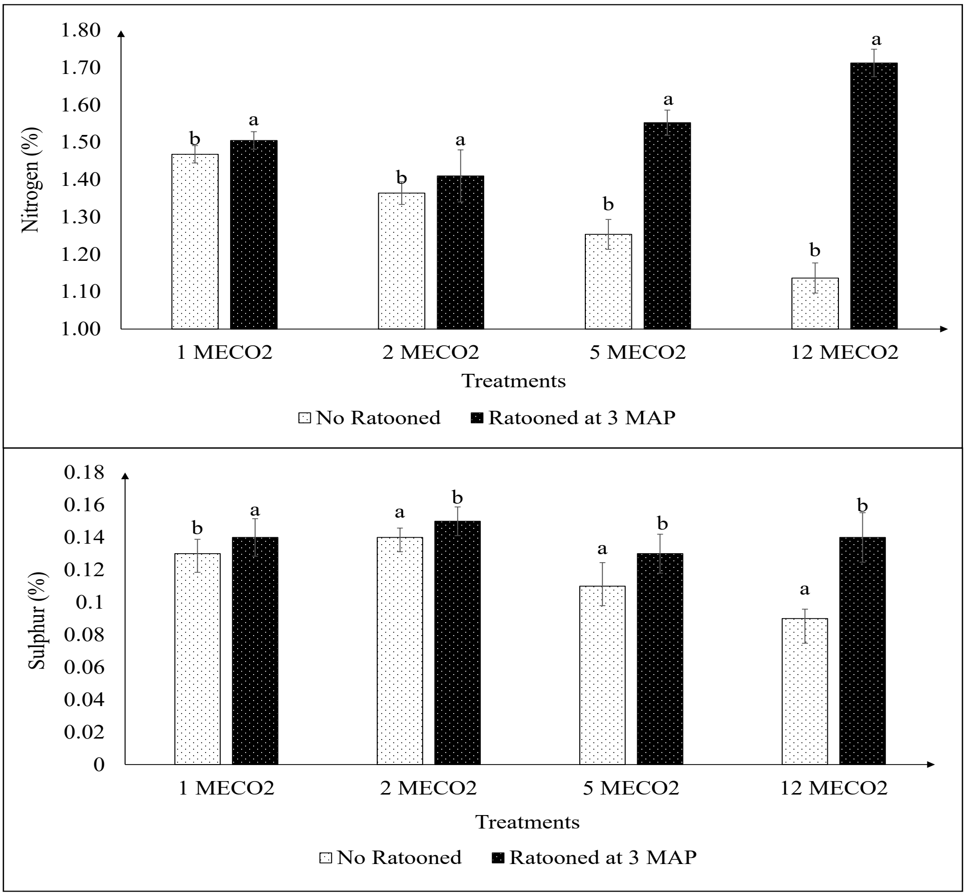

Figure 8. The interaction effect of ratooning and different periods of elevated carbon dioxide on (A) total nitrogen and (B) total sulphur of Napier grass (*Pennisetum purpureum* cv. Taiwan), respectively

Note. T1 = 1-month elevated CO_2 ($MECO_2$)-no ratooned (noR); T2 = 1 $MECO_2$-ratooned (R) at three months after planting (MAP); T3 = 2 $MECO_2$-noR; T4 = 2 $MECO_2$-R at 3 MAP; T5 = 5 $MECO_2$-noR; T6 = 5 $MECO_2$-R at 3 MAP; T7 = 12 $MECO_2$-noR; T8 = 12 $MECO_2$-R at 3 MAP; a and b indicate significant difference between treatment means using least significant difference at $p \leq 0.05$

potentially reducing nitrogen content (Zakaria et al., 2019). Additionally, elevated CO_2 can alter soil microbial communities' composition and abundance, affecting ecosystems' nitrogen availability and cycling (Leakey et al., 2009). These changes can indirectly influence the nitrogen content in organic materials (Mwendia et al., 2019). Elevated CO_2 can indirectly affect the sulphur content of organic materials by altering soil microbial activities and nutrient cycling (Norhaiza et al., 2009).

Based on Figure 8B, five treatments show the highest reading of total sulphur, which are 1 $MECO_2$-noR (T1), 1 $MECO_2$-R at 3 MAP (T2), 2 $MECO_2$-noR (T3), 2 $MECO_2$-R at 3 MAP (T4), and 12 $MECO_2$-R at 3 MAP (T8). Fertile soil contains enough macronutrients to meet plant requirements, which include nitrogen, potassium, phosphorus, calcium, magnesium, and sulphur (Manyawu et al., 2003). Sulphur is categorised as a secondary element of macronutrients, which are required in smaller amounts than primary nutrients (Marafon et al., 2021). However, unlike carbon and nitrogen, sulphur is not a limiting nutrient in terrestrial ecosystems (Mason et al., 2008). Difference periods of elevated CO_2 can affect soil microbial communities and biological activities (Said et al., 2019). Microbes play a crucial role in the decomposition of organic matter and involve sulphur release through the sulphur cycle (Rengsirikul et al., 2013).

Table 3
The effect of elevated carbon dioxide (CO_2) period on carbon, hydrogen, nitrogen, and sulphur of Napier grass (Pennisetum purpureum *cv. Taiwan*)

Treatments	Carbon (%)	Hydrogen (%)
Ratooned (R)		
noR	44.34a	5.68a
R	43.42a	5.69a
LSD (P<0.05)	NS	NS
Elevated CO_2 (ECO_2)		
T1: 1-month ECO_2	44.95a	5.68ab
T2: 2-month ECO_2	44.67a	5.85a
T3: 5-month ECO_2	44.81a	5.76a
T4: 12-month ECO_2	41.10b	5.45b
LSD P<0.05	1.84	0.27
R x ECO_2	NS	NS

Note. Means followed by the same letter within a column are not significantly different at P>0.05 by least significant difference (LSD) test with n = 32; NS = Not significant; noR = No ratooned

Total Biomass

In Figure 9, the highest total biomass of Napier grass was recorded at 1 $MECO_2$-noR (T1), which increased by 74.34% (T2), 10.08% (T3), 78.69% (T4), 91.86% (T5), 86.11% (T6), 23.59% (T7), and 88.59% (T8), respectively, for every period of harvest after planting followed by 2 $MECO_2$-noR (T2), 12 $MECO_2$-noR (T7), and other treatments. Elevated CO_2 levels typically stimulate photosynthesis and carbon assimilation in plants, leading to enhanced growth (Hampton et al., 2013). It can result in greater aboveground biomass, including stems, leaves, and fruits (Mukhtar, 2006). The increased availability of CO_2 allows plants to fix more carbon dioxide and produce higher amounts of carbohydrates, such as sugars and starches, which are the building blocks for biomass production (Zailan et al., 2016). However, Napier grass acclimates to elevated CO_2 levels, resulting in reduced growth response compared to initial exposure (Thompson et al., 2017). The reason behind this result was that Napier grass can adjust its physiological processes according to the new carbon dioxide environment (Sawasdee & Pisutpaisal, 2014). Ibrahim et al. (2011b) and Rambau et al. (2016) reported that crops exposed to elevated CO_2 positively increased the total biomass at week 12 by 6.08 t/ha. However, increasing the period of elevated CO_2 will

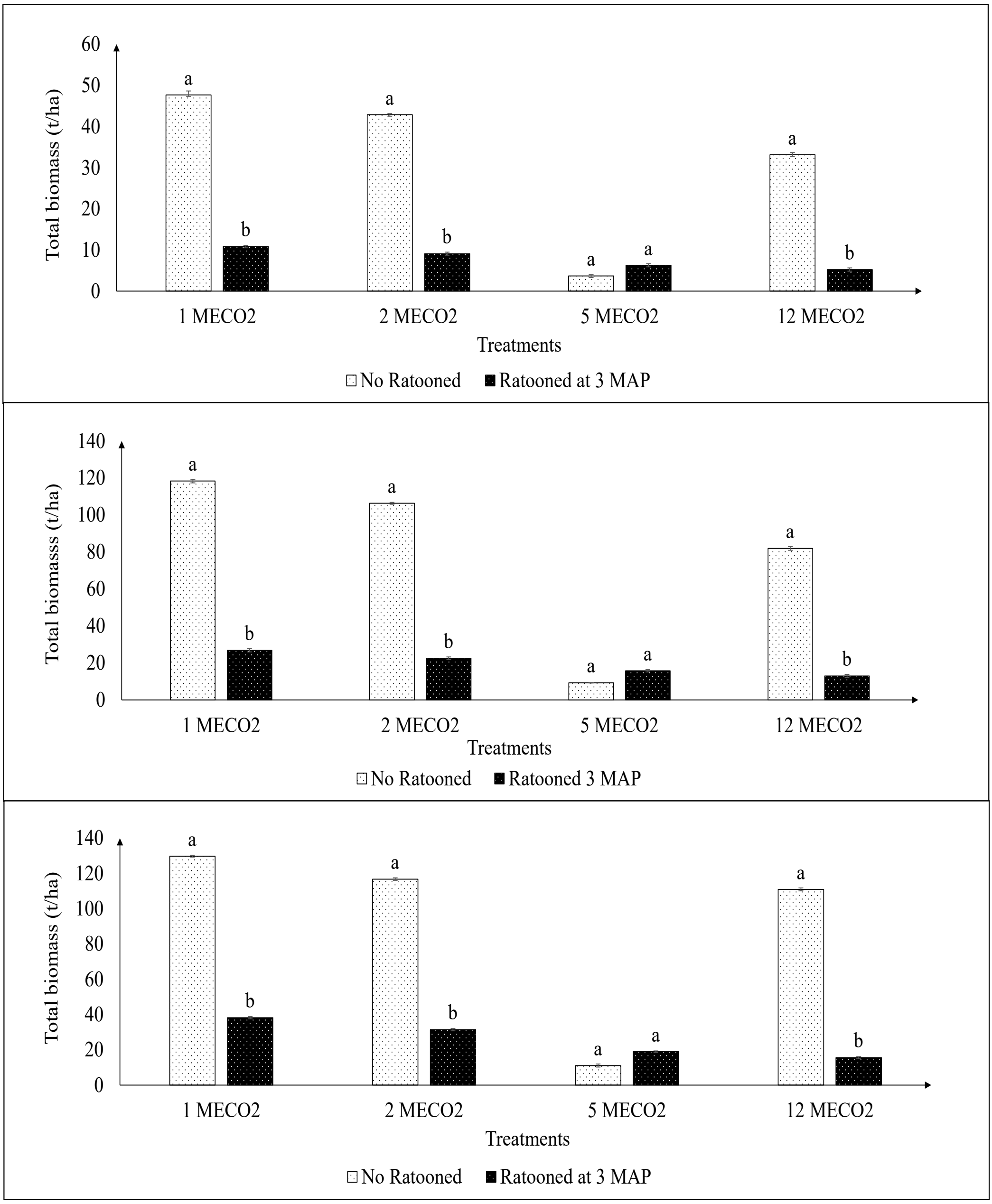

Figure 9. The interaction effect of ratooning and different periods of elevated carbon dioxide on total biomass of Napier grass (*Pennisetum purpureum* cv. Taiwan) at (A) 6 months after planting (MAP), (B) 8 MAP, and (C) 12 MAP, respectively; a and b indicate significant difference between treatment means using least significant difference at $p \leq 0.05$

Note. T1 = 1-month elevated CO_2 ($MECO_2$)-no ratooned (noR); T2 = 1 $MECO_2$-ratooned (R) at three months after planting (MAP); T3 = 2 $MECO_2$-noR; T4 = 2 $MECO_2$-R at 3 MAP; T5 = 5 $MECO_2$-noR; T6 = 5 $MECO_2$-R at 3 MAP; T7 = 12 $MECO_2$-noR; T8 = 12 $MECO_2$-R at 3 MAP not eventually increase the total biomass at a certain time (Namiki, 1990). The plant initially responds positively to elevated CO_2 levels by exhibiting increased rates of photosynthesis (Akah & Onweluzo, 2014). It can lead to enhanced plant growth and increased biomass production, especially in the early stages of exposure (Niinemets & Valladares, 2006). This response is referred to as the "CO_2 fertilisation effect" (Collatz et al., 1992). However, the longer exposure of Napier grass to the high carbon dioxide percentage will not help increase the total biomass as it reaches maximum growth (Negawo et al., 2017).

CONCLUSION

Experiment 1 showed that Napier grass with no ratooned resulted in the highest readings in all parameters compared to ratooned in plant height, stem diameter, total bunch circumference, plant biomass, total nitrogen, and sulphur in the ultimate analysis. The ultimate analysis did not significantly affect tiller number, total carbon, or nitrogen.

Experiment 2 proved that Napier growth, such as plant height, stem diameter, tiller number, tiller bunch circumference, plant biomass, total nitrogen, and sulphur in the ultimate analysis, showed significant interactions between ratooning and elevated CO_2. The treatment that implied the highest result in all parameters was 1 $MECO_2$, which was able to promote similar results as the plant received treatments from 2, 5, and 12 $MECO_2$, which is better than control treatments. However, in Experiment 2, all treatments that had no ratooned showed the highest reading compared to ratooned treatments.

These results prove that crops were facing a slow recovery process after being ratooned, thus affecting crop productivity. In conclusion, Napier grass production will decrease with increasing crop age, either ratooned or no ratooned treatments. Long exposure under elevated CO_2 conditions did not significantly benefit plant growth in the study. Meanwhile, the results suggested that shorter exposure periods (1 and 2 $MECO_2$) led to more favourable outcomes in terms of growth performance, biomass quality, and biochemical accumulation. Napier grass has been considered for carbon dioxide sequestration due to its fast growth and high biomass production. The suggestion for utilising Napier grass in carbon dioxide sequestration areas is to build agroforestry systems that integrate or plant trees alongside Napier grass, creating a more diverse and resilient ecosystem and capturing carbon in the grass biomass and the trees. Besides, mixed cropping with other fast-growing plants or cover crops should be considered to enhance the overall carbon sequestration potential. Diverse plantings can also improve soil health and fertility. Next, Napier grass, such as biogas or bioethanol, can be utilised for bioenergy production. While burning biomass releases carbon dioxide, using it as a renewable energy source can potentially displace fossil fuels, contributing to a net reduction in atmospheric carbon.

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