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INFLUENCES OF MANURE AND BIOCHAR ON BIOMASS YIELD AND NUTRIENT VALUE OF *PENNISETUM PURPUREUM* CV. MOTT GROWN ON POST-NICKEL-MINING SOIL

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ABSTRACT

The objectives of this study were to determine the effects of biochar and manure additions to growth performance, nutrient value, *in vitro* digestibility, and rumen fermentation characteristics of *Pennisetum purpureum* cv. Mott grown in soil remaining after nickel-mining activities. The goat manure and iron wood biochar were used in this study. Treatments were arranged 4 x 2 factorially in a completely randomized block design. The factors were four levels of biochar (0, 5, 10, and 15 metric ton/ha) and two levels of manure (0 and 10 metric ton/ha). The plants were harvested at 70 days after sowing (first harvest), 128 days (first ratoon) and 193 days (second ratoon). Results showed that at first and second ratoon, plant height and tillering number were increased after 5 metric ton/ha biochar and 10 metric ton/ha manure additions ($p < 0.05$). Manure treatment resulted in higher crude protein content ($p < 0.05$) than controls at first and second ratoon stages. Combinations of biochar ($p < 0.01$) and manure ($p = 0.09$) improved *in vitro* gas production from the insoluble fraction (*b*). The study concludes that treatment with 5 metric ton/ha biochar and 10 metric ton/ha manure fertilizers on post-nickel-mining soil increases tillering number, height, CP content and *in vitro* digestibility of *P. purpureum* cv. Mott at ratoon stages.

Keywords: Biochar, manure, nutrient value, *Pennisetum purpureum* cv. Mott, post-mining

INTRODUCTION

In 2019, Southeast Sulawesi produced 23,967,146 metric tons of nickel (Ni) (Statistics Indonesia, 2020). Nickel minings in Southeast Sulawesi are located in North Konawe, South Konawe, Konawe, North Kolaka, Kolaka, Bombana and Buton Regencies, across a total area of 313,788.77 ha (Kadir *et al.,* 2020). In terms of reclamation efforts, post-mining areas such as these offer several opportunities for management, including cattle farming, that can be developed and profitable (Kodir *et al.,* 2017). Nickel is an essential micronutrient required for the growth and development of plants, but Ni accumulations are toxic and lead to physiological disorders (Rehman *et al.,* 2016). Appropriate strategies should be practiced to enable the cultivation of animal forage on post-Ni-mining soils. As rehabilitation agent, grasses are highly adapted to poor soils because they have extensive rhizome systems and, in some cases, underground tubers (Hapsari *et al.,* 2020). *Pennisetum purpureum* cv Mott (dwarf Napier grass) is a fast-growing forage that can be grown in harsh environments (Sarwanto *et al*., 2019; Kowitwiwat and Sampanpanish, 2020).

Over the past few years, combinations of organic and inorganic agents have been used in applications for in-situ stabilization for Ni contaminated soils (Shahbaz *et al.,* 2018; Shahbaz *et al.,* 2019). Biochar and animal manure are inorganic and organic agents, respectively, that suitable for rehabilitation application in such situations (Gardner *et al.,* 2012). Biochar is carbon-rich material that can increase organic carbon availability, soil pH, and cation exchange capacity (Cardenas-Aguiar *et al.,* 2020). Biochar also has high retention capacity that increases total nitrogen concentrations (Visconti *et al.,* 2020). Of organic fertilizers, farm manure has been shown to be most effective in reducing Ni toxicity in maize (Rehman *et al.,* 2016).

Animal manure and biochar are soil rehabilitation agents commonly used to cope with metallic trace-element contamination. Application of biochar and manure slightly reduced the uptake and aggregation of heavy metals in aboveground parts of dwarf napier grass (Kowitwiwat and Sampanpanish, 2020). In a previous study, a combination of biochar and cationic zeolite was shown to efficiently reduce Ni accumulations in the grain, shoots, and roots of maize (Shahbaz *et al.,* 2018). Furthermore, in terms of feed safety, Harmini *et al.* (2019) reported that napier grass from ex-mining can replace native grass from farmers because it has the same nutrient and allowable mineral content. No previous studies have been conducted to assess the effects of animal manure and biochar on biomass yield, nutrient yield, and *in vitro* digestibility of *Pennisetum purpureum* cv. Mott in post-Ni-mining soil. *In vitro* digestibility could be measured by *in vitro* gas production system as reported by Ayaşan *et al.* (2020). The objectives of this study are therefore to determine the effects of biochar and manure amendments on the growth performance, nutrient value, *in vitro* digestibility, and rumen fermentation characteristics of *Pennisetum purpureum* cv. Mott.

MATERIALS AND METHODS

Soil sample, biochar, and manure preparation: Soil samples at depths of 0–30 cm from the surface layer were collected from Palangga Ni-mining field, located in South Konawe, Southeast Sulawesi, Indonesia (4°24'11.1"S 122°19'14.4"E, elevation 180 m). Characteristics of the soil are represented in Table 1. The goat manure used in this study was obtained from Mulya Jaya Toari, a local organic fertilizer producer in Kolaka Regency. Manures were collected from local goat and all impurities (grasses and stones) were taken off manually. Manures were milled to pass through a 2-mm sieve. Biochar produced from iron wood (*Eusideroxylon zwageri*) was obtained from a forest on the outskirts of Konawe. Chemical properties of soil, manure, and biochar samples were analyzed using Fourier Transform Mid-Infrared (FTIR) equipment from Shimadzu®, Japan. Characteristics of biochar and manure are represented in Figure 1.

Table 1. Chemical characteristics of post-Ni-mining soil in Konawe, Southeast Sulawesi, Indonesia

Characteristics	Soil
N (%)	0.14
P (ppm)	98.6
K (cmol/kg)	0.22
C (%)	0.52
pH	5.28
CEC (cmol/kg)	18.28

CEC (cation exchange capacity).

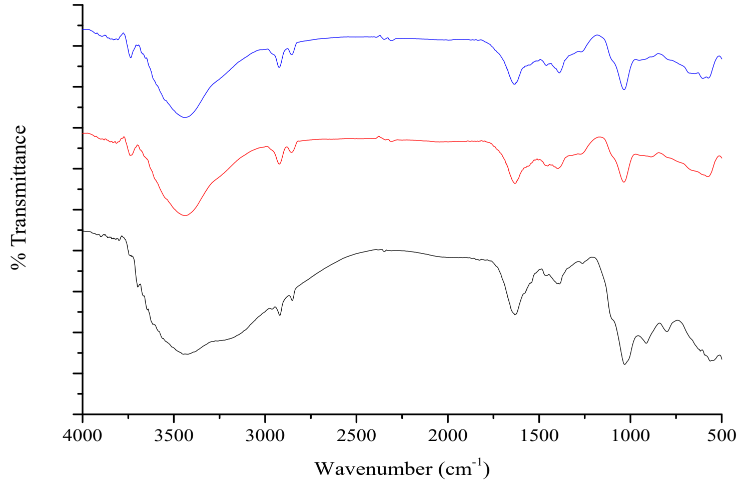

Fig 1. FTIR spectra of post-Ni-mining soil (—), biochar (—) and manure (—).

Plant cultivation: The *P. purpureum* cv. Mott used in the study was obtained from the agrostology unit of the Nutrition and Feed Science Laboratory of the Animal Science Faculty, Universitas of Halu Oleo. Pot experiments were carried out in a greenhouse from February 10 to August 20, 2018. The pots were arranged 4 x 2 factorially in a completely randomized block design. The factors were four biochar (B) fertilizer levels (0, 5, 10, and 15 metric ton/ha) and two levels of manure fertilizer (M) (0 and 10 metric ton/ha). One *P. purpureum* cv. Mott stem (with four internodes on each) was sown in each pot at 3–4 cm depth. Plants were watered twice a day and allowed to grow under normal environmental conditions (sunlight during the day and dark at night) at an average temperature of 29^{o}C. The plants were harvested at 70 days after sowing (first harvest), 128 days (first ratoon), and 193 days (second ratoon). Plant growth measurements such as plant height (cm), tillering number, biomass yield, leaf yield, stem yield, and leaf-to-stem ratio were recorded. All edible parts (leaves and stems) were placed into individual paper bags and dried at 60^{o}C for 48 h until a constant weight was achieved (Wahyono *et al.* 2019). The total biomass yield was calculated by fresh biomass yield x dry matter (DM) percentage. Dry samples were then ground and passed through a 1 mm sieve and prepared for nutrient value and degradability analysis.

Nutrient value determination: The value of organic matter (OM), crude protein (CP), ether extract (EE), and crude fiber (CF) were determined following AOAC guidelines (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest *et al.,* (1991). For second ratoon plants, hemicellulose, cellulose, non-fiber carbohydrate (NFC), dry matter intake (DMI), dry matter digestibility (DMI), and relative feed value (RFV) were calculated as follows:

$$\text{Hemicellulose (\%)} = \text{NDF (\%)} - \text{ADF (\%)}$$

$$\text{Cellulose (\%)} = \text{ADF (\%)} - \text{ADL (\%)}$$

$$\text{NFC (\%)} = \text{OM (\%)} - \text{CP (\%)} - \text{NDF (\%)} - \text{EE (\%)}$$

$$\text{DMI (\% LW)} = 120/\text{\% NDF}$$

$$\text{DMD (\%)} = 88.9/(\text{\% ADF} \times 0.779)$$

$$\text{RFV} = (\text{DMD} \times \text{DMI})/1.29$$

(Rohweder *et al.,* 1978)

According to the quality grading standards established The Hay Marketing Task Force of the American Forage and Grassland Council (Rohweder *et al.*, 1978), the nutrient quality grading standards for RFV are prime (> 151), premium (151–125), good (124–103), fair (102–87), poor (86–75) and reject (< 75).

The net energy of lactation (NE_L) and total digestible nutrients (TDN) were calculated according to Undersander *et al.,* (1993):

$$\text{NEL (Mcal/lb)} = 1.0876 - (0.0127 \times \text{ADF})$$

$$\text{TDN (\%)} = 4.898 + (89.796 \times \text{NEL})$$

(Pennsylvania State equation)

$$\text{NEL (Mcal/lb)} = 1.085 - (0.0150 \times \text{ADF})$$

$$\text{TDN (\%)} = 34.9 + (53.1 \times \text{NEL})$$

(New York State equation)

Mcal/lb converted to Mcal/kg

The 3rd TDN estimation also calculated according to Jayanegara *et al.,* (2019), as follows:

$$T\ (\%) = (0.479 \times N\ ) + (0.704 \times N\ ) + (1.594 \times E\ ) + (0.714 \times C\ )$$

***In vitro* degradability assay:** This experiment was carried out in accordance with Research Center for Isotope and Radiation Application Technology Animal Ethics Committee (Aproved Protocol number 001/KEPPHP-BATAN/X/2020). *In vitro* degradability was determined for second ratoon plants according to the *in vitro* gas production technique used by Menke and Steingass (1988). Rumen fluid was collected before morning feeding from two Holstein Friesian crossbred cattle with rumen cannulas fed with native grass and concentrates (2:1) at maintenance level. *In vitro* gas production was observed at 0, 3, 6, 9, 12, 24, 48, and 72 h of incubation. Total gas production values were fitted to Ørskov and McDonald's (1979) exponential equation as follows: $Y = a + b\ (1-e^{-ct})$, where Y is the gas production at time t (ml), a is the gas production from the soluble fraction (ml), b is the gas production from the insoluble fraction (ml), c is the gas production rate constant, $(a+b)$ is the potential gas production (ml),and t is the incubation time (h).

The gas production characteristics caused by soluble (GPSF) and insoluble (GPNSF) fractions was calculated from the amount of gas produced after 3 and 24 h incubation (El-Waziry *et al.,* 2016; Van Gelder *et al.,* 2005) as follows:

$$\text{GPSF (ml)} = (\text{gas at 3 h} \times 0.99 \times 5) - 3$$

$$\text{GPNSF (ml)} = (1.02 \times ((\text{gas at 24h} \times 5) - (\text{gas at 3 h} \times 5))) + 2$$

Approximately 10 ml of *in vitro* fermentation medium was collected after 72 h incubation to determine pH, NH_3 concentration, and total volatile fatty acids (TVFA). Measurement of pH using Hanna Instrument® pH meter (Rhode Island, US), NH_3 measurement using Conway (1951) method and TVFA measurement based on Kromann *et al.* (1966) method. *In vitro* organic matter digestibility (IVOMD), metabolizable energy (ME) and microbial protein (MP) were calculated using Menke and Steingass (1988), Czerkawski (1986) and Ayasan et al., (2020a), respectively:

$$\text{IVOMD (\%)} = 14.88 + (0.889 \times \text{GP}) + (0.45 \times \text{CP}) + (0.0651 \times \text{A})$$

$$\text{ME (MJ/kg DM)} = 2.2 + (0.136 \times \text{GP}) + (0.057 \times \text{CP}) + (0.0029 \times \text{EE}^2)$$

$$\text{MP (g/kg IVOMD)} = \text{IVOMD} \times 10.3 \times 6.25$$

Where:

GP is comulative gas production after 24 h incubation, CP is crude protein, A is ash and EE is ether extract. Mj converted to kcal.

Statistical analyses: Growth parameters, nutrient value, and *in vitro* assay data were analyzed using one-way analysis of variance (ANOVA). Duncan Multiple Range Test were used to separate the means value at significance level of $p < 0.05$ (Steel and Torrie, 1960) and performed using SPSS version 22.0.

RESULTS

Soil, biochar, and manure characteristics: Post-Ni-mining soil, biochar, and manure were analyzed to evaluate the structure of functional groups, as presented in Figure 1. In soil samples, the infrared peaks were analyzed according to previous studies (Smidt *et al.,* 2011; Abdulrazzaq *et al.,* 2014; Pattnaik *et al.,* 2018; Sarfaraz *et al.,* 2020). Peak 563 is related to metal presence, while peaks 800 and 1013 are related to C-O out-of-plane bending (carbonates) and Si-O stretching (clay minerals). Peaks 1388 and 1633 illustrate N-O stretching (nitrate leachate) C=O, and COO stretching (amide I, carboxylates). Peaks 2850 and 290 are related to C-H stretching (*s*) and C-H stretching (*as*) (aliphatic methylene group). Biochar spectra from Figure 1 are as follows: 576: inorganic metal presence; 1035: C-O-C stretch of the esters reflected in lignin or Si-O-Si stretch; 1633: C=C ring stretching (aromatic); 2922 and 2856: cm^{-1} asymmetric and symmetric C-H stretching vibrations for aliphatic groups; and 3439: O-H stretching, cellulose, and hemicellulose components. The manure spectra from Figure 1 are as follows: 574: inorganic metals (K and Ca); 650: out-of-plane ring deformation or weak vibration $-CH_2$ rocking; 1035: C-O-C stretching of the ethers present in lignin or Si-O-Si stretch; 1460 and O–H groups (carboxyl). We also analyzed the chemical characteristics of soil, biochar, and manure (data not shown). The pH values of soil, biochar, and manure were 7.65, 8.60, and 7.74, respectively, while biochar and manure had P contents of 0.13 and 2.37 %, respectively.

Influence of biochar and manure fertilizer on growth performance of *P. purpureum* cv. Mott: Results regarding the influence of manure and biochar additions on the height, tillering number, and weight of *P. purpureum* cv. Mott are illustrated in Figure 2. Biochar and manure amendment had no effect on height and tillering number at first harvesting stage. At first and second ratoon harvesting, the plant height and tillering number were increased after the 5 metric ton/ha biochar and 10 metric ton/ha manure additions ($p < 0.05$). However, there were no significant differences between the fertilizer treatments ($p > 0.05$). There was no significant interaction in terms of growth performance between the biochar and manure treatments ($p > 0.05$).

Influence of biochar and manure fertilizer on nutrient composition of *P. purpureum* cv. Mott: Data on nutrient composition and fiber fraction as affected by biochar and manure additions are summarized in Table 2. Except for CF ($p < 0.05$), no significant difference was observed for nutrient contents at first harvesting ($p > 0.05$). At first ratoon, The CF concentration decreased after manure addition ($p < 0.05$) compared to the control. Conversely, the CF concentration increased after manure addition ($p > 0.01$) at second ratoon. The manure treatments resulted in higher CP content ($p < 0.05$) than control at first and second ratoon harvesting. At second ratoon, all additions tended to cause a decrease in fiber fractions (NDF and ADF) of *P. purpureum* cv. Mott, although the decreases were not always significant ($p > 0.05$).

Influence of biochar and manure fertilizer on nutrient value of *P. purpureum* cv. Mott: The nutrient value of *P. purpureum* cv. Mott after biochar and manure treatments is shown in Table 3. Biochar and manure additions do tend to increase RFV and TDN values. According to nutrient quality grading standards with respect to the RFV index, it was found that manure addition combined with 0–5 metric ton/ha^{-1} biochar could improve forage quality from "fair" to "good".

***In vitro* gas production and digestibility of *P. purpureum* cv. Mott:** The *in vitro* gas production and digestibility data for *P. purpureum* cv. Mott are shown in Table 4. A significant increase in cumulative gas production after 10 metric ton/ha manure fertilization was observed at 6 ($p < 0.01$), 9, and 12 h ($p < 0.05$). A significant increase was also observed in optimum gas production (parameter $a + b$) ($p < 0.05$) as a result of biochar addition. Combination of biochar ($p < 0.01$) and manure ($p = 0.09$) (quasi-significant) improves *in vitro* gas production from the insoluble fraction (*b*). Manure addition increases GPSF ($p < 0.05$) but does not affect GPNSF. As shown in Table 4, increasing levels of biochar fertilizer (max 10 metric ton/ha^{-1}) results in increases in IVOMD, ME, and MP parameters ($p < 0.05$). There was no effect of the treatments on pH, NH_3, or TVFA products ($p > 0.05$), and there was no significant interaction on *in vitro* digestibility between the biochar and manure treatments.

DISCUSSION

Improving soil fertility to support improvements in animal production in Indonesia is an important issue, especially in post-mining areas such as Southeast Sulawesi. In this study, the soil observed was a subsoil layer from which the surface area had been mined. To improve soil fertility, it is necessary to introduce grass plant varieties such as *P. purpureum* that are able to grow in marginal and critical soils (Harmini *et al.,* 2019). However, low organic and nutrient content is a consequence of this ability. We hypothesized that biochar additions at 5, 10, and 15 metric ton/ha could improve soil fertility. As reported by Blanco-Canqui (2017), biochar application generally improves the quality of soil structure, alters water transmission characteristics, increases water retention capacity, and can moderate soil temperature. The combination of biochar and animal manure could increase soil aggregation and physical properties to a greater extent than biochar alone (Blanco-Canqui, 2017). Biochar and organic manure are simple to produce and do not require sophisticated equipment, and so could be appropriate for use by rural communities in post-mining areas.

The greatest height and tillering number were reported for treatments with 5 metric ton/ha of biochar and 10 metric ton/ha manure, but were not significantly different to other treatments. Despite there being no significant interaction, biochar manure has a positive effect on plant growth. It is interesting to note that height and tillering number at the ratoon stages is higher than at first harvesting. This is related to the more complete root system developing after plants are harvested for the first time (Sumiyoshi *et al.,* 2017). Biochar application can improve marginal soils that have low nutrient and OM content (El-Naggar *et al.,* 2018). Biochar treatment significantly increases soil pH, total organic carbon (TOC), and total nitrogen (TN), and stabilizes OM (Visconti *et al.,* 2020).

Organic manure has positive effects on increasing soil pH, P, N concentration, and exchangeable K, Ca, and Mg (Han *et al.,* 2016). In the case of post-Ni-mining soil management, organic fertilizers are the most efficient application for reducing Ni concentration in soil; nevertheless, their effectiveness depends on the choice of plant species (Álvarez-López *et al.,* 2016). Unfortunately, data on the changes in soil conditions are not reported in this study as we only report on plant performance after biochar–manure additions. Contrary to our predictions, despite biochar and manure application increasing height and tillering number, it did not changes plant yields. The reason for this is not clear, but we assume it may be due to 1) there being an energy partition to increasing tillering number and 2) the short-term nature of the experiments. Liu *et al.* (2020a) reported that at high biochar treatment levels the inhibition of plant growth becomes more obvious with the increase in animal manure. Similarly to our findings, Cardenas-Aquiar *et al*. (2020) reported that biochar addition does not cause any direct changes to plant yield. Plant yield has been shown to increase over time, thus longer-term investigation is needed to reach a more representative conclusion about the effects of the combination of biochar and manure addition on yields of *P. purpureum cv.* Mott. The differences between the results of various studies into the use of biochar can be caused by a number of factors: 1) soil properties and texture; 2) agroclimate; 3) biochar feedstock type; 4) pyrolysis temperature during production; 5) placement methods; and 6) other combination variations (Blanco-Canqui, 2017; Cardenas-Aguiar *et al.,* 2020). However, the added organic manure could supply nutrients (N and P) while the high adsorption capacity of biochar could help retain these nutrients in marginal soils.

At first ratoon, adding 10 metric ton/ha of manure (without biochar) increased CP content by 38.37%. At second ratoon, 10 metric ton/ha of manure combined with 5 and 15 metric ton/ha of biochar increased CP content by 28.09 and 34.71%, respectively. Increases in CP content may be related to the increase of N and P uptake due to manure addition. This assumption is clarified by the statistically significant value obtained from the manure treatment factor. Liu *et al.* (2020b) reported that organic manure without biochar increased the N, P, K, Ca, and Mg content of soybean shoots. The CP content in forage is influenced by the nutrients available in the soil (Yudiastari *et al.,* 2019).

Agegnehu *et al.* (2016) demonstrated that application of biochar and organic fertilizer (without mineral fertilizer) increased N concentration of barley grain and straw by 13%. Shahbaz *et al.* (2019) also reported that biochar addition increased the protein content of wheat grain by 11%. Indirectly, the increase in CP content may be associated with high photosynthetic performance after biochar addition (Shahbaz *et al.,* 2018). However, this assumption needs to be explored further. Furthermore, increased availability of nutrients and good microbial communities in soil increases plant growth and nutrition yield (Liu *et al.,* 2020b). Han *et al.* (2016) demonstrated that there were no significant effects on N concentration in stems and leaves of yellow poplar (*Liriodendron tulipifera* Lin.) from organic manure addition.

An interesting point is demonstrated by the change in CF content. At first harvest, treatments reduced CF content, while at first ratoon, the CF content tended to increase. This may be explained by 1) the dynamics of the influence of manure and biochar at each harvesting stage; 2) the relationship between the effect of amendments on the maturity rate of plants; and 3) the dynamics of plant structure development (stems and leaves). Yudiastari *et al.* (2019) reported that there are complex interactions influencing the fiber content of plants, including soil effects, plant effects, and agroclimatic effects. Longer-term further study is needed to gather more evidence, because the reason for the dynamics of CF content is not clearly understood. To understand better the influence of biochar, more above–below ground biotic overview is required from long-term studies (McCormack *et al.,* 2019). At second ratoon, fiber fraction represented by NDF and ADF tended to decrease after treatments. We speculate that the effect of manure is more dominant as a supplier of organic N, thereby reducing the percentage of plant defense in the form of lignin. One of the defense mechanisms of plants in extreme environments is lignin content. Despite not being itself a carbohydrate, lignin is associated with carbohydrates in plant cell walls (McDonald *et al.,* 2010). Lignin combines with hemicellulose and cellulose in the form of NDF (Wahyono *et al.,* 2019). A previous study reported that NDF, ADF, and acid detergent lignin (ADL) concentration decreased with increasing N fertilization (Balabanli *et al.,* 2010).

Decreasing in fiber fraction (NDF and ADF) (Table 2) will increase the nutrient quality represented by the RFV and TDN predicted values. The NDF content, particularly lignocellulose, has negative effect on ruminal degradation (Jayanegara *et al.,* 2019). Furthermore, NDF makes lower contribution to energy sources for ruminants, and thus has a negative relationship with TDN. Generally, TDN is highly correlated with DMD and digestible energy (Undersander *et al.,* 1993). Relative feed value (RFV) represents the nutrient value and quality of forage, and it is therefore important to determine nutrient utility, especially of fiber (Wahyono *et al.,* 2019), as predicted from the Alfalfa standard value; thus, *P. purpureum* cv. Mott will be included in Alfalfa quality classes of “fair” and “good”. Manure addition combined with 0–5 metric ton/ha biochar increases the nutrient value from “fair” to “good”. In this study, the RFV prediction for *P. purpureum* cv. Mott is between 99.32 and 106.41 (Table 3).

Cumulative gas production, rumen fermentation products, and *in vitro* digestibility are parameters related to the digestibility of plants, while total gas production is a reflection of nutrient profiles (Wahyono *et al.,* 2019; Ayasan *et al*., 2020b). Our hypothesis is that the changes in nutrient fraction resulting from biochar and manure amendment will affect the digestibility of plants. In this study, CP and fiber fractions are factors that tend to change after amendments. Total gas production is predominately from fermentation of carbohydrates, followed by protein, and finally fat content (Zailan *et al.,* 2018). As reported in Table 4, *P. purpureum* cv. Mott treated with biochar and manure produced high cumulative gas production. Because the increase of gas production was indicated by the *b* fraction, we can conclude that changes in fiber fractions (NDF and ADF) in plants may play a role in improving plant digestibility. Albores-Moreno *et al.* (2018) demonstrated that the highest digestibility of forage is related to greater digestion of structural carbohydrates. The primary chemical component of substrates that determines their digestion rate is NDF, which is itself a measure of cell-wall components (McDonald *et al.,* 2010). Total gas production and IVOMD have negative correlation with NDF and ADF (Adejoro and Hassen, 2018; Wahyono *et al.,* 2019). Despite the concentration of NH_3 not being significantly different between forages, the high IVOMD found in biochar and manure additions may be related to higher CP content (Table 2). Zailan *et al.* (2018) reported that protein fermentation is the second most important factor influencing fermentation in the rumen after fermentation of fiber. However, concentration of NH_3 is not only influenced by CP level on substrates. Differences in the amounts of proteins with various characteristics (soluble, quickly, moderately, slowly degraded, or undegradable) in forage also effect the extent of NH_3 concentration in the rumen (Jayanegara *et al.,* 2016; Wahyono *et al.,* 2019).

Conclusion: The current study concludes that treatments of 5 metric ton/ha of biochar and 10 metric ton/ha of manure on post-Ni-mining soil increases tillering number and height of *P. purpureum* cv. Mott at ratoon stages. The CP content of *P. purpureum* cv. Mott at ratoon harvesting time increased with application of biochar and manure to the soil. Decrease in fiber fraction at second ratoon stage contributed to higher RFV and TDN estimation values. Furthermore, application of 5 metric ton/ha biochar and 10 metric ton/ha manure to soil increased *in vitro* GPNSF and OM digestibility of *P. purpureum* cv. Mott. Future work may help to specify how the biochar and manure additions impact on heavy-metal accumulation in plants and soil microbial populations in post-NI-mining remediation. However, this study has highlighted the importance of considering biochar and manure in the context of improving post-Ni-mining soil in Southeast Sulawesi, Indonesia.

Conflict of interest: The authors declare no conflict of interest with any personal, financial or other relationships with organization related to the content in the manuscript.

Table 2. **Table of means for effects of biochar and manure addition on nutrient and fiber composition of *Pennisetum purpureum* cv. Mott.**

Harvesting time	Treatment	% DM								
		OM	ash	CP	CF	EE	NDF	ADF	Hemicellulose	NFC
1st Harvest	B0M0	80.11	19.89	11.56	24.45^{b}	-	-	-	-	-
	B5M0	79.90	20.10	11.42	25.74^{b}	-	-	-	-	-
	B10M0	79.43	20.57	11.79	25.40^{b}	-	-	-	-	-
	B15M0	81.19	18.81	12.64	23.73^{ab}	-	-	-	-	-
	B0M10	79.50	20.50	11.75	23.66^{ab}	-	-	-	-	-
	B5M10	79.09	20.91	10.96	21.03^{a}	-	-	-	-	-
	B10M10	79.55	20.45	12.66	21.18^{a}	-	-	-	-	-
	B15M10	79.37	20.63	12.09	22.07^{ab}	-	-	-	-	-
	SEM	0.301	0.232	0.238	0.529	-	-	-	-	-
	B	ns	ns	ns	ns	-	-	-	-	-
	M	ns	ns	ns	.01	-	-	-	-	-
	B*M	ns	ns	ns	ns	-	-	-	-	-
1st Ratoon	B0M0	81.61	18.39	7.14^{a}	25.91^{abc}	-	-	-	-	-
	B5M0	81.72	18.28	7.88^{ab}	24.59^{abc}	-	-	-	-	-
	B10M0	81.04	18.96	8.79^{ab}	23.43^{ab}	-	-	-	-	-
	B15M0	80.26	19.74	7.58^{ab}	23.00^{a}	-	-	-	-	-
	B0M10	82.12	17.88	9.88^{b}	26.58^{abc}	-	-	-	-	-
	B5M10	81.77	18.23	7.57^{ab}	28.03^{c}	-	-	-	-	-

	B10M10	81.79	18.21	9.80^{ab}	26.42^{abc}	-	-	-	-	-
	B15M10	81.36	18.64	8.66^{ab}	26.91^{bc}	-	-	-	-	-
	SEM	0.250	0.206	0.305	0.458	-	-	-	-	-
	B	ns	ns	ns	ns	-	-	-	-	-
	M	ns	ns	.05	<0.01	-	-	-	-	-
	$B^{*}M$	ns	ns	ns	ns	-	-	-	-	-
2nd Ratoon	B0M0	82.68	17.32	7.26^{a}	-	8.38	60.09	31.86	28.23	8.48
	B5M0	85.09	14.91	8.77^{ab}	-	10.20	59.47	31.50	27.96	8.63
	B10M0	83.42	16.58	8.12^{ab}	-	9.75	59.47	30.69	28.78	7.90
	B15M0	83.00	17.00	9.78^{b}	-	9.52	58.59	30.66	27.94	7.04
	B0M10	85.55	14.45	8.08^{ab}	-	10.18	57.51	30.08	27.43	11.71
	B5M10	83.55	16.45	9.30^{b}	-	10.73	58.14	30.81	27.33	7.43
	B10M10	86.22	13.78	8.74^{ab}	-	10.10	59.56	31.15	28.40	9.78
	B15M10	83.18	16.82	9.24^{b}	-	10.05	58.97	31.49	27.48	7.00
	SEM	0.528	0.471	0.253		0.226	0.324	0.206	0.205	0.572
	B	ns	ns	.05		ns	ns	ns	ns	ns
	M	ns	ns	ns		ns	ns	ns	ns	ns
	$B^{*}M$	ns	ns	ns		ns	ns	ns	ns	ns

OM (organic matter); CP (crude protein), CF (crude fiber), EE (ether extract), NDF (neutral detergent fiber), ADF (acid detergent fiber). SEM (standard error of Mean); ns (non-significant).

a (gas production from the immediately soluble fraction, ml); b (gas production from the insoluble fraction, ml)); c (gas production rate constant); a+b (potential gas production, ml). GPSF (the gas production characteristics caused by soluble fraction; GPNSF (the gas production characteristics caused by insoluble fraction); TVFA (total volatile fatty acids); NH_3 (ammonia); IVOMD (*in vitro* organic matter digestibility); ME (metabolizable energy); MP (microbial protein). SEM (standard error of Mean); ns (non-significant).

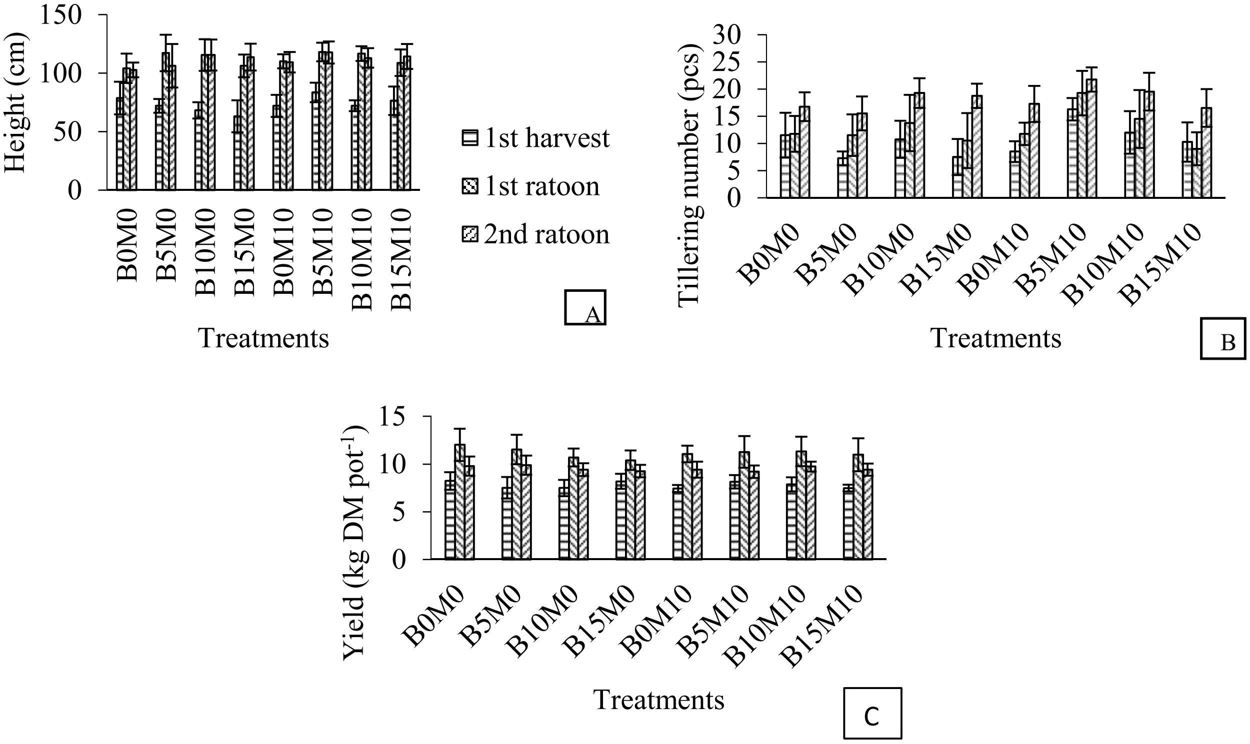

Figure 2. **Effects of biochar and manure addition on *Pennisetum purpureum* cv. Mott height (A), tillering number (B) and yield (C) at three different harvesting time.**

Table 3. **Table of means for effects of biochar and manure addition on nutrient value predictions of *Pennisetum purpureum* cv. Mott.**

Treatment	DMI (% live weight)	DMD (%)	RFV	NE$_{L}$* (Mcal/kg)	TDN* (%)	NE$_{L}$** (Mcal/kg)	TDN** (%)	TDN*** (%)
B0M0	1.99	64.08	99.32	0.68	66.23	0.61	67.14	53.29
B5M0	2.02	64.36	100.76	0.69	66.63	0.61	67.42	57.09
B10M0	2.02	64.99	101.65	0.69	67.55	0.63	68.06	55.39
B15M0	2.05	65.02	103.25	0.69	67.59	0.63	68.09	55.18
B0M10	2.09	65.46	106.41	0.71	68.25	0.63	68.55	57.79
B5M10	2.06	64.89	103.88	0.69	67.42	0.62	67.97	56.83
B10M10	2.01	64.63	100.95	0.69	67.03	0.62	67.70	57.73
B15M10	2.04	64.37	101.62	0.69	66.65	0.61	67.43	55.79
SEM	0.012	0.161	0.823	0.003	0.235	0.003	0.164	0.561
B	ns	ns	-	ns	-	Ns	-	-
M	ns	ns	-	ns	-	Ns	-	-
B*M	ns	ns	-	ns	-	Ns	-	-

DMI (dry matter intake); DMD (dry matter digestibility); RFV (relative feed value); NEL (net energy of lactation); TDN (total digestible nutrients). *Pennsylvania state equation; **New York state equation; ***Jayanegara et al (2019) equation. SEM (standard error of Mean); ns (nonsignificant).

Table 4. **Table of means for effects of biochar and manure addition on *in vitro* digestibility of *Pennisetum purpureu cv.* Mott.**

	B0M0	B5M0	B10M0	B15M0	B0M10	B5M10	B10M10	B15M10	SEM	B	M	B*M
Incubation time (h)												
3	6.25^{b}	5.55^{ab}	4.96^{a}	5.21^{ab}	6.22^{b}	6.39^{b}	5.93^{ab}	6.08^{ab}	0.151	ns	.02	ns
6	10.30^{ab}	9.58^{ab}	9.41^{a}	10.09^{ab}	11.10^{ab}	11.44^{b}	10.84^{ab}	11.00^{ab}	0.170	ns	.01	ns
9	15.36	15.30	14.12	14.63	16.48	17.00	16.10	15.77	0.322	ns	.03	ns
12	21.09	21.01	19.66	20.01	22.36	22.72	22.53	20.70	0.397	ns	.04	ns
24	36.78	39.85	37.98	36.98	36.82	40.39	39.80	37.78	0.626	ns	ns	ns
48	53.16^{ab}	56.49^{b}	55.45^{ab}	53.29^{ab}	49.52^{a}	57.38^{b}	55.54^{ab}	52.56^{ab}	0.763	.04	ns	ns
72	57.72^{ab}	63.22^{b}	58.98^{ab}	56.31^{ab}	51.86^{a}	62.41^{b}	60.11^{b}	56.51^{ab}	1.004	.02	ns	ns
Gas kinetics												
A	-2.02	-3.64	-4.36	-3.48	-2.65	-2.50	-3.25	-2.36	0.275	ns	ns	ns
B	65.10^{abc}	72.98^{c}	69.24^{bc}	64.76^{abc}	57.86^{a}	70.12^{bc}	67.70^{bc}	63.21^{ab}	1.201	.01	.09	ns
a+b	63.08^{ab}	69.34^{b}	64.88^{b}	61.28^{ab}	55.20^{a}	67.62^{b}	64.45^{b}	60.84^{ab}	1.143	.01	ns	ns
C	0.037	0.036	0.038	0.040	0.049	0.039	0.41	0.040	0.017	ns	ns	ns
Gas characteristics												
GPSF	27.93	24.45	21.53	22.80	27.81	28.65	26.34	27.08	0.748	ns	.02	ns
GPNSF	157.73	176.93	170.40	164.03	158.03	175.37	174.73	163.69	3.111	ns	ns	ns
Fermentation characteristics												
pH	6.86	6.85	6.87	6.91	6.86	6.92	6.92	6.90	0.011	ns	ns	ns
TVFA (mM)	51.67	58.33	50.00	56.67	98.33	51.67	55.00	51.67	5.579	ns	ns	ns
NH$_3$ (mg/100 ml)	23.33	23.33	24.17	23.42	23.33	23.42	19.75	21.00	0.513	ns	ns	ns
IVOMD (%)	60.65^{ab}	65.81^{b}	62.04^{ab}	60.38^{ab}	55.48^{a}	65.51^{b}	63.12^{b}	60.35^{ab}	0.908	.02	ns	ns
ME (kkal/kg DM)	3134.49^{ab}	3686.45^{b}	3423.08^{ab}	3538.11^{ab}	3059.87^{a}	3634.06^{b}	3554.73^{b}	3498.69^{ab}	81.792	.02	ns	ns
MP (g/kg IVOMD)	7316.04^{ab}	7938.42^{b}	7483.70^{ab}	7283.42^{ab}	6691.83^{a}	7901.62^{b}	7613.57^{b}	7280.22^{ab}	109.542	.02	ns	ns

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