

Effects of Fresh and Composted *Azolla* on Soil Chemical Properties

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ABSTRACT

The rise in chemical fertilizer use in Malaysia raises concerns about soil degradation and potential long-term yield reductions, highlighting the importance of using organic matter for soil restoration. *Azolla* has been extensively studied as an alternative soil amendment due to its high nitrogen and nutrient content, as well as its rapid growth. However, the effects of fresh and composted *Azolla* amendments on soil chemical properties are not yet fully understood. A soil incubation study was thus conducted to determine the effects of fresh and composted *Azolla* on soil chemical properties over a 3-month incubation period. The soil treatments consisted of non-amended soil (control); fresh *Azolla* at 3, 6, and 9% w/w; and composted *Azolla* at 1, 2, and 3% w/w, with soil water holding capacity maintained at 55% throughout the incubation period. The collected soil samples were analyzed for soil pH and electrical conductivity (EC), total carbon (C) and nitrogen (N), available phosphorus, exchangeable bases—potassium (K), calcium, and magnesium, using inductively coupled

plasma optical emission spectrometry, and cation exchange capacity (CEC). All data were subjected to variance analysis for statistical analysis. The study revealed significant effects of interaction between soil treatments and incubation periods for all soil parameters. At the end of the incubation period, the soil treated with 3% composted *Azolla* exhibited higher soil EC, total C and N, exchangeable K, and CEC compared to

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other soil treatments. The 3% fresh *Azolla* treatments were also observed to improve the soil's exchangeable calcium by the end of the incubation period. In conclusion, 3% composted *Azolla* is best to help restore soil nutrient levels for crop uptake.

Keywords: *Azolla microphylla*, clay soil, soil amendment, soil incubation, soil nutrients

INTRODUCTION

Soil is a crucial natural resource that is vital for plant growth. It serves as a growth medium, supporting the plant's root system while simultaneously providing essential nutrients and moisture (Laruna et al., 2020). However, increasing soil degradation and agricultural waste outputs have become serious global challenges. Commonly, repetitive and unbalanced fertilizer applications promote organic matter mineralization and lead to a decline in overall soil fertility, such as decreased soil carbon reserves and increased soil acidity (B. Singh, 2018; Karam et al., 2021). Increasing environmental concerns related to the excessive use of chemical fertilizers highlight the need for thorough research into potential sustainable methods to address the associated risks.

Various soil amendments, including mineral, organic, and synthetic, are employed to improve soil fertility for crop growth. Long-term application of soil amendments improves many soil variables, including soil texture, organic C, nutrient availability, crop growth and its environment, and microbes that are useful for crop production,

compared to chemical fertilizers (V. K. Singh et al., 2022). Organic amendments, such as compost or green manure, are common amendments for boosting soil performance and crop productivity, where these amendments have low production costs (Trupiano et al., 2017; V. K. Singh et al., 2022). For instance, amendments on clay soil can be advantageous as clay's greater surface area and chemical bonding capacity make it an ideal site for the formation of macro- and micro-aggregates, preserving the organic matter (Bronick & Lal, 2005; Ge et al., 2019; Oades, 1988). Hence, the increasing production of eco-friendly amendments has made *Azolla* a viable option for enhancing clayey soil properties (Marzouk et al., 2023).

Farmers in certain limited areas of China and Vietnam have been using *Azolla* for centuries. These countries began conducting research to expand the utilization of *Azolla* in crop production as far back as the early sixties (van Hove & Lejeune, 1996). Indonesia has also studied the *Azolla* amendment extensively due to its high nitrogen (N) content and rapid growth (Setiawati, Damayani, et al., 2018; Widiastuti et al., 2018). In Asia, it is commonly grown as an intercrop in lowland paddy fields or as a pre-season crop before planting (Thapa & Poudel, 2021). Further, according to the Malaysian Agricultural Research and Development Institute (MARDI), local farmers have integrated *Azolla* into paddy fields (Shafiee et al., 2021). However, the utilization of the *Azolla* amendment in Malaysia, particularly

in the state of Sabah on Borneo Island in East Malaysia, remains an area that has not yet been thoroughly studied.

Despite its apparent beneficial qualities, *Azolla* is considered one of Europe's most harmful invasive aquatic plants (Pinero-Rodríguez et al., 2021). Djojokuswito (2000) stated that spreading about 500 kg of *Azolla* seed per ha in a paddy field led to *Azolla* biomass increasing to 20,000 kg/ha within 2 weeks, indicating its rapid multiplication potential. Regular harvesting of the *Azolla* biomass for conversion into alternative soil amendments, such as compost, can help prevent the formation of such dense mats in paddy fields or other areas where it is cultivated. Interestingly, *Azolla* can be used in several forms, such as extracts, compost, green manure, and biochar.

Generally, in a day, *Azolla* fixes 75 mg N/g per dry weight and yields fresh weight of approximately 347 tonnes/ha in a year with about 868 kg N content, which is equivalent to 1,900 kg of urea (Yadav et al., 2014). *Azolla* is reported to be able to supply approximately 35–50% of fixed N to paddy fields, rendering it a perfect organic fertilizer for other crops as well, such as leafy vegetables, either in fresh or composted form (Pereira, 2018; Setiawati, Damayani, et al., 2018). Further, the study by Lestari et al. (2019) demonstrated that applying *Azolla* compost increased mustard green yield by improving the soil properties. The introduction of *Azolla* into the soil also improves other soil chemical properties such as CEC, exchangeable bases, and acidity, hence efficiently improving the nutrient

uptake by crops (Barus et al., 2018; Sanjay-Swami & Singh, 2019b; Setiawati, Suryatmana, et al., 2018). Hence, *Azolla* is a viable alternative to reduce reliance on synthetic fertilizers in agriculture.

While the agronomic potential of using *the Azolla* amendment has been widely proposed, the effects of a single application of the *Azolla* amendment (i.e., fresh and composted *Azolla*) alone on soil properties over time have yet to be thoroughly studied. Thus, this study was conducted to determine the effects of fresh and composted *Azolla* on soil chemical properties over a three-month incubation period.

MATERIALS AND METHODS

Composted *Azolla* Preparation

A soil incubation study under field conditions was conducted in an insect-proof net house at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. Fresh *Azolla* harvested from the insect-proof net house was cleaned and washed before being sundried for a few days prior to composting. Composted *Azolla* was prepared according to the method suggested by Jumadi et al. (2014), with some modifications to fit the study conditions. For the compost-making, a mixture of dried and fresh *Azolla* biomass and molasses was used at 9:6:2 of total weight, respectively. The mixture was placed in a black bin with dimensions of 40 cm diameter and 50 cm height (45 L). Water was added throughout the composting process to maintain the moisture content at 50–60% of the total weight. The composting process spanned 14 days, with the compost

undergoing the mesophilic stage for 3 days, the thermophilic stage for 5 days, and the curing phase for 6 days (Figure 1).

The matured compost was ready for harvest once the temperature was found to remain constant even after turning (30–34°C), the color changed to dark brown, easily crumbled, the pH was almost neutral, and EC was below 4 dS/m (Bernai et al., 1998; El-mrini et al., 2022; Kalamdhad & Kazmi, 2009; Khalib et al., 2020). The fresh and composted *Azolla* were characterized by pH, EC, C, N, C/N ratio, phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) content before applying it to the soil. The pH and EC readings were taken with a pH/EC meter (PC 2700, Eutech Instruments, Singapore). The C and N of the *Azolla* biomass were determined with a CHN elemental analyzer (FP628, LECO Corporation, USA). Nutrient content (P, K, Ca, and Mg) of the fresh and composted *Azolla* was determined using the dry ashing method suggested by the AOAC

International (2002), where the filtrate was analyzed using an inductively coupled plasma optical emission spectrometry (ICP-OES) instrument (Optima 2000DV, Perkin Elmer, USA).

Soil Incubation: Experimental Design, Treatments, Laboratory Tests, and Statistical Analysis

This completely randomized design soil incubation study was carried out using plastic containers (20 cm diameter × 9.5 cm height), each filled with 2.5 kg of Typic Paleudults soil (Silabukan series). *Azolla* was applied to the soil as an amendment in the form of fresh biomass or compost at different rates at the beginning of the study, as shown in Table 1. Each soil treatment was replicated five times. At the beginning of the experiment, about 500 ml of deionized water was added to the soil. The soil’s water-holding capacity was maintained at 55% using a soil sensor reader (WaterScout SMEC 300, Spectrum Technologies Inc.,

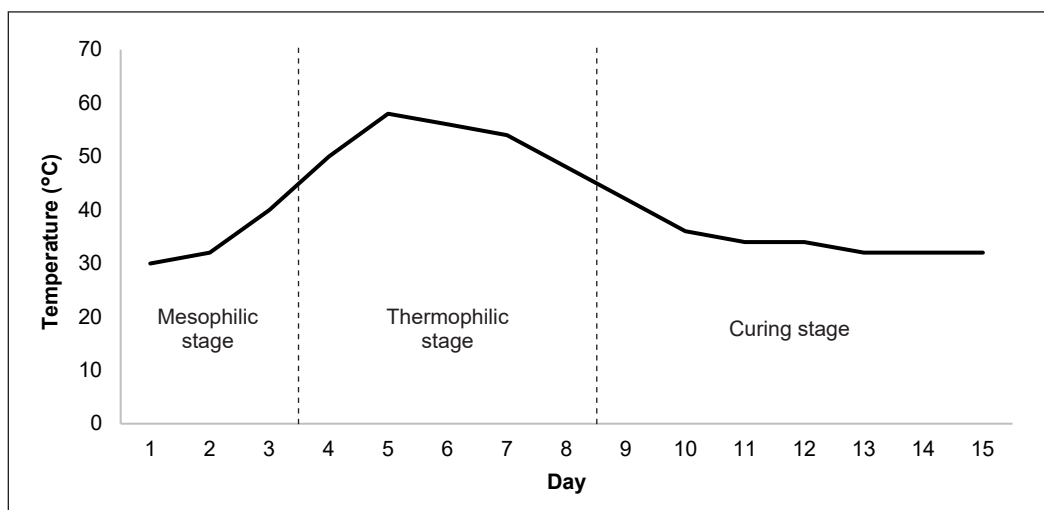


Figure 1. Temperature variation over a composting period

USA) throughout the incubation period by adding deionized water (Jumadi et al., 2014). Soil samples were collected at the beginning of the experiment and every one-month interval for up to three months. At the beginning of the study, the soil texture was determined using the hydrometer method suggested by D. Sarkar and Haldar (2010). The percentage of silt, clay, and sand was calculated, and the results were used to determine the textural class of the soil using the International Society of Soil Science (ISSS) textural triangle. The soil samples were analyzed for pH, EC, total C and N, available P, and exchangeable bases — K, Ca, Mg, and CEC. The pH and EC readings were measured using a pH/EC meter (PC 2700, Eutech Instruments, Singapore). The total C and N were determined using a CHN elemental analyzer (FP628, LECO Corporation, USA); soil available P was determined by mixing with concentrated hydrochloric acid (HCl) (System, Malaysia) and sulphuric acid (System, Malaysia) (Bray & Kurtz, 1945); then, the soil filtrate was mixed with Reagent B and analyzed by UV-Vis spectrophotometer (Genesys 10S, Thermo Scientific, USA) at 882 nanometer.

The soil exchangeable bases were determined by extracting the soil with 1 N ammonium acetate (Merck, Germany) (Soil Survey Staff, 2014). The extraction was then measured for the exchangeable bases using an ICP-OES instrument. The procedures for soil exchangeable bases determination were continued to determine the soil CEC. Ethanol (System, Malaysia) was added to a leaching tube with 0.05 M

potassium sulfate (Merck, Germany) before extracting the soil. The resulting extraction was mixed with sodium hydroxide (Merck, Germany) in a distillation glass, with boric acid (Merck, Germany) used as an indicator in a conical flask. The distillation process was carried out using a unit (K350, Büchi, Switzerland), lasting 4 min (with a color change from purple to green). The green solution was then titrated with 0.01 N HCl for neutralization, changing the color to purple. The amount of HCl used was recorded. The Statistical Analysis System (SAS) software (version 9.4) was used for all data analysis. Where significant interaction effects between the factors were observed, the simple effects of the incubation period on the measured variables were determined for each soil treatment using analysis of variance (ANOVA) and mean comparison by the least significant difference test (LSD) at a 95% confidence level.

RESULTS

There were significant differences between fresh and composted *Azolla* for the pH, EC, C, C/N, P, K, and Ca content but not for the N and Mg content (Table 2). Due to the addition of molasses, the composted *Azolla* had higher P, K, and Ca contents than the fresh *Azolla*. The soil texture was typical clay (clay: 74.96%, silt: 21.04%, sand: 4.00%). The initial soil pH was 6.93, placed within the slightly acidic to neutral range, with an EC of 0.12 dS/m. Meanwhile, the total C and N content were 2.75 and 0.66%, respectively. The soil contained 6.23 mg/kg available P, with exchangeable K, Ca, and

Mg values of 1.04, 9.31, and 9.22 cmolc/kg, respectively. Further, the initial CEC of the soil was reported to be 20.52 cmolc/kg. The soil amendment (SA) and incubation period (IP) affected the soil parameters significantly, as shown in Table 3.

Table 1
Azolla amendment treatments on soil

Treatment	Rate of application (%)	Weight of Azolla amendment (g/container)
Non-amended (control)	0	0
Fresh Azolla	3	75
Fresh Azolla	6	150
Fresh Azolla	9	225
Composted Azolla	1	25
Composted Azolla	2	50
Composted Azolla	3	75

Table 2
Chemical properties of fresh and composted Azolla

Parameter	Fresh Azolla	Composted Azolla	t-test
pH	6.75	7.67	***
EC (dS/m)	3.31	3.02	**
C (%)	38.34	35.35	***
N (%)	4.28	4.48	ns
C/N ratio	9.19	7.92	*
P (mg/L)	44.08	68.20	*
K (mg/L)	366.60	468.70	***
Ca (mg/L)	110.70	171.20	**
Mg (mg/L)	69.65	76.32	ns

Note. * Significant at $P \leq 0.05$ probability level; ** Significant at $P \leq 0.01$ probability level; *** Significant at $P \leq 0.001$; ns = Not significant

Table 3
Summary of main and interaction effects of soil amendments on soil properties throughout the incubation period

Factor	pH	EC (dS/m)	Total (%)		Available P (mg/kg)	Exchangeable (cmolc/kg)			CEC (cmolc/kg)
			C	N		K	Ca	Mg	
Soil amendment	***	***	***	***	***	***	***	***	***
Incubation period	***	***	**	***	**	*	***	***	***
Soil amendment × Incubation period	***	***	***	***	***	***	***	***	***

Note. * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$; EC = Electrical conductivity; CEC = Cation exchange capacity

pH

As shown in Figure 2, all soil treatments showed a gradual reduction in soil pH from month 1 to month 3, except for the 1% composted *Azolla* treatment, which showed increased pH. At the end of the IP, the control resulted in significantly lower soil pH than the other soil treatments, except for the 3% fresh *Azolla* and 3% composted *Azolla* treatments.

Electrical Conductivity

Figure 3 shows a continuous increase in soil EC during the IP for the 3% composted

Azolla, which registered the highest soil EC compared to the control and other treatments at the end of the IP. The soil treated with 3, 6, and 9% fresh *Azolla* showed no significant differences throughout the IP.

Total Carbon

Figure 4 shows no significant differences between the fresh *Azolla* treatments for soil total C at the end of the IP. However, the soil treated with 3% composted *Azolla* showed the highest total C throughout the IP. At the end of the IP, 3% composted *Azolla* resulted in the highest soil total C, followed

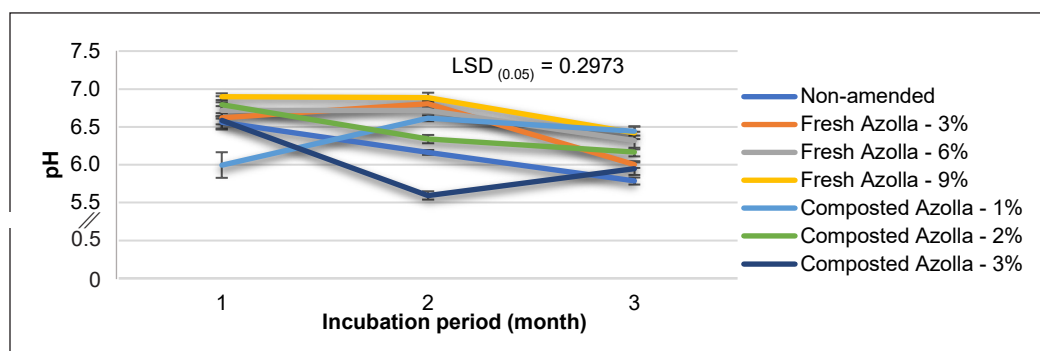


Figure 2. Interaction effects of soil amendments on soil pH during the incubation period
 Note. Error bars represent the standard errors of the means

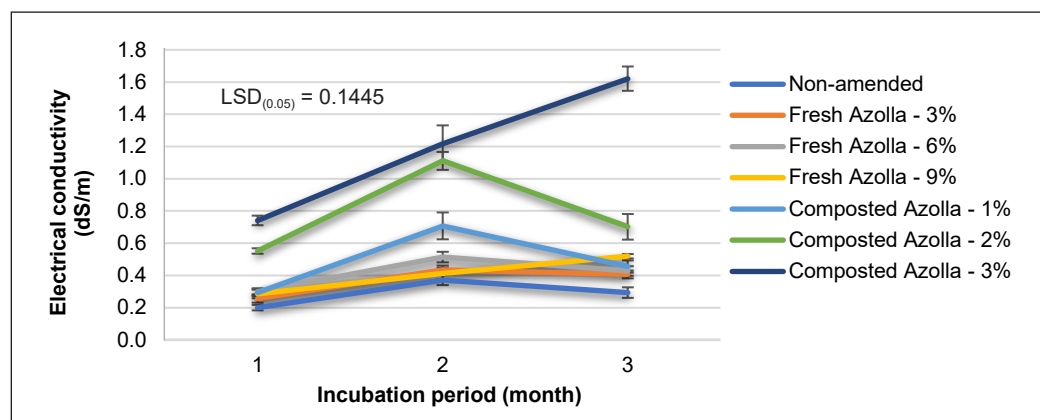


Figure 3. Interaction effects of soil amendments on soil electrical conductivity during the incubation period
 Note. Error bars represent the standard errors of the means

by the control and 2% composted *Azolla* treatments.

Total Nitrogen

Figure 5 shows that although there was a reduction in the total N of soil for all soil treatments at the end of the IP, the values were still above the initial total N values. The soil total N for the control and all soil treatments was reported to increase from the first month to the second month but decreased significantly in the third month

of the IP. The 9% fresh *Azolla* treatment showed the highest total N compared to the other soil treatments in the second month of the IP. At the end of the IP, the soil treated with 3% composted *Azolla* showed significantly higher total N than the control and other soil treatments.

Available Phosphorus

Figure 6 shows no significant differences between treatments for soil available P, except for the soil treated with 3% fresh

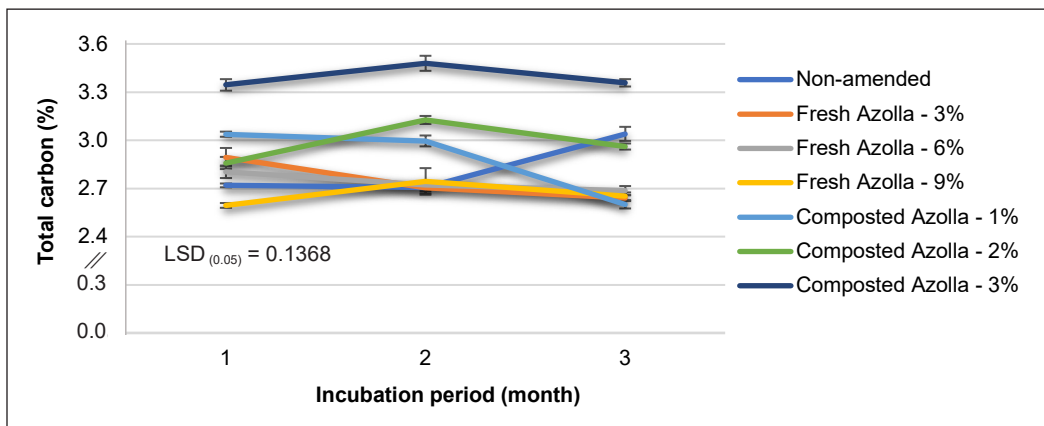


Figure 4. Interaction effects of soil amendments on soil total carbon during the incubation period

Note. Error bars represent the standard errors of the means

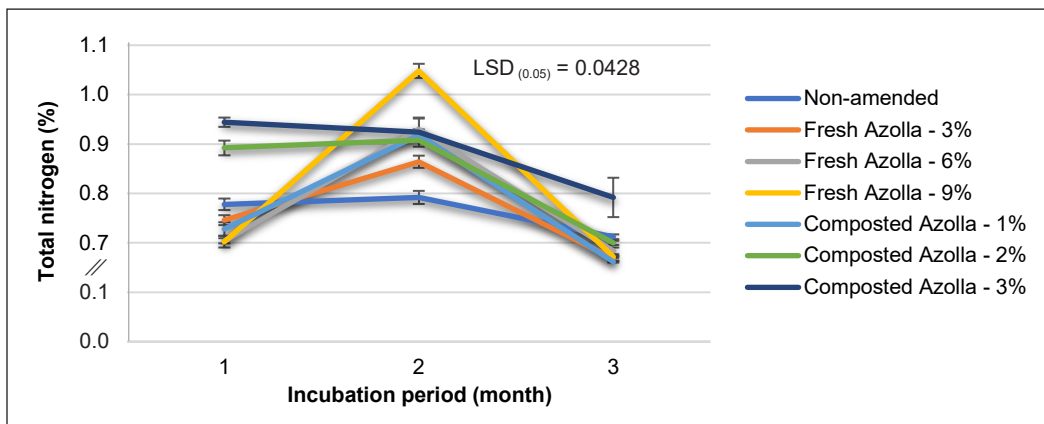


Figure 5. Interaction effects of soil amendments on soil total nitrogen during the incubation period

Note. Error bars represent the standard errors of the means

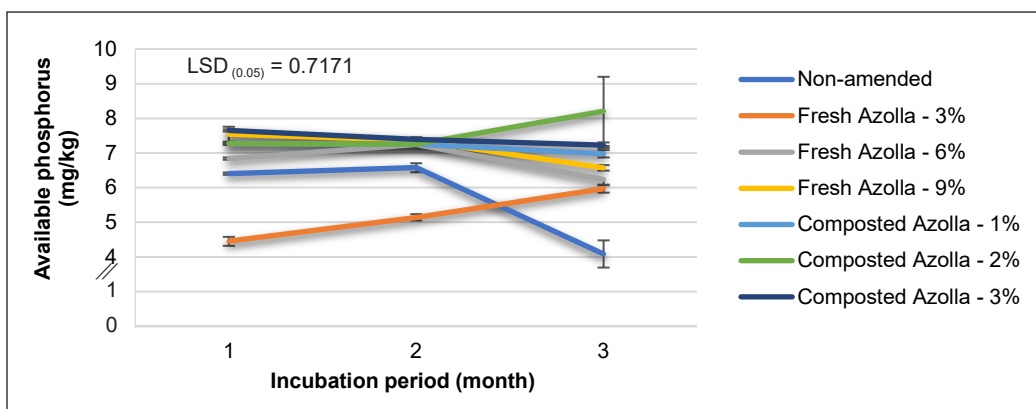


Figure 6. Interaction effects of soil amendments on soil available phosphorus during the incubation period
 Note. Error bars represent the standard errors of the means

Azolla in the first and second months of the IP. At the end of the IP, the soil treated with 2% composted *Azolla* showed the highest available P compared to the control and other soil treatments, with an increasing trend observed throughout the IP.

Exchangeable Potassium

As shown in Figure 7, during the first two months of the IP, the 3 and 6% fresh *Azolla* treatments showed no significant difference in exchangeable K as compared to the control, while the 1, 2, and 3% composted *Azolla* treatments showed the opposite. At the end of the IP, the 3% composted *Azolla* treatment resulted in significantly higher soil exchangeable K than the control, followed by the 2% composted *Azolla* treatment.

Exchangeable Calcium

As seen in Figure 8, the control and all the *Azolla* treatments, except for the 3% fresh *Azolla* and 1% composted *Azolla* treatments, reduced exchangeable Ca throughout the IP. At the end of the IP, the 3% fresh *Azolla*

treatment exhibited significantly higher exchangeable Ca than the non-amended soil, with an increasing trend throughout the IP, followed by the 9% fresh *Azolla* and 3% composted *Azolla* treatments.

Exchangeable Magnesium

Figure 9 shows that exchangeable Mg for the fresh *Azolla* and 1% composted *Azolla* treatments was significantly reduced throughout the IP. In the third month of the IP, the non-amended soil had the highest exchangeable Mg compared to the other soil treatments. Further, the 2% and 3% composted *Azolla* treatments resulted in higher exchangeable Mg than the fresh *Azolla* treatments by the end of the IP.

Cation Exchange Capacity

Figure 10 shows that in all soil treatments, except for the control, 6% fresh *Azolla* and 2% composted *Azolla*, the CEC increased from month 1 to month 3. At the end of the IP, however, the soil treated with 3% composted *Azolla* resulted in significantly

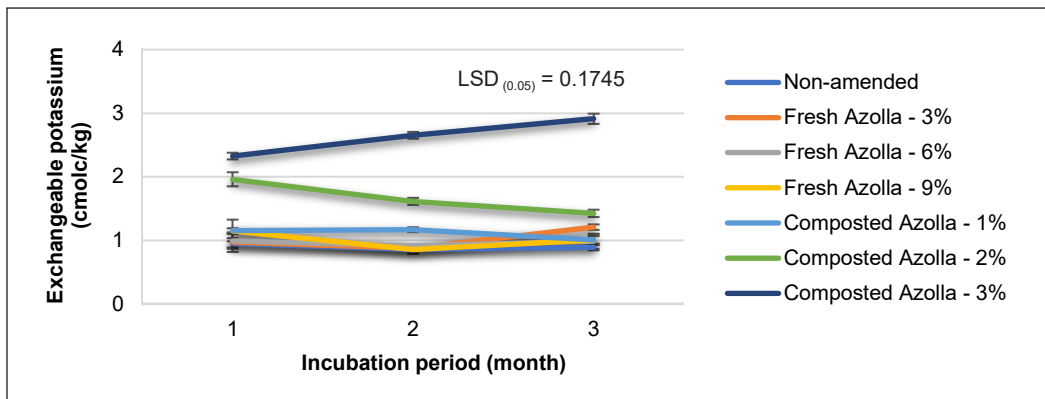


Figure 7. Interaction effects of soil amendments on soil exchangeable potassium during the incubation period
 Note. Error bars represent the standard errors of the means

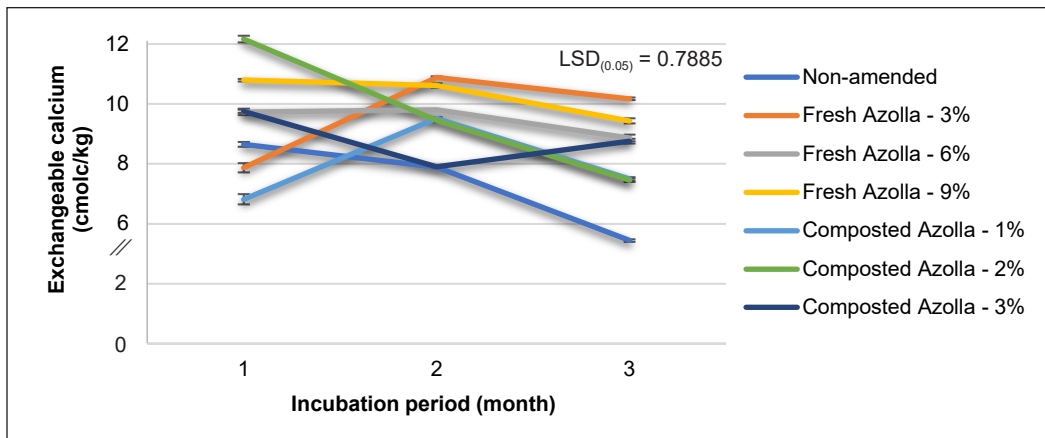


Figure 8. Interaction effects of soil amendments on soil exchangeable calcium during the incubation period
 Note. Error bars represent the standard errors of the means

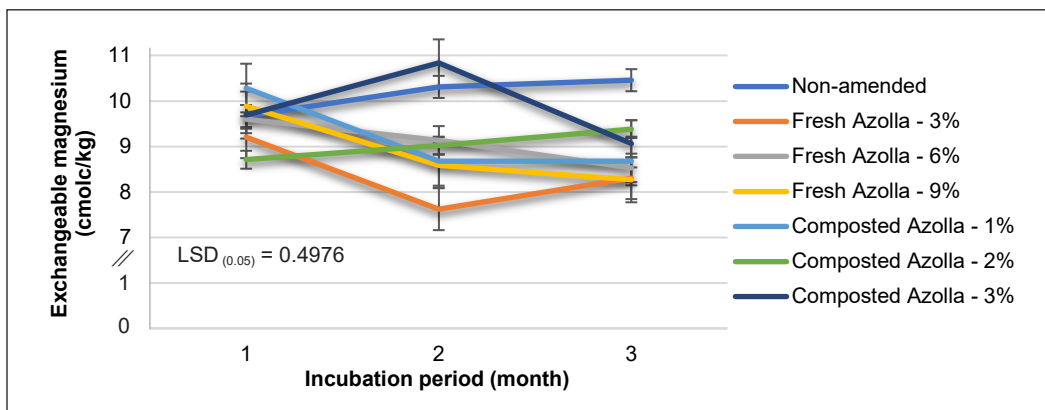


Figure 9. Interaction effects of soil amendments on soil exchangeable magnesium during the incubation period
 Note. Error bars represent the standard errors of the means

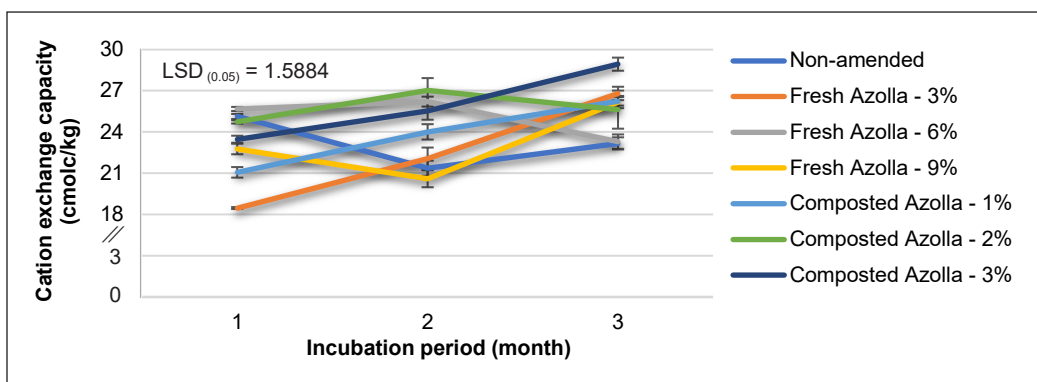


Figure 10. Interaction effects of soil amendments on soil cation exchange capacity during the incubation period
 Note. Error bars represent the standard errors of the means

higher CEC, with a range of 7.36 to 19.46%, compared to the control and other soil treatments.

DISCUSSION

Commonly, soil amendments promote plant growth and development in farming by supplying organic and inorganic nutrients to the soil and enhancing soil organic matter and water-holding capacity (Clements & Bihn, 2019). High organic matter content improves soil chemical properties, suppressing the solubility of aluminum (Al) and iron (Fe) in the soil through the process of complexation, pH buffering, precipitation, and competitive sorption (B. Sarkar et al., 2018; Ifansyah, 2013; Sung et al., 2017). Further, soil's physical and chemical characteristics are also influenced by clay minerals, the most reactive particles in soil. Clay minerals have abundant specific surface area and a net negative surface charge, allowing them to bond with and chemically stabilize organic matter (B. Sarkar et al., 2018). Generally, C components from organic matter adsorb

onto clay minerals through mechanisms such as electrostatic attraction, hydrophobic attraction, ligand exchange, and π -bonding, protecting it from microbial decomposition (Baldock & Skjemstad, 2000; M. Singh et al., 2018). Consequently, applying compost to soils with high clay content will likely enhance C stabilization (Bolan et al., 2012). It explains the improvements in the clay soil's properties in this study.

Changes in soil pH are influenced by the release of basic cations such as Ca, Mg, K, and Na from weathered minerals, which leaves hydrogen (H^+) and Al ions as the dominant exchangeable cations; humic residues from the humification of soil organic matter, that results in large numbers of carboxyl and phenolic groups, which break down to release H^+ ions, nitrification of ammonium to nitrate resulting in the presence of H^+ ions, and the elimination of N in plant and animal products (Adeleke et al., 2017; Hong et al., 2019; White, 2005). Further, soil nutrients' solubility and availability greatly influence the soil's EC. The increase in soil EC throughout the

IP with the composted *Azolla* treatments was due to higher nutrient release from the composted organic matter than the fresh *Azolla* treatments. It resulted in more salts and ions in the soil and liquid phase, influencing soil EC over time (do Carmo et al., 2016). Thus, the higher the rates of organic matter applied, the higher the number of salts and ions released into the soil. As Iacomino et al. (2022) reported, soil EC can increase over time through compost applications alone.

Soil C and N are negatively associated with soil pH, indicating that a low pH promotes organic matter accumulation (Zhou et al., 2019). It explains the increase of total C and N and the associated decrease in pH of soil treated with *Azolla* biomass over the first two months of the IP. The soil total C and N for the 3% composted *Azolla* treatment was the highest at the end of the IP. These results are in line with Bharali et al. (2021), Benny et al. (2020), Novair et al. (2020), and Setiawati, Suryatmana, et al. (2018), where the incorporation of *Azolla* biomass, fresh or composted, increased soil total C and N compared to when there was no *Azolla* biomass applied. The *Azolla* biomass's high C and N content increases soil total C and N upon decomposition (Benny et al., 2020). Commonly, after four weeks of *Azolla* incorporation into the soil, 50% of the *Azolla* decomposes, releasing organic matter and nutrients into the soil (Ventura et al., 1992). Furthermore, soil C is positively correlated to soil N (Zhou et al., 2019). It explains the parallel increase of total C and N values in the second month

of the soil IP. During the mineralization of organic matter, there is a constant and slow release of the fixed N stored in the *Azolla* leaves into the soil by the associated cyanobacteria (Seleiman et al., 2022).

On the other hand, the decrease in soil total N for all treatments at the end of the incubation period could be attributed to N losses through ammonia volatilization, denitrification or absorption of N by the moss plants that grew on the soil (Laruna et al., 2020).

As illustrated in Figure 6, at the end of the IP, the 2% composted *Azolla* treatment resulted in the highest amount of soil available P compared to the other soil treatments. Similar results were obtained by Sanjay-Swami and Singh (2019b) and Setiawati, Damayani, et al. (2018), who reported that *Azolla* biomass significantly increased soil P. The lower soil available P resulting from treatment with fresh *Azolla* may be related to the lower proliferation of microorganisms during the early stages of degradation and the potential immobilization of P by these microorganisms (Muktamar et al., 2020). Additionally, the *Azolla* biomass raised the value of P desorbed due to a primary reaction between the *Azolla* biomass and the soil, known as the acid neutralization reaction. This reaction lowers P fixation in acid soils and also encourages optimal nutrient utilization through timely nutrient delivery for maximum crop output (Johan et al., 2022).

Some soil chemical properties, such as the level of nutrients (N, P, K, Ca, Mg), show changes within a short period (<3

years) with all organic matter applications (Bhogal et al., 2018). Throughout the IP, the exchangeable K and Ca values of soil treated with 2 and 3% composted *Azolla* were higher than those for the fresh *Azolla* treatments. Further, soil treated with 3 and 9% fresh *Azolla* had higher exchangeable Ca compared to the other treatments at the end of the IP. Similar results were obtained by Rani et al. (2020), where *Azolla* biomass resulted in increased exchangeable K of about 50.91% from day 0 to day 35 of incubation and 3.31 and 4.05% from day 35 to 70 and day 70 to 105 of incubation, respectively. Moreover, Emam et al. (2022) stated that *Azolla* biomass organic amendment also increased the soil Ca and Mg levels.

Azolla biomass can supply nutrients as it has high N content and other minerals such as Ca, P, K, and Mg (Setiawati, Damayani, et al., 2018). It could be due to the decomposition of the fern, along with the effects on pH caused by the incorporation of *Azolla* biomass, which improved the solubility of these elements (Bhuvaneshwari & Singh, 2015). A further accelerated decrease in pH caused a reduction in the availability of exchangeable Ca and Mg. Throughout the IP, the soil pH range was 5.6 to 6.9, whereby the most abundant amount of Ca and Mg is found at pH <7.2. Additionally, the variations in soil exchangeable Mg and Ca concentrations were due to the soil exchangeable Al content (Miyazawa et al., 2001). The improved soil physical properties enhanced the mineralization of organic matter by the microorganisms in the soil pool, which

possibly increased the exchangeable bases in the soil (Sanjay-Swami & Singh, 2019a, 2019b).

The soil treated with 3% composted *Azolla* showed the highest CEC compared to the other treatments at the end of the IP. This result is comparable with that obtained by Rani et al. (2020) and Sanjay-Swami and Singh (2019b). Changes in soil CEC are closely related to fluctuations in soil C content and the formation of negative charges in the soil organic matter and humified chemicals present in organic matter. Furthermore, clay soil is good at storing nutrients due to its high CEC (Pal & Marschner, 2016).

CONCLUSION

Organic matter is crucial for restoring soil fertility and allaying concerns about soil damage from excessive chemical fertilizer use. The application of composted *Azolla*, especially 3% composted *Azolla*, improved the soil's chemical properties, which was most noticeable with soil EC, total C and N, exchangeable K, and CEC, compared to that of the non-amended soil and other *Azolla* amendments. Further, despite increasing application rates, fresh *Azolla* showed no substantial improvement in soil chemical properties, except for soil exchangeable Ca at the end of the IP. Based on the results from this experiment, composted *Azolla* helped the soil recover appropriate nutrient levels with only a single application over a 3-month incubation period. In future studies, it is recommended to consider other relevant soil analyses, such as soil bulk

density, to assess the effects of the applied *Azolla* amendments on the compactness of clay soils. Understanding how soil quality improvements affect crop growth is also essential in enhancing the reliability of the findings.

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