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# Effects of Black Pepper-Groundnut Intercropping on Soil Total Nitrogen and the Physiological Characteristics of Black Pepper (*Piper nigrum* L.)

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## ABSTRACT

The utilisation of leguminous groundnut (*Arachis hypogaea*) has been recognised for its advantageous effects, including nitrogen (N) fixation, which consequently enhances the soil N availability. The objective of this study was to analyse the impact of a mixed intercropping system involving pepper (*Piper nigrum*) and groundnut on soil total N. Additionally, the study aimed to assess the influence of N on various parameters, including the Normalised Difference Vegetation Index (NDVI), chlorophyll content, and gas exchange rate of black pepper. The study employed a randomized complete block design in a field experiment, with two treatments that were reproduced 15 times. The treatments consisted of the control group (M0), which involved a pepper plot without groundnut intercropping, and the mixed intercropping system (M1), which involved a pepper plot with groundnut intercropping. The soil total N content exhibited a statistically significant increase ($p < 0.05$) in the context of a mixed intercropping system. The intercropping treatment resulted in considerably higher values ($p < 0.05$) for pepper NDVI, foliar chlorophyll concentration, photosynthetic rates (*A*), stomatal conductance (*gs*), and transpiration (*E*) compared to the control treatment. Moreover, a positive correlation has been seen between the chlorophyll content of pepper leaves and the total N content in the soil. Similarly, a positive association has been established between the *A* of pepper plants and the total N content in the soil. As a result, the observed elevation in *A* under high N circumstances was ascribed to an augmentation in chlorophyll concentration. Consequently, the integration of groundnut within pepper cultivation systems over an extended period has the potential to enhance crop productivity through the amelioration of soil conditions.

**Keywords**: *Arachis hypogaea*, chlorophyll, intercropping, *Piper nigrum*, soil nitrogen.

## INTRODUCTION

*Piper nigrum* Linn. also known as pepper, is a widely recognised condiment that holds significant prominence in various culinary traditions across the globe. It is worth noting that pepper holds particular significance in terms of its economic importance (Shanmugapriya et al., 2012). The cultivation of pepper in the Sarawak state of Malaysia can be traced back to the year 1856, with more widespread planting initiatives commencing in the 1900s. In 2021, the state of Sarawak exported a total of 7,407 tonnes of pepper, which had an estimated value of RM154 million. This data underscores the significance of pepper as a crucial cash crop that sustains the livelihoods of approximately 36,000 rural inhabitants residing in upland regions of Sarawak (Malaysian Pepper Board, 2023).

The groundnut plant, *Arachis hypogaea* L., is a crop of significant economic importance due to its high value. It serves as a valuable source of protein and cooking oil, as highlighted by Davis and Dean

(2016). Additionally, peanut plants could fix nitrogen (N) from the atmosphere, and the residues left behind by peanut harvests can serve as a source of N for other intercropping crops. Soil N is widely recognised for its role in influencing the green coloration of leaves. In soil environments with ample N availability, there is a notable enhancement in leaf growth and development. This is particularly evident in the creation of chloroplasts and the accumulation of chlorophyll within these cellular structures (Khaliq et al., 2016).

Integration of economic crop such as groundnut with pepper may optimise land utilisation and provide additional income for the growers. Previously, the documentation on groundnut intercropping in pepper farms in Sarawak was still lacking, which needs attention if this practice were to be undertaken seriously. Hence, it is imperative to further study the appropriateness of the groundnut-pepper intercropping system in Malaysia, specifically focusing on soil enhancement and the physiological properties of pepper. Therefore, this study was conducted with the objectives to investigate soil total N in a pepper-groundnut intercropping system, to investigate the normalized difference vegetation index (NDVI), chlorophyll concentration and gas exchange properties of pepper grown in a groundnut intercropping system, and to examine the relationship of soil total N on the NDVI and chlorophyll concentration of pepper.

## MATERIALS AND METHODS

### Experimental Site

A field experiment was conducted on a 485.6 $m^2$ plot located at the Bengoh Resettlement Scheme, Padawan, Sarawak, Malaysia (1°19’42” N, 110°14’39” E). The soil series at the study location was the Bemang Series. The Bemang series is an alluvial soil composed of fine loamy/silty, siliceous/mixed, acidic, isohyperthermic, *Typic Dystruptes*, and alluvium from sedimentary rocks (Paramanathan, 2020). The soil profile is generally friable and somewhat well-drained. The climate is tropical and moderately hot, with an average annual rainfall of 3500 - 4000 mm. The temperature in Padawan ranges from 20 to 36 °C, with an average of 23 °C in the early morning and rising to 32 °C by mid-afternoon (Sarawak Tourism Board, 2024). At the planting site, the permeability condition of the sandy clay loam soil ranges from low to moderate.

Pepper and groundnut were the crops examined in this study. Cuttings of pepper of the Kuching variety were rooted on a sandy area. The pepper cuttings were then moved to the planting location after 4 weeks. To prevent notable fluctuations in the data, only vines that were planted on level terrain with a gradual slope of no more than 10° steepness were chosen. In this study, pepper vines that were grown according to standard farm practices recommended by the Malaysian Pepper Board (MPB) were used.

For a duration of 1 week, groundnut seeds were sown (immersed in water for 2 days, then moved to potting media until day 7). Next, in a mixed intercropping system, the groundnut seeds were sown in between the pepper vine inter-rows. The planting depth was 5 cm, and the planting hole distance was 15 cm for groundnuts. The study was carried out from January 2023 to July 2023.

### Experimental Design and Treatments

Two treatments were replicated 15 times in a randomized complete block design (RCBD) experiment. The two treatments were pepper plot without groundnut intercropping (control; M0) and pepper plot with groundnut intercropping (mixed intercropping system; M1).

### Soil Total N Determination

At 4 months after the initiation of treatment, the soil samples were collected with an auger at a depth of 0 – 15 cm and analysed for total N using the Kjeldahl method (Edwards, 2010).

### Pepper NDVI, Chlorophyll, and Gas Exchange Rate Measurements

Measurements were undertaken on pepper leaves to determine the normalised difference vegetation index (NDVI), foliar chlorophyll concentration, net photosynthesis rate ($A$), leaf stomatal conductance ($gs$), and transpiration rate ($E$). The methods employed for these measurements were based on the approach described by DiCristina and Germino (2006).

### Statistical Analysis

Data were analysed statistically by independent t-test to detect treatment effect. The statistical software used was the Statistical Package for Social Science software (SPSS version 15, IBM, Armonk, New York, USA) The relationship of pepper leaf chlorophyll content – soil total N and pepper leaf $A$ – soil total N were determined using trend analysis.

## RESULTS AND DISCUSSION

### Soil Total N

Table 1 presents the impact of groundnut intercropping on the total N content in the soil. The observed elevation in soil total N content suggested that it was likely attributable to the assimilation of atmospheric nitrogen gas ($N_2$) by N-fixing bacteria residing in the root nodules of groundnut, resulting in its deposition into the soil. This result was similar to a study by Tang et al. (2020) who studied that groundnut intercropping raises the amount of rhizospheric microorganisms in the soil, which then increases the amount of N that is accessible for use. According to Ngome et al. (2011), the introduction of a legume cover crop into the soil typically results in a significant mineralization of N, wherein organic forms of N are converted into plant-accessible forms within a relatively short period. Indicators such as soil N play a significant role in assessing the productivity and sustainability of an agricultural production system (Kifuko et al., 2007). Furthermore, Witcombe and Tiemann (2022) observed that N-fixing bacteria and soil N stocks provided the groundnuts with N, which may have enhanced yields and food output.

Table 1. Effect of groundnut intercropping on Total N in the soil

| Treatment | Total N (% oven dry) |
|---|---|
| M0 | $0.18 \pm 0.01^{b}$ |
| M1 | $0.50 \pm 0.01^{a}$ |

Note: Means with different alphabets within column indicate significant difference between treatments by independent t-test at $p < 0.05$. Treatments are M0 – control and M1 – mixed intercropping system

### Pepper NDVI, Chlorophyll, and Gas Exchange Rate

According to the normalized difference vegetation index (NDVI) results presented in Table 2, it can be observed that the NDVI value in M1 was greater than for M0. This disparity may be attributed to a greater abundance of N in the soil. Cechin and Fumis (2004) conducted both field and laboratory research, which revealed that augmenting the supply of N in soil might lead to an increase in leaf greenness, thereby resulting in higher NDVI values. Moreover, Tucker (2004) postulated that the NDVI is contingent upon the N concentration present in the soil. A study by Farias et al. (2023) reported that the foliar NDVI are a good indicator to determine the effect of high soil total N in plants. Farias et al. (2023) added that with high N availability in soil, plants will generally demonstrate vigorous and greener foliar growth.

Table 2. Effect of pepper-groundnut mixed intercropping system on pepper NDVI, foliar chlorophyll concentration and selected gas exchange properties

| Treatment | NDVI | Chlorophyll concentration (µmol per $m^2$ of leaf) | *A* (µmol $CO_2$ $m^{-2}s^{-1}$) | *gs* (mol $H_2O$ $m^{-2}s^{-1}$) | *E* (mmol $H_2O$ $m^{-2}s^{-1}$) |
|---|---|---|---|---|---|
| M0 | $0.79 \pm 0.02^b$ | $315.16 \pm 14.21^b$ | $7.84 \pm 0.08^b$ | $0.10 \pm 0.01^b$ | $2.75 \pm 0.1^b$ |
| M1 | $0.92 \pm 0.01^a$ | $578.11 \pm 32.25^a$ | $8.54 \pm 0.13^a$ | $0.13 \pm 0.01^a$ | $3.26 \pm 0.17^a$ |

Means with different alphabets within column indicate significant difference between treatments by independent t-test at $p < 0.05$. Treatments are M0 – control and M1 – mixed intercropping system. The physiological properties of pepper are NDVI – normalized difference vegetation index, *A* – net photosynthesis rate, *gs* – stomatal conductance, and *E* – transpiration rate

The observed increase of 83% in foliar chlorophyll concentration in the pepper-groundnut mixed intercropping system, compared to the control, could be primarily attributable to two factors namely higher N content in the soil and the presence of cytoplasmic fluid at the cellular level of the leaves (Table 2). According to Cabrera (2004) and Sulok et al. (2012), there is a correlation between higher N content and the existence of adequate fluid in plants, which is associated with the manifestation of deeper green leaves. Fathi (2022) reported that the excess of soil N encourages the formation of new leaves from the terminal meristem of the stem and the lateral buds of older leaves. Moreover, N is the primary component of protein and chlorophyll in plant cells. The amount of chlorophyll is essential for regulating the pace of photosynthetic reaction and dry matter generation. Since 70% of leaf N accumulates in chloroplasts, which make the pigments that make up chlorophyll, there is a close relationship between the amount of chlorophyll and N in plants (Fathi and Zeidali, 2021). Nitrogen is the primary constituent of proteins, nucleic acids, amino acids, and the structure of chlorophyll (Mendoza-Tafolla et al., 2019).

The M1 treatment resulted in a 9% increase in the *A*, indicating that the intercropping system of pepper and groundnut enhanced the gas exchange rate of pepper. Lawlor (2002) conducted a study that highlighted the crucial role of soil N in facilitating the photosynthetic process, which subsequently promotes reproductive growth and enhances output. According to the findings of Hak et al. (1993), a significant proportion of leaf N, up to 75%, is located within the chloroplasts. Most of this N is primarily allocated to the ribulose-biphosphate carboxylase enzyme. Photosynthesis occurs within chloroplasts that contain chlorophyll which plays a crucial role in facilitating the photosynthesis process. Olszewski et al. (2014) mentioned that the efficiency of photosynthesis, assimilation, and distribution determines crop yield. Nitrogen is therefore crucial to these processes. Nitrogen influences growth patterns and leaf longevity in addition to increasing leaf area, which eventually affects photosynthetic efficiency.

Table 2 presents the data indicating that the *gs* exhibited a 20% increase in the treatment group when pepper was intercropped with groundnut, compared to the control group. The findings aligned with those of Sulok et al. (2013), indicating that plants with greater NDVI values exhibit a tendency to open their stomata, which serves as an indicator of a robust and thriving green plant. In addition, Fathi (2022) reported that any increase in soil N leads to an increase in *gs* which leads to an increase in the photosynthesis process. According to the pepper transpiration rate results shown in Table 2, it can be observed that the *E* exhibited a higher value in pepper plants that were subjected to mixed intercropping. Specifically, there was an increase of 13.6% compared to the control value. The findings on leaf *E* aligned with the study conducted by Ashizawa et al. (2003), who observed a similar trend of increasing *E* rates in response to rising *gs*.

The pepper chlorophyll and soil total N relationship results in Figure 1 illustrates the correlation between the concentration of chlorophyll in the leaves and the total N content in the soil. The correlation between chlorophyll and leaf total N, irrespective of treatments, was shown by a polynomial cubic regression line with a zero intercept. The coefficient of determination ($R^2$) for this relationship is 0.65, suggesting that an elevated N content in the soil leads to an increased concentration of chlorophyll in pepper plants. The findings of Tucker (2004) and Daughtry et al. (2000) indicated that the presence of N has a significant impact on the development of chloroplasts and the accumulation of chlorophyll within them, as N serves as a structural component of chlorophyll. Moeinirad et al. (2021) reported that the consumption of N by plants improved pigments as the rate of chlorophyll (a, b) was increased. They also reported a significant and positive correlation between leaf chlorophyll indexes and soil N content.

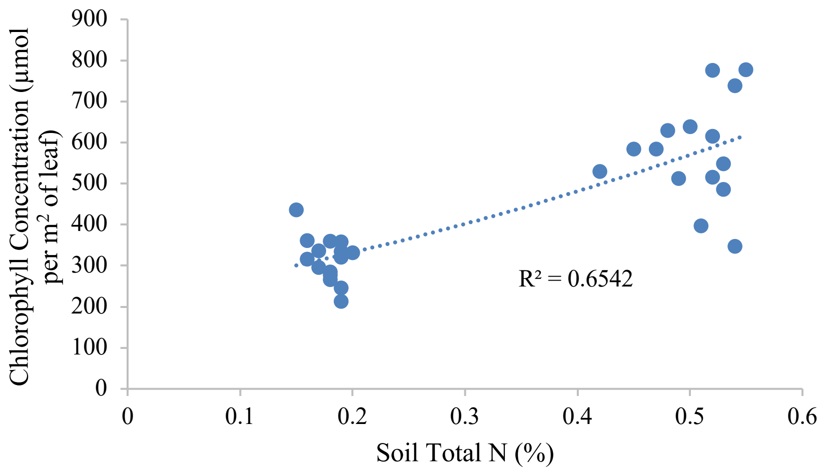


Figure 1. Relationship between pepper leaf chlorophyll concentration and soil total N subjected to different treatments. Values are means ± s.e. of 15 leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determination coefficient are included.

In a similar vein, there exists a correlation between the leaf *A* of pepper and the soil total N content under various treatments, with an R-squared value of 0.64 (Figure 2). The association between the two variables, irrespective of the treatments used, was most accurately represented by a polynomial cubic regression line with a zero intercept. This model accounted for approximately 64% of the observed variability in leaf photosynthesis. This observation demonstrated a strong correlation between the two variables, whereby the growth rate of leaf *A* exhibited a positive trend with the increasing N levels in the soil. According to Ndukwe et al. (2011), the level of N in the soil was found to have an impact on the chlorophyll content present in the leaves. This, in turn, influenced the rate at which photosynthesis occurred, as chlorophyll is responsible for absorbing light energy necessary for the progression of the photosynthetic reactions. As a result, the occurrence of elevated levels of photosynthesis in the presence of increased soil N availability is frequently ascribed to the synthesis of chlorophyll photosynthetic pigments and the activity of Rubisco (Toth et al., 2002). Arvin (2019) found that N stimulates vegetative growth and plant branching because it boosts meristem cells and cell division. Consequently, the plant produces additional leaves, raising its degree of photosynthetic capacity.

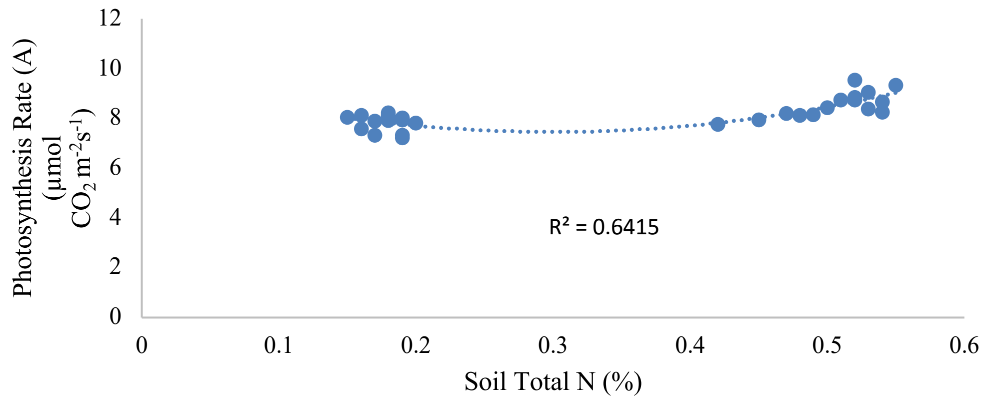


Figure 2. Relationship between leaf *A* rate of pepper means ± s.e. of 15 leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determination coefficient are included.

The amount of chlorophyll in the reaction centres of photosystems is closely correlated with the fluorescence of chlorophyll. Nitrogen absorbed by the plant is one of the key components in the synthesis of chlorophyll, and any disruption or alteration in the structure and pigments of photosystem II results in a decreased quantum performance of the photosystem under dark conditions (Fracheboud and Leipner, 2003). Due to a drop in the synthesis of critical enzymes in the photosynthesis process – the most significant of which is the RuBisCO enzyme – the plant photosynthetic ability has diminished under N deficient conditions, which has resulted in a fall in quantum yield (Qi et al., 2013). Furthermore, having enough N in the plant maintains high efficiency in energy conversion and the photosynthetic electron transfer chain while improving the leaf area index and subsequently carbon assimilation during photosynthesis in the chloroplast thylakoid, which raises quantum yield (Moeinirad et al., 2021).

## CONCLUSION

The concentration of soil N was significantly influenced by the implementation of pepper and groundnut intercropping systems. The leaf normalized difference vegetation index (NDVI), chlorophyll concentration, photosynthetic rate (*A*), stomatal conductance (*gs*), and transpiration rate (*E*) of pepper cultivated in the mixed intercropping system exhibited statistically significant increases compared to the control group. Additionally, a strong correlation was observed between the concentration of leaf chlorophyll and the *A* of pepper, with soil total N being the influencing factor. It can be argued that the practice of intercropping groundnut and pepper can yield advantageous outcomes, including the fixation of N and the enhancement of soil N levels. Hence, the use of groundnut in the growth of pepper has the potential to enhance the photosynthetic rate of pepper through the improvement of soil N availability.

## ETHICAL APPROVAL

No ethical approval was required.

## AUTHORS CONTRIBUTION

K.M.T.S., A.T.H.G., A.J.B., D.B.A., and W.C.M. performed and completed the experiments. K.M.T.S., A.T.H.G., A.J.B., D.B.A., K.P.E., C.Y.S., and W.C.M. acquired and/or interpreted the data and K.M.T.S.

performed the statistical analysis. K.M.T.S. drafted the manuscript. Each author participated in the critical revision and final approval of the manuscript.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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