Growth response of cowpea (Vigna unguiculata), sunn hemp (Crotalaria juncea), and green gram (Vigna radiata) on Acrisol, Gleysol, and Andosol fertilized with CaCO_3 and phosphate

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Growth response of cowpea (*Vigna unguiculata*), sunn hemp (*Crotalaria juncea*), and green gram (*Vigna radiata*) on Acrisol, Gleysol, and Andosol fertilized with $CaCO_3$ and phosphate

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Declaration

I hereby declare that this submission is my own work in its entirety and that to the best of my knowledge, it contains no material previously published or written by other persons or materials extend has been accepted for the award of any other degree or diploma of the university of higher learning, except where due acknowledgement has been made in the text. The chapter 1 of the thesis was published in the Journal of Tropical Agriculture and Development and accepted their agreement to use in this graduation thesis.

Ei Phyu Thae Date

Abstract

To maximize agricultural production for the growing global population, agrochemicals play a significant role. The extensive use of chemical fertilizers has been crucial in boosting cereal production to meet global food demands. However, the improper application of these fertilizers and pesticides by many farmers has detrimental effects on ecosystems, leading to soil degradation, posing risks to human health, and harming the environment. Consequently, leguminous green manure plants are essential for enhancing crop production in infertile soils, as they improve soil quality with minimal environmental impact. While numerous studies have identified suitable green manure plants for temperate regions, information for tropical areas remains limited.

The growth response of three common tropical leguminous green manure plants: cowpea (*Vigna unguiculata*), sunn hemp (*Crotalaria juncea*), and green gram (*Vigna radiata*) on two different soil types: a highly acidic Haplic Acrisol with low available phosphate (P) and a slightly acidic Eutric Gleysol with a P deficit were investigated in the chapter 1. By controlling soil pH and P availability, cowpea produced the highest dry matter and absorbed the most P, with its performance differing by soil type. The Gleysol supported better growth than the Acrisol. Specifically, cowpea absorbed significantly more P from the Gleysol (6-7 times more than from the Acrisol). However, cowpea's P acquisition on the Acrisol was insufficient, even with the addition of $CaCO_3$ and P fertilizers.

Japanese Andosols are popular for their high acidity and phosphorus fixation capacity, and which could not supply the required P for the plant growth. The growth responses of Japanese native and invasive plants on non-allophanic Andosol were reported by many scientists however the study on the growth responses of tropical leguminous green manure plants on non-allophanic Andosol were limited. The authors would like to know the response of tropical green manure plants on Andosol and want to confirm the P acquisition ability of

tropical green manure plants on Andosol as well. Therefore, the soils were controlled by adding $CaCO_3$ and P fertilizers to set up favorable conditions for plant growth of tropical leguminous green manure plants in chapter 2. Cowpea showed the highest dry matter production and P acquisition among the three green manure plants with high tolerance against soil acidity. However, the total dry matter production and P acquisition were suppressed in the plants grown on this soil in compares with the previous experiment although the soils were amended with $CaCO_3$, and P fertilizer even in cowpea. Those suppression would be caused by differences in P acquisition mechanisms of green manure plants and P adsorption ability of Andosol. The results of the experiments in Chapters 1 and 2 revealed that cowpea is the best in both strongly and slightly acidic soils but its P acquisition abilities still quite low. Therefore, it would be necessary to clarify the P acquisition mechanism of green manure plants on Andosol.

The author studied the P concentration in the rhizosphere area of cowpea, sunn hemp and the other new green manure species, yellow lupin and checked their P acquisition mechanism by controlling soil pH in chapter 3. The results showed that yellow lupin can absorb the greatest P from soil whereas, cowpea and sunn hemp's P absorption are nothing. However, the P concentration in rhizosphere of yellow lupin and cowpea was the same but the P concentration in both plant-growing and blank conditions were depleted with time due to strong P adsorption of Andosols. Therefore, the author estimated that there will be same affinity of P transporter on the surface of the roots of these two plants but different in P uptake activity by yellow lupin's extensive root system and its organic P solubilization effect.

The results of this study revealed that the growth response of tropical leguminous green manure plants differed depending on soil types. Among them, cowpea showed the best performance in terms of total dry matter production and has a high tolerance to soil acidity and P acquisition ability across three types of soil: Gleysol > Acrisol > Andosol. The growth inhibition and the lower in P acquisition of green manure plants on Andosol is due to its high affinity to phosphorus. Yellow lupin is more suitable green manure which has the highest P acquisition ability on Andosol even under without P addition due to its P solubilization effect, however the P depletion in the rhizosphere was observed due to P adsorption of non-allophanic Andosol. Therefore, it would be confirmed that the growth inhibition and limitation in P acquisition of tropical leguminous green manure plants on Andosol is due to differences in P uptake activity between temperate and tropical green manure plants and strongly P fixation ability of Andosol.

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Chapter 1

Growth Response of three tropical leguminous green manure plants to soils fertilized with $CaCO_3$ and Phosphate

Abstract

Leguminous green manure plants are crucial for enhancing crop production in infertile soils due to their ability to improve soil quality with minimal environmental impact. While many studies have identified suitable green manure plants for temperate regions, there is limited information for tropical areas. This study examined the growth response of three common tropical leguminous green manure plants; cowpea (*Vigna unguiculata*), sunn hemp (*Crotalaria juncea*), and green gram (*Vigna radiata*), on a Haplic Acrisol, which is highly acidic and low in available phosphate (P), compared to a Eutric Gleysol with slightly acidic P deficit soil by controlling soil pH and P availability. Among these plants, cowpea produced the highest dry matter and absorbed the most P, with performance varying by soil type: Gleysol > Acrisol. Cowpea effectively absorbed P from the Gleysol (6-7 times more than from other soils), but its P acquisition on the Acrisol was insufficient, even with the addition of CaCO3 and P fertilizers. Therefore, it is recommended to either identify other green manure species with better P acquisition for Acrisols or increase P fertilization to counteract the P adsorption in these soils, despite cowpea being the most effective among the tested plants.

Introduction

1.1Research Background

1.1.1 Uses of Chemical Fertilizers in Agriculture

To meet ever growing population of the world, the modern agricultural technologies have been adopted for volumizing food grain production (Abril et al. 2007) because nearly 99% of the world's food supply comes from the agricultural production of the arable land. In response to severe food shortages in many developing countries during the late 1950s, efforts were made to enhance the yield of major grain crops like rice, wheat, and corn to alleviate hunger. Plant breeders created hybrid, short-stemmed varieties that showed significant improvements in productivity when high levels of fertilizer were applied (Kendall and Pimentel 1994). Chemical fertilizers supply vital nutrient such as nitrogen (N), phosphorus (P), and potassium (K) for the plant growth and contribute to 30-60% of the total increase in food production globally.

For the previous 40 years, massive chemical fertilizer and pesticide have applied, with a corresponding increase in crop yields (Richardson, 1991). In the past, chemical fertilizers have been used sources for supplying nutrients to the crop's ecosystems (Mappaona et al. 1995, Hall et al. 1996, Fageria et al., 2005).

Many years ago, in developing country, like Myanmar chemical fertilizers were supplied as subsidies to encourage the farmers for maximum usage of fertilizers for their crop production. Therefore, the low cost readily available chemical fertilizers have been occupied in the place of organic manures including green manures. In recent years, the global use of fertilizers has surged dramatically, leading to significant environmental issues. The application of fertilizers can contribute to the buildup of heavy metals in both soil and plant systems. As plants absorb these fertilizers from the soil, the contaminants can enter the food chain. Consequently, fertilization contributes to pollution of water, soil, and air.

Over the next 30 years, the use of fertilizers is expected to increase significantly to boost agricultural production. However, the excessive application of chemical fertilizers in agriculture will lead to numerous environmental problems because of heavy metals, such as cadmium and chromium, as well as high levels of radionuclides contained in certain fertilizers. Consequently, these fertilizers become the primary source of heavy metals and radionuclides in agro-ecosystems, causing the accumulation of inorganic pollutants in plants. (Savci, 2012). The injudiciously use of inorganic fertilizer in agriculture worldwide for ensuring the world food security caused so many health problems and unrecoverable environmental pollution. Although, the benefits of chemical fertilizers on increasing food grain production are remarkable, its negative impacts on public health and environmental problems are serious especially in developing countries (Pimentel, 1996).

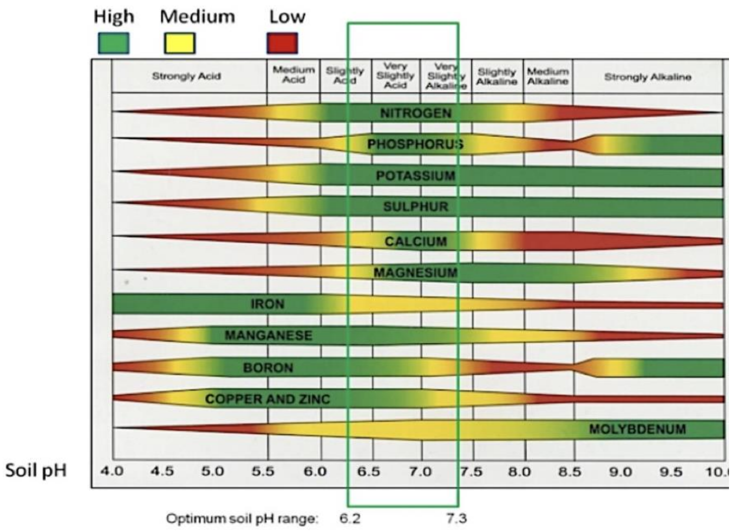

Figure 1.1 Effect of pH on the availability of important nutrients in plant growth

(Troug 1946 b)

1.1.2 Soil acidity problems for crop production

The two fundamental factors which interfere the fertility of the acidic soils are impoverishment of available nutrients such as phosphorus and the presence of certain toxic elements for instance, aluminum (Al), Iron (Fe), and manganese (Mn) at toxic levels. Acid soils in temperate and tropical regions often hinder dry matter production due to the above mentioned two main factors (Haynes and Ludecke 1981). Soil acidity impacts crop production by affecting nutrient availability (Figure 1.1), root growth, and altering various physical, chemical, and biological properties (Guo et al. 2010). Studies have revealed that acidic soils limit root growth and nutrient uptake (Schroder et al. 2011). Low soil pH is also associated with low water uptake, limited availability of nutrients (nitrogen, phosphorus, magnesium, molybdenum), and stunted plants (Kemmitt et al. 2006). Depletion of base cations hampers an ecosystem's acid-buffering capacity that leads to greater susceptibility of crops to environmental stresses, such as extreme temperature, salinity, and drought (Tian and Niu 2015).

Crop production in most area of the world, especially in tropical region is mainly limited by soil acidity and soil infertility (IAEA 2000, Fageria et al. 2009). Almost one-third of the world's land is problematically acidic, and most of the soils in tropical and subtropical regions of Southeast Asia are naturally acidic and low in P availability (von Uexkull and Mutert, 1995; IAEA, 2000; Kochian *et al*., 2004; Haruna 2011; Rahman *et al*., 2018). Soil acidity is not a single factor problem, but very complicated and related with many factors that may affect the growth of plants (Dugganna et al. 1995). Soil acidity (soil pH) is one of the most important chemical properties to understand the ability of soils to grow plants because it regulates the nutrient status and toxicities of the soils (Fageria and Zimmermann, 1998). Strongly acidic soils ($pH < 5.5$) greatly reduce plant production due to aluminum toxicity (Sparks, 2003). Most of the soils in upland areas of Southeast Asia are aluminum-toxic and low in P fertility, and those soils are typically classified as Acrisols by World Reference Base for Soil Resources (FAO, 2022). Acrisols occupy 50.4% of the terrestrial area of mainland Southeast Asia, and the soils cover 70.7 % of Laos, 54.2 % of Thailand, 48.9% of Vietnam, 48.2% of Cambodia, and 41.9% of Myanmar (Elstner, 2017). Low P availability in strongly acidic soils is also a major problem for crop growth. According to Aye (2001), in Southern Shan State, Myanmar, where most soils are classified as Acrisols, the growth of leguminous plants is limited because of the natural low P availability (e.g., Olsen P value of 8 mg kg^{-1}) of the soils. Acid soils and low soil fertility are common production problems in Myanmar. Soil acidity and low P status are major limiting to growing crops in red earths and yellow earths soils, Acrisol in Southern Shan State and Eutric Gleysol in Ayeyarwaddy Region of Myanmar. The upland red earths and yellow earths soils highly leached and weathered seriously due to shifting cultivation, thus plant available nutrients including P becomes low (IAEA 2000, Aye 2001). In this condition, the application of phosphorus fertilizer and liming is indispensable to improve crop yield on these soils. Larger amount of chemical fertilizers has been used in developed countries such as Japan to correct soil acidity. But, in the tropics, developing countries, the acidic soil area is vast and most of the farmers are poor, and they cannot afford to buy liberal amount of fertilizers (Tanaka et al. 1984, Hunter 1997).

1.1.3 Importance of Green Manures in Agriculture

To avoid the notorious effects of synthetic fertilizers on human and soil health including the environment, nowadays the new agricultural technologies have been adopted and practiced such as organic, sustainable, and ecofriendly agriculture using such as green manures, biofertilizers and biopesticides etc. (Chandini et al., 2019). Green manuring refers to the agricultural technique of incorporating green manure crops into the soil through plowing while the plants are still green or shortly after they begin to flower (Yadav et al., 2021). The plants used for this purpose are known as green manure plants.

Green manuring is primarily cost-effective, easily available than chemical fertilizers and it could provide organic matter which is the basic of the soil fertility that enrich nutrients status in soil and improve the soil structure. Green manure plants also suppress weed emergence and control soil erosion by their soil coverage. Rising costs of chemical fertilizers and worsening environmental issues due to their overuse have prompted the promotion of green manure legumes as bio-fertilizers. On the other hand, inorganic fertilizers are known for their high cost and their deleterious effects if managed improperly and resultant in crop yield reduction due to soil degradation and nutrients imbalance (Hijbeek et al., 2018).

Green manuring is one of the oldest agricultural practices for soil improvement. In India and Greece, green manure has been used for thousands of years. In China grasses and weeds were used as green manure for soil nourishment since hundreds of years. Japan, Korea, and China have been using green manure like clover since during early 20th century before rice cultivation (King 1911). Although green manure is as old as agriculture, the interest in its uses has been reviving due to its soil improving quality with low cost means of conditioning and maintaining soil ability in depleted soils and its adaptability on poor soil (Bunch 1985, Jannink 1996, Fageria et al. 2005, Patrick et al,2019, Adekiya et al. 2019). Green manure is not only valuable to subsistence farmers, who has difficulty for purchasing expensive mineral fertilizers (Meena et al. 2014) but also important for larger scale farmers as chemical fertilizers tend to cause economically (Singh et al. 2013).

Leguminous green manure plants become popular for their response to soil factors than other crops especially in acidic soils and they can promote soil chemical properties differently due to its different nutrients supply into the soil. Leguminous green manure can increase agricultural production due to its effects on enhancing nutrient retention (Dinnes et al. 2002), improving soil fertility by reducing soil erosion and minimizing global warming (Fageria et al., 2005).

1.1.4 Uses of tropical leguminous green manures

In Myanmar, various leguminous species have been utilized as green manure plants before rice cultivation to ensure long-term soil sustainability. In current situation, chemical fertilizer-rice price ration may be unfavorable, and the diminishing returns of chemical fertilizers would make them impossible to continue using the maximum amount. Thus, leguminous green manure plants would alleviate socioeconomic considerations for the subsistence farmers.

Among the leguminous green manure plants, cowpea (*Vigna unguiculata*), sunn hemp (*Crotalaria juncea*), and green gram (*Vigna radiata*) are the most common. These tropical leguminous green manure plants are typically grown in the summer and incorporated into the soil before rice planting, with cowpea and sunn hemp being more frequently used than green gram. Additionally, these plants are grown as intercrops with cash crops to control pests and weeds. In orchards, they are grown to enhance soil fertility as an alternative to chemical nitrogen fertilizers due to their rapid growth and high nutrient content. Furthermore, cowpea and green gram serve as important edible legumes, while sunn hemp is valued as a fiber crop. For these reasons, the selection of green manure species tolerant to low pH and low P is great important for Myanmar farmers to achieve reasonable yields with minimum inputs.

Despite many leguminous green manure species were used for soil fertility upgrading purpose in Myanmar, effective green manure species such as cowpea, sunn hemp and green gram can be suitable with Myanmar`s acidic soil. The investigation on the response of suitable green manure species on different acidic soils are urgently needed (Poolpipatana and Hue 1994). Besides, the growth response and the performance of green manure species in acid soils has not been vigorously tested. (Mappaona and Yoshida, 1993) and there is little information about the growth of green manure under acidic low fertile soil (Warman, 1991).

Although there are many effective studies on the screening of suitable green manure species for the temperate region, little is known about the growth response of the tropical green manure species in strongly acidic and low P-fertility tropical soils (Poolpipatana and Hue, 1994; Fageria *et al*., 2009; Fageria *et al*., 2013; Patrick *et al*., 2019). Investigation of effective green manure species suitable for the tropical soils can be especial and beneficial.

1.2 Hypothesis

The dry matter production and P acquisition ability of the three green manure plants would or would not change depend on different soil acidity and available phosphorus rates.

1.3 Objectives of this study

The objectives of the present study were to clarify the growth response and P acquisition ability of the three green manure plants, cowpea, sunn hemp, and green gram, on an Acrisol and the other acidic soil, a Gleysol for comparison.

1.4 Materials and Methods

1.4.1 Soils and seeds

The purpose of the present study was to clarify the growth response of green manure plants on Acrisols as affected by $CaCO_3$ and P fertilization. Gleysol was used as ordinal and P deficient soils, for comparison. The three leguminous green manure plants were cultivated on two different soils, i.e., a Haplic Acrisol and a Eutric Gleysol according to the World Reference Base for Soil Resources (FAO, 2022). The Acrisol was collected from the Ap horizon of a field in Aungban Agricultural Research Farm in Shan State, Myanmar (20° 40′ 0″ N and 96° 38′ 0″ E). The Gleysol was collected from the Ap horizon of a field in Myaungmya Agricultural Research Farm in Ayeyarwaddy Region, Myanmar. Figure 1.2 shows the location of the soil sampling area in Myanmar. Gleysols are predominantly found in lowland areas where rice is a major crop. These soils are often rich in organic matter but maybe deficient in certain nutrients due to prolonged water saturation. Proper managements including controlled drainage and fertilization is essential to maintain productivity. Acrisols are typically found in tropical and subtropical regions with high rainfall, including the upland areas of Myanmar. They are important for growing a variety of crops, including upland rice, maize, cassava, and other cereals. However, their fertility is low due to leaching of nutrients, so sustainable management practices such as, application of green manuring, liming, and proper fertilizer application to improve soil fertility and soil health. These two soils were passed through a 5 mm sieve for cultivation experiments and a 2 mm sieve for chemical analyses. The basic physicochemical properties of the three soils were listed in Table 1.1.

The seeds of the three green manure plants were supplied from the Legumes and Pulses Section, Department of Agricultural Research (DAR), Myanmar. The green manure plants used in this study were shown in Figure 1.3.

Table 1.1 Physicochemical properties of Acrisol and Gleysol.

Soil	pH		EC (μS /cm)	Exchangeable acidity‡ (mmol/kg)	Available P†‡ (mg P_2O_5/kg)	Total content		C/N
	H_2O	KCl				C§‡ (%)	N⁋‡ (%)	
Acrisol	5.1	3.6	45	26	58	1.18	0.09	13.1
Gleysol	5.8	5.5	87	1.2	40	0.89	0.06	14.8

† Bray II P. § Carbon. ⁋ Nitrogen. ‡ Calculated based on oven dry weight.

Table 1.2 The application rate of $CaCO_3$ (L) and phosphate (P) in the growth experiments on Acrisol and Gleysol.

Fertilizer levels	**Acrisol**	**Gleysol**
$CaCO_3$	Application rate (g $CaCO_3$/kg soil) ‡	
L_0	0.0	0.0
L_1	1.0	1.65
L_2	4.0	-
Phosphate	Application rate (g P_2O_5/kg soil) ‡	
P_0	0.0	0.0
P_1	0.19	0.46
P_2	0.74	1.14

‡Calculated based on oven dry weight.

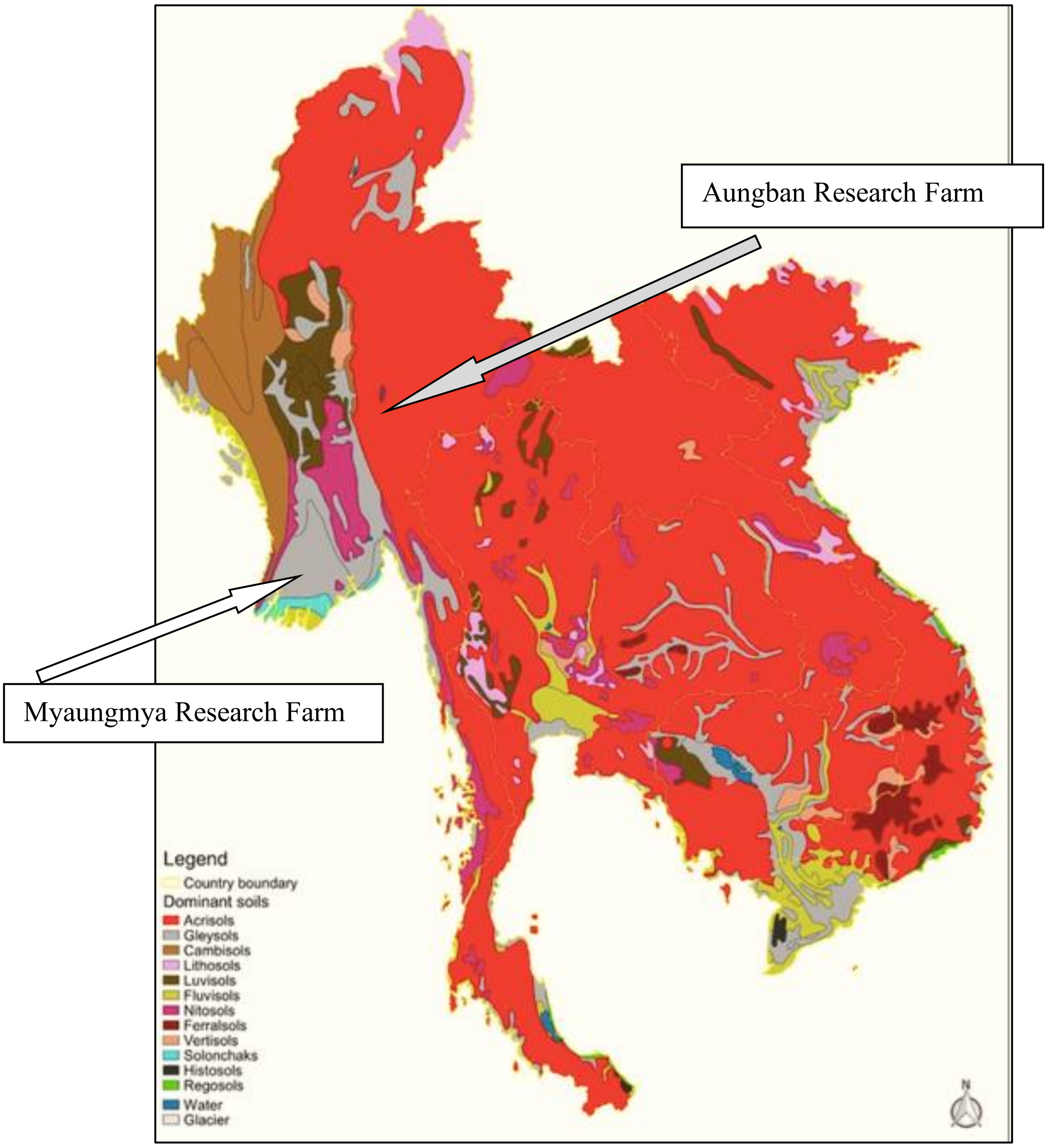

Figure 1.2 Map of the soil sampling area in Myanmar (FAO, 2007)

(a) Cowpea (b) Sunn hemp (c) Green gram

Figure 1.3 Three tropical leguminous green manures plants; (a) cowpea, (b) sunn hemp, and (c) green gram

1.4.2 Chemical analysis of soils and plants

For the measurement of soil pH values in H_2O (pH(H_2O)) and 1M KCl (pH(KCl)), a 2.0 g portion of the air-dried soil sample was mixed with 5.0 ml of deionized water and 1.0 M KCl, respectively. The mixture was allowed to stand for one day and the pH values were measured with a standard pH meter (F-51 HORIBA Ltd., Kyoto, Japan) in the suspension.

To determine exchangeable acidity, an 8.0 g portion of the air-dried soil sample and 20.0 mL of 1.0 M KCl were mixed, shaken for one day, and filtered through a filter membrane (disposable syringe filter unit DISMIC 25CS045AN, pore size 0.45 μm, cellulose acetate, Advantec Toyo, Tokyo, Japan). The extracted acid was determined by titration of the filtrate with 0.01 M NaOH with an auto titrator (Comtite COM-550, Hiranuma Sangyo Co., Ltd., Tokyo, Japan).

For the determination of electrical conductivity (EC), an 8.0 g of soil sample was mixed with 20.0 mL of deionized water, shaken for one day, and filtered through a filter membrane (disposable syringe filter unit DISMIC-25CS, Advantec Toyo). The filtrate was subjected to EC determination by using an EC meter (CM-25 R, TOA Corporation, Tokyo, Japan).

Plant-available P of soils was determined by following the Bray II method; 1.0 g of air-dried soil sample and 20.0 mL of 0.03 M NH_4F in 0.10 M HCl were mixed, shaken for one min, and filtered through a filter paper (Type 5B, Advantec Toyo). The concentration of extracted phosphate in the filtrate was determined by the molybdenum blue method (Murphy and Riley 1962) at a wavelength of 710 nm using a spectrophotometer (UV-Vis Spectrophotometer, Shimadzu Corporation, Tokyo, Japan).

For the determination of total C and N contents, visible plant residues in the soil samples were carefully removed by using tweezers. The soil sample was thoroughly ground using a mortar and a pestle and then analyzed for C and N content with a combustion method (MT-5 and MT-6, CHN coder, Yanaco, Tokyo, Japan).

To determine the amount of P in plants, an oven-dried plant sample was ground, added with concentrated HNO_3, and kept overnight. The sample was digested by heating for three hours at 110°C and again heating for another five hours at 110°C after adding H_2O_2 on a heating unit (DigiPREP MS, SCP SCIENCE, Canada). The digested solution was subjected to total P concentration analysis with the inductively coupled plasma-optical emission spectrometer (Agilent 5800 ICP-OES, Agilent Technologies, USA).

1.4.3 Control of soil pH and P availability

For the growth experiment on the Acrisol, the soil pH(H_2O) values were increased from 5.1 to around 5.5 and 6.5 by adding $CaCO_3$ (Analytical grade, Tianjin Zhiyuan Chemical reagents Co. Ltd., China), and the available P (Bray II P) contents of the soil were also increased from 58 to around 200 and 500 mg P_2O_5 kg^{-1} by adding triple super phosphate (Comet Hi P, 46% P_2O_5, Myanma Awba Co. Ltd., Yangon, Myanmar), giving nine soils with different soil pH and available P values (3 soil pH levels × 3 available P levels). Similarly, for the growth experiments on the Gleysol, the soil pH(H_2O) values were increased from 5.8 to 6.8 by adding $CaCO_3$ (Analytical grade, Tianjin Zhiyuan Chemical reagents Co. Ltd.), and the available P was also increased from 40 to around 200 and 500 mg P_2O_5 kg^{-1} by adding the triple super phosphate (Comet Hi P, 46% P_2O_5, Myanma Awba Co. Ltd.), giving six soils with different soil pH and available P values (2 soil pH levels × 3 available P levels). For the growth experiment on the Andosol, the soil pH(H_2O) values were increased from 4.8 to around 5.5 and 6.5 by adding $CaCO_3$ (granulated reagent, NACALAI TESQUE, INC., Tokyo, Japan), and the available P (Bray II P) values of the soils were also increased from 40 to around 200 and 500 mg P_2O_5 kg^{-1} by adding phosphoric acid (85 % H_3PO_4, NACALAI TESQUE, INC., Tokyo, Japan), giving nine soils with different soil pH and available P values (3 soil pH levels × 3 available P levels). The amount of the reagent applied to each soil was summarized in Table 1.2.

After the soil was mixed well with $CaCO_3$ and P reagent in a plastic bag, it was incubated for 7 days before filling it into a pot. A part of the soil sample was taken, dried, and subjected to chemical analysis.

The untreated Acrisol and Andosol were strongly acidic ranging from 4.8 to 5.1 in soil pH(H_2O) and low in available P ranging from 40 to 58 mg P_2O_5 kg^{-1} in Bray II P, and untreated Gleysol was weakly acidic with soil pH(H_2O) value of 5.8 and low in available P of 40 mg P_2O_5 kg^{-1} in Bray II P. As shown in Figure 1.4, the soil pH(H_2O) and the available P (Bray II P) were successfully controlled by the addition of P fertilizer.

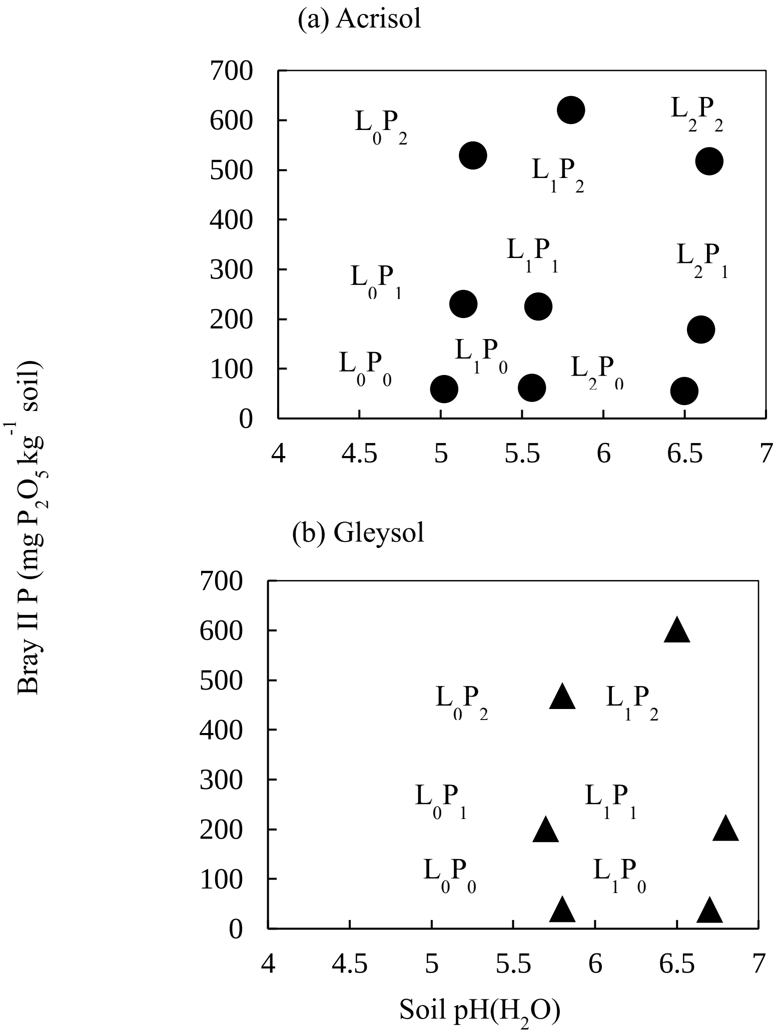

Figure 1.4 Soil $pH(H_2O)$ and available phosphate (Bray II P) values of three soil samples from (a) a Haplic Acrisol and (b) a Eutric Gleysol, fertilized with and without $CaCO_3$ (L) and phosphate (P) addition. (Thae et al., 2024)

1.4.4 Plant growth experiment

The growth experiment was conducted at Yezin Agricultural University in Myanmar (19° 49′ 59″ N, 96° 16′ 30″ E) using Acrisol and Gleysol soils. In these experiments a split-split plot design was utilized with three replicates for Acrisol and four replicates for Gleysol. The main plots were designated for different green manure species, sub-plots for varying CaCO3 levels, and sub-sub plots for different phosphate fertilizer levels.

For both experiments, a 2.0 kg portion of the soil was placed into a 4 L volume pot (top diameter; 200 mm, base diameter; 155 mm, height; 165 mm). The moisture content of the pots was maintained at 80% of the maximum water holding capacity by adding deionized water. Nitrogen and potassium fertilizers were used as urea (46% N, Shanxi JMGM Chemical Fertilizer Co. Ltd., China) and KCl (Comet Hi-K, K_2O 60%, Myanma Awba Co. Ltd., Yangon, Myanmar) for basal application with the ratio of 0.1 g N and K_2O kg^{-1} soil. Ten seeds of the green manure species were sown in each pot, and the weight of each pot was recorded. After three days of germination, the number of plants grown in a pot was reduced to three. Deionized water was supplied daily to maintain 80% of water holding capacity by adjusting the weight of the pots to match the initial weight after sowing. The cultivation period was lasted from August 8^{th} to September 6^{th} (30 days). During the cultivation period, the recorded average maximum, and minimum temperatures according to the data collected at the Agrometeorology Station, Department of Agronomy, Yezin Agricultural University, were 28°C and 25°C, respectively.

Harvesting was carried out 4 weeks after sowing. As soon as harvesting has done, the roots of the green manure plants were washed with the tap water to remove soil particles, then the above ground part (shoot) and the underground part (root) were separated. They were dried in an oven at 70°C for three days to reach the constant dry weight and then the dry weights were recorded. The summary of growth condition of the experiments was mentioned in Table 1.3.

Table 1.3 Summary of the conditions of cultivation experiments.

	Acrisol	Gleysol
Growing condition	Greenhouse (YAU†, Myanmar) (30-day cultivation)	Greenhouse (YAU†, Myanmar) (30-day cultivation)
Experimental design	Split-split plot design	Split-split plot design.
Soil weight/pot	2000 g	2000 g
Number of plant/pot	3	3

†Yezin Agricultural University

1.5 Statistical analysis

Using Statistix Version 10 (Analytical Software, Tallahassee, FL., USA), the data of total dry matter and relative dry matter were processed for the analysis of variance (ANOVA) in a split-split plot design to check the statistical significance among the treatments (green manure species, $CaCO_3$ levels, and phosphate fertilizer levels). Mean comparisons were analyzed by using Tukey's honestly significant difference (HSD) test at a 5% probability level.

1.6 Results and Discussion

1.6.1 Total dry matter production of cowpea, sunn hemp, and green gram

In the Acrisol soil, total dry matter production was significantly influenced by the addition of CaCO3, phosphate (P) fertilizer, and the type of green manure species used. Additionally, all interactions between these factors were highly significant ($p < 0.001$; Table 1.4). Among the green manure species, cowpea produced the highest total dry matter, followed by sunn hemp and green gram, which had similar production levels (Figure 1.5 a).

In the Gleysol soil, total dry matter production was significantly influenced by the addition of phosphate (P) fertilizer and the type of green manure species used. The interactions between these factors were also significant ($p < 0.001$). However, $CaCO3$ addition did not have a significant effect ($p > 0.05$; Table 1.4). Similar to the results from the Acrisol soil experiment, cowpea produced the highest total dry matter, followed by sunn hemp and green gram, which had comparable production levels (Figure 1.5 b).

Table 1.4 The probability values obtained from a 3-way analysis of variance (ANOVA) of total dry matter and relative total dry matter† as affected by three green manure species (GMS), level of $CaCO_3$ addition (L), and level of phosphate fertilizer addition (P). (Thae et al., 2024)

Treatments	Total dry matter	Relative total dry matter†
Acrisol		
GMS	< 0.001	< 0.001
L	< 0.001	< 0.001
GMS × L	< 0.001	< 0.001
P	< 0.001	< 0.001
GMS × P	< 0.001	0.02
L × P	< 0.001	< 0.001
GMS × L × P	< 0.001	< 0.001
CV (%)	9.29	6.63
Gleysol		
GMS	< 0.001	ns
L	ns	ns
GMS × L	0.002	0.02
P	< 0.001	< 0.001
GMS × P	< 0.001	ns
L × P	< 0.001	< 0.001
GMS × L × P	< 0.001	< 0.001
CV (%)	5.61	6.77

†Calculated by converting the highest total dry matter into 100.

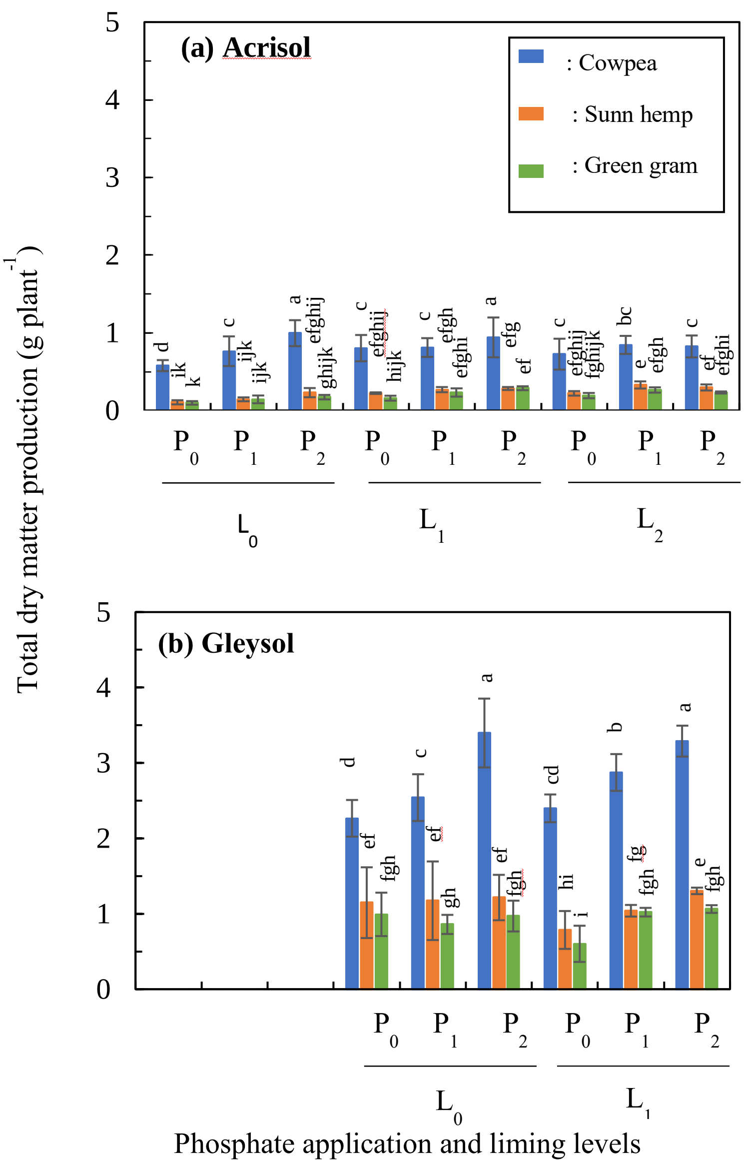

Figure 1.5 Total dry matter production of cowpea, sunn hemp, and green gram grown on the soils from (a) a Haplic Acrisol and (b) a Eutric Gleysol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition. (Thae et al., 2024)

For the application rate, see Table 1.2 Mean values with the same letter on bars in each of the three graphs are not significantly different in Tukey's HSD tests (p < 0.05). Error bars are the standard deviation of three replicates.

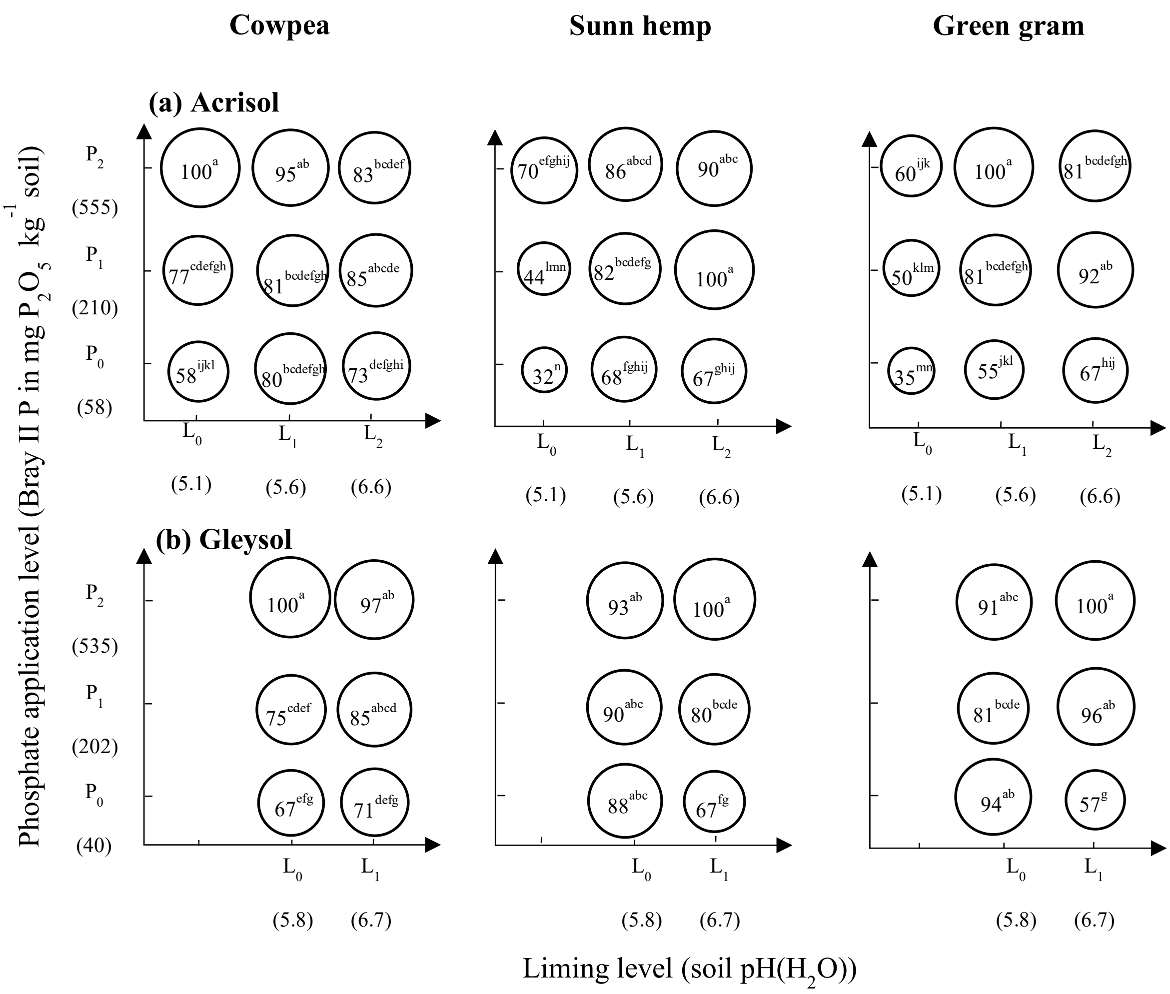

Figure 1.6 Relative total dry matter of cowpea, sunn hemp, and green gram grown on the soils from (a) a Haplic Acrisol and (b) a Eutric Gleysol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition. (Thae et al., 2024)

For the application rate, see Table 1.2 Mean values with the same letter in circles in each of graphs are not significantly different in Tukey's HSD tests ($p < 0.05$). The relative total dry matter values are calculated by converting the highest total dry matter into 100 in each graph and shown with values in circles, which correspond to the area of the circles.

Green manure plants were well grown and developed on the Gleysol, yielding the highest total dry matter production. In contrast, the dry matter production on the Acrisol was limited despite $CaCO_3$ and phosphate (P) fertilizer were added.

In this study, to clarify the tolerance of the plants against the soil acidity and low P conditions, the relative total dry matter values were calculated, which is the percentage of total dry matter value to the highest total dry matter in each green manure species (Figure 1.6). In the experiment on Acrisol, the relative total dry matter values of the three green manure plants were significantly affected by the addition $CaCO_3$ and P fertilizer (Table 1.4). On the soils without $CaCO_3$ and P fertilizer addition (L_0P_0), in Acrisol, cowpea (58 ± 2.4 %) showed the highest relative dry matter values than sunn hemp (32 ± 2.7 %) and green gram (35 ± 2.6 %), indicating that cowpea is highly tolerant against soil acidity and low P fertility (Figures 3a and 3c). Poolpipatana and Hue (1994) suggested controlling soil pH 5.5 to 6 to achieve high dry matter production of leguminous green manure plants grown in acid sulfate soil (Sulfic Tropaquept). Dugganna *et al*., (1995) noted out that cowpea demonstrated an outstanding ability to tolerate Al toxicity, although this finding was derived from a solution culture experiment. Therefore, they recommended additional research using natural soil conditions to validate the acidity tolerance of cowpea. The current study found that cowpea exhibited the highest acidity tolerance in the Acrisol compared to sunn hemp and green gram. The relative dry matter values of green manure plant species in Gleysol (Figure 1.6 b) were significantly affected by phosphorus addition but not by $CaCO_3$ addition (Table 1.4). The soil acidity in Gleysol would not be toxic because it has a mildly acidic pH.

Based on the total dry matter production and the relative total dry matter values from the current study, cowpea showed the best performance among the three green manure plants under two types of soils. However, although the soil pH and P fertility in the Acrisol being improved to match those of Gleysol, the total dry matter production in the Acrisol remained relatively low (Figure 1.6 a).

The typical symptom of N deficiency is chlorosis in older leaves (Marschner, 2012), but this was not observed in our study. Potassium deficiency generally results in chlorosis and necrosis in mature leaves and stems (Marschner and Cakmak, 1989), however these symptoms were absent in this study. Phosphorus deficiency, on the other hand, significantly reduces important plant growth parameters, such as the number of leaves and the shoot/root (SR) ratio (Lynch *et al.*, 1991). Additionally, increased carbohydrate partitioning to P deficit roots leads to decrease shoot/root (SR) ratio (Fredeen et al., 1989; Marschner, 2012). In this study, the leaf age and SR ratio of cowpea, sunn hemp and green gram grown on the Acrisol were 2-3 times lower than those grown on the Gleysol (Table 1.5). The author, therefore, assumed that the reduction in leaf age and SR ratio on Acrisol was due to P deficiency. It would be possible that P acquisition is limited by the strong P adsorption of the Acrisol. Nanzyo (2002) indicated that Andosols which has the similarity soil acidity properties with Acrisol, have a high affinity to P and leading to P deficiency in plants. It would be possible that P acquisition is inhibited by the strong P adsorption of Acrisol. Consequently, the amount of P absorbed by the plants will be analyzed in the next section.

Table 1.5 Leaf age and shoot/root (S/R) ratio of cowpea, sun hemp, and green gram at harvest stage (30 days after sowing) grown on an Acrisol and a Gleysol. (Thae et al., 2024)

	Soil type		Cowpea	Sun hemp	Green gram
Leaf age	Acrisol (n=27)		2.80 ± 0.11	3.78 ± 0.17	1.29 ± 0.13
	Gleysol (n=24)		6.93 ± 0.60	10.2 ± 0.7	3.51 ± 0.02
		P-value	< 0.001	< 0.001	< 0.001
S/R ratio	Acrisol (n=27)		3.94 ± 0.41	3.87 ± 0.73	4.47 ± 0.87
	Gleysol (n=24)		6.47 ± 0.89	9.95 ± 0.93	11.1 ± 2.2
		P-value	< 0.001	< 0.001	< 0.001

The data were calculated regardless of soil pH and soil available P levels and expressed as mean ± standard deviation. Probability values (P-values) were calculated between the mean values of two soils, Acrisol and Gleysol by using Student’s t-test.

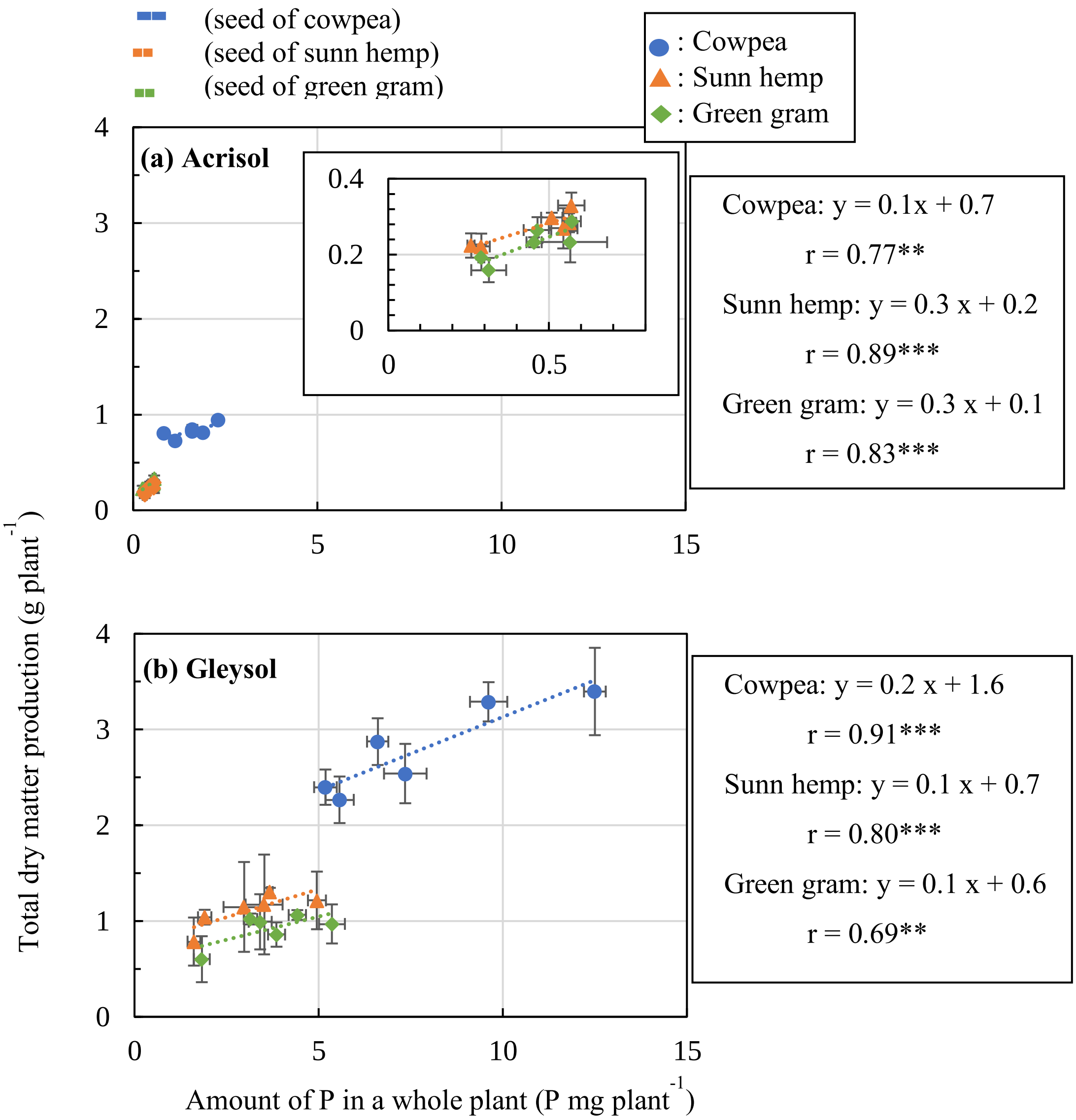

Figure 1.7 Relationship between amount of P in a whole plant and total dry matter production for cowpea, sunn hemp, and green gram grown on the soils from (a) a Haplic Acrisol and (b) a Eutric Gleysol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition. Only the data for weakly acidic pH(H_2O) range between 5.3 to 6.8 were plotted in the graphs to exclude the plant growth inhibition observed in strongly acidic soils. The horizontal bars at the top of this graph show the means and standard deviations of seed P for cowpea (0.58±0.08), sunnhemp (0.23±0.02), and green gram (0.23±0.01). **, and ***: significant at 0.01 and 0.001 levels, respectively. (Thae et al., 2024)

1.6.2 Phosphorus acquisition by cowpea, sunn hemp, and green gram

The amount of phosphorus (P) acquired by a plant from a soil was determined by calculating the difference between the P content in the plant and that in the seed, and both P contents were shown in Figure 1.7. The amounts of P in the whole plant of the three green manure plants were varied among plant species. Cowpea accumulated more phosphorus in the plant and produced greater total dry matter compared to sunn hemp and green gram on each type of soil (Figure 1.7 a and b). The greater P accumulation would be crucial to maximize the total dry matter production. Matsunaga *et al*. (2008) indicated that the amount of P accumulated in the shoot was closely correlated with shoot biomass in cowpea.

Depending on soil types, the amounts of P in the whole plant of the green manure plants were also different. On the Acrisol, the P contents in the whole plant of the green manure plants were 0.15 to 2.31 mg P plant^{-1} (Figure 1.7 a). On the Gleysol, the maximum P accumulations in the whole plant of cowpea, sunn hemp, and green gram were obtained among the three soil types, ranging from 1.61 to 12.5 mg P plant^{-1} (Figure 1.7 b). The amount of P in the whole plant grown in Gleysol was 6-7 times higher than that of Acrisol. The green manure plants grown on Acrisols would be suffered from P deficiency severely.

To clarify the P deficiency level of the three green manure plants, P concentrations in the plants were presented alongside the total dry weight in Figure 1.6. In this figure, the data within a slightly acidic pH range of 5.3 to 6.8 were shown to exclude the inhibitory effect of soil acidity. Green manure plants typically maintain a specific range of P concentration, but under P deficient conditions, the P concentration in plants decreases, leading to stunted plant growth. Therefore, the P concentration in plants can indicate as an index of P starvation, but not reliable to measure as an indicator for total dry matter production. Typically, plants require a P concentration range of 0.3 to 0.5 % for their optimal growth during the vegetative plant growth stage (Marschner, 2012). On the Acrisol and Gleysol soils, the P concentration in the dried plants ranged from 0.10 to 0.24 % and 0.18 to 0.55 %, respectively. Consequently, the plants grown on the Acrisol under all treatments were suffered from P deficiency. On the Gleysol soil, the plants grown without P addition had lower P concentrations than the standard P concentration range. However, with P fertilization condition, P deficiency was alleviated, and the P concentrations in plants increased to within the standard range. In both soils, the P concentrations of the three plant species were similar, but cowpea consistently had the highest the total dry weights, indicating that cowpea grows best among the three green manure plants.

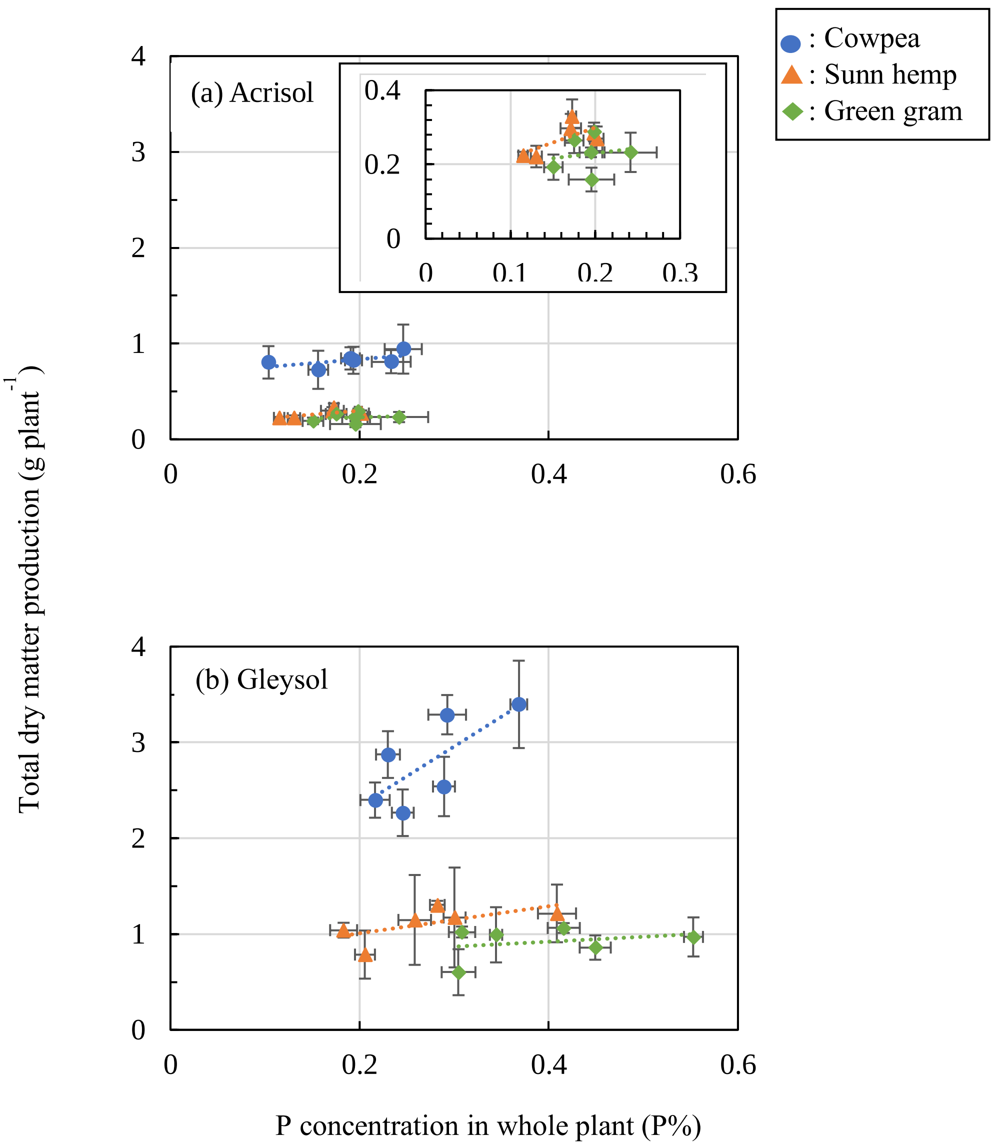

Figure 1.8 Relationship between P concentration in whole plant and total dry matter production of cowpea, sunn hemp, and green gram grown on the soils from (a) a Haplic Acrisol and (b) a Eutric Gleysol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition. (Thae et al., 2024)

Only the data for weakly acidic pH(H_2O) range between 5.3 to 6.8 were plotted in the graphs to exclude the plant growth inhibition observed in strongly acidic soils.

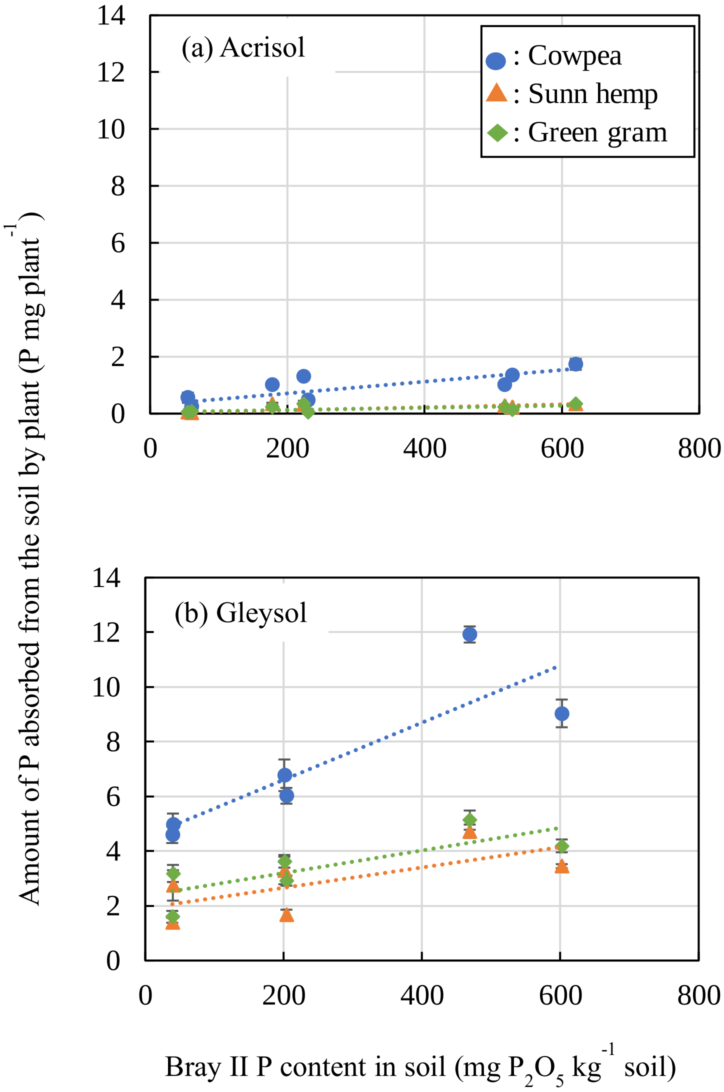

Figure 1.9 Relationship between Bray II P content in soil and amount of P absorbed from soil by cowpea, sunn hemp, and green gram grown on the soils from (a) a Haplic Acrisol and (b) a Eutric Gleysol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition. (Thae et al., 2024)

Figure 1.9 illustrated the amounts of P absorbed by the plants in relation to the available soil P (measured by Bray II P). It demonstrated that despite receiving the same amount of Bray II P, the soil differed in the abilities to supply P. Specifically the green manure plants absorbed more P absorbed from the Gleysol compared to Acrisol, indicating that their P acquisition abilities varied depending on soil types.

The study found that cowpea had a significantly higher ability to acquire P compared to sunn hemp and green gram in both Acrisol and Gleisoil soils. Cowpea grown on Gleysol could acquire 6-7 times greater P than that of Acrisol. However, on the Acrisol, even though the available phosphorus (Bray II P) was increased to 200 to 500 mg P_2O_5 kg^{-1} soil, which is in the recommended range of P-fertilization rate in Japan (MAFF, 2020), the P acquisition by the green manure plants including cowpea was too low. The P acquisition by green manure plants and plants growth were quite lower than that of in Gleysol. This indicates that the Bray II P is not a suitable indicator of available P for the three green manure plants, because it fails to accurately estimate the amount of available P for these plants in those soils. Kato *et al.* (1995) also pointed out that Troug P and Bray II P methods estimated two to three times higher available P in Andosols than the amount of P uptake by maize, and both methods were not correlated with plant P uptake. Sugito and Shinano (2013) also reviewed the results of many studies and stated that the estimation of available P by chemical extraction methods, including Troug P and Bray II P, is not a suitable indicator for plant P uptake. In the present study, it was expressed that the Bray II P would overestimate the available P for the three green manure plants, especially in Acrisols. In the current study, the P acquisition by cowpea in Acrisol was notably low. While cowpea acquire sufficient amount of P from Gleysols, its P acquiring ability would be inadequate on Acrisols even with Bray II P levels are increased to 200 to 500 mg P_2O_5 kg^{-1} soil. Based on this finding, it would be possible to suggest two options; (1) identify other green manure species which have higher P acquisition abilities enough to grow well on Acrisols, and (2) increase the P fertilization rate to counteract P adsorption of these soils.

1.7 Conclusion

In this study, the author examined how three tropical leguminous green manure plants i.e. cowpea, sunn hemp, and green gram respond to growth on two soil types, Acrisol and Gleysol, when fertilized with $CaCO_3$ and phosphorus (P) fertilizer. Cowpea demonstrated the highest dry matter production and phosphorus uptake among the three plants, showing strong tolerance to soil acidity. Growth responses varied depending on the soil type, with better performance on Gleysol compared to Acrisol. Despite the amendments with $CaCO_3$ and P fertilizer, plants grown on Acrisol exhibited reduced dry matter production and phosphorus acquisition, even in cowpea. This suppression is likely due to phosphorus deficiency. The phosphorus uptake ability of these plants appears inadequate for growth on Acrisols. Therefore, it might be necessary to either identify other green manure species with higher phosphorus acquisition capabilities suitable for Acrisols or increase the phosphorus fertilization rate to counteract the high phosphorus adsorption characteristic of these soils.

According to the present results, the author would recommend the farmers in Ayeyarwaddy region where, the massive rice growing area of Myanmar and in Shan State, the important region for upland rice and vegetables production area of Myanmar to choose cowpea as the suitable green manure crop to grow on Gleysol and Acrisol. The findings would be not only helpful for the farmers to reduce their production cost but also educate how to enhance soil quality with less environmental concerns.

Chapter 2

Growth Response of three tropical leguminous green manure plants on Non-allophanic Andosol fertilized with $CaCO_3$ and Phosphate

Abstract

Non-allophanic Andosols are known for their high acidity and phosphorus fixation capacity, which can make difficulties in management for crop production. Specific managements like liming and significant P fertilization are needed to ensure nutrients availability for plants. Leguminous green manure plants play a vital role in boosting crop yields in poor soils by enhancing soil quality with minimal environmental impact. The growth response of some invasive and Japanese native plants was clarified by many scientists. However, the responses of tropical leguminous green manure plants on non-allophanic Andosols are not well understood. The results of the experiment 1 revealed that phosphorus acquisition ability is the key factor of the total dry matter production of green manure plants. In Japan, Andosol is a problematic soil which could not supply required P for the plant growth. Therefore, the authors want to know the P acquisition of tropical green manure plants on Andosol and in this study, the author investigated the growth response of three tropical leguminous green manure plants, i.e., cowpea, sunn hemp, and green gram on an Andosol fertilized with $CaCO_3$, and P fertilizer. Cowpea showed the highest dry matter production and P acquisition among the three green manure plants with high tolerance against soil acidity. The growth responses also varied depending plant species; cowpea > sunn hemp > green gram; the total dry matter production and P acquisition were suppressed in the plants grown on this soil in compares with the previous experiment although the soils were amended with $CaCO_3$, and P fertilizer even in cowpea. Those suppressions would be caused by P deficiency. The P acquisition ability of these plants would be insufficient for growing on Andosols. It would be necessary to confirm P acquisition mechanism of green manure plants to clarify why these plants cannot absorb P from Andosol.

Introduction

2.1 Research Background

2.1.1 Importance of Non-allophanic Andosol in Japan

The term "volcanic ash soils" refers to "Kurobokudo" in Japanese, which means black-fluffy soils. These soils are classified as "Andosols" in the World Reference Base for Soil Resources (WRB) and "Andisols" in the US Soil Taxonomy. The names Andosols and Andisols originate from "Ando soils," with "An" meaning dark and "do" meaning soils in Japanese. These terms emphasize the soil's volcanic origin and distinctive dark color, which is significant in soil classification and agricultural practices.

Japanese soils except those on flood plain have been influenced by Andosol and they occupy a large proportion (31%) of Japanese terrestrial area (Saigusa and Matsuyama, 1998; Obara et al. 2016). Andosols are widely distributed in the greater part of Japan, of which Hokkaido, Tohoku, Kanto, and Kyushu represent the important regions.

Andosols are crucial for agricultural production in Japan due to their unique properties derived from volcanic ash. These soils, characterized by excellent physical properties suitable for crop production, such as high water-holding capacity, permeability, high organic matter content, good tilth, friability, resistance to water erosion, stable aggregation, low bulk density, high porosity with good aeration (Dahlgrenet al., 2004; Shoji and Takahashi 2002). Andosols are important agricultural soils in Japan, covering 50% of Japan's arable land. The slightly acidic and strongly acidic Andosols are typically classified into allophanic (silandic) and non-allophanic (aluandic) Andosols covering 20% and 63% of Japanese Andosols, respectively (Obara et al. 2016). Non-allophanic Andosol showed very strong acidity ($pH<5$), high Al saturation and Al toxicity to plants.

The phosphorous (P) fixation capacity of Andosol is often problematic in agricultural productions (Dahlgren et al., 2004), especially in non-allophanic Andosols, where their strong soil acidity inhibits plant growth because of the high level of exchangeable Al (Al toxicity).

Because of the above challenges, Andosol has negative attribute to crop productivity by limiting nutrient availability for plants. Effective management practices, such as the application of phosphorus fertilizers and soil conditioners are necessary to maximize their agricultural potential. These issues require careful soil management practices, such as lime application to adjust pH and the use of specific fertilizers to overcome nutrient limitations in commercial crop production (Takahashi et al. 2006; Dahlgren et al., 2008).

2.1.2 Crop production in Non-allophanic Andosol

Aluminum ions are the most plant growth inhibiting factor under strongly acidic condition in non allophanic Andosols. Its toxicity is extremely strong, and it has been reported that root elongation is strongly inhibited by approximately 50 μM Al^{3+} in a Ca^{2+} solution (Matsumoto, 2000). Aluminum is the most abundant metal element in soil, accounting for approximately 7%, and it dissolves as highly toxic ions, and especially at pH below 4.5, most of it exists as Al^{3+}, which is the most toxic to plants, so most of the plants become unable to grow. Specific symptoms include inhibition of nutrient absorption caused by inhibition of root elongation (Yamamoto et al., 2003).

The primary issues with non-allophanic Andosols are their high phosphate fixation capacity and acidity. These soils tend to bind phosphate tightly, making it less available to plants, which necessitates significant phosphate fertilization to support crop growth. Additionally, the soil acidity often requires liming to neutralize pH levels and reduce aluminum toxicity. Liming could reduce aluminum toxicity and creating a more favorable environment for root growth and nutrient uptake. Due to high phosphorus fixation capacity, large amounts of phosphorus fertilizers are needed to ensure sufficient availability for crops and to overcome the soil's natural ability to lock away phosphate.

When uncultivated Andosols are converted into arable lands for agricultural use, phosphate fertilizers equivalent to 10% of the phosphate absorption coefficient (PAC) of the soil are applied to ameliorate the Andosols, as described by Yamamoto and Miyasato in 1971. Following World War II, phosphate fertilizers and lime have been used in the agricultural fields of non-allophanic Andosol. Phosphorus (P) is the second most critical macronutrient for plant growth, playing essential roles in plant systems (Hou et al 2020). Low phosphorus availability in agricultural soils significantly affects crop productivity globally. Although farmers often apply heavy doses of P fertilizers, phosphate is quickly immobilized, leading to environmental issues such as eutrophication and a subsequent decrease in biodiversity (Sharpley et al., 2001; Jarvie et al. 2013), particularly in aquatic ecosystems (e.g., Simic et al., 2017; Howell and Dovei 2017).

Alleviating soil pH and enhancing the P availability by green manuring might be a feasible way to improve soil quality with low cost and less environmental concerns. The growth responses of tropical leguminous green manure plants on non-allophanic Andosols are not well understood. The response of some Japanese native and invasive plants was clarified by many scientists. Hiradate et al., (2012) reported that non-allophanic Andosols were deeply related to the difference in habitat between exotic and native plants. Their results showed that Japanese native plants are suited to highly acidic, low-nutrient soils, and exotic plants are suited to weakly acidic, nutrient-rich soils. According to Takehara (2020), Tsumori (2021), Shibata (2021) and Ikeda (2022), invasive plants grow poorly in highly acidic and low nutrients soils. In our previous study, the three tropical leguminous green manure plants could survive on the two Myanmar's slightly and strongly acidic soils; Eutric Gleysol and

Haplic Acrisol. The author would like to understand their response on non-allophanic Andosol which has the same acidity condition with Myanmar's Acrisol. Therefore, in this study, the response of tropical leguminous green manure plants on non-allophanic Andosol will be clarified by alleviating soil pH and available phosphorus (Bray II P) content.

2.2 Hypothesis

The dry matter production and P acquisition ability of the three tropical leguminous green manure plants would vary depending on different soil acidity and available P content of non-allophanic Andosol.

2.3 Objectives of this study

The objectives of the present study were to clarify the growth response and P acquisition ability of the three tropical leguminous green manure plants; cowpea, sunn hemp, and green gram, on non-allophanic alluandic Andsol.

2.4 Materials and Methods

2.4.1 Soil and Seeds

The plant growth response and P availability of Japanese invasive plants on non-allophanic Andosol were severely limited. In my previous study, I clarified the response of tropical leguminous green manure plants on two Myanmar soils; Haplic Acrisol which is strongly acidic and Eutric Gleysol which is slightly acidic. Green manure plants respond to soil pH and Bray II P levels differently, among them cowpea showed the best performance in terms of total dry matter production and P acquisition ability. To check their ability on Japanese non-allophanic Andosol, the soil samples were collected from the A horizon of secondary forest in the Field Science Center, Graduate School of Agricultural Science, Tohoku

University in Osaki, Miyagi prefecture, Japan. The soil was passed through a 5 mm sieve for cultivation experiments and a 2 mm sieve for chemical analyses. The basic physicochemical properties of the soil were listed in Table 2.1.

The seeds of the three green manure plants supplied from the Legumes and Pulses Section, Department of Agricultural Research (DAR), Myanmar were used in this experiment.

Table 2.1 Physicochemical properties of original soil.

Soil	pH		EC (µS /cm)	Exchangeable acidity‡ (mmol/kg)	Available P†‡ (mg P_2O_5/kg)	Total content		C/N
	H_2O	KCl				C§‡ (%)	N⁋‡ (%)	
Andosol	4.5	4.0	122	28	40	16.5	0.8	19.4

† Bray II P. § Carbon. ⁋ Nitrogen. ‡ Calculated based on oven dry weight.

2.4.2 Chemical analysis Soil and Plant

For the measurement of soil pH values in H_2O (pH(H_2O)) and 1M KCl (pH(KCl)), a 2.0 g portion of the air-dried soil sample was mixed with 5.0 ml of deionized water and 1.0 M KCl, respectively. The mixture was allowed to stand for one day and the pH values were measured with a standard pH meter (F-51 HORIBA Ltd., Kyoto, Japan) in the suspension.

To determine exchangeable acidity, an 8.0 g portion of the air-dried soil sample and 20.0 mL of 1.0 M KCl were mixed, shaken for one day, and filtered through a filter membrane (disposable syringe filter unit DISMIC 25CS045AN, pore size 0.45 µm, cellulose acetate, Advantec Toyo, Tokyo, Japan). The extracted acid was determined by titration of the filtrate with 0.01 M NaOH with an auto titrator (Comtite COM-550, Hiranuma Sangyo Co., Ltd., Tokyo, Japan).

For the determination of electrical conductivity (EC), an 8.0 g of soil sample was mixed with 20.0 mL of deionized water, shaken for one day, and filtered through a filter membrane (disposable syringe filter unit DISMIC-25CS, Advantec Toyo). The filtrate was subjected to EC determination by using an EC meter (CM-25 R, TOA Corporation, Tokyo, Japan).

Plant-available P of soils was determined by following the Bray II method; 1.0 g of air-dried soil sample and 20.0 mL of 0.03 M NH_4F in 0.10 M HCl were mixed, shaken for one min, and filtered through a filter paper (Type 5B, Advantec Toyo). The concentration of extracted phosphate in the filtrate was determined by the molybdenum blue method (Murphy and Riley 1962) at a wavelength of 710 nm using a spectrophotometer (UV-Vis Spectrophotometer, Shimadzu Corporation, Tokyo, Japan).

For the determination of total C and N contents, visible plant residues in the soil samples were carefully removed by using tweezers. The soil sample was thoroughly ground using a mortar and a pestle and then analyzed for C and N content with a combustion method (MT-5 and MT-6, CHN coder, Yanaco, Tokyo, Japan).

To determine the amount of P in plants, an oven-dried plant sample was ground, added with concentrated HNO_3, and kept overnight. The sample was digested by heating for three hours at 110°C and again heating for another five hours at 110°C after adding H_2O_2 on a heating unit (DigiPREP MS, SCP SCIENCE, Canada). The digested solution was subjected to total P concentration analysis with the inductively coupled plasma-optical emission spectrometer (Agilent 5800 ICP-OES, Agilent Technologies, USA).

2.4.3 Control of soil pH and P availability

For the growth experiment on the Andosol, the soil pH(H_2O) values were increased from 4.8 to around 5.5 and 6.5 by adding $CaCO_3$ (granulated reagent, NACALAI TESQUE, INC., Tokyo, Japan), and the available P (Bray II P) values of the soils were also increased from 40 to around 200 and 500 mg P_2O_5 kg^{-1} by adding phosphoric acid (85 % H_3PO_4, NACALAI TESQUE, INC., Tokyo, Japan), giving nine soils with different soil pH and available P values (3 soil pH levels × 3 available P levels). The amount of the reagent applied to each soil was summarized in Table 2.2.

Table 2.2 The application rate of $CaCO_3$ (L) and phosphate (P) in the growth experiments on Andosol.

Fertilizer levels	**Andosol**
$CaCO_3$	Application rate (g $CaCO_3$/kg soil) ‡
L_0	0.0
L_1	3.89
L_2	15.59
Phosphate	Application rate (g P_2O_5/kg soil) ‡
P_0	0.0
P_1	0.23
P_2	0.94

‡Calculated based on oven dry weight.

After the soil was mixed well with $CaCO_3$ and P reagent in a plastic bag, it was incubated for 7 days before filling it into a pot. A part of the soil sample was taken, dried, and subjected to chemical analysis.

The untreated soil was strongly acidic ranging from 4.8 to 5.1 in soil pH(H_2O) and low in available P ranging from 40 to 58 mg P_2O_5 kg^{-1} in Bray II P. As shown in Figure 2.1, the soil pH(H_2O) and the available P (Bray II P) were successfully controlled by the addition of P fertilizer.

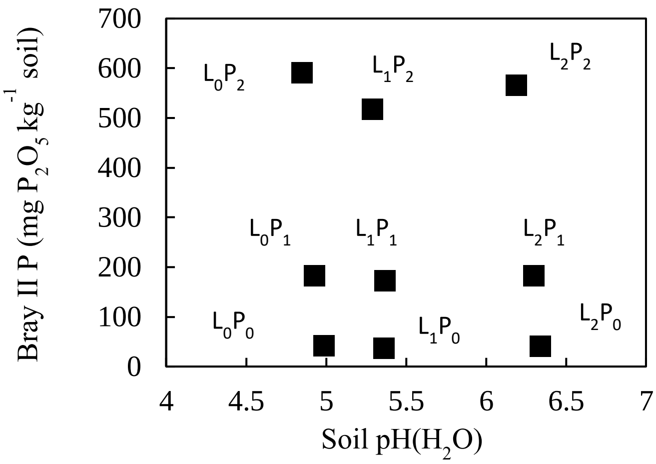

Figure 2.1 Soil pH(H_2O) and available phosphate (Bray II P) values of an Alludaic Andosol, fertilized with and without $CaCO_3$ (L) and phosphate (P).

2.4.4 Crop growth experiment

The crop growth experiment was conducted in a growth chamber (MLR-352H, PHC Co. Ltd., Tokyo), in soil science laboratory, Kyushu University, Japan. Pot experiment was conducted with a split-split plot design (3 × 3 × 3), where three green manure plants, three $CaCO_3$ levels, and three phosphate fertilizer levels were imposed as main plots, sub-plots, and sub-sub plots, respectively. A 52.5 g portion of soil was put into a 100 mL volume pot (top diameter; 50 mm, base diameter; 40 mm, height; 75 mm). The pots were watered with 80% of the maximum water holding capacity. In each pot, three seeds of each green manure species were sown. The weight of each pot was recorded, and deionized water was supplied daily to maintain the weight of each pot constant after sowing. After three days of germination, the number of plants grown in a pot was reduced to one.

Harvesting was carried out 4 weeks after sowing. As soon as harvesting has done, the roots of the green manure plants were washed with the tap water to remove soil particles, then the above ground part (shoot) and the underground part (root) were separated. They were dried in an oven at 70°C for three days to reach the constant dry weight and then the dry weights were recorded.

2.5 Statistical analysis

Using Statistix Version 10 (Analytical Software, Tallahassee, FL., USA), the data of total dry matter and relative dry matter were processed for the analysis of variance (ANOVA) in a split-split plot design to check the statistical significance among the treatments (green manure species, $CaCO_3$ levels, and phosphate fertilizer levels). Mean comparisons were performed by using Tukey's honestly significant difference (HSD) test at a 5% probability level.

2.6 Results and Discussion

2.6.1 Total dry matter production of cowpea, sunn hemp, and green gram

The total dry matter yield of cowpea, sunn hemp and green gram affected by different soil properties of Andosol were shown in Figure 2.2. Total dry matter yield of three green manures were significantly affected by $CaCO_3$ application ($p<0.001$; Table 2.3) but no significant differences were found with response to phosphorus application ($p>0.05$, Table 2.3. In cowpea, the highest total dry matter was produced in L_1P_0, followed by L_1P_1 which is not different from L_2P_0 while the lowest was obtained from L_0P_0. For sunn hemp, the highest dry matter yield was resulted in L_2P_2, followed by L_2P_1 but the lowest were produced in L_0P_0 and L_0P_2. The highest dry matter yield of green gram was observed in L_1P_2, followed by L_2P_2 and that of the lowest were recorded in L_0P_1, L_0P_2, and L_0P_0. The effect of green manures species, $CaCO_3$ application, and phosphorus addition were dependent each other. Among three green manure species, cowpea showed the best performance in term of total dry matter yield which is one of the most important agronomic characters and related to total P and total N as well.

Moreover, cowpea is tolerance to soil acidity and no response to phosphorus application. On the other hand, sunn hemp and green gram were very sensitive to soil acidity, and they cannot grow very well at high acidity and P deficit soil. These two green manures also respond to P application and the highest yield were resulted in higher ratio of lime and phosphorus.

The total dry matter production of cowpea, sunn hemp, and green gram significantly different among three green manure species with affected by different lime levels ($p<0.001$; Table 2.3), however no significant differences were observed with the effects of phosphorus levels ($p>0.05$, Table 2.3).

Cowpea also gave the highest total dry matter production like in the previous experiments on Gleysol and Acrisol, but the yields of three green manure plants were much smaller than the previous experiments (Figure 2.2). The dry matter production was limited even under the addition of $CaCO_3$ and P.

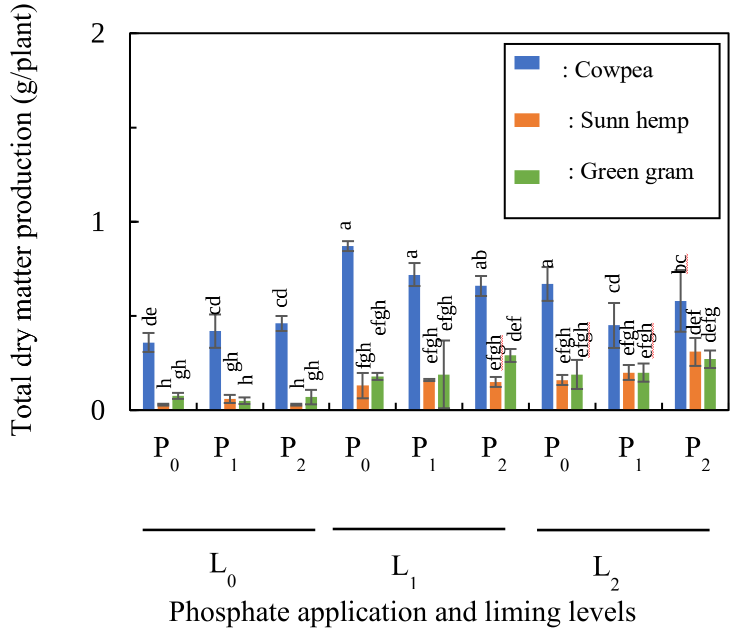

Figure 2.2 Total dry matter production of cowpea, sunn hemp, and green gram grown on the soil from an Aluandic Andosol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition.

For the application rate, see Table 2.2. Mean values with the same letter on bars in each of the three graphs are not significantly different in Tukey's HSD tests ($p < 0.05$). Error bars are the standard deviation of three replicates.

In this study, to clarify the tolerance of the plants against the soil acidity and low P conditions, the relative total dry matter value, which is the percentage of total dry matter value to the highest total dry matter in each green manure species were calculated (Figure 2.3). In this experiment, the relative total dry matter values of the three green manure plants were significantly affected by $CaCO_3$ and P addition (Table 2.3). On the soils without $CaCO_3$ and P addition (L_0P_0), cowpea showed the highest relative dry matter values than sunn hemp and green gram, indicating that cowpea is highly tolerant against soil acidity and low P fertility (Figure 2.3). Poolpipatana and Hue (1994) recommended controlling soil pH 5.5 to 6 for yielding high dry matter production of leguminous green manure plants in acid sulfate soil (Sulfic Tropaquept). Dugganna *et al.*, (1995) pointed out that cowpea showed an outstanding ability to tolerate Al toxicity, although this conclusion is obtained from a solution culture experiment. Therefore, they recommended further research with natural soil conditions to confirm the acidity tolerance of cowpea. The present study revealed that cowpea has the highest acidity tolerance in the Andosol compared with sunn hemp and green gram.

The leaf age of the three green manure species with response to different soil properties of Andosol soil was shown in Table 2.3. The leaf ages of three green manures were significantly affected by $CaCO_3$ and phosphorus levels ($P<0.001$, Table 2.3). Leaf age of green manure plants varied with plant species, obtaining the maximum in sunn hemp, followed by cowpea and the least in green gram. In this experiment, soil acidity tolerance of cowpea was observed but not in sunn hemp and green gram were susceptible. However, the maximum leaf age was produced by sunn hemp, and it was not related to soil acidity tolerance. The leaf age of a plant is influenced by a combination of factors categorized into (1) environmental factors such as light intensity and duration, temperature, water availability and nutrients availability (Chabot and Hicks, 1982; Wright, 2004; Bohnert and Jensen, 1996; Aerts and Chapin, 2000) (2) genetic factors such as specific-specific traits and leaf structure

(Reich et al., 1992; Larcher 2003) (3) physiological factors such as photosynthetic efficiency and hormonal regulation(Field and Mooney 1986; Gan and Amasino, 1997), and (4) biotic factors such as herbivory pathogens and symbiotic relationships (Agrawal, 1998; Smith and Read, 2008). Based on those factors, the leaf age can be varied. However, in our study, under the same growth condition, the leaf age of sunn hemp is larger than cowpea and green gram. The difference might be possible due to the different varietal character of green manure species.

Table 2.3 The probability values obtained from a 3-way analysis of variance (ANOVA) of total dry matter, leaf age, and relative total dry matter† as affected by three green manure species (GMS), level of $CaCO_3$ addition (L), and level of phosphate fertilizer addition (P) on Andosol.

Treatments	**Total dry matter (g/pot)**	**Leaf age**	**Relative total dry matter†**
Species (GMS)			
Cowpea	0.577 a	2.759 b	66.778 a
Sunnhemp	0.168 b	3.537 a	44.407 b
Green Gram	0.137 b	2.330 c	57.148 a
CaCO3 (L)			
L0	0.174 c	1.974 b	27.889 b
L1	0.372 a	3.189 a	69.667 a
L2	0.337 b	3.463 a	70.778 a
Phosphorus (P)			
P0	0.296 a	2.519 b	52.889 b
P1	0.273 a	3.026 a	52.259 b
P2	0.313 a	3.082 a	63.185 a
Source of Variance (Pr>F)			
Spp	0.000	0.000	0.005
L	0.000	0.000	0.000
GMS x L	0.000	0.000	0.001
P	ns	0.000	0.003
GMS x P	0.01	0.000	0.003
L x P	ns	0.000	ns
GMS x L x P	0.01	0.000	0.007
CV (%)	17.69	11.56	21.78

†Calculated by converting the highest total dry matter into 100.

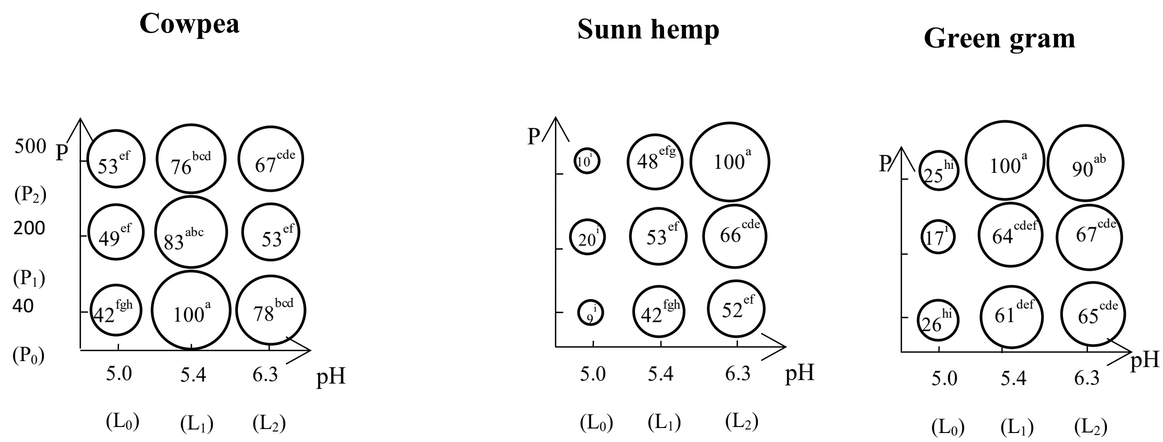

Figure 2.3 Relative total dry matter of cowpea, sun hemp, and green gram in response to three levels of $CaCO_3$ addition (L) and three levels of P fertilizer addition (P) in a growth experiment on Aluandic Andosol

0: no application, 1: low level of application, 2: high level of application. The total dry matter with the highest yield in each green manure species was converted into 100.

2.6.2 Phosphorus acquisition by cowpea, sunn hemp, and green gram

The present study compared the phosphorus acquisition ability of different tropical leguminous green manure plants grown on the different soil properties of Andosol. The total P contents in the whole plant of cowpea, sunn hemp, and green gram were positively and significantly correlated with dry matter production. The amount of phosphorus in the plant of cowpea was the highest among the three green manure plants under different soils properties (Figure 2.4). In this experiment, the same finding was observed like the experiments on Acrisol and Gleysol; cowpea obtained the highest total P than sunn hemp and green gram. The lowest P contents were observed with the plant P contents of 0.14 to 0.92 mg P plant^{-1} and the low P uptake would have caused the low total dry matter production of three green manure plants. The P deficiency would be very severe on the Andosol.

To clarify the P deficiency level of the three green manure plants, P concentrations in the plants were shown with the total dry weight in Figure 2.5. In this figure, the authors compared the data within a weakly acidic pH range between 5.3 and 6.8 to exclude the inhibitory effect of soil acidity. In the pH range, the P concentrations in the dried plants 0.07 to 0.17%. In general, plants need a P concentration range from 0.3 to 0.5% for their optimum growth during the vegetative plant growth stage (Marschner, 2012). It would be possible that the green manure plants grown on the Andosol suffered from severe P deficiency. The P concentrations of the three plant species were almost the same levels, but the total dry weights of cowpea were always the highest, meaning that cowpea can grow the best among the three green manure plants.

Phosphorus acquisition by three leguminous green manure plants is positively and significantly correlated with the P availability in soil (Bray II P), except in sun hemp (Figure 2.6). Cowpea showed the best P acquisition among three green manure plants on Andosol under the same Bray II P improvement. On the Andosol, even though Bray II P addition was increased to 200 to 500 mg P_2O_5 kg^{-1} soil, which is in the range of recommended P-fertilization rate in Japan (MAFF, 2020), the P acquisition by the plants including cowpea was not increased.

In this study, when the three green manure species were cultivated, the growth of cowpea is much higher than the other two species. Except for the low pH soil, the growth of green manure plants will depend on P concentration in plants, but the P acquisition ability for the plant yield will depend on the plant species (Figure 2.4). This means that even though the P concentration range in plants is almost the same level in all green manure species, cowpea can grow well. Besides, the other reason for cowpea growing well is cowpea can acquire P from the soil very strongly. Cowpea produced a good yield under low soil P as it has high P acquire capability (Fagbola et al., 2011). That is why the ability of cowpea to acquire phosphorus is much higher than sun hemp and green gram. Moreover, cowpea obtained the highest P content in the plant among the three green manure plants for all the experiments although the same levels of Bray II P were improved (Figure 2.6).

The green manure plant cannot grow very well even under weakly acidic conditions with P fertilization. The phosphorus acquisition ability of the three green manure plants was significantly different among the plant species, indicating cowpea has the highest ability. Although under the same Bray II P levels and soil pH conditions, the amount of P acquired by cowpea in Andosol were significantly lower than that in Gleysol and Acrisol, the previous experiment. Sun hemp and green gram could not acquire a larger amount of P from the soil in comparison with cowpea The results of this study revealed that cowpea showed the best performance in terms of total dry matter, total P content, and P acquisition among the three green manure plants species, thus it would be the most suitable and important green manure species to correct the low fertile acidic soil.

However, the P acquisition ability of the three green manure plants in non-allophanic Andosol was very low even in cowpea in comparison with the experiments of two Myanmar soils: Gleysol and Acrisol. The Andosol would have a strong affinity to P and limit P acquisition for the tropical leguminous green manure plants. It is lined with findings of many researchers that the invasive and alien plants could not survive well on non-allophanic Andosol compared with Japanese native plant species. To confirm this finding, the author will continue to study the P concentration in the rhizosphere area of the green manure plants.

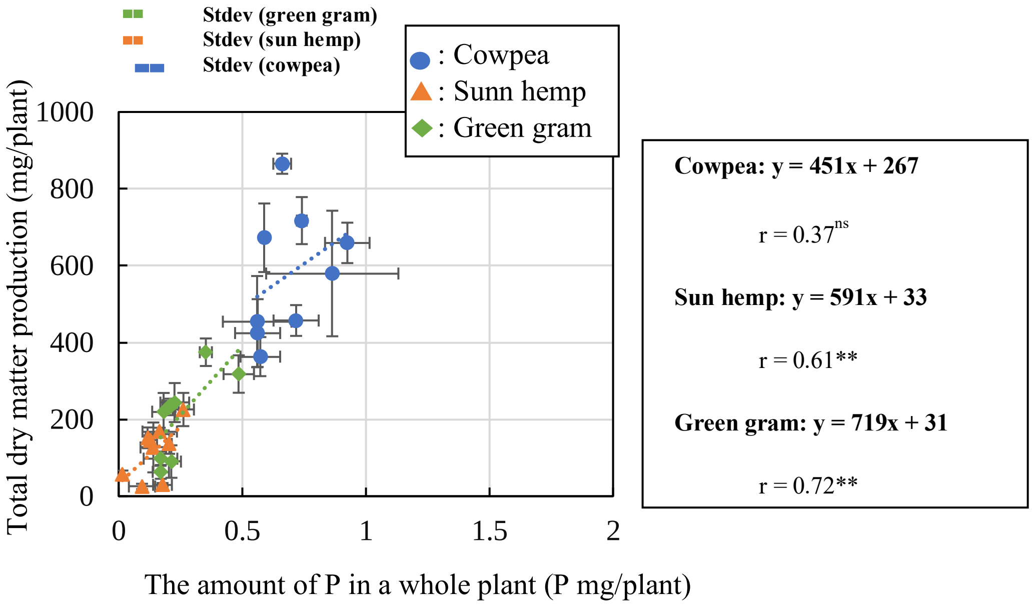

Figure 2.4 Correlation between total phosphorus content in a plant and total dry matter production in a plant of each green manure species with response to different soil properties of an Aluandic Andosol.

The horizontal bars indicated at the top of the graph showed the standard deviation and seed phosphorus content of cowpea (0.58±0.08), sun hemp (0.23±0.02) and green gram (0.23±0.01). *, **, ***: significant at 0.05, 0.01, 0.001 levels (r) respectively.

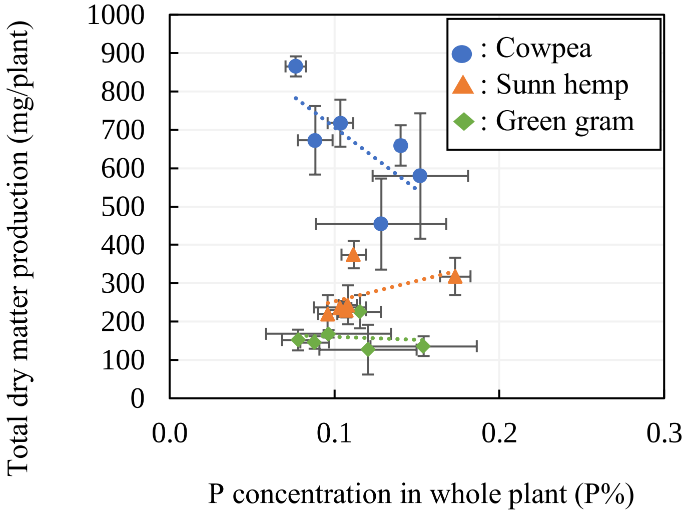

Figure 2.5 Relationship between phosphorus concentration (P%) in a plant and total dry weight in a plant of cowpea, sun hemp, and green gram grown on the soils from an Aluandic Andosol

Only the data for weaky acidic pH(H_2O) range between 5.3 to 6.8 were plotted in the graphs to exclude the plant growth inhibition observed in strongly acidic soils.

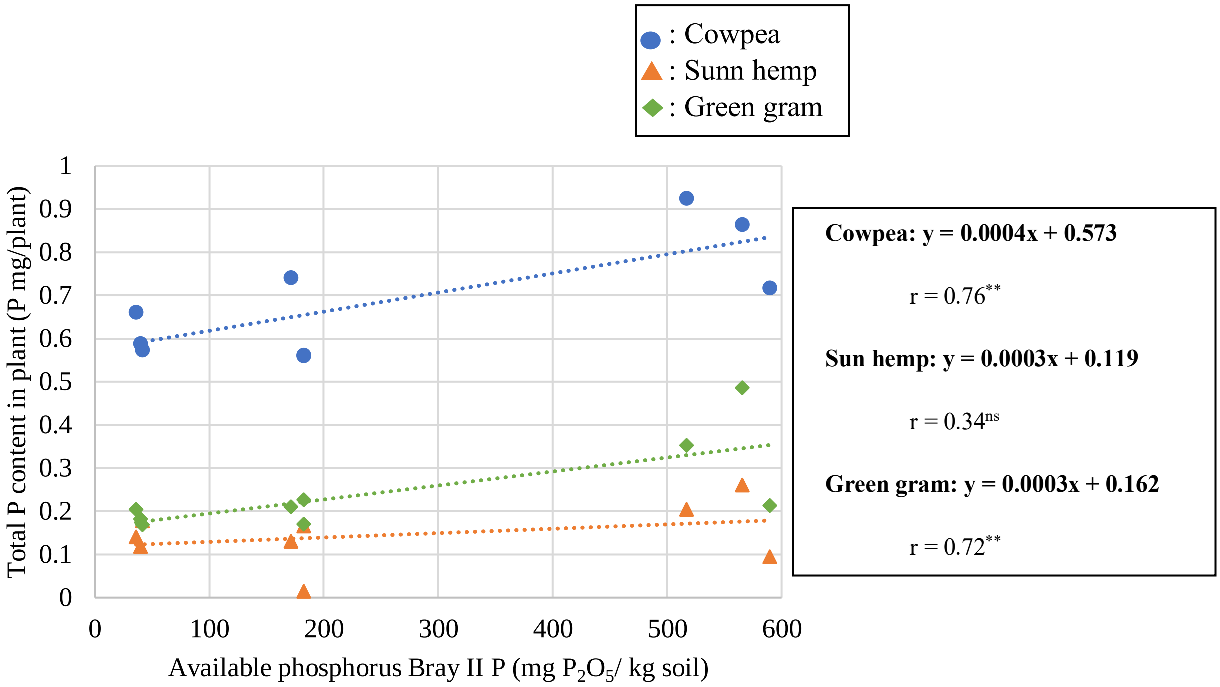

Figure 2.6 Relationship between Bray II P content in soil and amount of P absorbed from soil by cowpea, sunn hemp, and green gram grown on the soils from an Aluandic Andosol fertilized with and without $CaCO_3$ (L) and phosphate (P) addition.

2.7 Conclusion

In this study, the author investigated the growth response of three tropical leguminous green manure plants, i.e., cowpea, sunn hemp, and green gram on a non-allophanic Andosol fertilized with $CaCO_3$, and P fertilizer. Cowpea showed the highest dry matter production and P acquisition among the three green manure plants with high tolerance against soil acidity. The growth responses also varied depending on plant species; cowpea > sunn hemp > green gram; the total dry matter production and P acquisition were suppressed in the plants grown on this soil in compares with the previous experiment although the soils were amended with $CaCO_3$, and P fertilizer even in cowpea. Those suppressions would be caused by P deficiency. The P acquisition ability of these three green manure plants would be insufficient for growing on Andosols. It would be necessary to clarify the P acquisition mechanism of green manure plants to confirm why these plants cannot absorb P from Andosol.

Chapter 3

Study on P concentration in the rhizosphere of leguminous green manure plants on non-allophanic Andosol

Abstract

Nutrient deficiency in most terrestrial ecosystems constrains the global primary productivity. Rhizosphere nutrient availability directly regulates plant growth and is influenced by many factors, including soil properties (pH) and plant characteristic. A quantitatively comprehensive understanding of the role of these factors in modulating rhizosphere nutrient availability remains largely unknown. By conducting growth experiments on Acrisol, Gleysol, and Andosol, the authors found that tropical green manure plants cannot absorb P from Andosol even though available P levels were increased to 500 mg P_2O_5 kg^{-1} soil. Therefore, the author compared and clarified the P acquisition mechanism of the green manure plants and studied the nutrients concentration in the rhizosphere soil solution of three leguminous green manure plants; yellow lupin (*Lupinus luteus*), a temperate green manure, cowpea (*Vigna unguiculata*), sunn hemp (*Crotalaria juncea*) and bare soil (blank) with response to different soil pH of non-allophanic Andosol over time. We observed that P concentration in the rhizosphere of plant growing, and blank conditions varied depending on soil pH levels and they depleted with time. P concentration in the rhizosphere of yellow lupin and cowpea is almost the same although P acquisition ability of yellow lupin is greater than cowpea. The authors estimated that there will be same affinity of P transporter on the surface of the roots of these two plants but different in P uptake activity by yellow lupin's extensive root system and its organic P solubilization effect. Further studies are recommended to clarify more detail about the P acquisition mechanism of green manure plants on Andosol.

Introduction

3.1 Research Background

3.1.1 P deficiency problem in crop production

Global agricultural productivity must increase by up to 70% to meet the food demand of nearly 10 billion people expected to populate Earth by 2050 (Hunter et al., 2017). This challenge must be addressed without expanding arable land and with reduced inputs of fertilizers and pesticides, as failing to do so could lead to greater loss of natural ecosystems and the depletion of rock phosphate reserves (Chowdhury et al., 2017; Sattari et al., 2012; Tilman et al., 2011). Despite soil phosphorus (P) plays crucial role and one of the major nutrients, P deficiency is one of the most common nutritional problems worldwide, threatening plant production and ecosystem stability (Elser et al., 2007; Hou et al., 2020).

The results of our previous crop growth experiments on Acrisol and Andosol revealed that although soil pH and the available phosphorus (Bray II P) contents were alleviated to a reasonable level for the crop growth, the green manure plants could not grow well, and the P acquisition ability of the plants were also too low. Less P acquisition was occurred due to the green manure plants suffering from P deficiency severely. It might be the low concentration of P in the rhizosphere area of the green manure plants especially in the non-allophanic Andosol.

Because the rhizosphere is one of the most pivotal microbial hotspots determining the dynamics and cycling of nutrients; therefore, the plant-microbial interactions are very intense in the rhizosphere (Kuzyakov and Blagodatskaya, 2015). The interaction between roots and microorganisms influences nutrient availability by regulating three key processes: (1) the rate at which plants absorb nutrients, microorganisms immobilize them, or they are released through the decomposition of organic matter; (2) nutrient mobility within the soil; and (3) the transformation between forms of nutrients that are unavailable and those that are accessible. (Darrah, 1993). The original soil properties, plant characteristics, and climate factors are interrelated and influence the above three process groups and modulate the rhizosphere nutrient status (Jones et al., 2004). For example, in low-fertile soil environments, an exudation of organic compounds and protons are activated to maximize the nutrients solubilization in the rhizosphere (Rengel and Marschner, 2005).

Phosphorus (P) concentration in plants varies from 0.05% to 0.50% of their dry weight. This element is essential for numerous functions such as energy production, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme regulation, redox reactions, signaling, carbohydrate metabolism, and nitrogen fixation. The concentration gradient from soil solution phosphorus to plant cells is more than 2000-fold, with the average inorganic phosphate (Pi) concentration about 1 μM in the soil solution (Schachtman et al., 1998). This level is not sufficient for the plant uptake. Crop production on 30-40% of the world's arable land is limited by P availability (Runge-Metzger, 1995; von Uexküll & Mutert, 1995). Phosphorus becomes unavailable because it quickly forms insoluble complexes with cations, especially aluminum and iron, in acidic conditions. The acid-weathered soils in tropical and subtropical regions are particularly susceptible to phosphorus deficiency (Sanchez &Uehara, 1980).

3.1.2 Phosphorus availability in the rhizosphere affected by soil acidity and plant species.

Soil pH is probably the most important chemical soil parameter which reflects the overall chemical status of the soil and influences a whole range of chemical and biological processes occurring in the soil (Bloom, 2000). During plant growth, roots exchange several substances with their environment, thus ultimately modifying the physiochemical conditions of the soil solution and its pH in particular. The pH changes mainly result from the two processes: (1) The buildup of CO_2 partial pressure generated by the roots and rhizosphere microorganisms which can contribute to soil acidification. (2) The major cause of pH changes in the rhizosphere is related to the release of H^+ and OH^- to counterbalance a net excess of cations or anions, respectively, entering the roots. These processes can be affected by environmental stresses; Iron (Fe) and Phosphorus (P) deficiency and the latter is more relevant for acid soil which enhances H^+ release by plant roots.

Phosphorus is one of the major limiting factors for plant growth in many soils. Plant availability of inorganic phosphorus can be limited by adsorption to Fe^- and Al^- oxide/hydroxide surfaces in acid soils, by formation of Fe/Al-P complexes with humic acids (Greke,1992, and Hinsinger, 2001, McDowell and Condron 2000, Gessa et al. 2005), precipitation as Al^-, or Fe^- phosphates as well as sorption to clays and organic matter (Asomaning 2020). Liming is a commonly used agricultural practice on acid soils to maintain an appropriate pH for plant growth and decreasing Al phytotoxicity (Morton and Moir 2018; Bouray et al. 2020).

Phosphorus deficiency can significantly alter the composition of root exudates in away, which is at least some plant species related to an increased ability for mobilization of sparingly soluble P sources (Jones, 1998). In acidic soil, plants elevate pH and optimize rhizosphere nutrient availability by releasing various root exudates, enhancing the uptake of anions or excreting OH^- from nitrate reduction (Sugihara et al., 2016). In contrast, in alkaline soils, roots secrete organic anions (e.g., citrate and malate) and protons, leading to rhizosphere acidification (Sun et al., 2020). One of the ways by which plants attempt to overcome this stress is by reinforcing nutrient availability in the rhizosphere (Hinsinger et al., 2003; Kuzyakov and Razavi, 2019).

Phosphorus (P) availability in the rhizosphere can differ based on the plant species (Richardson et al., 2009; Lambers et al., 2012). Different plants have evolved various strategies to improve P acquisition from the soil, including the exudation of organic acids by roots, modifications in root architecture, and forming associations with mycorrhizal fungi. These variations in root morphology, exudation patterns, and symbiotic relationships can greatly influence P availability and uptake (Richardson et al., 2009).

Growth responses of tropical green manure plants on Andosol was limited although the soil was ameliorated with $CaCO_3$ and P fertilizers. The P acquisition ability of green manure plants also quite low even in P fertilized condition. The P availability in the rhizosphere soil solution will be checked to know pH-P relationship.

3.2 Hypothesis

Investigating the relationship between pH and phosphorus (P) availability within the rhizosphere can offer valuable insights into how soil pH adjustments through liming and different plant species influence P uptake by plants.

3.3 Objectives of this study

This study aimed to investigate changes in phosphorus availability in the rhizospheres of three leguminous green manure plants; cowpea (Vigna unguiculata), sunn hemp (Crotalaria juncea), and yellow lupin (Lupinus luteus) in response to elevated soil pH.

3.4 Materials and Methods

3.4.1 Soil and Seeds

The aim of this experiment is to study the relationship between pH- P availability in the rhizosphere of the green manure plants grown on non-allophanic Andosol which would limit

P availability. The Andosol was collected from the A horizon of secondary forest in the Field Science Center, Graduate School of Agricultural Science, Tohoku University in Osaki, Miyagi prefecture, Japan. The soil was passed through a 5 mm sieve for cultivation experiments and a 2 mm sieve for chemical analyses. The basic physicochemical properties of the soil were listed in Table 2.1.

In this experiment, the seeds of the three green manure plants; yellow lupin supplied from Sakata seed cooperation, Japan and cowpea and sunn hemp supplied from the Legumes and Pulses Section, Department of Agricultural Research (DAR), Myanmar were used. The author selected the yellow lupin as the new leguminous green manure plants due to its popularity on P solubilization under low fertile acidic soil condition. Besides, the P acquisition ability of white lupin and blue lupin are reported in many articles whereas the information for the yellow lupin is limited. Therefore, yellow lupin was used as a new candidate in this experiment to clarify its P acquisition ability.

3.4.2 Soil and Plant Analysis

The soil solution collected from the rhizosphere were subjected for soil solution pH. The 1 mL of each soil solution were measured for pH values with a standard pH meter (F-51 HORIBA Ltd., Kyoto, Japan) in the suspension.

The extracted rhizosphere soil solutions were subjected to total phosphorus, calcium, magnesium, potassium, aluminum, and iron concentration analysis with the inductively coupled plasma-optical emission spectrometer (Agilent 5800 ICP-OES, Agilent Technologies, USA).

To determine the amount of P in plants, an oven-dried plant sample was ground, added with concentrated HNO_3, and kept overnight. The sample was digested by heating for three hours at 110°C and again heating for another five hours at 110°C after adding H_2O_2 on a heating unit (DigiPREP MS, SCP SCIENCE, Canada). The digested solution was subjected to total P concentration analysis with the inductively coupled plasma-optical emission spectrometer (Agilent 5800 ICP-OES, Agilent Technologies, USA).

3.4.3 Control of soil pH

For the growth experiment on the Andosol, the soil pH(H_2O) values were increased from 4.8 to around 5.5 and 6.5 by adding $CaCO_3$ (granulated reagent, NACALAI TESQUE, INC., Tokyo, Japan), giving three soils with three different soil pH levels. The amount of the $CaCO_3$ applied to Andosol was summarized in Table 2.2.

After the soil was mixed well with $CaCO_3$ in a plastic bag, it was incubated for 7 days before filling it into a pot. A part of the soil sample was taken, dried, and subjected to chemical analysis.

3.4.4 Plant growth experiment

The crop growth experiment was conducted in a growth chamber (MLR-352H, PHC Co. Ltd., Tokyo), in soil science laboratory, Kyushu University, Japan. Pot experiment was conducted with a split-split plot design (3 × 3 × 3), where sampling time, three green manure plants, and three $CaCO_3$ levels were imposed as main plots, sub-plots, and sub-sub plots, respectively. An 80 g portion was put into a 100 mL volume pot (top diameter; 50 mm, base diameter; 40 mm, height; 75 mm). The pots were watered with 80% of the maximum water holding capacity. In each pot, five seeds of each green manure species were sown. The weight of each pot was recorded, and deionized water was supplied daily to maintain the weight of each pot constant after sowing. After three days of germination, the number of plants grown in a pot was reduced to three.

The first soil solution data collection was done two weeks after sowing (Figure 3.1). Firstly, the pots were watered with 10:2 hours interval and then the soil solution was extracted from porous cap inserted into the rhizosphere area of plants by the syringe. The sampling was done weekly until 7 weeks after sowing (7WAS).

Harvesting was carried out 7 weeks after sowing. As soon as harvesting has done, the roots of the green manure plants were washed with the tap water to remove soil particles, then the above ground part (shoot) and the underground part (root) were separated. They were dried in an oven at 70°C for three days to reach the constant dry weight and then the dry weights were recorded.

(a) Lupin (b) Cowpea (c) Sunn hemp

Figure 3.1 Crop growth condition of the three green manure at two weeks after sowing (2 WAS)

3.5 Statistical Analysis

Using Statistix Version 10 (Analytical Software, Tallahassee, FL., USA), the data of total dry matter and relative dry matter were processed for the analysis of variance (ANOVA) in a split-split plot design to check the statistical significance among the treatments (sampling time, green manure species, and $CaCO_3$ levels). Mean comparisons were performed by using Tukey's honestly significant difference (HSD) test at a 5% probability level.

3.6 Results and Discussion

3.6.1 Total dry matter production and P acquisition by green manure plants

The growth response of the three green manure plants, yellow lupin, cowpea, and sunn hemp were shown in Figure. 3.2. Under the same $CaCO_3$ (L) level, the green manure plants responded to the soil acidity differently, where the cowpea produced the maximum dry matter in L_2, which is not significantly different from cowpea (L_1) and Lupin (L_2). The least total dry matter was produced from L_0 of sunn hemp. Under strongly acidic condition, the growth response of cowpea grow well, and sunn hemp was sensitive to soil acidity as the same results in our first and second experiment. In this experiment, we observed that lupin is more tolerant to soil acidity than cowpea by producing larger total dry matter at the strongly acidic condition, but the values are not significantly different each other.

Tang and Robson (1993) demonstrated that lupins are particularly well-suited to acidic soils where other crops might struggle. It would be the ability of lupin which are well-adapted to low pH conditions and can efficiently utilize phosphorus (P) even when it is not readily available in the soils. The acidity tolerance of cowpea and lupin would depend on higher seed weight compared to sunn hemp. Fageria et al., (2009) reported that variation in shoot dry weight efficiency index is 86% correlated to seed weight, meaning higher seed weight is directly related to higher acidity tolerance.

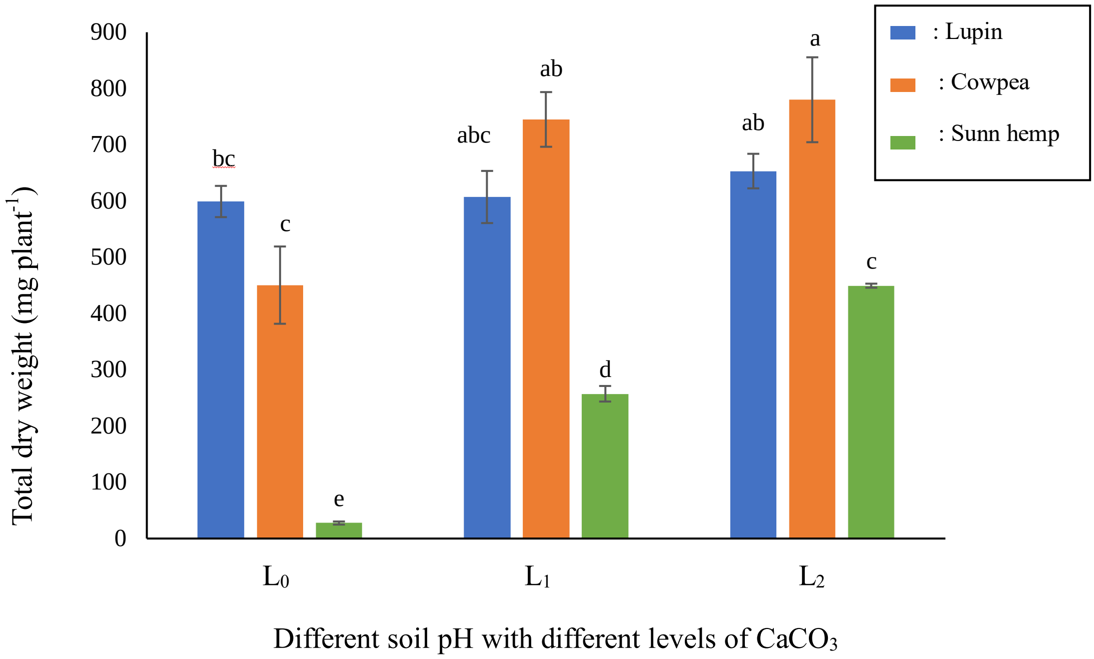

Figure 3.2 Total dry weight of Lupin, cowpea, and sunn hemp grown on the soil from an Aluandic Andosol fertilized with and without $CaCO_3$ (L) addition.

For the application rate, see Table 2.2 Mean values with the same letter on bars in each of the three graphs are not significantly different in Tukey's HSD tests ($p < 0.05$). Error bars are the standard deviation of three replicates.

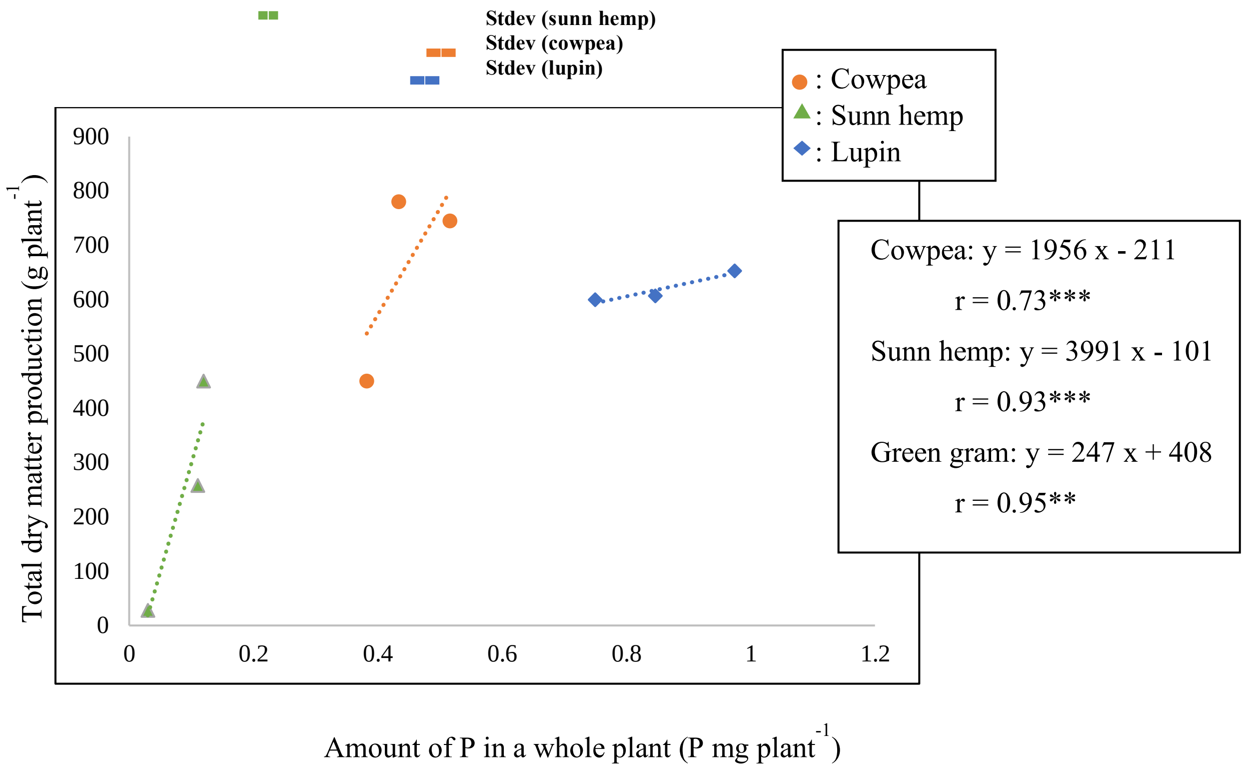

Figure 3.3 Correlation between total phosphorus content in a plant and total dry weight in a plant of lupin, cowpea, and sunn hemp with response to different soil properties of an Aluandic Andosol.

The horizontal bars indicated at the top of the graph showed the standard deviation and seed phosphorus content of cowpea (0.58±0.08), sun hemp (0.23±0.02) and Lupin (0.48±0.07). *, **, ***: significant at 0.05, 0.01, 0.001 levels (r) respectively.

This present study compared the phosphorus acquisition ability (PAA) of different leguminous green manure plants grown on the different soil pH levels of non-allophanic Andosol. Phosphorus acquisition ability refers to a plant's ability to take up phosphorus from the soil. The total P contents in the whole plant of yellow lupin, cowpea, and sunn hemp were positively and significantly correlated with dry matter production. The amount of phosphorus in the plant of lupin was the highest among the three green manure plants under different soil pH (Figure 3.3). In this experiment, the P contents ranges were observed with the plant contents of 0.03 to 0.97 mg P plant^{-1} and the low P acquisition would have caused the low total dry matter production of sunn hemp among three green manure plants. However, in the case of cowpea, the highest P acquisition was not directly related to the highest dry matter production, meaning although cowpea produced the highest total dry matter, its P acquisition ability was lower than lupin. Lupin has the highest P acquisition ability with the highest P uptake from the soil. High phosphorus acquisition ability would depend on the extensive root system of lupin and its solubilization of inorganic phosphate compounds in the soil, making more available P uptake (Vance et al., 2003 and Richardson et al., 2009). Although cowpea has lower phosphorus acquisition ability than lupin, its phosphorus use efficiency (PUE) is higher by producing the highest total dry matter (Figure 3.2). PUE is the ability of a plant to produce biomass or yield per unit phosphorus absorbed form the soil (Veneklaas et al., 2012 and Rose et al., 2013). The amount of P absorbed form the soil were listed in Table 3.1. Lupin can absorb more phosphorus from the soil but in cowpea and sunn hemp, they could not uptake any additional phosphorus from soil, and they could not even take all the phosphorus content in their seeds.

In this experiment, lupin acquired the maximum phosphorus from the soil (0.75 to 0.97 P plant^{-1}) and its phosphorus content in plant under strongly acidic condition (L_0) is 25 times greater than that of sunn hemp and 2 times larger than that of cowpea. It would be possible that the organic acids exudated from the lupin roots chelate with inorganic phosphorus compounds, thereby increasing their solubility and release phosphorus from iron, aluminum, and calcium phosphate complexes in the soil. This was coincided with Gardner et al., (1983), Tang and Robson (1993), Dinkelaker et al., (1995), Neumann et al., (1999) and Lambers et al., (2013).

Table 3.1 The phosphorus (P) content in whole plant, seed and the total P absorbed from the soil by the three leguminous green manures.

Plant species	$CaCO_3$ (L) levels	P content in whole plant (P mg plant^{-1})	P content in seed (P mg seed^{-1})	Total P absorbed from soil
Lupin	L_0	0.75	0.46(±0.07)	0.29(±0.08)
	L_1	0.85	0.46(±0.07)	0.39(±0.10)
	L_2	0.97	0.46(±0.07)	0.51(±0.12)
Cowpea	L_0	0.38	0.58(±0.08)	-0.19(±0.04)
	L_1	0.52	0.58(±0.08)	-0.06(±0.06)
	L_2	0.43	0.58(±0.08)	-0.15(±0.09)
Sunn hemp	L_0	0.03	0.23(±0.01)	-0.20(±0.07)
	L_1	0.11	0.23(±0.01)	-0.12(±0.01)
	L_2	0.12	0.23(±0.01)	-0.11(±0.01)

3.6.2 Available nutrients (P, K, Mg, Ca, Al) in the rhizosphere and blank soil as affected by soil pH

The author studied the nutrient contents and availability in the rhizosphere of the three leguminous green manure plants; yellow lupin, cowpea, and sunn hemp as affected by the soil acidity levels of non-allophanic Andosol. This studies mainly reported the changes in available nutrients; phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), aluminum (Al), and iron (Fe) concentration, in rhizosphere area of plants and in the bare soil (blank) conditions.

The P concentration in the rhizosphere of lupin, cowpea, sunn hemp, and blank were shown in Figure 3.4 (a, b, c, and d). The rhizosphere P concentration is the highest at 2 weeks after sowing (2WAS) in both crop growing and blank condition, however, the P concentration gradually decreased with time. The depletion of P in the rhizosphere soil solution would depend on plant uptake, for instance, at 2WAS, P concentration in lupin and cowpea were lower than that of in sunn hemp which is related with the least growth condition of sunn hemp. P depletion in the rhizosphere by plant uptake is obvious by seeing Figure 3.4 (d), the blank condition that has the highest rhizosphere P at all time interval, especially at 2WAS. Green manure plants absorb phosphorus from the soil for their growth and development. As the plants grow, their demand for phosphorus increases, leading to a depletion of phosphorus in the rhizosphere (Richardson and Simpson, 2011). Another possibility is due to P fixation process. Phosphorus in the soil can become adsorbed onto soil particles or precipitated as insoluble compounds, especially with high level of iron, and aluminum in acidic soils, thus P concentration was less available over time (Hinsinger, 2001).

Calcium concentration in the rhizosphere soil solution were varied depending on $CaCO_3$ addition as illustrated in Figure 3.5. Our data showed that Ca concentration in the rhizosphere also deplete with time. The depletion of Ca concentration would happen due to the plant uptake. As plants grow, their demand for calcium increases, leading to a gradual depletion of calcium in the rhizosphere (Marschner, 1995). Besides, the microbial activity can also impact calcium levels. Certain soil microbes solubilize calcium compounds, making calcium more available to plants, which then uptake it, leading to depletion (Hinsinger, 2001). Moreover, calcium can be lost due to soil exchange complex. Ca is held on the cation exchange sites of soil particles. Over time, other cations like hydrogen (H^+), potassium (K^+), and sodium (Na^+) can displace calcium from these sites, leading to its depletion (Brady and Weil, 2017).

In this study, the magnesium (Mg) and potassium (K) concentration in the rhizosphere showed the same response as illustrated in Figure 3.6 and Figure 3.7. Mg and K concentration were depleted with time in both plant-growing and bare soil conditions. Depletion was more prominent in the higher pH (L_1 and L_2) condition of the plant-growing condition. The depletion would occur due to plant uptake, moreover, the other chemical interactions and biological processes such as ion exchange processes, and increased precipitation and reduced solubility would also decrease the Mg concentration at higher soil pH (Mengel and Kirkby (2001), Brady and Weil (2002), Lindsay (1979). The reasons of K depletion at higher pH in plant-growing condition might be the plant uptake as plants tend to uptake potassium more efficiently at neutral to slightly acidic pH levels. At higher pH, the root membrane potential and the electrochemical gradient that drives potassium uptake can be altered, reducing the efficiency of potassium absorption by plant roots (Marschner, 1995). Other highlighting factors that lead to depletion of K concentration would be increased in cation exchange capacity (CEC) which causes to increased competition between different cations such as Ca, Mg, and K where K is less competitive than Ca and Mg, can be displaced K from the exchangeable sites that leads to K depletion in the soil solution (Brady and Weil, 2002). At higher soil pH, some K compounds may become less soluble, especially when the Ca concentration is high. This could lead to less availability of K in the rhizosphere soil solution for plant uptake (Lindsay, 1979). Moreover, depletion would happen due to microbial activity as some certain soil microorganisms may preferentially uptake K at higher pH and compete with plant roots for nutrients (Sylvia et al., 2005).

The iron (Fe) concentration in the rhizosphere of our study showed that Fe concentration in the slightly acidic condition (L_1, pH around 5.5) was higher than that of in strongly acidic (L_0, pH around 4.8) and nearly neutral (L_2, pH around 6.8) (Figure 3.8). Fe concentration in the rhizosphere is higher under slightly acidic conditions compared to strongly acidic or nearly neutral conditions due to the solubility dynamics, microbial interactions, and the formation of iron compounds. Fe solubility is highly dependent on pH where Fe is more soluble in slightly acidic conditions than nearly neutral or strongly acidic conditions (Lindsay, 1979). Precipitation of Fe compounds at strongly acidic and nearly neutral form insoluble hydroxides and oxides, significantly decreasing for plant available Fe (Schwertmann and Taylor, 1989). Slightly acidic conditions enhance the certain soil microbial activity which can produce siderophores compounds that bind and solubilize Fe and make more available for plants. Strongly acidic or nearly neutral conditions would not provide the optimal microbial activity for siderophore production (Marschner, 1995).

The aluminum (Al) concentration in the rhizosphere soil solution was almost stable at each sampling time in the bare soil condition as well as in the plant-growing conditions (Figure 3.9). The Al concentration did not vary drastically between strongly acidic, L_0 and slightly acidic, L_1 condition. It might be due to the consistent release and solubility of aluminum compounds in the strongly and slightly acidic soils, however, Al concentration in L_2 (nearly neutral) become smaller. Al is more soluble at strongly acidic soil solution pH less than 5, primarily as Al^{3+} ions. It still remains in enough amount even in slightly acidic (pH 5-6) to maintain high concentration. The relatively stable concentration of aluminum in the rhizosphere across strongly acidic and slightly acidic conditions is primarily due to the consistent solubility of aluminum compounds, the soil's buffering capacity, and the formation of aluminum complexes with organic matter. These processes ensure that aluminum remains available in the soil solution over a range of acidic pH levels, preventing significant variation in its concentration. (Lindsay, 1979, Brady and Weil, 2002). In our study, although the Al concentration was higher in L_0 (strongly acidic) and L_1 (slightly acidic), the dry matter production of lupin and cowpea in those pH conditions and L_2 (nearly neutral) condition were not significantly different. However, sunn hemp suffered from soil acidity severely, thus, the dry matter yield among L_0, L_1, and L_2 were significantly different (Figure 3.2). Generally, when soil pH declines below 5, the Al solubility and availability increase. However, the impacts of Al on plants would greatly depend on plant species, therefore, lupin and cowpea were resistant to Al toxicity, could grow well and did not reduce in dry matter production. In the next section, the author will discuss how the nutrients status in rhizosphere change depend on plant species.

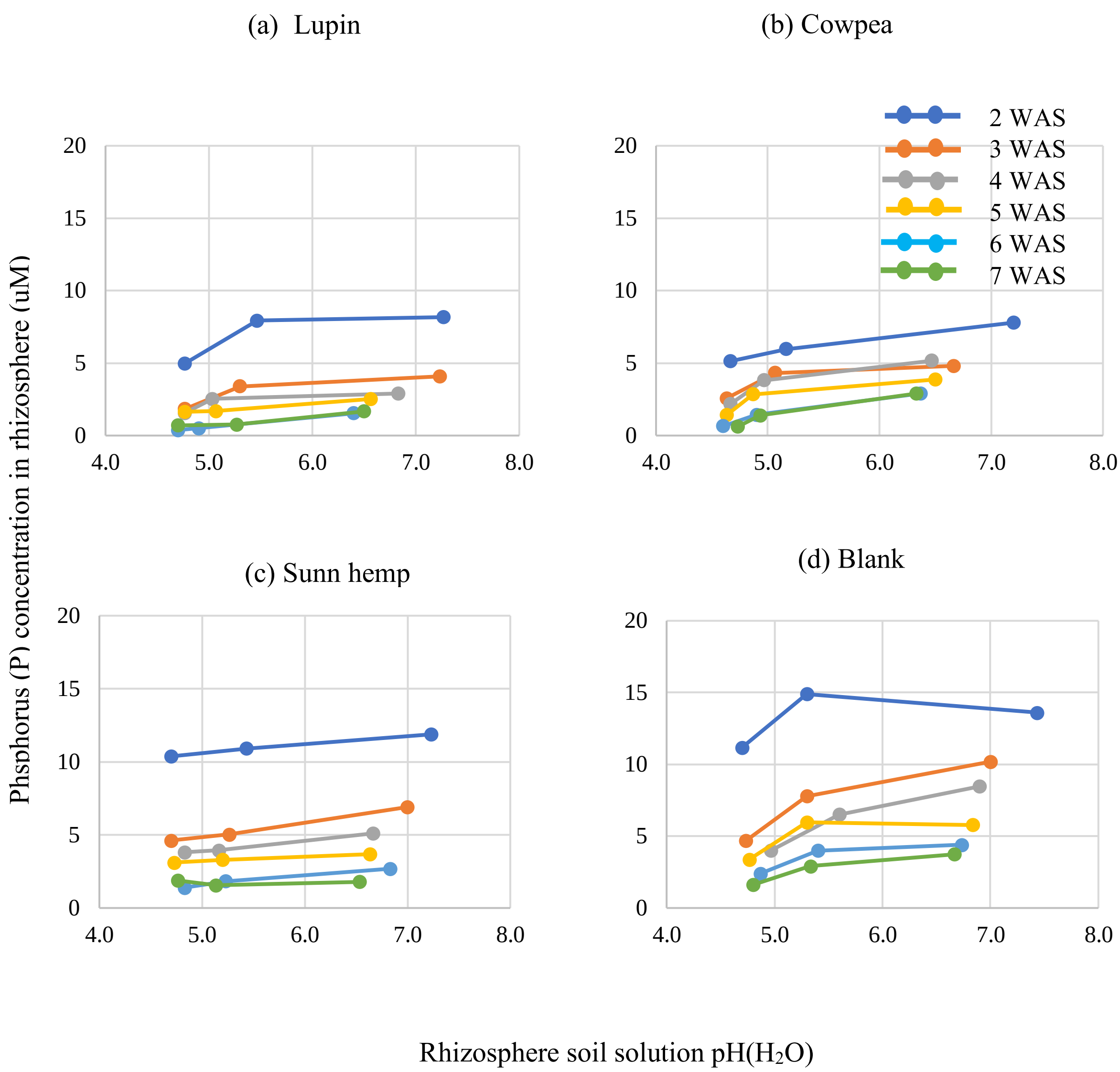

Figure 3.4 Changes in P concentration in the rhizosphere of green manure plants; lupin, cowpea, sunn hemp and blank as affected by rhizosphere soil pH with time.

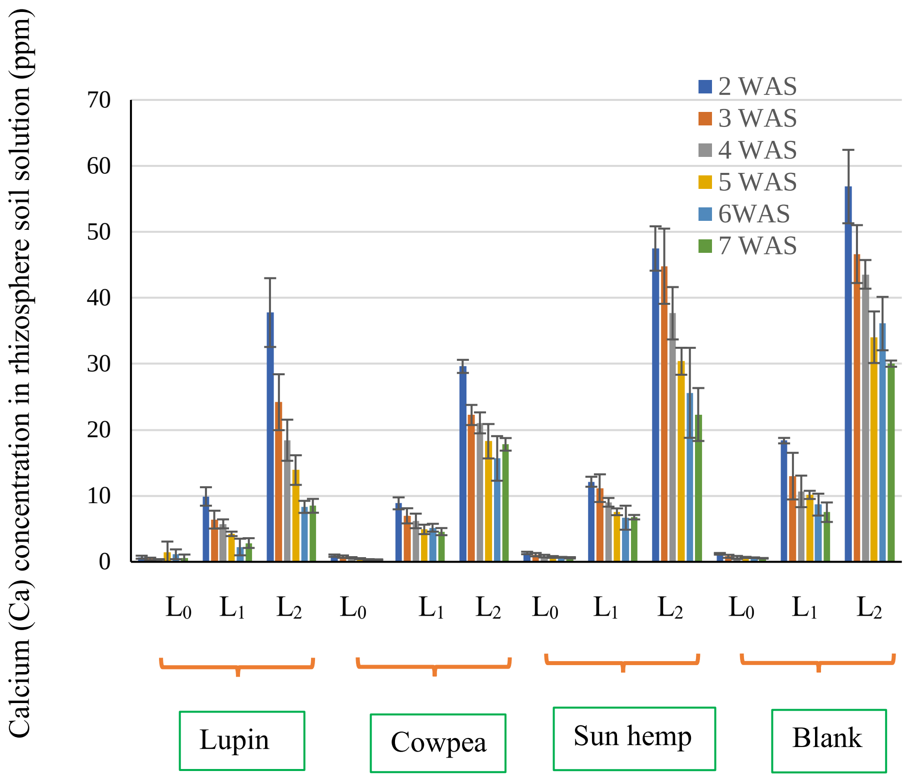

Figure 3.5 Changes in Ca concentration in the rhizosphere of green manure plants; lupin, cowpea, sunn hemp and blank as affected by rhizosphere soil pH with time.

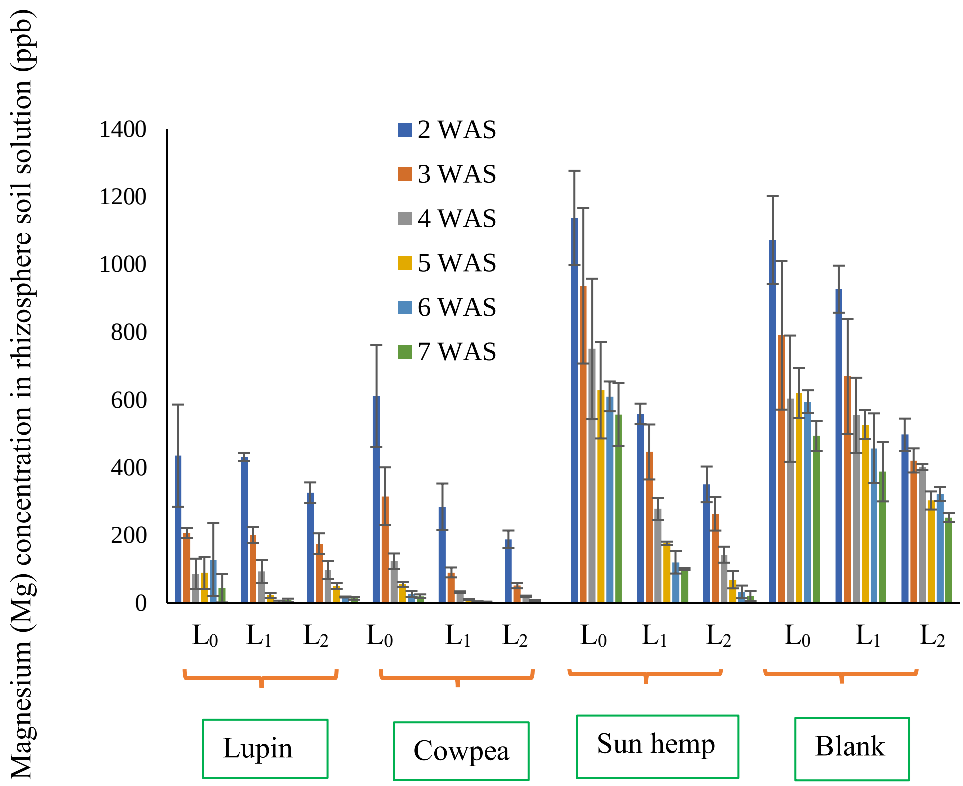

Figure 3.6 Changes in Mg concentration in the rhizosphere of green manure plants; lupin, cowpea, sunn hemp and blank as affected by rhizosphere soil pH with time.

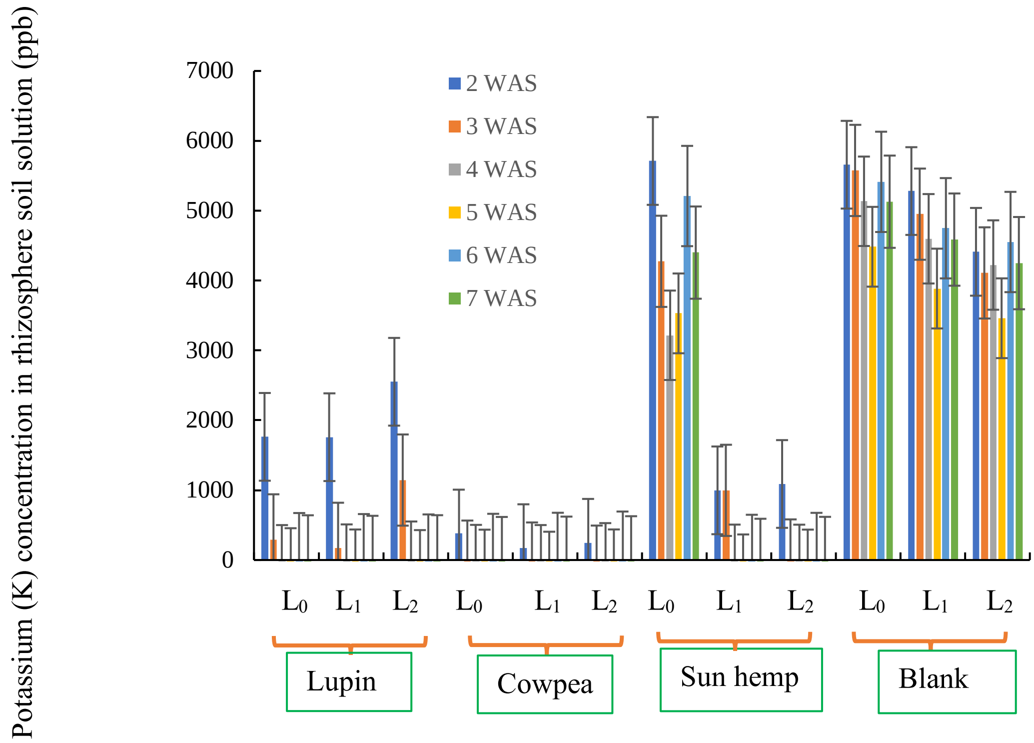

Figure 3.7 Changes in K concentration in the rhizosphere of green manure plants; lupin, cowpea, sunn hemp and blank as affected by rhizosphere soil pH with time.

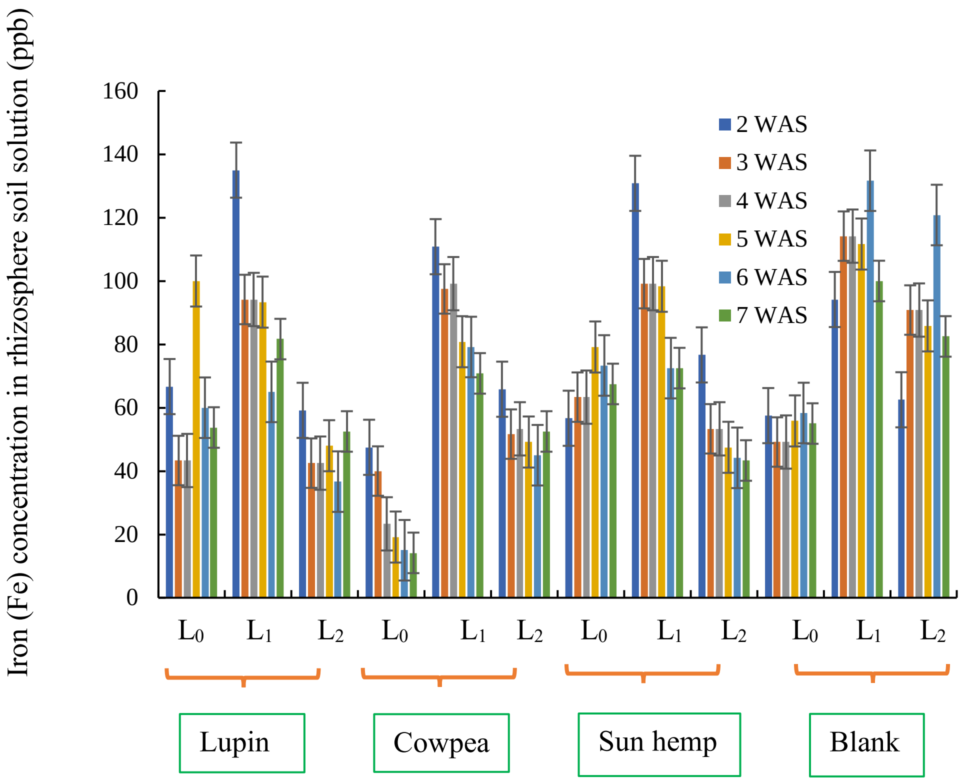

Figure 3.8 Changes in Fe concentration in the rhizosphere of green manure plants; lupin, cowpea, sunn hemp and blank as affected by rhizosphere soil pH with time.

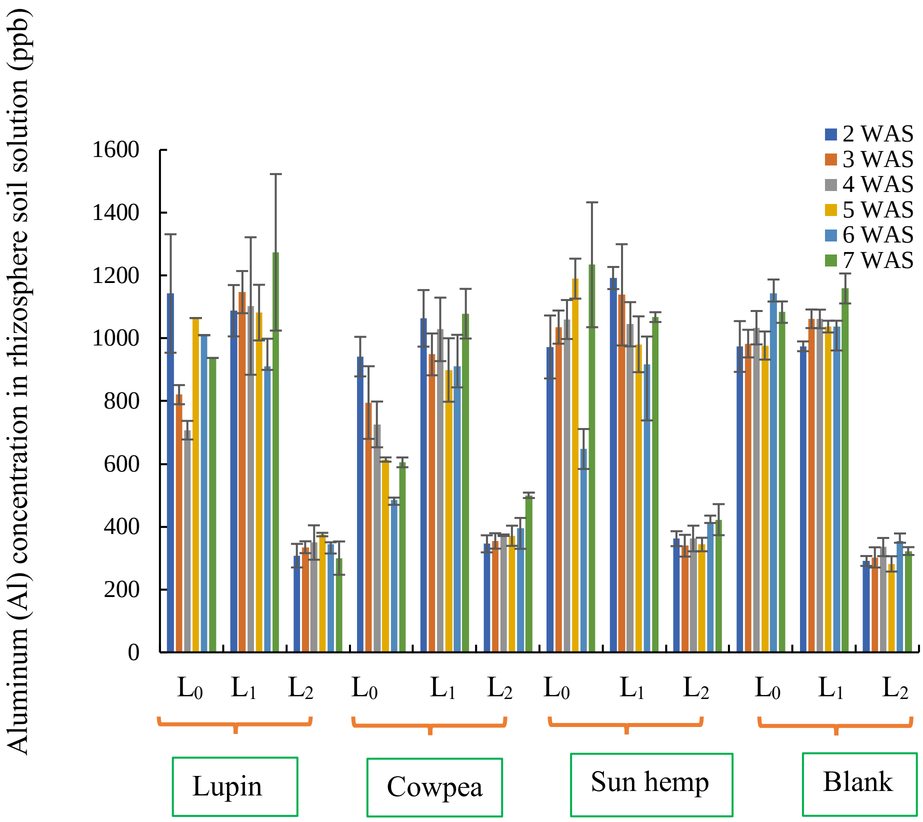

Figure 3.9 Changes in Al concentration in the rhizosphere of green manure plants; lupin, cowpea, sunn hemp and blank as affected by rhizosphere soil pH with time.

3.6.3 Rhizosphere nutrient status as affected by plant species

The rhizosphere is the crucial area for the availability of plant nutrients as it determines their availability. Generally, the availability of most nutrients in the rhizosphere of plant-growing condition is more than bare soil (blank). In our study, The P concentration in the rhizosphere was 2 times lower in comparison with bare soil for lupin and cowpea. However, the concentration in the sunn hemp was almost the same with the blank in L_0 and only 0.5 times lower than blank in L_1, and L_2. We found that sunn hemp cannot grow in strongly acidic condition (L_0), and it died a few days after germination (Figure. 3.10). Joner et al., (1995) and Wang et al., (2007) also stated that the nutrients in the rhizosphere was depleted compared to bare soil. However, in this study, the available P in the rhizosphere in both bare soil and plant-growing conditions were depleted but the depletion in the rhizosphere is more than bare soil.

The available P was drastically depleted in the rhizosphere of all three leguminous green manure plants, indicating extreme demand of P especially for legumes and microorganisms. Liu et al., (2022) interpreted that the available P in the rhizosphere of leguminous plants is more exhausted than non-leguminous plants. Legumes create symbiotic relationships with N-fixing bacteria to increase N availability. For the effective formation of symbiotic relationship with nitrogen fixing bacteria, leguminous plants need to acquire more phosphorus. (Magadlela et al., 2016; Stevens et al., 2019). This is because available P is more exhausted in the rhizosphere of legumes than of other plant species.

In this study, the three leguminous species were used, thus, the P concentration in the rhizosphere was depleted with time. Moreover, it was found that the P depletion was happened in the bare soil condition as well. Phosphorus in the soil in bare condition can become depleted due to the soil chemical reaction known as adsorption which involves phosphorus binding to the soil particles, especially in the presence of iron, aluminum, and calcium compounds (Sanyal and De Detta, 1991). This adsorption process would reduce the amount of available phosphorus in the soil solution with time (Barrow, 1983).

Plants absorb phosphorus primarily in the form of orthophosphate ions, specifically $H_2PO_4^-$ and HPO_4^{2-}, which are present in soil solutions at very low concentrations (0.1–10 µM) (Hinsinger, 2001). Raghothama (1999) pointed out that the optimal pH range for phosphate uptake is between 4.5 and 5.0, indicating that plants preferentially take up $H_2PO_4^-$ over HPO_4^{2-} where in our study optimal P was observed at pH around 5.5. Even though soil P content is larger after liming, its bioavailable P is often just a few ppm. Up to half of the soil phosphorus can be organic, originating from plant residues and soil organisms, and must be mineralized to be accessible to plants (Horst et al., 2001).The concentration of orthophosphate in the soil solution is regulated by several reactions, including the dissolution and precipitation of phosphorus-bearing minerals, the adsorption and desorption of phosphate on soil surfaces, and the hydrolysis of organic matter (Comerford, 1998; Hinsinger, 2001). It is challenging to distinguish between adsorbed and precipitated forms of phosphorus, but phosphorus retention or fixation is highest in the presence of iron and aluminum hydroxylated surfaces and, at higher pH levels, calcium carbonate (Matar et al., 1992; Comerford, 1998). The availability of phosphorus is influenced by the types and amounts of clay and metal oxides, as well as by factors such as soil pH, ionic strength, and the concentrations of phosphorus, metals (Fe, Al, Ca), competing anions, including organic acids (Sanyal & DeDatta, 1991; Hinsinger, 2001) and plant species.

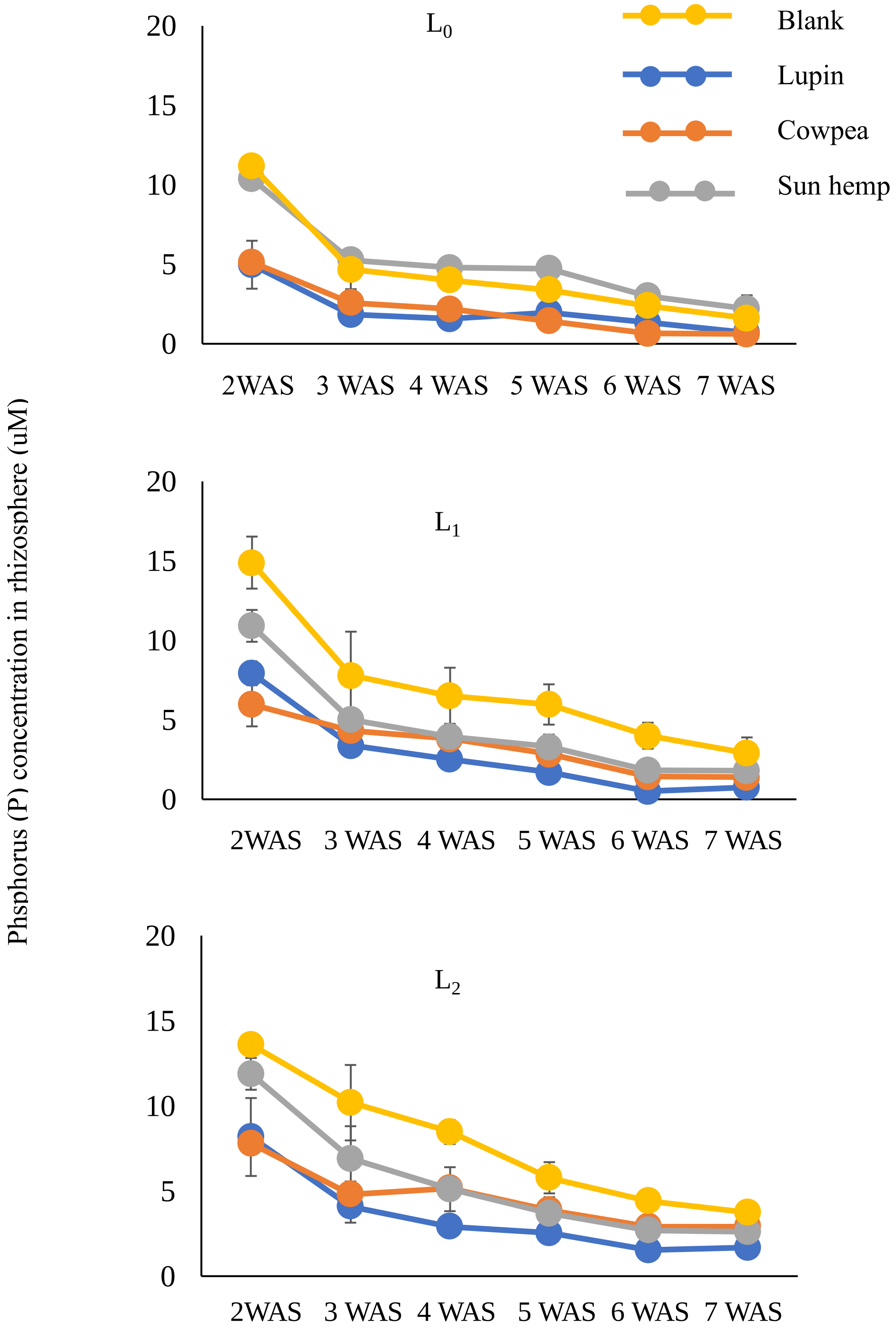

Figure 3.10 Depletion of P concentration in rhizosphere of lupin, cowpea, and sunn hemp at different soil pH

3.7 Conclusion

The aim of the present study was to investigate P availability in the rhizosphere of leguminous green manure plants; yellow lupin, cowpea, sunn hemp, and bare soil condition with response to different soil pH levels of non-allophanic Andosol over time. The result has been shown that the available P in the rhizosphere of green manure plants responded to soil solution pH, giving the highest concentration 5 – 12 and 11- 15 μM in the blank at the first-time sampling (2 WAS). The P concentrations were diminished with time especially in yellow lupin and cowpea directly related with maximum dry matter production of those two plant species. Although the total dry matter production of those two species were not drastically different, the phosphorus acquisition ability of yellow lupin is the greatest among three leguminous green manure plants. In sunn hemp, P depletion is not too much due to least crop growth development and P acquisition ability, and the values are not much different from blank condition. At the last sampling time (7 WAS), the P concentrations in the rhizosphere of both plant-growing and blank conditions were dramatically changed, however, the rhizosphere decreased 20% lower than blank.

The author concluded that depletions of P in the rhizosphere were mainly happened due to some deciding factors, for instance, plant uptake, microbial activities, and soil chemical reaction such as adsorption in the rhizosphere and due to adsorption in the blank. The reasons why tropical green manure plant could not absorb P from Andosol would be the differences in P uptake activity between temperate green manure, yellow lupin and cowpea and sunn hemp, tropical green manure due to genetic variations. Yellow lupin roots have extensive root system and solubilization effect. That would increase the P uptake activity which lead to maximum P acquisition by yellow lupin. However, the subject matter is very complex, and more research is needed to understand the interaction between the plant, the rhizosphere environment, nutrient availability and should clarify P acquisition mechanism more clearly. Therefore, the author will continue to study the P acquistion mechanism of non-allophanic Andosol in the future to confirm the above-mentioned assumption.

Chapter 4

General Conclusion and Future Recommendations

4.1 General Conclusion

The study in the chapter 1 investigated the growth responses of three common tropical leguminous green manure plants; cowpea (*Vigna unguiculata*), sunn hemp (*Crotalaria juncea*), and green gram (*Vigna radiata*) on two different soil types: a highly acidic Haplic Acrisol with low available phosphate (P) and a slightly acidic Eutric Gleysol with a P deficit. By adjusting soil pH and P availability, cowpea produced the highest dry matter and absorbed the most P, with performance varying by soil type. The Gleysol supported better growth than the Acrisol, with cowpea absorbing significantly more P from the Gleysol (6-7 times more than from the Acrisol). However, even with $CaCO_3$ and P fertilizers, cowpea's P acquisition on the Acrisol remained insufficient.

Chapter 2 explored the growth responses of tropical leguminous green manure plants on non-allophanic Andosol, known for high acidity and phosphorus fixation capacity. Despite amendments with $CaCO_3$ and P fertilizers, the total dry matter production and P acquisition on Andosol were lower compared to the previous experiment. This suppression was attributed to the P adsorption ability of Andosol, affecting even cowpea, which showed the highest dry matter production and P acquisition among the three plants with high tolerance to soil acidity. The results clearly showed that sunn hemp and green gram could not survive on strongly acidic soils whereas cowpea can grow with less growth and P acquisition. Therefore, the authors recommended to check P acquisition mechanism of the green manure plants on Andosol.

In Chapter 3, the P concentration in the rhizosphere of cowpea, sunn hemp, and a new green manure species, yellow lupin, was examined by controlling soil pH. Results indicated that although P concentration in the rhizosphere soil solution of yellow lupin and cowpea were the same, the P acquisition ability of yellow lupin is greater than cowpea due to the differences in P uptake activity of yellow lupin. P concentration in the rhizosphere of both plant-growing and blank condition diminished over time due to plant uptake, microbial activity, and the strong P adsorption of Andosols, highlighting the need to assess the P acquisition mechanism of green manure plants on Andosol in more detail.

Overall, the study revealed that the growth response of tropical leguminous green manure plants varies by soil type. Cowpea performed best in terms of total dry matter production and showed high tolerance to soil acidity and P acquisition ability across three soil types: Gleysol > Acrisol > Andosol. Growth inhibition and lower P acquisition in Andosol were due to its high phosphorus affinity. Yellow lupin emerged as a more suitable green manure plant on Andosol, with the highest P acquisition ability even without P addition, although P depletion in the rhizosphere was observed due to P adsorption. This confirms that the growth inhibition and limited P acquisition of tropical leguminous green manure plants especially on Andosol are due to differences in P uptake activity between temperate and tropical green manure plants as the former has extensive root system and soil organic P solubilization effect.

Enhancing the phosphorus use efficiency and acquisition ability in crop production can significantly reduce the environmental impact of P fertilizers and waste, boost the nutritional value of grains, and improve farm economies. More efficient P use within plants not only increases the amount of grain produced by improving P-acquisition efficiency but also minimizes P fluxes in crop land and the environment. The greatest yield benefits from improved phosphorus acquisition ability are anticipated for crops grown in soils with very low P content and where minimal or no P fertilizer is applied. Conversely, the greatest savings in P fertilizer are expected on highly productive land with near-optimal crop growth conditions. Based on the results of the current study, cowpea has higher phosphorus use efficiency on three types of soils, however its P acquisition ability on strongly acidic condition is quite low. Yellow lupin has the highest P acquisition ability from non-allophanic Andosol compared with cowpea and sunn hemp.

4.2 Future Recommendations

The findings indicated that the growth response of tropical leguminous green manure plants varied according to soil types. Cowpea demonstrated the highest performance in terms of total dry matter production and exhibited significant tolerance to soil acidity and phosphorus (P) acquisition across three soil types, ranking Gleysol > Acrisol > Andosol.

The author recommends that cowpea is the most suitable green manure plants for slightly acidic P deficit soil, Gleysol, however, it would be necessary to find out the more suitable green manure plant for strongly acidic Acrisol of Myanmar. For non-allophanic Andosol, tropical leguminous green manure plants; cowpea, sunn hemp, and green gram could not acquire P from that soil, whereas yellow lupin is more suitable green manure plants with obtaining the highest P uptake from Andosol. However, P depletion occurred in the rhizosphere starting from 21 days after sowing due to strong P adsorption capacity of Andosol. Therefore, to confirm P fixation of the Andosol, it would be necessary to conduct the experiments for checking the P adsorption mechanism of non-allophanic Andosol. Besides, searching green manure genotypes with greater P acquisition ability would be great importance in ensuring maximum crop production in in every region of the world, where the soil has low fertility.

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Appendix

(a) (b) (c)

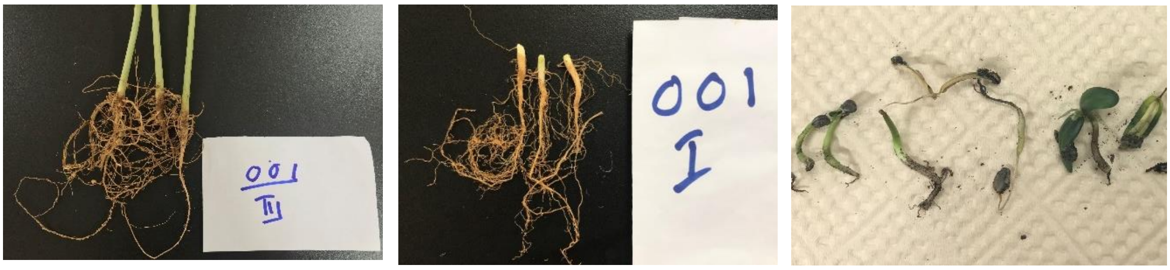
Crop growth conditions of (a) cowpea, (b) sunn hemp, and (c) green gram on a Haplic Acrisol.

(a) (b) (c)

Crop growth conditions of (a) cowpea, (b) sunn hemp, and (c) green gram on a Eutric Gleysol.

(a) (b) (c)

Crop growth conditions of (a) cowpea, (b) sunn hemp, and (c) green gram on an Aluandic Andosol.



(a) (b) (c)

Root development of green manure plants with response to the native soil pH of (a) a Eutric Gleysol (b) a Haplic Acrisol, and (c) and Aluandic Andosol.

Data

1. Soil characteristics of Acrisol, Gleysol, and Andosol (soil pH and available P)

LP	pH(H_2O)			BrayII P (mg P_2O_5/ Kg)		
	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	5.02	5.8	4.99	58	40	41.8
01	5.14	5.7	4.93	230	201	182.5
02	5.2	5.8	4.85	528	469	590
10	5.56	6.7	5.36	61	39	36.17
11	5.6	6.8	5.37	224	204	171.8
12	5.8	6.5	5.29	620	602	517
20	6.5	-	6.34	55	-	39.92
21	6.6	-	6.31	178	-	182.5
22	6.65	-	6.19	517	-	565.7

2. Total dry matter production of Cowpea on three types of soils.

LP	Root dry weight (g/plant)			Soot dry weight (g/plant)		
Cowpea	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	0.06±0.02	0.28±0.04	0.11±0.01	0.53±0.06	1.98±0.2	0.25±0.03
01	0.12±0.03	0.34±0.08	0.11±0.01	0.64±0.19	2.19±0.3	0.31±0.08
02	0.13±0.03	0.58±0.06	0.12±0.01	0.86±0.15	2.82±0.4	0.34±0.04
10	0.13±0.01	0.29±0.1	0.19±0.01	0.67±0.17	2.1±0.2	0.68±0.03
11	0.16±0.02	0.39±0.08	0.14±0.01	0.65±0.14	2.48±0.3	0.58±0.05
12	0.18±0.02	0.41±0.16	0.14±0.02	0.76±0.25	2.87±0.07	0.52±0.04
20	0.18±0.02		0.13±0.03	0.55±0.18		0.54±0.06
21	0.25±0.03		0.08±0.02	0.59±0.13		0.38±0.10
22	0.15±0.02		0.11±0.05	0.67±0.13		0.47±0.12

3. Total dry matter production of Cowpea on three types of soils.

LP	Root dry weight (g/plant)			Soot dry weight (g/plant)		
Sunn hemp	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	0.02±0.01	0.10±0.47	0.002±0.00	0.09±0.02	1.05±0.07	0.02±0.00
01	0.04±0.00	0.10±0.49	0.02±0.01	0.11±0.03	1.07±0.02	0.04±0.01
02	0.04±0.01	0.11±0.27	0.001±0.01	0.19±0.05	1.10±0.03	0.03±0.00
10	0.04±0.01	0.08±0.25	0.05±0.03	0.18±0.01	0.70±0.01	0.08±0.03
11	0.05±0.01	0.10±0.07	0.05±0.00	0.22±0.03	0.94±0.01	0.11±0.00
12	0.06±0.01	0.12±0.06	0.03±0.00	0.22±0.02	1.18±0.02	0.11±0.00
20	0.05±0.01		0.04±0.00	0.17±0.03		0.12±0.02
21	0.09±0.00		0.04±0.00	0.72±0.03		0.16±0.03
22	0.05±0.00		0.05±0.02	0.75±0.04		0.26±0.06

4. Total dry matter production of Cowpea on three types of soils.

LP	Root dry weight (g/plant)			Soot dry weight (g/plant)		
Green Gram	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	0.02±0.01	0.07±0.27	0.01±0.00	0.08±0.03	0.92±0.02	0.06±0.01
01	0.03±0.01	0.06±0.13	0.01±0.01	0.11±0.04	0.79±0.01	0.04±0.01
02	0.03±0.01	0.08±0.19	0.01±0.01	0.14±0.04	0.89±0.02	0.06±0.03
10	0.03±0.01	0.07±0.24	0.03±0.00	0.12±0.03	0.6±0.01	0.14±0.02
11	0.05±0.01	0.09±0.08	0.03±0.01	0.18±0.05	0.93±0.04	0.16±0.01
12	0.04±0.01	0.09±0.05	0.06±0.00	0.25±0.03	1.0±0.01	0.14±0.02
20	0.04±0.01		0.03±0.01	0.16±0.03		0.16±0.01
21	0.04±0.01		0.03±0.01	0.22±0.02		0.24±0.03
22	0.04±0.01		0.03±0.01	0.20±0.01		0.16±0.06

5. Phosphorus concentration and content in plants of cowpea under three types of soils

LP	P concentration in plant (P%)			Amount of P in plant (P mg/plant)		
Cowpea	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	0.11±0.01	0.25±0.01	0.16±0.01	0.63±0.06	5.57±0.38	0.57±0.08
01	0.14±0.01	0.29±0.01	0.13±0.03	1.06±0.14	7.35±0.58	0.56±0.09
02	0.20±0.01	0.37±0.01	0.16±0.03	1.95±0.04	12.50±0.29	0.72±0.09
10	0.10±0.01	0.22±0.02	0.08±0.01	0.83±0.06	5.19±0.31	0.66±0.04
11	0.23±0.02	0.23±0.01	0.10±0.01	1.89±0.08	6.60±0.29	0.74±0.02
12	0.25±0.02	0.29±0.02	0.14±0.01	2.31±0.19	9.62±0.51	0.92±0.09
20	0.16±0.01		0.09±0.04	1.14±0.18		0.59±0.01
21	0.19±0.01		0.13±0.03	1.61±0.10		0.56±0.01
22	0.19		0.15	1.60±0.09		0.86±0.3

6. Phosphorus concentration and content in plants of sunn hemp under three types of soils

LP	P concentration in plant (P%)			Amount of P in plant (P mg/plant)		
Sunn hemp	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	0.10±0.00	0.26±0.02	0.02±0.2	0.11±0.01	2.97±0.56	0.18±0.03
01	0.11±0.00	0.30±0.01	0.03±0.00	0.16±0.01	3.52±0.50	0.02±0.00
02	0.20±0.01	0.41±0.02	0.36±0.1	0.47±0.02	4.95±0.24	0.09±0.05
10	0.11±0.01	0.21±0.01	0.12±0.02	0.26±0.01	1.61±0.17	0.14±0.05
11	0.20±0.01	0.18±0.01	0.09±0.01	0.54±0.04	1.91±0.18	0.13±0.03
12	0.20±0.00	0.28±0.01	0.15±0.03	0.56±0.02	3.68±0.07	0.20±0.01
20	0.13±0.01		0.08±0.01	0.29±0.03		0.12±0.01
21	0.17±0.00		0.10±0.04	0.57±0.04		0.17±0.07
22	0.17±0.01		0.12±0.01	0.51±0.03		0.26±0.04

7. Phosphorus concentration and content in plants of green gram under three types of soils

LP	P concentration in plant (P%)			Amount of P in plant (P mg/plant)		
Green gram	Acrisol	Gleysol	Andosol	Acrisol	Gleysol	Andosol
00	0.15±0.01	0.34±0.01	0.20±0.05	0.15±0.01	3.41±0.31	0.17±0.07
01	0.20±0.01	0.45±0.02	0.35±0.2	0.29±0.04	3.86±0.23	0.17±0.03
02	0.21±0.01	0.55±0.01	0.32±0.1	0.36±0.01	5.36±0.35	0.21±0.03
10	0.20±0.02	0.31±0.02	0.11±0.01	0.31±0.05	1.83±0.22	0.20±0.01
11	0.24±0.02	0.31±0.01	0.10±0.02	0.57±0.11	3.15±0.18	0.21±0.03
12	0.20±0.00	0.42±0.02	0.11±0.01	0.57±0.03	4.42±0.24	0.35±0.02
20	0.15±0.01		0.10±0.01	0.29±0.01		0.18±0.05
21	0.18±0.01		0.11±0.01	0.46±0.04		0.23±0.05
22	0.19±0.01		0.17±0.01	0.45±0.02		0.49±0.06

8. Phosphorus (P) concentraiton (P µM) in the rhizosphere of three green manure plants.

		2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS
L_0	Lupin	4.97±0.51	1.83±0.20	1.56±0.41	1.96±0.03	1.34±0.62	0.70±0.05
L_1		7.93±0.75	3.39±0.66	2.53±0.55	1.69±0.32	0.51±0.12	0.75±0.38
L_2		8.17±0.29	4.09±0.95	2.90±0.21	2.53±0.17	1.53±0.14	1.67±0.54
L_0	Cowpea	5.13±0.58	2.58±0.61	2.18±0.16	1.42±0.20	0.65±0.16	0.62±0.17
L_1		5.97±0.38	4.33±0.62	3.82±0.93	2.85±0.19	1.42±0.38	1.40±0.25
L_2		7.80±0.47	4.81±0.75	5.16±0.14	3.87±0.77	2.90±0.45	2.90±0.82
L_0	Sunn hemp	10.38±0.40	5.27±0.61	4.78±0.41	4.73±0.44	3.01±0.63	2.20±0.85
L_1		10.91±1.00	5.03±0.40	3.95±0.58	3.31±0.77	1.83±0.38	1.80±0.40
L_2		11.88±0.93	6.91±0.90	5.11±0.29	3.68±0.68	2.69±0.28	2.61±0.81
L_0	Blank	11.18±0.31	4.68±0.24	4.01±0.46	3.39±0.21	2.39±0.17	1.61±0.21
L_1		14.89±0.64	7.80±0.75	6.51±0.77	5.97±0.27	4.01±0.82	2.90±0.99
L_2		13.60±0.49	10.19±0.92	8.47±0.70	5.78±0.92	4.41±0.52	3.74±0.23

9. Calcium (Ca) concentration (ppm) in the rhizosphere of three green manure plants.

		2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS
L_0	Lupin	0.69±0.23	0.44±0.18	0.37±0.04	1.42±1.65	1.14±0.74	0.53±0.58
L_1		9.91±1.39	6.39±1.35	5.75±0.68	4.24±0.34	2.24±1.26	2.84±0.74
L_2		37.77±5.2	24.19±4.2	18.43±3.1	13.90±2.2	8.33±0.91	8.49±1.05
L_0	Cowpea	0.93±0.18	0.78±0.18	0.63±0.09	0.48±0.08	0.34±0.07	0.31±0.07
L_1		8.86±0.91	6.97±1.15	6.19±1.11	4.91±0.72	5.17±0.59	4.58±0.54
L_2		29.62±1	22.25±1.5	21.06±1.6	18.27±2.6	15.67±3.4	17.80±1
L_0	Sunn hemp	1.34±0.19	1.09±0.26	0.86±0.20	0.73±0.14	0.69±0.06	0.61±0.10
L_1		12.14±0.76	11.16±2.09	9.02±0.66	7.58±0.52	6.69±1.82	6.76±0.34
L_2		47.48±3.4	44.79±5.7	37.67±3.9	30.39±2	25.61±6.8	22.32±4.0
L_0	Blank	1.22±0.13	0.83±0.23	0.66±0.23	0.67±0.08	0.62±0.05	0.54±0.05
L_1		18.36±0.4	12.99±3.5	10.67±2.4	10.15±0.6	8.67±1.66	7.52±1.48
L_2		56.87±5.6	46.64±4.4	43.56±2	34.04±4	36.10±4	30.03±0.47

10. Magnesium (Mg) concentration (ppm) in the rhizosphere of three green manure plants.

		2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS
L_0	Lupin	436±51	208±15	87±15	89±7	128±10	44±12
L_1		432±12	202±24	93±14	23±7	3±5	8±5
L_2		327±30	176±11	98±16	51±9	18±1	14±4
L_0	Cowpea	612±15	316±15	124±13	56±7	28±9	21±5
L_1		285±19	91±15	33±3	12±1	4±1	3±1
L_2		189±26	52±8	20±3	8±1	-1±1	-2±1
L_0	Sunn hemp	1138±39	938±13	751±8	629±4	611±4	558±9
L_1		559±30	447±11	278±3	177±3	121±3	102±3
L_2		351±13	264±15	143±4	69±19	33±19	22±15
L_0	Blank	1073±12	791±21	604±18	621±4	595±14	494±14
L_1		928±19	670±17	555±11	528±10	458±10	388±18
L_2		498±18	422±16	403±9	303±12	323±12	253±13

11. Potassium (K) concentraiton (ppb) in the rhizosphere of three green manure plants.

		2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS
L0	Lupin	1763±161	288±45	-140±5	-116±14	-45±3	-20±2
L1		1758±149	167±58	-130±4	-134±17	-60±3	-28±0
L2		2551±190	114436	-88±0	-143±20	-65±2	-19±2
L0	Cowpea	379±83	-88±0.5	-137±14	-137±7	-56±2	-44±12
L1		170±23	-115±2	-140±33	-166±14	-41±2	-39±8
L2		247±53	-159±6	-112±16	-133±6	-24±2	-33±5
L0	Sunn hemp	5711±0	4275±0	3217±0	35300	5210±0	4400±0
L1		997±80	997±91	-133±8	-205±2	-70±14	-70±2
L2		1089±95	-71±55	-134±12	-136±2	-43±10	-43±5
L0	Blank	5658±187	5576±41	5135±191	4484±191	5413±99	5128±106
L1		5281±115	4950±32	4598±188	3885±188	4749±94	4586±179
L2		4411±160	4110±21	4222±117	3461±117	4552±62	4249±118

12. Aluminum (Al) concentration (ppb)

		2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS
L0	Lupin	1143±88	821±13.49	707±29	1064±0	1010±0	938±0
L1		1088±82	1147±17	1103±22	1082±9	910±10.6	1273.8±25
L2		308±38	335±19	350±55	375±5	345±30	300±13
L0	Cowpea	942±63	795.4±15	726±73	614±7	486±16	605±16
L1		1064±29	949±16	1029±101	899±101	910±66	1078±79
L2		346±27	355±25	373±2	371±32	396±67	500±9
L0	Sunn hemp	972±20	1036±53	1060±62	1190±23	648±6	1234±19
L1		1192±35	1138±61	1045±70	981±29	917±8	1068±16
L2		362±24	340±35	362±41	343±22	414±2	423±15
L0	Blank	974±19	983±44	1034±53	977±45	1143±25	1083±34
L1		975±16	1062±30	1062±30	1037±19	1037±76	1159±48
L2		291±16	302±32	335±29	282±24	354±5	323±13

Iron (Fe) concentration (ppb)

		2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS
L0	Lupin	67±12	43±9	43±24	100±46	60±28	54±14
L1		135±30	94±7	94±15	93±10	65±16	82±20
L2		59±5	43±4	43±7	48±16	37±1	53±16
L0	Cowpea	48±7	40±22	23±4	19±3	15±3	14±3
L1		111±6	98±11	99±29	81±21	79±12	71±12
L2		66±5	52±6	53±1	49±6	45±9	53±3
L0	Sunn hemp	57±8	63±3	63±5	79±13	73±19	68±12
L1		131±8	99±5	99±17	98±20	73±4	73±5
L2		77±10	53±12	53±8	48±4	44±15	43±8
L0	Blank	58±5	49±15	49±17	56±5	58±9	55±9
L1		94±1	114±25	114±22	112±5	132±16	100±13
L2		63±4	91±9	91±43	86±19	121±18	83±13