BJNRD (2024), 11(2): 11-22
Bhutan Journal of Natural Resources & Development

Research Article

www.bjnrd.org

Open Access

ISSN 2409–2797 (Print)
ISSN 2409–5273 (Online)

DOI: https://doi.org/10.17102/cnr.2024.97

Effects of *Fagopyrum esculentum* (Buckwheat) and *Vigna radiata* (Mung Bean) Cover Crops (Monoculture and Mixture) on Soil Organic Matter, Total Nitrogen, and Available Phosphorus

Pema Tamang[1] and Tenzin Wangchuk[1]*

Abstract

Cover crops play a vital role in enhancing soil health and agroecosystem sustainability. This study evaluated the effects of monoculture and mixed cover crops on soil nutrient dynamics under dry subtropical conditions in Bhutan. The experiment followed a randomized complete block design with four treatments: buckwheat (*Fagopyrum esculentum*) monoculture, mung bean (*Vigna radiata*) monoculture, buckwheat-mung bean mixture, and control (no cover crops). The biomass of the cover crops was sampled destructively 45 days after sowing and then incorporated into the soil. Five composite soil samples were collected at intervals of 15 days to determine the changes in soil nutrients after the incorporation of cover crop residue. By 60 days after residue incorporation, soil organic matter levels were significantly higher in the mung bean (7.64 ± 0.66%) and mixture plots (6.85 ± 0.62%) compared to the control plots (5.41 ± 0.28%). Total nitrogen was also significantly higher in the mung bean (0.38 ± 0.03%), and mixture plots (0.34 ± 0.03%), compared to the control plots (0.27 ± 0.01%). Buckwheat (monoculture) consistently enhanced available phosphorus levels (0.23 ± 0.01 ppm), followed by the mixture (0.14 ± 0.01 ppm), both significantly higher than mung bean (0.06 ± 0.01 ppm) and the control plots (0.09 ± 0.01 ppm). These findings emphasize the potential use of buckwheat and mung bean as cover crop mixtures to enhance soil health through complementary nutrient contributions. This study provides valuable insights for promoting cover cropping practices to address soil fertility challenges in Bhutan.

Keywords: Buckwheat, Cover crop, Mixture, Mung bean, Soil properties

Introduction

Cover crops are grown to enhance farming practices (Treadwell et al., 2008), and are a crucial part of agroecosystems (Alonso-Ayuso et al., 2014). They are typically planted between rotations of main crops usually in the late summer or early fall (Treadwell et al., 2008; Gibson, 2021). Cover crops provide numerous benefits to soil health and increase the yield of succeeding main crops (Magdoff & Van Es, 2021) and are a long-term investment that gradually improves farm management. Utilizing cover crops in crop rotation cycles is one way to decrease soil degradation and sustainably manage soil and its nutrients for increased productivity (Dabney et al., 2001).

Integrating cover crops in the existing cropping system has the potential to enhance

[1] Department of Agriculture, College of Natural Resources, Royal University of Bhutan
*Corresponding author: tenzin.cnr@rub.edu.bt / tenxingwangchuk10@gmail.com
Received: May 15, 2024
Accepted: December 6, 2024
Published online: December 31, 2024
Editor: Rekha Chhetri

ecosystem services (Blanco-Canqui et al., 2015) such as an increase in water infiltration into the soil and reduced surface runoff due to reduced surface seal formation (Ruan et al., 2001). In addition, it increases soil porosity (Hao et al., 2022) and enhances the rates of soil nitrogen mineralization (Kaye & Delgado, 2017). Furthermore, incorporation of cover crops in the soil results in increased organic carbon content (Topps et al., 2021) and reduces pest and disease infestation (USDA, 2022).

However, the benefits of growing cover crops depend on several factors, one of the most important being the selection of cover crop species (Chu et al., 2017). Leguminous cover crops can fix nitrogen in the soil, help minimize erosions, enhance beneficial insect populations, and increase soil organic matter (Clark, 2007). Non-leguminous cover crops can effectively scavenge leftover nutrients from the previous crop and are known for their rapid growth, and high biomass production (Clark, 2007; Magdoff & Van Es., 2021) which can be incorporated into the soil. Since a single species cannot provide all the necessary benefits, using a mix of different cover crop species can be investigated to provide agroecosystems with enhanced multifunctional benefits (Tosti et al., 2014).

The integration of diverse cover crops has shown to optimize nutrient cycling and microbial activity, which are vital for soil health (Gentsch et al., 2024). Combining cover crop species in a mixture increases the benefits through greater complementarity in resource distribution (Finney & Kaye, 2016). Since plants have different resource requirements and functional capacities, a variety of plant species are better at exploiting available resources than its constituent species planted as monocultures (Cardinale et al., 2006). Combining multiple cover crops improves nutrient absorption because of the complementary nature of their root systems, given the varying root system among distinct species of cover crops (Brooker et al., 2014; Bukovsky-Reyes et al., 2019).

In recent years, farming practices in Bhutan have transitioned from traditional methods to more intensive approaches. The continuous cultivation of staple crops, coupled with limited organic matter inputs, can led to a decline in soil fertility and productivity (Imran, 2024). Cover crops, which are widely recognized for their capacity to improve soil health, offer a promising solution to this problem. However, to the best of the author’s knowledge, the practice of growing cover crops has not yet been widely adopted in the Bhutanese agro-ecosystem. Furthermore, research on the use of cover crops in Bhutan is limited, and little is known about how different species might synergistically enhance soil quality in the region’s unique agroecosystems. Although, the benefits of cover crops such as buckwheat (Bulan et al., 2015) and mung bean in monoculture have been studied elsewhere (Abaye, 2020; Jaya et al., 2021), no studies have quantified the benefits of these cover crops in mixture. When grown in mixture they may provide complementary benefits. Buckwheat’s capacity to improve soil organic matter and scavenge residual nutrients (Patel & Paul, 2024), paired with mung beans nitrogen-fixing properties (Favero et al., 2021; Pawar et al., 2024), could enhance soil organic matter, total nitrogen, and available phosphorus. Investigating the effects of this cover crop mixture could inform sustainable practices for Bhutanese farmers, promoting healthier soils and better yields without heavy reliance on chemical inputs. This study aimed to address the potential benefits of growing cover crop mixtures in improving the soil nutrition, offering insights that could support local farmers in adopting cover crop mixtures as a strategy for enhancing long-term soil health and productivity. The objective of this study was to investigate the effects of incorporating cover crops on soil organic matter, total nitrogen, and available phosphorus.

Materials and Method

Study site and experimental design

The study was conducted at the agricultural farm of the College of Natural Resources, Lobesa, Punakha, Bhutan (27°30'N, 89°52'E) from 5th July to 24th October 2023. The experimental site was located approximately 1480 masl under the dry subtropical region. The average air temperature during the study period was 24.4°C and a total rainfall of 320.2 mm was received throughout the experiment (Figure 1) (National Centre for Hydrology and Metrology, 2024). The soil texture of the site was sandy clay loam which contained 52.8% sand, 15.3% silt, and 31.9% clay. The total soil nitrogen content was 0.16%, available phosphorus was 0.22 ppm, and the soil contained 3.13% organic matter (Table 1). Maize was previously cultivated on this experimental site.

The field experiment was laid out in a randomized complete block design with three replications (blocks). The experiment consisted of four treatments i.e. Treatment 1 – Buckwheat monoculture (*Fagopyrum esculentum)*, Treatment 2 – Mung bean monoculture (*Vigna radiata*), Treatment 3 – Mixture (Buckwheat + Mung bean), and Treatment 4 – Control (No cover crops sown but weeds allowed to grow).

Cultural practices

The field was ploughed, harrowed, and labelled to create an ideal seedbed for planting. A total of 12 plots, each with an area of 7.5 m^2 (2.5 m x 3 m) were prepared and a spacing of 0.5 m was maintained between each plot. A total of 166.73 grams of buckwheat and 88.9 grams of mung bean were used for the experiment. This is according to the seed rate of 30 kg ac^{-1} of buckwheat (Myers, n.d.) and 16 kg ac^{-1} of mung bean (Henning & Kilian, 2017). The seed rate for the mixture treatment was determined using the replacement method, where 50% of the seed rate for each cover crop in sole stand was used. This resulted in a mixture containing 83.4 grams of buckwheat and 44.45 grams of mung bean.

The cover crops and the control plots were watered uniformly for the first two weeks and then irrigation was stopped. None of the plots in the study received supplemental fertilizers.

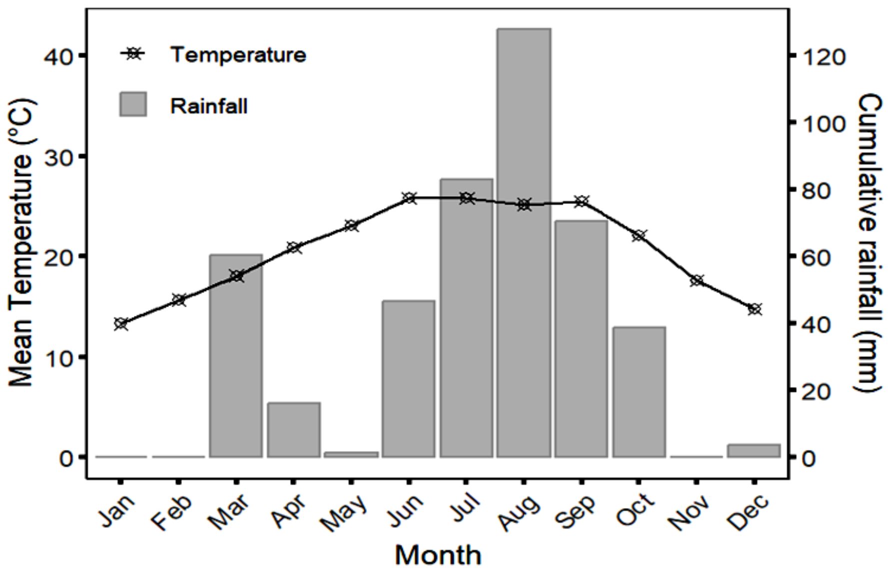

Figure 1: Mean air temperature and cumulative monthly rainfall during the experimental period (5th July - 24th October 2023).

Cover crop biomass

At 45 days after sowing (DAS), destructive sampling of above-ground biomass for all the treatments and control plots was done with the help of a 1 m^2 wooden quadrant to determine dry weight. All the plant materials rooted within the sampling area were clipped at the surface of the soil and were segregated as a cover crop and weed biomass into a labelled paper bag. All fresh plant materials from the 1 m^2 area were dried in the hot air oven at 105ºC for 48 hours and their dry weight was recorded. Following this, all the plant materials from the entire field were manually terminated (on 26th August) by cutting them at the surface of the soil and were incorporated into the soil after chopping them to a size of 5 cm.

Soil sampling and analysis

The soil samples were collected after the termination of all plants and before incorporating the cover crop residues into the soil from each plot. Four composite soil samples at 20 cm depth were taken from each plot every 15 days after the cover crop biomass was incorporated.

The soil analysis was done at the soil and water analytical laboratory, College of Natural Resources. Soil Organic Matter (SOM) content was determined by the Loss on Ignition method (Storer, 1984). Total nitrogen was determined by multiplying the SOM by 0.05 (Dwivedi & Baghel, 2015). Available phosphorus in the soil sample was determined with the Bray No 1 extract using the spectrophotometer (Bray & Kurtz, 1945).

Data analysis

Data were analysed using R statistical software version 4.4.2 (R-Core-Team, 2024). One-way Analysis of variance (ANOVA) was performed to compare differences in cover crop biomass caused by the treatments. Repeated measure anova using mixed model was used to determine the changes in SOM, total nitrogen, and available phosphorus levels between the treatments over sampling date. Whenever the treatment effect was significant, multiple comparisons were carried out to determine the treatment differences within sampling dates, using the Bonferroni post-hoc test using

Table 1: Summary of soil physical and chemical properties at the experimental site for the top 20 cm of the soil profile

Soil parameters	Units	Values
Soil Colour	Hue: 5YR Value: 6 Chroma: 4	Light reddish brown (Munsell colour chart)
Texture	-	Sandy clay loam (Hydrometric method)
Sand	%	52.77
Silt	%	15.27
Clay	%	31.94
pH (H_2O)	-	7.17
EC	µS/cm	124.06
Bulk Density	gcm^{-3}	1.65
Organic Matter	%	3.13
Organic Carbon	%	1.81
Total Nitrogen	%	0.16
Available Phosphorus	ppm	0.22

"lsmeans" package (Lenth & Lenth, 2018). All the statistical tests were performed at 5% level of significance.

Results and Discussion

Cover crop biomass

Dry weights for the aboveground biomass of the cover crop treatments and the control were measured at 45 DAS. The analysis revealed a significant difference in the dry weight of cover crop treatments (p = .002). The buckwheat cover crop produced 723 gm^{-2} of dry biomass, while the mung bean cover crop yielded 663 gm^{-2} of dry biomass (Figure 2). Both the buckwheat and mung bean cover crops showed significantly higher dry weight compared to the control treatment by 50.4% and 38%, respectively. However, the mixture treatment (buckwheat+mung bean), with 590 gm^{-2} of dry matter, did not show significant difference from other treatments in terms of biomass accumulation. Although not statistically significant, the mixture accumulated 22.8% higher biomass compared to the control.

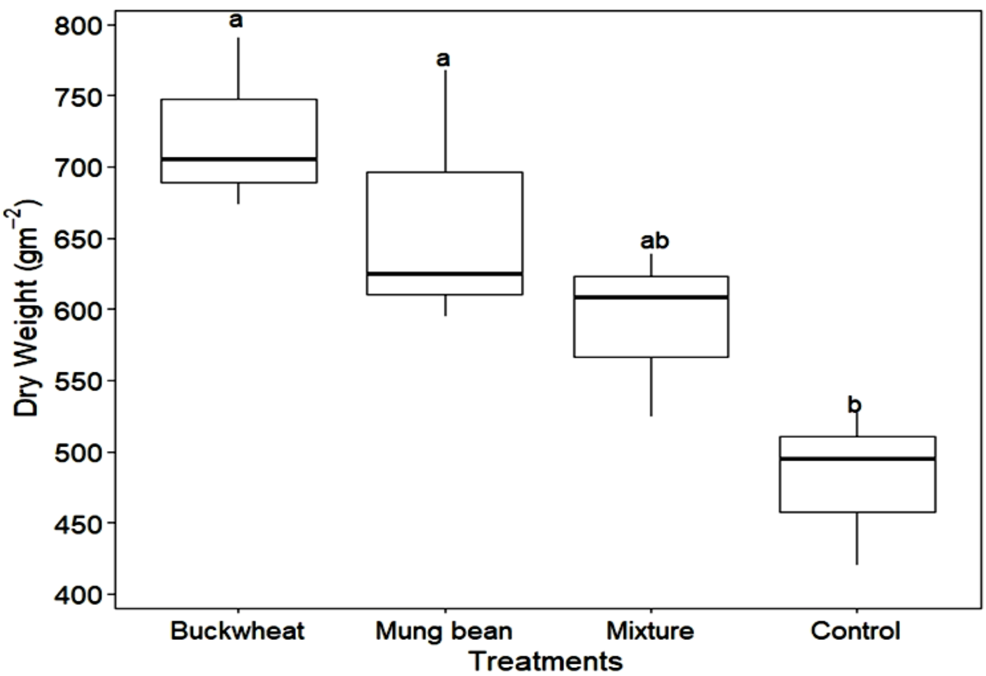

Figure 2: Above-ground dry weight of cover crop treatments and control (weeds) at 45 DAS. Different letters indicate significant differences between treatments (p < .05) as established by the Bonferroni post-hoc test

Combining cover crop species in a mixture increases their potential through greater complementarity in resource distribution (Finney and Kaye, 2016). However, the result contradicted this hypothesis with the mixture of cover crops not being able to outperform the monocultures. This might be the result of inter-species competition between the two species in the mixture due to higher seed rates (Biszczak et al., 2020). Buckwheat plants growing upright at a faster rate (Farooq et al., 2016) could have limited the amount of sunlight causing a shading effect on mung bean plants with vining growth habit. Holmes et al. (2017), in their study comparing the productivity (dry weight) of various types of single cover crop species and mixtures, found no significant difference in dry weight between buckwheat alone, a mixture of buckwheat and cowpea, and a mixture of buckwheat and soybean. Overall, the ability of buckwheat and mung bean cover crops to accumulate a higher quantity of biomass compared to the control showcases the potential benefits of cover cropping in the agroecosystem. The insignificant difference in biomass for the mixture compared to other treatments indicates a similar quantity of biomass production.

Soil organic matter (SOM)

The incorporation of plant residue significantly affected the SOM levels, with variations observed between the different cover crop treatments across the sampling dates ($p = 0.02$) indicating SOM accumulation potential by cover crops with time. Till Day 30 after incorporation, no difference in the SOM level was noted between the cover crop plot and the control plot. However, at 45 and 60 day, mung bean contributed significantly higher SOM levels (7.64±0.66%) compared to buckwheat (6.24±0.48%) and control (5.4±0.28%)(Figure 3). The mixture also contributed significantly higher SOM compared to the control at 60 day. The differences in SOM levels in the plots with buckwheat and mung bean cover crops suggest that different cover crop species may have distinct potentials for improving SOM content. Similar conclusions were also reported by several studies (Carvalho et al., 2011; Demir et al., 2019; USDA, 2023), which demonstrated higher capacity of legume cover crops to increase soil organic matter compared to other species. The mixture treatment did not outperform mung bean as it did in other studies (Florence & McGuire, 2020; Blanco-Canqui, 2022), which could be due to lower biomass production by the mixture in our study (Jaya et al., 2021). However, the insignificant difference in SOM levels compared to the monoculture treatments indicates the equal potential of mixtures to contribute to SOM while providing additional benefits.

Total Nitrogen

The total nitrogen level was also significantly affected by plant residue incorporation, with variations observed among the different cover crop treatments across the sampling dates ($p = .02$; Figure 4). During the first three sampling dates (Day 0, 15, and 30), the total nitrogen levels were similar across all treatment plots. However, by Day 45, total nitrogen was significantly higher in the plots incorporated

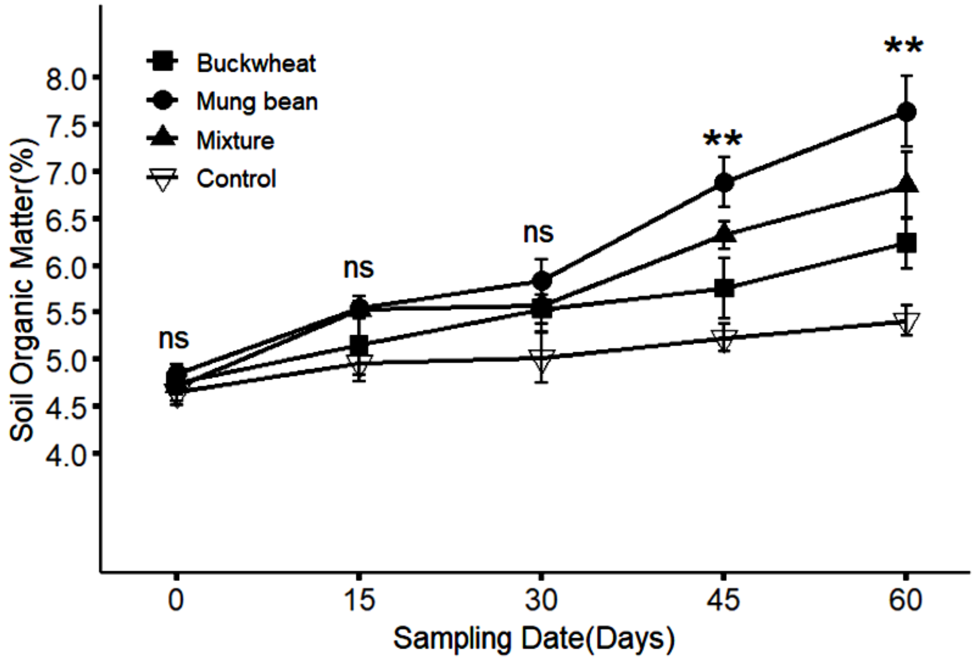

Figure 3: Soil organic matter (%) measured at five different sampling dates from 0-20 cm soil depth. The error bars are the standard error of the mean (n = 3). The asterisks indicate significant differences between the treatments within the same sampling date as established by the Bonferroni post-hoc test (ns = not significant, ** significant at $p < .01$)

with mung bean cover crop residues compared to the control plots. At day 60, total nitrogen was significantly higher in the plots with mung bean cover crop residues (0.38 ± 0.03%) compared to both the control (0.27 ± 0.01%) and buckwheat plots (0.31 ± 0.02%). Additionally, the mixture (0.34 ± 0.03%) contributed significantly higher level of total nitrogen compared to the control. The high levels of total nitrogen in plots incorporated with mung bean compared to buckwheat shows the unique capabilities of legumes to contribute to the soil nitrogen compared to other species of cover crops. This finding aligns with the established phenomenon of atmospheric nitrogen fixation by leguminous species. The effectiveness of leguminous cover crops over non-leguminous cover crops has been reported in various studies (Kuo et al., 1997; Bilenky et al., 2022; Blanco-Canqui et al., 2015; Finney et al., 2016). The mixture with significantly higher amount of total nitrogen compared to the control aligns with the fact that combining non-leguminous cover crops with leguminous cover crops in mixture can increase the total nitrogen content compared to the poorly performing single species (Finney & Kaye, 2016; Chu et al., 2017).

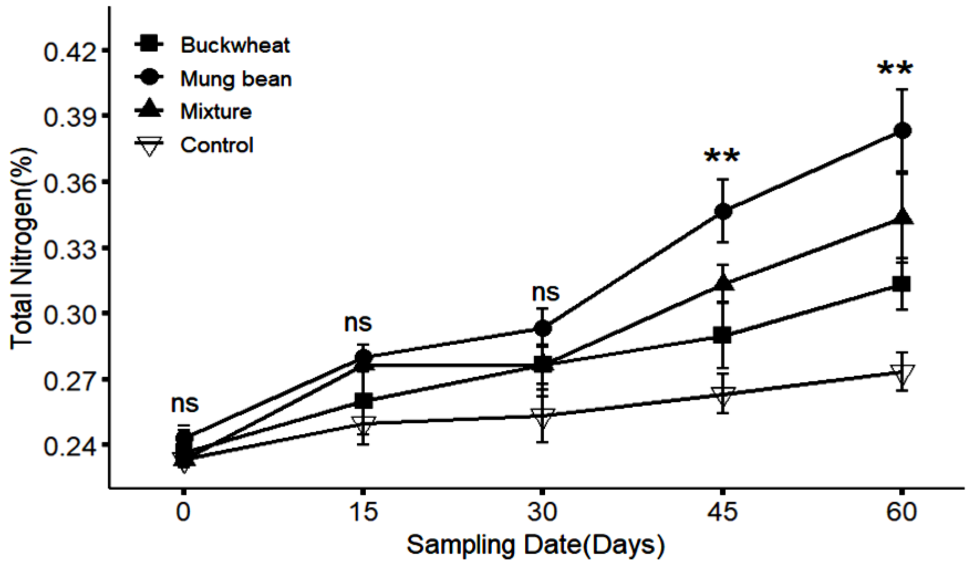

Figure 4: Total Nitrogen (%) measured at five different sampling dates from 0-20 cm soil depth. The error bars are the standard error of the mean (n = 3). The asterisks indicate significant differences between the treatments within the same sampling date as established by the Bonferroni post-hoc test (ns = not significant, ** significant at $p < .01$)

Available Phosphorus

The impact of plant residue incorporation on available phosphorus levels differed significantly among the cover crop treatments over the sampling dates ($p < .001$). The available phosphorus levels of the soil peaked before the incorporation (Day 0) of plant biomass compared to other sampling dates (15, 30, 45 and 60 days) for all the cover crop treatments. At Day 0, available phosphorus level was the highest in the plot cultivated with buckwheat cover crop (0.45 ± 0.01 ppm), followed by mixture (0.38 ± 0.02 ppm), control (0.32 ± 0.01 ppm), and then mung bean (0.21 ± 0.01 ppm) (Figure 5). Subsequently, the available phosphorus level of the soil decreased constantly across the four remaining sampling dates after the incorporation of biomass for all the treatments. Despite this decrease in the levels of available phosphorus across the sampling dates, the plots incorporated with buckwheat cover crop residues consistently maintained the highest available phosphorus levels compared to the other treatments. The plot incorporated with mixture residues also showed significantly higher available phosphorus levels compared to plots incorporated with mung bean and control residues for all the sampling dates. At the 60 day sampling, plots incorporated with buckwheat cover crop residues consistently displayed the highest levels of available phosphorus (0.23 ± 0.01 ppm), followed by the mixture plot (0.14 ± 0.01 ppm). Both the plots incorporated with buckwheat and mixture residues showed higher available phosphorus levels compared to control and mung bean plots (0.09 ± 0.01 and 0.06 ± 0.01 ppm, respectively). The higher

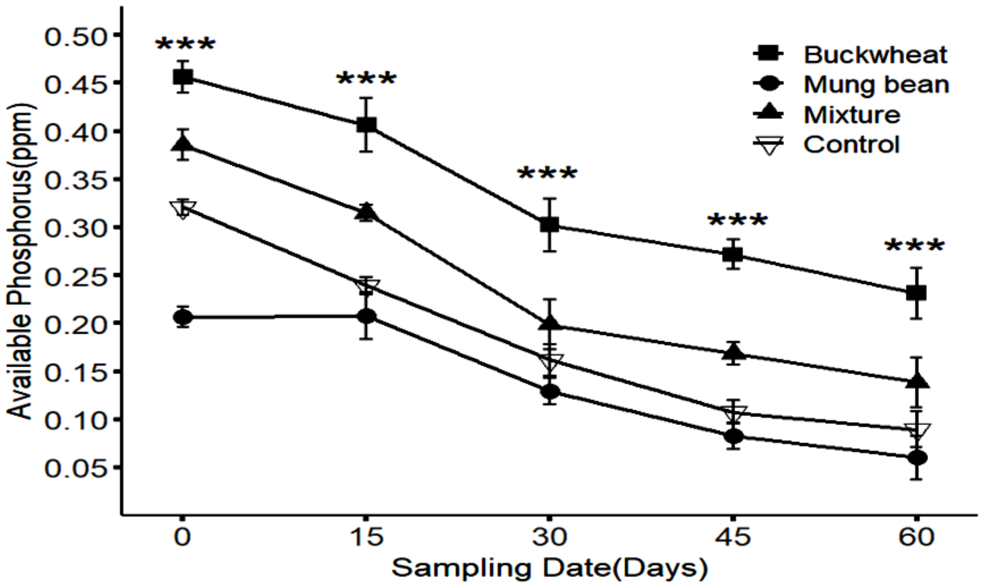

Figure 5: Soil available phosphorus measured at five different sampling dates from 0-20 cm soil depth. The error bars are the standard error of the mean (n = 3). The asterisks indicate significant differences between the treatments within the same sampling date as established by the Bonferroni post-hoc test (*** significant at $p < .001$)

level of available phosphorus in the plots cultivated with buckwheat indicates its efficacy in increasing soil phosphorus availability in the early stages of decomposition, likely attributed to the root exudates such as organic acids and enzymes, facilitating phosphorus solubilization and mobilization (Amann & Amberger 1989; Zhu et al., 2002; Possinger et al., 2013). The decline in available phosphorus after the initial peak suggests that the phosphorus that was released during the breakdown of cover crop biomass was either slowly adsorbed or immobilised in the soil matrix (Kaur et al., 2024). Factors such as runoff, erosion, and leaching also could have contributed to its declining level (Prasad & Chakraborty, 2019). This decrease in the available phosphorus level after crop residue incorporation was also reported by Mahmood et al. (2013) who attributed this decline to the increased phosphorus fixation due to the presence of more active sites for phosphorus adsorption.

Buckwheat contributing to the highest phosphorus level could be related to its roots' ability to acidify the rhizosphere mildly during the growth phase and therefore to release nutrients from the soil (Zhu et al., 2002). Also, the movement and relocation of phosphorus from the sub-surface soil layers through a well-developed root system to the surface soil with the decomposed residues could have increased phosphorus availability (Boglaienko et al., 2014). The mixture contributing more to available phosphorus compared to mung bean can be explained by the presence of buckwheat as one of its components which is a great phosphorus scavenger. This indicates the potential benefit of mixing legumes with phosphorus-scavenging cover crops such as buckwheat to improve phosphorus availability.

Conclusions

This study demonstrates the potential benefits of incorporating buckwheat and mung bean cover crops, as well as their mixture, into agricultural systems to improve soil nutrition. Buckwheat and mung bean cover crops showed significantly higher biomass accumulation compared to the control, with mung bean exhibiting superior contributions to soil organic matter (SOM) and total nitrogen, highlighting its role as a leguminous cover crop in nitrogen fixation. Buckwheat consistently enhanced available phosphorus levels, likely due to its phosphorus scavenging ability and rhizosphere acidification. Although the mixture of buckwheat and mung bean did not outperform the monocultures in biomass production, it demonstrated comparable contributions to SOM, total nitrogen, and phosphorus, underscoring the potential for complementary benefits when combining species. These findings highlight the value of cover cropping in enhancing soil health, offering practical insights for farmers to adopt cover crop strategies that align with the goals of sustainable agriculture and long-term productivity.

Acknowledgments

The authors express sincere appreciation to the College of Natural Resources for the invaluable opportunity and resources extended for this research endeavour. Gratitude is extended to Mr. Sonam Moktan, Mr. Ugyen Tenzin, Dr. Rekha Chhetri, and Ms.Yangchen Ghishing for their significant contributions.

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