SOIL ORGANIC CARBON AND FUNGAL POPULATION IN DIFFERENT LAND USE TYPES OF CHAPAGAUN, LALITPUR

A THESIS

SUBMITTED FOR THE

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THE MASTER'S DEGREE IN BOTANY IV SEMESTER

BY

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DECLARATION

I, *Sabina Thokar*, hereby declare that this work is wholly my own, except where explicitly cited by references or acknowledgements. It has neither been published nor submitted in full or in part for any other degree or professional qualification. All sources of literature, data, or other works cited in this thesis have been given due acknowledgement and are included in the reference list.

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ACRONYMS AND ABBREVATIONS

SOC: Soil Organic Carbon

LUC: Land Use Change

SR: Soil respiration

CO2: Carbon-dioxide

IVI: Importance Value Index

C: Carbon

N: Nitrogen

TN: Total Nitrogen

AGTB: Above Ground Total Biomass

CFU: Colony Forming Unit

PDA: Potato Dextrose Agar

GHGs: Green House Gases

F: Forest land

Ag: Agriculture land

P: Pasture land

Figure: Figure

GF: Ginseng Farmland

YF: Oak Young Forest

mg: Milligram

Kg: Kilogram

MBC: Microbial Biomass Carbon

SOM: Soil Organic Carbon

AGSB: Above Ground Soil Biomass

DEF: Dry Evergreen Forest

CRP: Conservation Reserve Program

AC: Active Carbon

DHM: Department of Hydrology and Metereology

WSA: Water Stability of Soil Aggregate

FAO: Food and Agriculture Organization

PP: *Dalbergia* Plantation

GL: Grassland

RP: Rubber Plantation

PF: Pineapple Field

Ca: Calcium

cm: Centimeter

CMI: Carbon Management Index

POC: Particulate Organic Carbon

MOC: Mineral Organic Carbon

TOC: Total Organic Carbon

VC: Non-cultivated Trees

AF: Artificial Forest

NS: Natural Shrub

AG: Artificial Grassland

NG: Natural Grassland

SC: Slope Cropland

mm: millimeter

ASCOL: Amrit Campus

D: Density

RD: Relative Density

F: Frequency

RF: Relative Frequency

C: Coverage

RC: Relative Coverage

HP: Tree Garden

NF: Pine mixed forest

SP: Spruce Plantation

CL: Cropland

VF: VegeTable Field

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ABSTRACT

Soil serves as the best carbon pool and land use change is one of the major factors that can affect soil carbon with its nutrient contents, soil texture, and pH. Changing land use patterns specifically from forest to pasture or cultivated land is a most common occurrence. The main threats of land use change is on the soil carbon and also soil basal respiration. Effects of land use change on soil organic carbon (SOC), basal respiration and fungal colony is poorly understood. Hence this study was carried out to determine the effects of land use change in soil organic carbon, basal respiration, pH, and fungal colony forming unit (CFU) with forest plant diversity. Soil organic carbon, basal respiration, soil pH and fungal colony forming unit were studied in three selected types of land use such as agricultural land, forest land and pasture land. In addition, plant diversity of the same forest was also studied. A total of 75 soil samples were studied of agricultural lands, forest lands and pasture lands. The results showed that soil organic carbon content and basal respiration was significantly higher in agricultural land followed by forest and pasture land. Simultaneously, higher values of fungal colony-forming unit were detected in agricultural land in all dilution factors than in forest and pasture basal respiration The value of pH was less (acidic) in agricultural land and more (alkaline) in forest and pasture land. Statistical analysis showed there were significant differences in SOC, basal respiration, CFU, and pH among different land use types studied. There was a negative relation between SOC and basal respiration. Higher values of SOC content and basal respiration might be due to major factors such as the high amount of carbon input in agricultural land through green manure, organic fertilizers, and advanced management practices. This study can provide data for evaluating SOC, basal respiration, and fungal population across different land use types and compelled us to think of agricultural land as a carbon sink after forest land.

Keywords: SOC, basal respiration/basal respiration, colony forming unit, land use types

सोध सार

माटोले उत्कृष्ट कार्बन पोखरीको रूपमा काम गर्दछ र भूमिको प्रयोग परिवर्तन मुख्य कारकहरू मध्ये एक हो जसले माटोको कार्बनलाई यसको पोषक तत्व, माटोको बनावट र pH मा असर पार्न सक्छ। विशेष गरी जङ्गलबाट चरन वा खेती गरिएको जमिनमा भूमि प्रयोगको ढाँचा परिवर्तन गर्नु सामान्य घटना हो। भूमि प्रयोग परिवर्तनको मुख्य खतरा माटोको कार्बन र माटोको आधारभूत श्वासप्रश्वासमा पनि छ। माटोको जैविक कार्बन (SOC), बेसल श्वासप्रश्वास र फंगल कोलोनीमा भूमि प्रयोगको परिवर्तनको प्रभावलाई राम्ररी बुझिएको छैन। तसर्थ यो अध्ययन माटोको जैविक कार्बन, बेसल श्वासप्रश्वास, pH, र फङ्गल कोलोनी गठन इकाई (CFU) मा वन बिरुवाको विविधतामा हुने परिवर्तनको प्रभाव निर्धारण गर्न गरिएको थियो। माटोको जैविक कार्बन, बेसल रेस्पिरेसन, माटोको पीएच र फङ्गल कोलोनी बनाउने एकाइको अध्ययन गरी तीन प्रकारका भू-उपयोगहरू जस्तै कृषि भूमि, वन जग्गा र चरन भूमिमा अध्ययन गरियो। साथै, एउटै वनको वनस्पति विविधता पनि अध्ययन गरिएको थियो। कृषियोग्य जमिन, वनजङ्गल र चरन जग्गाको कुल ७५ वटा माटोको नमूना अध्ययन गरिएको थियो । नतिजाहरूले देखाए कि माटोमा जैविक कार्बनको मात्रा र आधारभूत श्वासप्रश्वास कृषि भूमिमा उल्लेख्य रूपमा उच्च थियो र त्यसपछि वन र चरन भूमिमा। यसका साथसाथै, फङ्गल कोलोनी बनाउने एकाइको उच्च मान कृषि भूमिमा वन र चरन आधारभूत श्वासप्रश्वासको तुलनामा सबै पातलो कारकहरूमा पत्ता लगाइएको थियो। pH को मूल्य कृषि भूमिमा कम (अम्लीय) र वन र चरन भूमिमा बढी (क्षारीय) थियो। सांख्यिकीय विश्लेषणले अध्ययन गरिएका विभिन्न भू-उपयोग प्रकारहरूमा SOC, बेसल रेस्पिरेसन, CFU, र pH मा महत्त्वपूर्ण भिन्नताहरू रहेको देखाएको छ। SOC र बेसल श्वासप्रश्वासको बीचमा नकारात्मक सम्बन्ध थियो। SOC सामग्री र बेसल श्वासप्रश्वासको उच्च मूल्यहरू हरियो मल, जैविक मल, र उन्नत व्यवस्थापन अभ्यासहरू मार्फत कृषि भूमिमा कार्बन इनपुटको उच्च मात्रा जस्ता प्रमुख कारकहरूको कारण हुन सक्छ। यस अध्ययनले विभिन्न भू-उपयोग प्रकारहरूमा एसओसी, आधारभूत श्वासप्रश्वास र फंगल जनसंख्याको मूल्याङ्कन गर्न डेटा प्रदान गर्न सक्छ र हामीलाई कृषि भूमिलाई वन भूमि पछि कार्बन सिङ्कको रूपमा सोच्न बाध्य तुल्यायो।

कीवर्डहरू: SOC, बेसल रेस्पिरेसन/बेसल रेस्पिरेसन, कोलोनी बनाउने एकाइ, भूमि प्रयोग प्रकारहरू

1. INTRODUCTION

1.1 Background

One of the major factors that can affect soil carbon, soil nutrient contents, soil texture, and pH is land use change (Post & Mann, 1990). Changing land use patterns specifically from forest to pasture or agricultural land is a most common practice in many landscapes (Sala *et al*., 2000). Land use changes also change the composition of plant species and associated management practices across (Lauber *et al*., 2008). During 1980- 2000, more than half of the tropical forests were converted to agricultural land all over the world (Gibbs *et al*., 2010). This change has consequently changed major terrestrial ecosystems (Chen *et al*., 2022). Soil organic carbon (SOC) mainly comprised of plant roots inputs, microbes and their metabolites, plant, and animal decayed materials, and decomposed materials. However, LUC on both regional and local levels has made a huge impact on SOC accumulation (Abraha *et al*., 2019). There is a net loss of SOC after deforestation and converting it into cropland or grazing land (Watanabe *et al*., 2001) and this accelerates emission of CO_2, degrades soil structure and quality and also decrease ecosystem services provisioned by soil (Don *et al*., 2011). In soil-plant ecosystem, SOC plays a crucial role as it affects soil quality such as its properties, processes, nutrient buffering, supply etc. (Nsabimana *et al*., 2004). SOC also functions a significant role in the carbon cycle. LUC determines SOC content in the soil as it brings changes in biogeochemical cycles, soil aggregates, and soil respiration (Nsabimana *et al*., 2004). Due to this effect of LUC (converting natural forest to agricultural or pasture land), SOC has been lost which is because of a decline in organic matter inputs, alteration in moisture, and other climate regimes (Zhang *et al*., 2007). SOC and microbial biomass (MBC) is reduced in changed agriculture land uses relative to the native forest (Pandey *et al*., 2010). Quantifying SOC under different LUCs entails the potential to sequester carbon reduce GHG emissions and foster to development of land management scenarios (Toru and Kibret, 2019). Though agricultural land shows tremendous loss of soil carbon, it emits as well as sequester CO_2 (Toru *et al*., 2019). Intense decomposition, removal of above-ground biomass, and soil eradication may result in SOC loss but practice of proper agricultural land use and management measures then agricultural land also serves as a carbon sink (Lal *et al*., 2006).

Moreover, land use change has also affected basal respiration (basal respiration rate i.e. CO2 evolution) which are directly or indirectly relevant to SOC circulation (Nsabimana

et al., 2004, Shi *et al*., 2009). Determining SOC and basal respiration changes can provide valuable information on effect of LUC on carbon pool and soil quality. Basal respiration in the soil can be determined by the rate of carbon dioxide production (Doran *et al*., 2012). Soil basal respiration can therefore be measured through the rate of CO_2 production. Soil respiration (CO_2 production) is an important process that controls the loss of soil carbon from land ecosystems to the atmosphere (Bond-Lamberty and Thomson, 2010). LUC effects on soil carbon or substrate inputs that ultimately alter the soil microbial respiration (Yang *et al*., 2021). Microbial respiration or basal respiration increases in soil when there is a high amount of recalcitrant carbon which comes from a large amount of altered Carbon (C) and Nitrogen (N) input (Mori *et al*., 2018). Similarly, soil respiration is significantly affected due to fluctuations in temperature and soil moisture (Yan *et al*., 2018).

Soil microbial community composition is one of the major components of soil which is regulated by different factors of soil substrates. Microbial resources such as C, N, and P contents are altered by plant residue and plant root exudation inputs which are ultimately influenced by LUC (Xu *et al*., 2021). In addition, the microbial community is also greatly driven by the soil pH (Fierer and Jackson, 2006). LUC is one of the significant environmental changes that alter not only soil environmental conditions but also microbial communities in it (Englhardt *et al*., 2018; Wang *et al*., 2019). Converting from woodland (forest) to pasture or agricultural land might alter soil nutrient cycling which might lead to a decrease in the diversity and richness of the microbial community (Merloti *et al*., 2019; Shrestha *et al*., 2019). There is a high difference in the microbial community in intensive management and unmanaged soil (Drenovsky *et al*., 2010). Furthermore, management measures that include adding of chemical fertilizers and herbicides could lead to more intense and diverse microorganisms in the soil (Szoboszlay *et al*., 2017; Santos *et al*., 2020). However, pasture land with overgrazing can reduce plant diversity and complexity ultimately affecting the microbial community by reducing the quality and quantity of soil inputs (Dassen *et al*., 2017; Han *et al*., 2018).

Forests store 70-90% of terrestrial above and below-ground biomass which plays a crucial role in the biogeochemical cycle as forests are associated with plant diversity and serve as crucial carbon sink (Aponte *et al*., 2020; Besnard *et al*., 2021). Forest soil usually contains a high amount of soil carbon due to litter production and plant root

exudation (Cheng *et al*., 2013). Eradications of plant biomass, the addition of fertilizers and herbicides, and the use of pesticides frequently is practiced in agricultural lands and grasslands (Szobaszlay *et al*., 2017), while in forests, carbon inputs mainly occur from the exudates of roots and also through litter layer on the land surface. This depicts that forestland, cropland, and grassland have characteristic variances in soil structure, quality and quantity of nutrients, potential as carbon sink, and sources for micro-organisms (Szobaszlay *et al*., 2017). Hence these LUC harbor structurally and functionally distinct microbial communities (Szobaszlay *et al*., 2017).

Plant diversity influences carbon sequestration which is a key ecosystem function (Bartelt-Ryser *et al*., 2005, Isbell *et al*., 2018). SOC storage, sequestration of carbon and restorations of ecosystem functions can be increased through vegetation restoration (Zhao *et al*., 2015; Pandey *et al*., 2023; Shahi *et al*., 2023). Plant diversity not only improves plant productivity but also increases plant carbon input in soil and ultimately increases SOC storage (Chen *et al*., 2020).

Hence, quantifying SOC and soil basal respiration along with fungal population under different LUCs entails soil potential to sequester carbon reduce GHG emissions and foster to development of land management scenarios. Research on SOC sequestration and how it is impacted by LUC may aid in the assessment of soil potential as a sink or a source of CO_2 emissions in the atmosphere. It also provides information to reduce greenhouse gas emissions and mitigate climate change effects along with soil management (Sommer and Bossio, 2014).

1.2 Rationale of Study

Increasing CO_2 concentration in the atmosphere and its direct and indirect effect on global climate change has promoted wide research on different aspects of the carbon-geochemical cycle globally. Increasing anthropogenic activities in Nepal mainly urbanization have caused changes in land use (LUC) over time. This has consequently caused changes in the soil's different physical, chemical, and biological properties along with the plant diversity and microbial community. However, the main threats of LUC are on the soil carbon and also on the soil basal respiration. With the major anthropogenic disturbances, there might have been changes in the vegetation pattern and composition that consequently have impacted on soil organic carbon and its basal respiration which could be beneficial (if LUC is forested) but could be detrimental (if

LUC is pasture land). Over the years, not much research has been done to relate the land use types and their impact trend on soil organic carbon, soil respiration, and fungal colony.

In the context to Nepal, a wide range of research have not been conducted yet relating to land use changes and their impact on soil organic carbon and basal respiration together. Various researchers are concerned with the land use changes and their effects on different aspects such as soil carbon stock, nutrient contents, carbon cycles, microbial community, and so on. However, the LUC effect on soil organic carbon, basal respiration, and microbial colony is poorly understood. Also, in Nepal there is practice of collecting litter fall from forest and use them in different livestock purposes which could bring variation in the soil organic carbon content and other soil nutrients both in forest and agriculture land. In addition, determining plant diversity of forest land use type may support the reason behind high or low amounts of soil organic carbon and basal respiration. Research on the effect of LUC on SOC and basal respiration not only provides insights into ecosystem functioning due to LUC but also fosters the mitigation measures and conservation practices across different land use patterns to maintain the potential of soil as a carbon sink and provision of ecosystem services (Van Leewen *et al*., 2017).

Moreover, determining and comparing soil organic carbon and basal respiration of different land use patterns not only provides details into the effects of LUC on the soil properties and vegetation but also provides us with the relationship among soil basal respiration, soil carbon organic and microbial colony which will ultimately emphasize on the mitigation measures of LUC on soil properties. Also, understanding and quantifying SOC and basal respiration along with fungal population in different land use types might compelled researchers to think which land use type has great potential as a carbon sink amid global climate change issues.

1.3 Research Questions

- How do land use types affect SOC?
- What are the effects of LUC on soil basal respiration/ soil respiration?
- How does plant diversity of forest land aid soil organic carbon and basal respiration?

1.4 Objectives

The underlying objectives of this study are to determine the plant diversity, soil organic carbon, and soil basal respiration of three different land use types.

The major objectives of this study are as follows:

- To estimate the soil organic carbon and pH across different land use types
- To estimate soil basal respiration across different land use types
- To quantify fungal population and their identification

2. LITERATURE REVIEW

2.1 Soil organic carbon across land use types

Xiangmin *et al*. (2014) studied five types of land use, including natural broad-leaved Korean pine mixed forests (NF), spruce plantations (SP), cropland (CL), ginseng farms (GF), and a five-year-old Mongolian oak young forest (YF). They discovered that SOC levels were relatively higher in NF compared to SP, CL, GF, and YF. Their findings also indicated that basal respiration was notably lower in SP, CL, GF, and YF compared to NF.

Ferreira *et al*. (2014) found that carbon losses occur when the native forest was converted to cropland however when converted to pastures or grazed pastures there was more carbon and high SMB which functioned as C sinks. They concluded that grazed pastures can have more potential for C sequestration than cropland land use. They studied five land use changes in a semi-arid region of northeast Brazil to find the effect of land use type on SOC and microbial characteristics.

Poudel (2019) examined and quantified SOC across various land use types, including community forests, leasehold forests, and agricultural land, in the Chitwan district of Nepal. He found that the above-ground total above biomass (AGTB) and above-ground soil biomass (AGSB) of community forests were higher than leasehold forests. They concluded that properly maintained community forests were more contributing to reducing global carbon emissions as they had the highest carbon stock than leasehold forests and agricultural land.

Wooliver *et al*. (2022) reviewed five case studies to find out links among crop diversification, microbial diversity and SOC. They highlighted that SOC accumulation could be high in agricultural land or cropland by selecting ideal crop functional types considering diversification options. Also authors highlighted that SOC response to crop diversification fluctuated annually and influenced by the length of crop cover.

Lepcha *et al*. (2020) studied that SMB and Soil carbon could differ across land use and influenced by season and soil depth. They found that with the alterations in land use, soil depth and seasonal conditions, as well as soil characteristics automatically changes which consequently changes MBC as well. They also found that SOC was higher in Cardamom agroforestry. Their study indicated that microbial C immobilization helped

in enhancing soil fertility and SOC sequestration through the restoration of the litter layer.

Alias *et al*. (2022) investigated how abandoning cropland impacts soil carbon stocks within an agroforestry system in Southwestern Spain. They found that there was an increase in C sequestration and SOC in the soil profiles (upper 30cm) after the abandonment of agricultural activity. Hence, they concluded that abandonment of agricultural land could increase the potential of land as a carbon sink.

According to Sainepo (2018), land use types had a significant influence on SOC pools and also on the carbon management index (CMI) as they assessed SOC and CMI across different land use types in Kenya. Their results showed that soil organic carbon varied across different land use types notably. They found that TOC (Total organic carbon), POC, and MOC were higher in shrub lands than grasslands and bare lands.

Ilboudo *et al*. (2022) measured carbon content in non-cultivated tress (VC), SOC and soil Nitrogen content across various land uses in forests of West Africa. They found that VC and SOC had a positive relation with the annual croplands, perennial croplands, grasslands, and bush lands. They concluded that land uses were determined by soil properties and topography that consequently affects SOC, TN and VC.

Toru and Kibret (2019) evaluated carbon stock across various land use and land cover types in Eastern Ethiopia. Their results indicated that SOC level declined with soil depth even if higher amount of carbon was found in lower depth. Their study indicated that SOC stock was more in natural forest than cropland. Hence, they concluded that land uses such as woody perennials had more carbon stock compared to cropland and grazing land. Hence, this study suggested that inclusion of woody perennials could enhance carbon sequestration in sub watershed.

Szoboszlay *et al*. (2017) investigated the effects of land use changes and quality of soil organic carbon on soil microbial diversity across Europe. They discovered that shifts of croplands to grasslands or forests, as well as from grasslands to croplands or forests, were associated with variations in microbial abundance. They found that quantity of SOC only explained 5% of the variation and the quality of SOC with 22%. Through network analysis, they identified connections between specific SOC fractions and particular bacterial taxa.

Rhoades *et al*. (2000) assessed soil carbon variations across the three land use types: forest, agriculture, and secondary vegetation in Equador. They estimated the loss of forest-derived soil carbon and accumulation of carbon from sugarcane and pasture vegetation. They found that total soil carbon was lost in sugarcane vegetation than old growth forests.

Recognizing soil aggregation as a crucial process for carbon sequestration, Shrestha and his colleagues (2006) investigated soil aggregate and particle-associated organic carbon across various land uses in Nepal. They assessed the water stability of soil aggregates (WSA) and the soil organic carbon (SOC) associated with aggregates and primary particles in cultivated and forested lands within a mountainous watershed in Nepal. They found that forest soils contained more macro-aggregates and cultivated soils with more micro-aggregates. Mean WSA was also higher in forest soils than in cultivated soils. In their results, though macro-aggregates (more in forest soils) hold less SOC than micro-aggregates (more in cultivated soils), total SOC content was higher in forest lands than in cultivated soils. This is because cultivated soils contain higher amounts of clay than forest soils which hold less amount of SOC than cultivated lands.

2.2 Soil basal respiration

Chen *et al*., 2022, researched on impacts of land use changes on soil microbial properties with their controls. They concluded that SOC had a positive impact on basal microbial respiration. They observed that cropland with human disturbance had less basal microbial respiration as it had lower SOC content. Their results concluded that microbial attributes were dependable on LUC type and it could foster new insight for land use management.

Mazetto *et al*. (2015) compared effects of various land use changes on soil microbial respiration (CO_2 emmisions) in south western Amazon. Their results indicated that substrate induced microbial respiration was highly correlated with different land use type not to the soil type or climate.

2.3 Microbial community across land use types

Puangsombat *et al*. (2010) assessed and related fungal diversity of different land use types with soil parameters including physical and chemical properties. They investigated five different land use types including secondary dry evergreen forest

(DEF), *Cochinchinensis* plantation (PP), grassland (GL), rubber plantation (RP) and a pineapple field (PF). They observed that the highest Shannon-Wiener diversity index for soil fungi was found in PF, followed by DEF, PP, GL, and RP, respectively. They also found that the diversity of soil fungi had a positive correlation with the levels of P, Ca, and Mg in the soil, although this relationship varied across different land use types.

Lauber *et al*. (2008) studies on impacts of LUC soil types on structure of bacterial and fungal communities. They investigated four types of land use in the Southeastern US—hardwood forests, pine forests, cultivated lands, and livestock pastures—and found that, apart from differences in soil texture and nutrient levels, there were not significant variations in soil properties across these land use types. Similarly, they found similar types of fungal and bacterial communities across distinct land use types. In this study, they concluded that land use type might not necessarily determine the variations in microbial community attributes in different landscapes.

Grestner et al. (2014) reviewed the effects of land use on plant diversity in a review paper. They discovered that land use impacts plant diversity in both direct and indirect ways, which can result in either local reductions or increases in diversity.

Thakur *et al*. (2015) studied soil microbial biomass carbon in grasslands influenced by plant diversity irrespective of global environmental change factors. Their results indicated that plant diversity had positive effects on SMB and can influence SOC dynamics.

Yang *et al*. (2017) investigated how soil fungal diversity relates to plant and soil properties on the Loess Plateau. They found that fungal communities structure in soil were effected by variations in land use types such as Artificial forest (AF), Natural shrub (NS), Artificial grassland (AG), Natural grassland (NG) and Slope cropland (SC) with the dominant phyla: Ascomycota, Basidiomycota and Zygomycota due to vegetation restoration. Shannon's diversity was in order NS>AF>NG>AG>SC. They found that SOC and plant richness were strong driving factors for fungal diversity.

2.4 Soil pH across land use types

The soil pH levels around 7 are ideal for overall nutrient availability, crop tolerance, and soil microorganism activity. The primary factors that impact SOC and pH include climatic factors such as precipitation, gully density, forested land, and grain yield (Ou *et al*., 2017).

According to Zhou *et al*. (2019), the pH values increase with depth and again decrease as they found pH ranges from 4.2 to 6.1 in topsoil due to rich in organic matter and the decomposition of more organic acids leading to lowering pH of topsoil. Similarly, they found that soil carbon, nitrogen, and the C/N (TC/TN ratio) are negatively correlated with soil pH which demonstrated that relatively low pH has positive effects on the accumulation of organic matter. In their study, they also found that pH values are more affected by precipitation and evaporation. Soil pH is affected by various factors such as climate, buffering system, plants, etc. which ultimately controls many soil properties (Hong *et al*., 2019). Additionally, soil pH is crucial in influencing the variation in the structure and diversity of microbial communities, which in turn impacts the processes of decomposition and nitrification. The increment in carbon loss could be due to land use intensification in low-pH soils resulting in low acid retardation of microbial growth. They also observed that in nearly neutral pH soils, which are subject to fewer management practices, there is a greater capacity for carbon storage due to enhanced microbial growth efficiency. In contrast, acidic soils, with their reduced microbial growth, significantly affect decomposition rates.

2.5 Effects of plant diversity on SOC and soil respiration

According to Zhang *et al*. (2019), forest restoration could change soil microbial biomass (SMB), community composition and enzyme activities. They examined soils from two afforested sites and two reference sites from South China. Their results showed that SMB, community composition and enzyme activities were high after afforestation. They also demonstrated afforestation with mixed species plantation was more effective than monoculture plantation.

Similarly, Amoakwah *et al*. (2022) studied how deforestation and changes in land use over time effects on soil organic carbon (SOC) storage and quality. Their results demonstrated that SOC, TN (Total Nitrogen), AC (Active carbon), and FA (Fulvic acid) were comparatively higher in CRP (Conservation reserve program) than in converted agricultural land from forest.

Management strategies for grassland ecosystems could be easier by understanding how soil carbon storage is related to species diversity (Anacker *et al*., 2021). Anacker *et al*. (2021) studied 141 grassland transects to find out the relationship between soil carbon and plant species richness, grassland type, soil texture, and prairie dog presence. Their

results showed that soil carbon and plant species richness were positively related but not among all studied grassland types. Furthermore, this study showed that soil carbon and soil clay were negatively related and also prairie dogs had a negative impact on soil carbon. Hence their study suggested that management for species richness provides advantage for soil carbon storage.

From the above all studies it can be depicted that SOC and basal respiration might show positively relation with each other and they might highly differ across different land use types. Understanding SOC content and basal respiration in different land use types not only supports in promoting management practices of different land use covers but also promotes in the mitigation measures of GHGs in atmosphere with the more carbon sequestration in soil.

3. MATERIALS AND METHODS

3.1 Study Area

The research was carried out in three distinct types of land use of Chapagaun, Godawari Municipality-10 (Figure 1). The three different land use types were forest land, Agricultural land and Pasture land. The study area was located in between 27°35'7"N and 85°18' 55" E. These lands consist of moderate to steep slopes with altitude ranging from 1442m to 1465m from sea level for agricultural land, 1550m to 1750m from sea level for forest and 1495m to 1500m from sea level for pasture land. Predominant soils in agriculture land and pasture soils comprise of cambisols and luvisols and forest land comprises leptosols according to the FAO classification system.

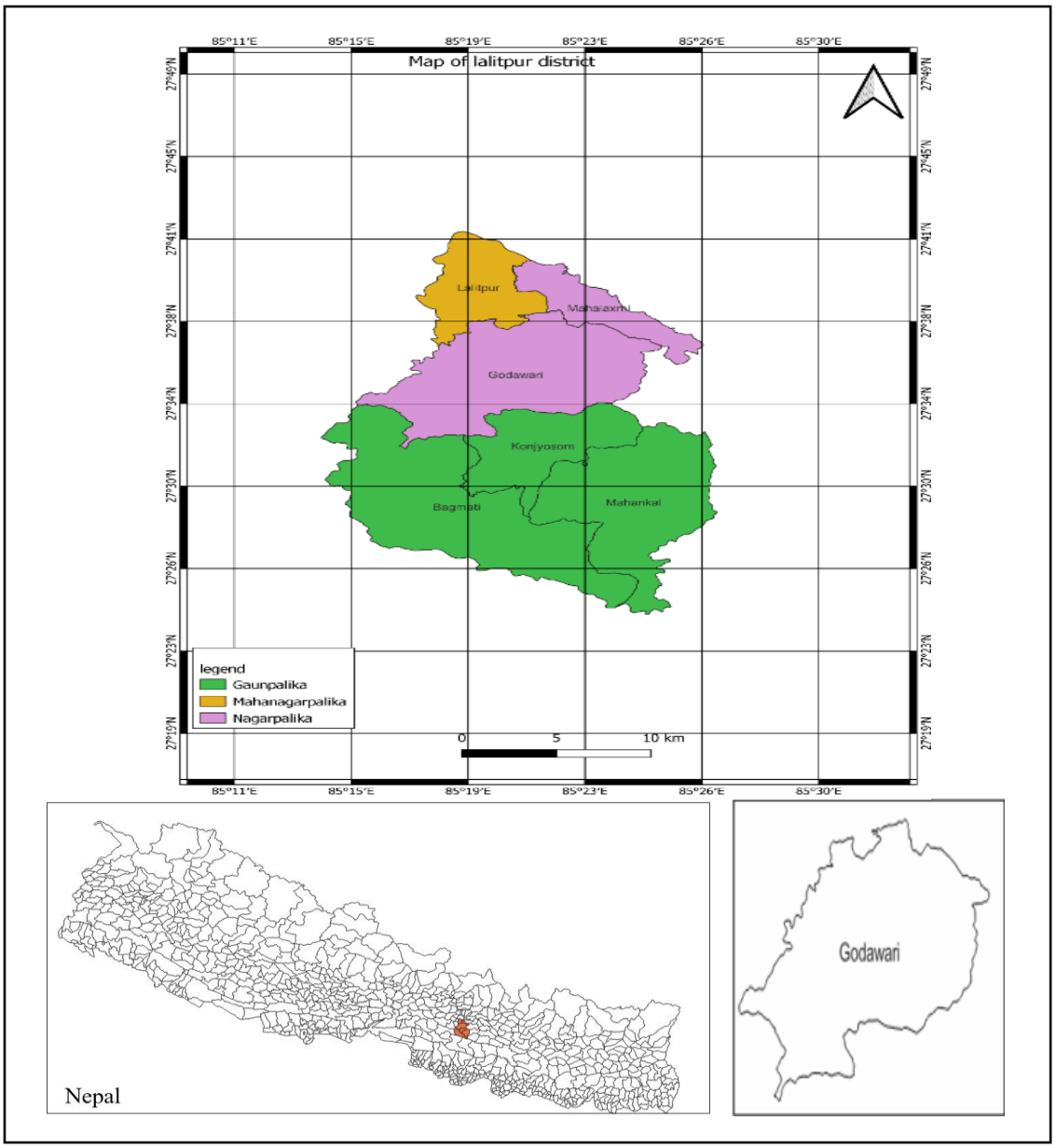


Figure 1: Map showing the location of the study area (source: QGIS)

3.1.1 Climate

Climatic data from 1990 to 2023 showed monthly average maximum and minimum temperatures were 23.83°C in June and 5.9°C in January respectively (DHM, 2023). The average maximum temperature reached peak in 2010 and 2017 with 24.12°C and 24.042°C respectively.

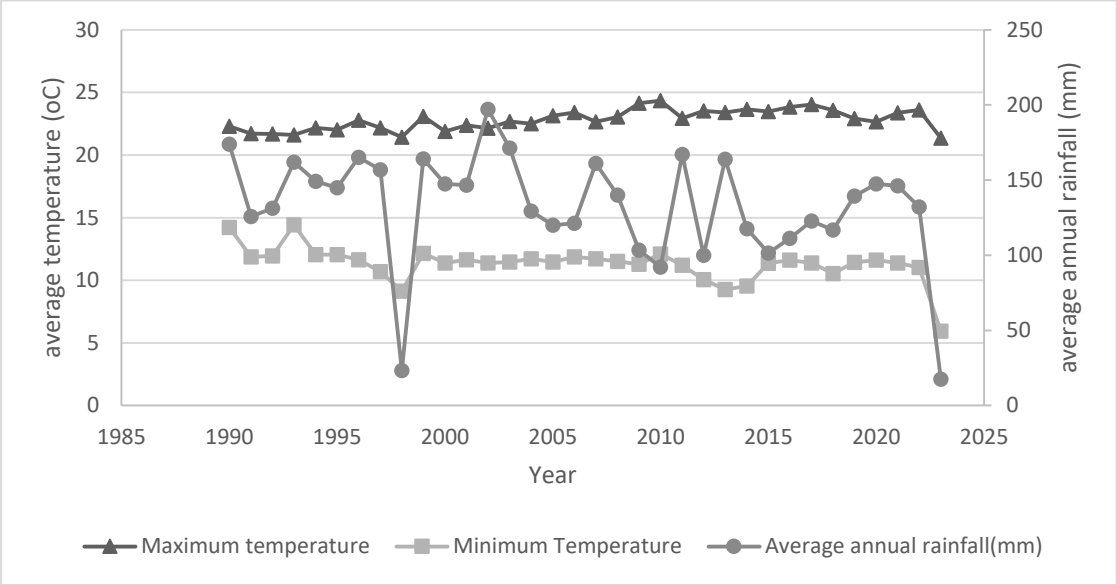


Figure 2: Climatic data (maximum temperature, minimum temperature and annual rainfall) trend in last 30 years

In 2023 during the time of study was conducted, the average temperature was 21.36°C. From the graph above it is clear that there is increasing trend of both maximum and minimum temperatures. Similarly, the highest and lowest mean annual rainfall in the study area was 197.21mm over the 20 years. During the time of study was carried the mean rainfall was 17.6mm in the month of March.

3.1.2 Vegetation

The community forest of Chapagaun covers an area of 1215 square Km and altitude ranging from 1550m to 1750m above from sea level. The forest was mixed with dominant species of *Schima wallichii, Pinus* sp., *Rhododendron* sp. and *Alnus* sp. The forest studied consisted of invasion of a number of species such as *Ageratina* sp., *Bidens* sp. and *Lantana* sp. The forest was sources of fodder and timber for some community of people living around there. So there was slight disturbance in the lower area of forest near to the livelihoods.

Similarly, agriculture primarily relies on irrigation, although some areas may also be rain-fed. The irrigation systems include both traditional methods and modern techniques, depending on the specific crops and farming practices used in the region. The main crops typically include paddy, wheat, millet, maize and vegetables (potato, tomato, leafy greens etc.). The specific crops vary based on season and farming practices. Local farmers often practice mixed cropping to optimize yields and maintain soil health.

Likewise, pasture land constitutes species such as *Stellaria media, Imperata* sp., *Trifolium* sp., *Bidens* sp., *Paspalum* sp. and *Artimesia* sp. The pasture lands now were agriculture lands before almost seven years ago which clarifies agriculture lands were converted to pasture land after 7 years of abandonment. Local cattle such as cow, goat, buffalo etc. are being reared by local farmers in pasture land.

3.2 Sampling Design

3.2.1 Soil Sampling

Soil sampling was taken in March 2023. Soil samples were collected by stratified random sampling using square plots of $100m^2$ (10x10m) (Lauber *et al*., 2008). Each land use area (Agricultural, Forest and Pasture) was subdivided into five subplots (replicates) (Ferreira *et al*., 2014). From each subplots five subsamples (four corners and center) (Toru and Kibret, 2019) were gathered using a spade at soil depths, 0-30cm, and pooled to form a composite sample. Finally, 25 samples (5 sites x 5 samples) were collected for each land use types and a total of 75 soil samples (3 land use types x 25 soil samples) were collected from study sites. Visible roots and plant debris were immediately removed after sampling. The soil samples were placed in individual plastic bags with the labeling of each plots and transported to the laboratory of Amrit Campus. In the laboratory, each soil samples were sieved (2mm mesh) to remove plants, fine roots, and gravel; and were partitioned into subsamples: one was air dried to incubate fungal growth and other was stored in freeze to determine soil organic carbon and basal respiration.

3.2.2 Vegetation Sampling

Vegetation sampling in forest was done by stratified random sampling using circular plots of radius 10m for trees, subplots of radius 5m for saplings/shrubs and1m radius for seedlings/herbs. Altogether twenty-five plots were sampled in five different transects of

altitude ranging from 1550m to 1750m. Radius for each plots and subplots were determined using species-area curve.

3.2.3 Plant Identification

Most plants were identified during the field survey. Flowering plants were preserved as herbarium specimens for further identification, and photographs were taken of unidentified species in the field. Unknown species were subsequently identified with the help of teachers and by consulting reference books such as Plants of Nepal (2022) and Fern and Fern Allies (2016).

3.3 Data collection

3.3.1 Estimation of SOC

In this study, soil organic carbon (SOC) content was estimated using the Walkey-Black chromic acid oxidation method. This technique involves oxidizing the soil's oxidizable matter with a 1N $K_2Cr_2O_7$ (dichromate) solution. The oxidation reaction is facilitated by the heat produced when two volumes of H_2SO_4 (sulfuric acid) are mixed with one volume of dichromate. The excess dichromate is then titrated with ferrous sulfate. The amount of titrant used is inversely proportional to the carbon content in the soil sample (Walkey and Black, 1934). This method is based on the principle of wet oxidation, which includes digesting organic carbon in an acid dichromate solution and then determining the remaining dichromate through back titration with ferrous ammonium sulfate.

In this experiment, 0.5gm of soil was taken in a dry 500ml conical flak and 100ml of 1N $K_2Cr_2O_7$ was pipetted in and swirled a little. Then 20ml of H_2SO_4 was added and swirled again two or three times. This flask was allowed to stand for 30 minutes and then 200ml of distilled water was added. 10ml of phosphoric acid and 1ml of diphenylamine indicator was added to this solution. The content was titrated with 0.5N ferrous ammonium sulfate solution till the color was changed from blue-violet to green. Simultaneously, a blank titration was run without soil. This titration consumed more than 7ml of dichromate solution, hence taking 0.5gm (small quantity) of soil was appropriate. Five replicates of each soil samples were prepared so altogether 25 replicates of land use types were titrated.

Calculation:

Weight of soil taken = 0.5 gm = S

Volume of ferrous ammonium sulfate solution required to neutralize dichromate solution (blank titration) = B

Volume of ferrous ammonium sulfate solution needed for titration with soil sample = C

Volume of dichromate solution used for the oxidation of soil carbon =10(B-C)/B ml= D

Amount of carbon present in 0.5 gm soil = 0.003 x D x 1.3 = E

Amount of carbon present in 100gm soil (% of carbon in soil) = (E / 0.5) x 100

3.3.2 Estimation of soil basal respiration

We estimated basal respiration or soil respiration (SR) by the traditional method using alkali for titration. In this method, CO_2 released from basal respiration is measured by trapping it with alkali solutions. Carbonates are then precipitated using barium chloride, and any remaining hydroxide is titrated with standardized acid (Stotzky, 1965). A key benefit of this technique is that the analysis of the alkali solution can be delayed for a short time, rather than needing to be performed immediately.

In this experiment, 50ml standardized 0.1N KOH was placed in a 100ml beaker and 5ml $BaCl_2$ solution was added with 3-4 drops of phenolphthalein. Simultaneously, 100gm air-dried soil sample was placed in a petri-dish and the soil was moisten with water. The beaker with KOH solution was placed on the petri-dish along with soil sample. The system (dish and beaker) was covered with the bell jar and the base of the jar was sealed with grease. A similar control system was set up without soil sample. After 24 hours, contents of the beaker was titrated with standardized 0.1N HCl. Five replicates of each soil samples were prepared and altogether 25 replicates were prepared for each land use type.

Calculation:

CO_2 in the bell jar = f = (difference in volumes of acid and KOH) x 2.2

CO_2 present in jar with soil = fa

CO_2 present in control = fb

CO_2 produced by soil sample = (fa- fb) mg/100gm

3.3.3 Estimation of Colony Forming Unit (CFU) and Fungal Population

The serial dilution plating method was used to dilute the soil sample to minimize the fungi in the soil in each dilution. Initial soil sample solution (10ml) was diluted five times in different test tubes and they were labeled as 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ and 10^{-5} respectively corresponding to their dilution factor. To grow fungal colonies we used sample solutions of 10^{-3}, $10^{-4,}$ and 10^{-5} concentrations. 1ml from each solution samples chosen were transferred using a pipette to sterilize Petri dishes containing Potato Dextrose Agar (PDA) media and was gently spread over media. There were 5 replicates of each concentration and for each land use type soil, there were altogether 75 petri dishes containing soil samples. All the prepared Petri dishes with samples were incubated in an incubator maintaining a room temperature of 27^{o}C for five days and the fungal growth was observed.

Before the identification of the fungal colony, colony morphology in terms of color and number was observed and noted down. Fungal colonies were identified by observing their hypha growth and spores under an electro-microscope. Their spores under the microscope were photographed for further identification. Identification was performed with the help of experts and also by tallying the pictures from the books (Pictorial Atlas of Soil and Seed Fungi) with their detail descriptions. In addition, a fungal colony of each species were calculated by using the CFU formula as mentioned below:

Colony Forming Unit (CFU)/g = Number of colonies x dilution reverse

3.3.4 Estimation of soil pH

The pH of each soil samples from different land use types were determined using pH meter with electrodes. Freshly collected soil samples were weighed and mixed with dilute water in the ratio of 1:2 (soil: water) and left for some minutes for soil suspension. The glass electrode pH meter was initially standardized at pH 7 inside the buffer solution. This standardized electrode of pH meter was then gently dipped inside soil suspension and pH was displayed on pH meter which was noted down for each soil samples of different land use types. There were five replicates of each soil samples collected.

3.3.5 Estimation of plant community attributes

Diversity analysis was done by estimating cover, calculating density, frequency, IVI and diversity indices (Zobel et al. 2000).

a. Density (D) and Relative Density (RD)

D = Total no. of individual species/ Total quadrate studied

RD (%) = (Density of species/ Density of all species) X 100

b. Frequency (F) and Relative Frequency (RF)

F (%) = (No. of the quadrat in which species occurred/ Total no. of quadrate studied) X 100

RF (%) = (Frequency of one species/ Total frequency of all species) X 100 %

c. Coverage (C) and Relative coverage (RC)

RC (%) = (Coverage of one species/ Total cover of all species) X 100%

Do = Total basal area of a species/ No. of tree

d. Importance value index (IVI)

IVI (%) = RD + RC + RF

e. Shimson and Shanon-Weiner Index

Shimson's Index (D) = $1-(\sum ni(ni-1)/N(N-1))$

where, ni = number of individual

N = Total number of individual

Shanon Index (H) = $-\sum pi\ (lnpi)$

where, pi = relative abundance

3.4 Statistical Analysis

Before the analysis, the data were assessed for normality using Shapiro-wilk test of normality. One-way ANOVA and Tukey's HSD test was used to analyze the effects of LUC on SOC, MA and pH. Similarly, two-way ANOVA and Tukey's HSD test was again used to analyze the effects of MA and pH on SOC across different LUC. Pearson's correlation coefficients were calculated to determine the correlation between SOC and MA; SOC and pH and MA and pH. Likewise, relations among IVI and Species richness with soil organic carbon and basal respiration were also performed by testing Pearson's correlation coefficient. All these statistical analysis were performed in R version 4.3.3. (RStudio team, 2024).

4. RESULTS

All data were assessed for normality by applying the Shapiro-Wilk test. The tested data fit a normal distribution as their P-values were more than 0.05 ($P>0.05$) at a 5% level of significance. The least significant difference $P<0.001$ and $P<0.05$ for ANOVA and Tukey's test respectively, were used to separate the means when differences were significant.

4.1 Soil Organic Carbon

From the analysis, we found a statistically significant difference in SOC across different land use types studied at $F(2)=20.41$, $P<0.001$. This meant soil organic carbon (SOC) varied significantly across different land use types studied. The soil organic carbon of composite agricultural soil samples was comparatively higher than forest and pasture composite soil samples (Table 1). The soil organic carbon (SOC) content in composite agriculture soil samples ranged from 5.05% to 3.32% in the 100 gm soil samples studied. Similarly, SOC content in composite forest soil samples ranged from 4.41% to 2.21%. In the context of composite pasture soil samples, SOC ranged from 2.19% to 0.10%. The agriculture soil samples had the highest content of soil organic carbon followed by forest soil samples and then pasture soil samples.

Table 1: Soil organic carbon (SOC) in 100 gm soil samples across three land use types

Land use types	Composite Soil Samples	SOC %	Mean
Agriculture	A1	5.05	4.57±0.93a
	A2	4.28	
	A3	4.37	
	A4	5.83	
	A5	3.32	
Forest	F1	2.22	3.30±0.92a
	F2	3.59	
	F3	4.41	
	F4	4.07	
	F5	2.21	
Pasture	P1	0.93	0.85±0.82b
	P2	0.10	
	P3	0.77	
	P4	0.28	
	P5	2.19	

Note: A1, A2, A3, A4 and A5 are composite soil samples of agriculture land use type; F1, F2, F3, F4 and F5 are composite soil samples of forest land use type; P1, P2, P3, P4 and P5 are composite soil samples of pasture land use type; values after ± is standard deviation; values with different letters are significantly different at *P<0.05* (according to Tukey HSD post hoc test)

Soil organic carbon (SOC) of pasture and agriculture lands and SOC of pasture and forest lands were statistically significant at P<0.05 but SOC of forest and agricultural lands were not. This showed that the soil organic carbon of pasture soil samples greatly reduced than that of agriculture and forest soil samples. However, soil organic carbon of forest and agriculture soil samples did not vary significantly which meant soil organic carbon content in forest and agriculture soil samples were somewhat the same.

4.2 Soil basal respiration

Basal respiration (CO_2 efflux) notably varied across different types of land use studied at F(2)=766.4, P<0.001.This analysis clearly indicated microbial respiration was variedly different across land use types studied. The CO_2 produced from soil samples was measured in mg/100gm. Among three different land use types studied, agriculture soil samples had the highest soil respiration as compared to forest and pasture soil samples, among which pasture soil samples had the least soil respiration (Table 2).

Table 2: Soil basal respiration (Soil Respiration) in 100 gm soil samples

Land use types	Composite Soil Samples	CO_2 efflux (mg/100 gm)	Mean
Agriculture	A1	3.2	3.6±0.45a
	A2	4.3	
	A3	3.7	
	A4	3.2	
	A5	3.4	
Forest	F1	1.3	1.3±0.25b
	F2	0.8	
	F3	1.4	
	F4	1.5	
	F5	1.4	
Pasture	P1	0.6	0.9±0.3c
	P2	0.7	
	P3	1.1	
	P4	1.3	
	P5	1.0	

Note: A1, A2, A3, A4 and A5 are composite soil samples of agriculture land use type; F1, F2, F3, F4 and F5 are composite soil samples of forest land use type; P1, P2, P3, P4 and P5 are

composite soil samples of pasture land use type; values after ± is standard deviation; values with different letters are significantly different at *P<0.05* (according to Tukey HSD post hoc test)

Basal respiration across different types of land use studied was statistically significant at P<0.05. This analysis indicated that CO_2 produced from each land use type varied significantly. Soil respiration (CO_2 production) in agriculture soil samples ranged from 4.3 mg/100 gm to 3.2 mg/100 gm soil. The maximum soil respiration was from soil samples A2 while the minimum was from A1 and A4. Similarly, CO_2 produced from forest soil samples ranged from 1.5 to 0.8 mg/100 gm soil. The highest soil respiration was found to be from soil sample F4 and that of minimum was to be from soil sample F2. Moreover, in the context of pasture land, CO_2 produced ranged from 1.3 to 0.6 mg/100 gm soil with the maximum being of soil sample P4 and the minimum of P1 soil samples.

4.3 Colony Forming Unit (CFU)

Colony forming unit (CFU) was calculated for all soil samples (agriculture, forest and, pasture) in three different dilution factors such as 10^{-3}, 10^{-4} and, 10^{-5} (Table 3).

Table 3: Colony forming unit (CFU)/g in three dilution factors

Land use types	**10^{-3}**	**10^{-4}**	**10^{-5}**
Agriculture	3.5±2.9a	4.5±3.7a	5.5±5.3a
Forest	3.1±3.08a	4.6±4.5a	5.6±5.2a
Pasture	2.7±2.8b	3.7±3.6b	4.6±4.5b

Note: values in Table are log transferred with base 10; values after ± is standard deviation; values with different letters are significantly different at *P<0.05* (Tukey HSD post hoc test)

Colony forming unit (CFU)/g in three dilution factors (10^{-3}, 10^{-4} and 10^{-5}) were significantly different at P<0.05 across different types of land use studied. Comparatively agriculture soil samples had maximum CFU in 10^{-3} dilution factor whereas forest had maximum CFU in both 10^{-4} and 10^{-5} dilution factors.

4.4 Population of soil fungi

Ten different fungal genera were observed from five different agricultural soil samples incubated. They were *Penicillium* sp., *Fusarium* sp., *Paecilomyces* sp., *Phytophthora* sp., *Cladosporium* sp., *Rhizoctonia* sp., *Mucor* sp., *Chetomium* sp., *Gliocladium* sp. and *Microsporum* sp.. *Penicillium* sp. had highest number of colony formed followed by

Paecilomyces sp. for all agricultural soil samples. Similarly, from forest soil samples seven different fungal genera were observed and they were *Mucor* sp., *Rhizoctonia* sp., *Paecilomyces* sp., *Penicillium* sp., *Phytophthora* sp., *Gliocladium* sp. and *Fusarium* sp. Among all of the genera identified *Mucor* sp. had the highest number of colony formed. Pasture soils also had eight different fungal genera identified and they were *Penicillium* sp., *Fusarium* sp., *Chetomium* sp., *Microsporum* sp., *Phytophthora* sp., *Paecilomyces* sp., *Mucor* sp., *Penicillium* sp. and *Aspergillus* sp. Among these fungal genera *Mucor* sp. had higher number of colony formed than other fungal genera found.

Table 4: Occurrences of fungal species in each land use type

Fungal Species	Occurrence in land use types
Penicillium sp.	A, F, P
Fusarium sp.	A, F, P
Paecilomyces sp.	A, F, P
Phytophthora sp.	A, F, P
Cladosporium sp.	A
Rhizoctonia sp.	A, F
Mucor sp.	A, F, P
Chetomium sp.	A, P
Gliocladium sp.	A, F
Microsporum sp.	A, P
Aspergillus isp.	A, P

Note: A, F and P refers Agricultural land, Forest land and Pasture land respectively.

The Table 4 above clearly showed that highest number of colony was found to be in agricultural soil samples followed by pasture soil samples and forest soil samples. This meant that pasture soil samples had least number of fungal colony formed in each dilution factor incubated compared to other two land use types.

4.5 Plant Diversity of Forest land

Pinus roxburghii tree had maximum IVI (60.44) followed by *Castonopsis* sp. and *Alnus sp.* with IVI value 48.83 (Figure). But *Quercus* sp. and *Myrica* sp. showed minimum IVI (17.16) value among tree species of Chapagaun forest. The tree species found there were *Pinus roxburghii*, *Schima wallichii*, *Alnus* sp., *Castonopsis* sp., *Rhododendron* sp., *Quercus* sp., *Myrica esculenta* and *Spondias* sp.

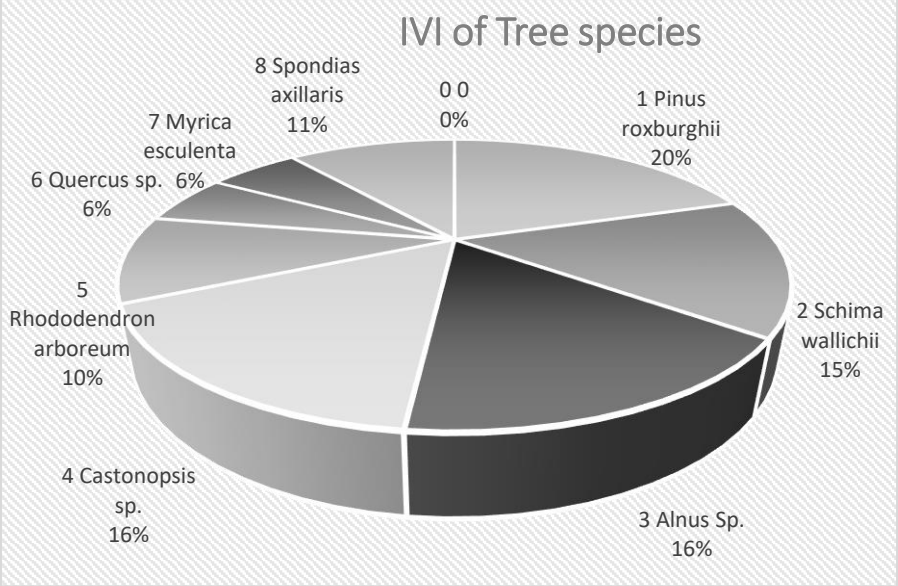


Figure 3: Importance Value Index (IVI) of tree species

For saplings/shrubs, *Ageratina adenophora* showed maximum IVI (37.91) followed by *Reinwarditia indica* (31.95) and *Bidens pilosa* (30.35) (Figure 4) while *Schima wallichii* saplings had least IVI (6.57) (Table7). Other species of saplings and shrubs to be found were *Urtica dioca*, *Daphne* sp., *Smilax* sp., *Ziziphus indica*, *Lonicera incurva*, *Anisomeles indica*, *Ageratina conyzoides*, *Parthenocissus unifoliolator* and *Lantana camara.*

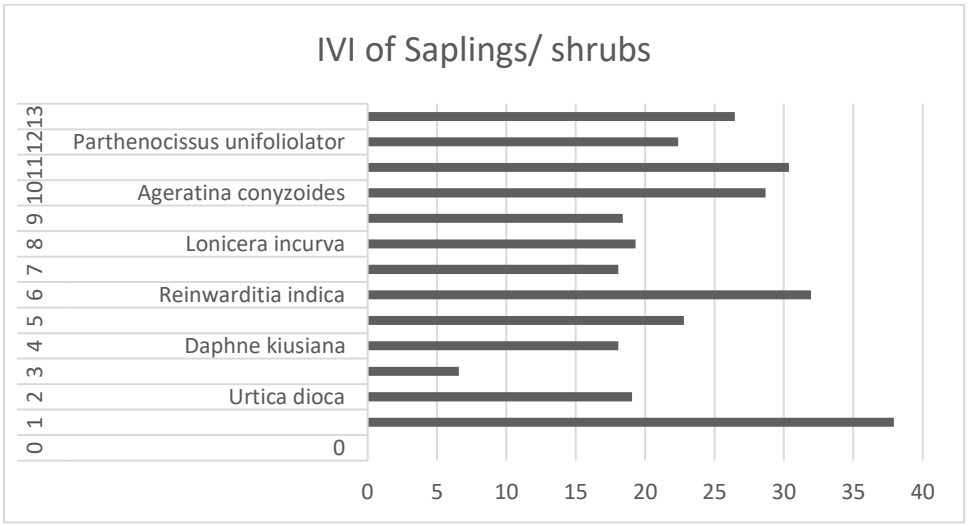


Figure 4: Importance Value Index (IVI) of saplings/shrubs

Likewise, *Oplismenus compositus* has maximum IVI (39.05) among seedlings and herbs found in study sites followed by *Dryopteris* sp. (Figure 5). While species with least IVI was *Quercus* sp. seddlings. Other seedlings and herbs found there were *Crassocephalum*

sp., *Salvia* sp., *Desmodium* sp., *Isodon* sp., *Chenopodium* sp., *Diplazium* sp., *Dennstaeditia* sp., *Pteris* sp., *Tectaria* sp., *Thelypteris* sp. and *Polystichum* sp.

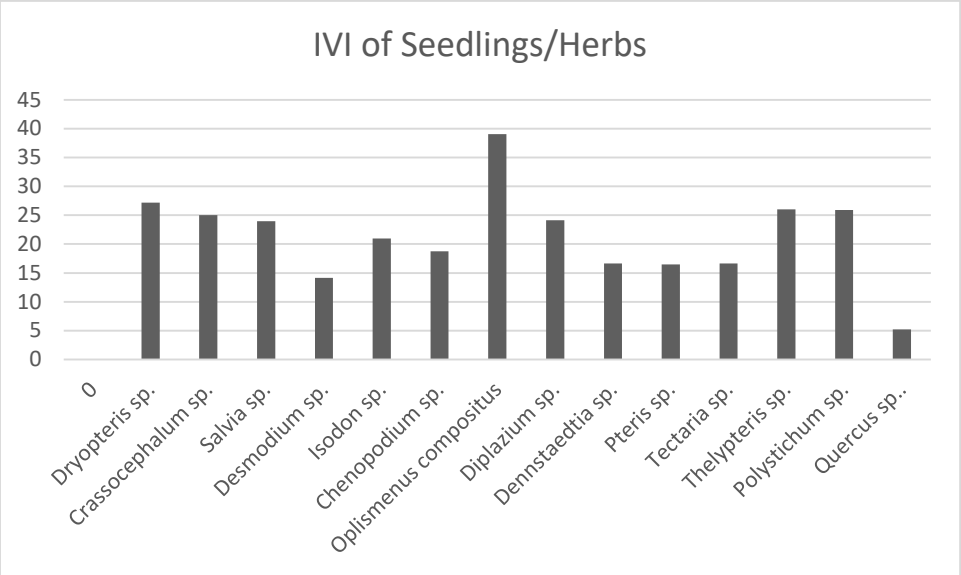


Figure 5: Importance Value Index (IVI) of seedlings/herbs

Among three different plant types (tree, saplings/shrubs and seedlings/herbs), seedlings/herbs had maximum Simpson's (D) and Shannon diversity (H) index. This indicated that seedlings/herbs had both species abundance and species richness while trees had least Simpson's (D) and Shannon diversity index (H) which indicated trees had least species abundance and richness (Figure 6).

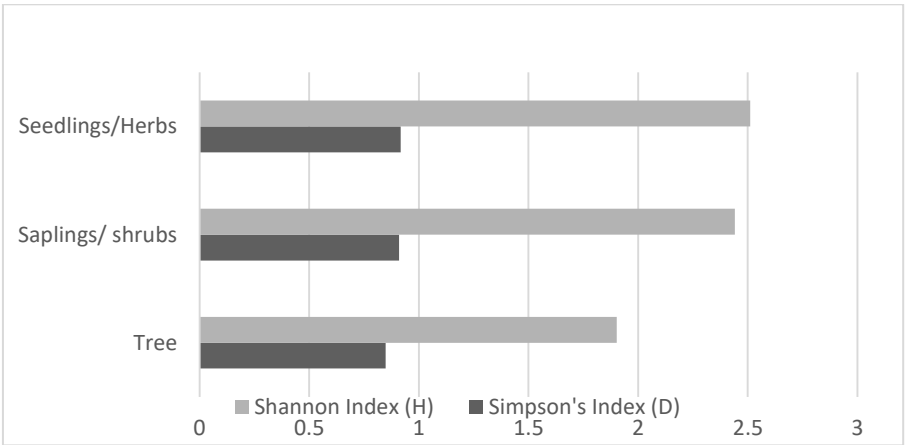


Figure 6: Simpson's and Shannon diversity index of tree, saplings and seedlings

However, Figure 8 showed that all three plant types (tree, saplings/shrubs and seedlings/herbs) had Simpson's index (D) value near to 1 which depicted high plant diversity and abundance in the forest. In addition saplings/shrubs and seedlings/herbs had

Shannon index (H) value near to 3 which showed high species diversity but in contrary trees showed least Shannon index (H) value that showed least species diversity compared to saplings/shrubs and seedlings/herbs.

4.6 Soil pH

The pH values were notably distinct across different types of land use studied at $F(2)=15.39$, $P<0.001$. This analysis clearly indicated pH values were also variedly different across land use types studied. From the post-hoc test (Tukey's HSD test) results, we found that pH of forest and agricultural lands and pH of pasture and agricultural lands were statistically different at $P<0.05$. This illustrated pH of forest and agriculture soil samples were varied significantly. But pH of pasture and forest lands were not statistically significant which meant that pH of pasture and forest soil samples did not varied significantly.

Table 5: pH of different soil samples of agriculture, pasture and forest soil samples

Land use types	Soil Sample	pH	Mean
Agriculture	A1	4.9	4.99±0.25b
	A2	4.9	
	A3	4.7	
	A4	5.03	
	A5	5.4	
Forest	F1	6.67	6.61±0.7a
	F2	5.55	
	F3	6.523	
	F4	6.8	
	F5	7.5	
Pasture	P1	6.28	6.08±0.33a
	P2	6.0	
	P3	5.54	
	P4	6.21	
	P5	6.37	

Note: A1, A2, A3, A4 and A5 are composite soil samples of agriculture land use type; F1, F2, F3, F4 and F5 are composite soil samples of forest land use type; P1, P2, P3, P4 and P5 are composite soil samples of pasture land use type; values after ± is standard deviation; values with different letters are significantly different at $P<0.05$ (Tukey HSD post hoc test)

The pH values of agriculture soil samples were quite low as compared to forest and pasture soil samples. The pH values ranged from 4.72 to 5.4. However, average minimum pH value of forest soil samples were found to be 5.55 (F2 soil samples) exceeding to 7.53

(F5 soil samples). Similarly, pasture soil samples also had the same range of pH as that of forest soil samples which is 5.54 to 6.37 (Table 5).

4.7 Correlation among SOC, MA, CFU and pH

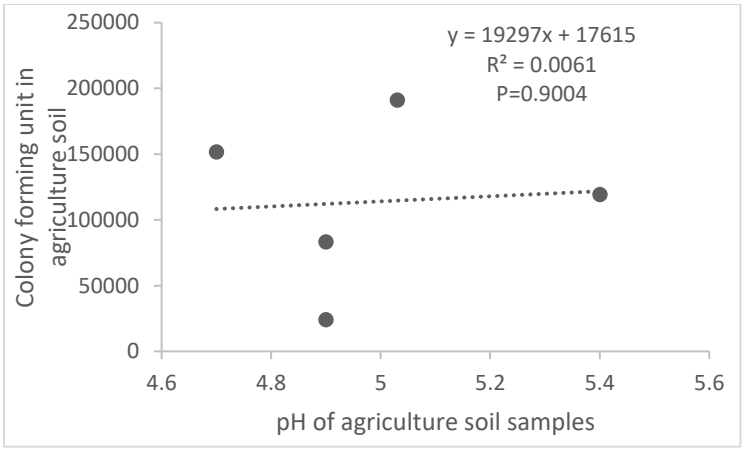
Correlation coefficient ranges from -1 to 1 and indicated the direction of relationship between two variables and the strength of the relationship between two variables.

Table 6: Correlation among SOC, SR, CFU and pH in different land use types studied

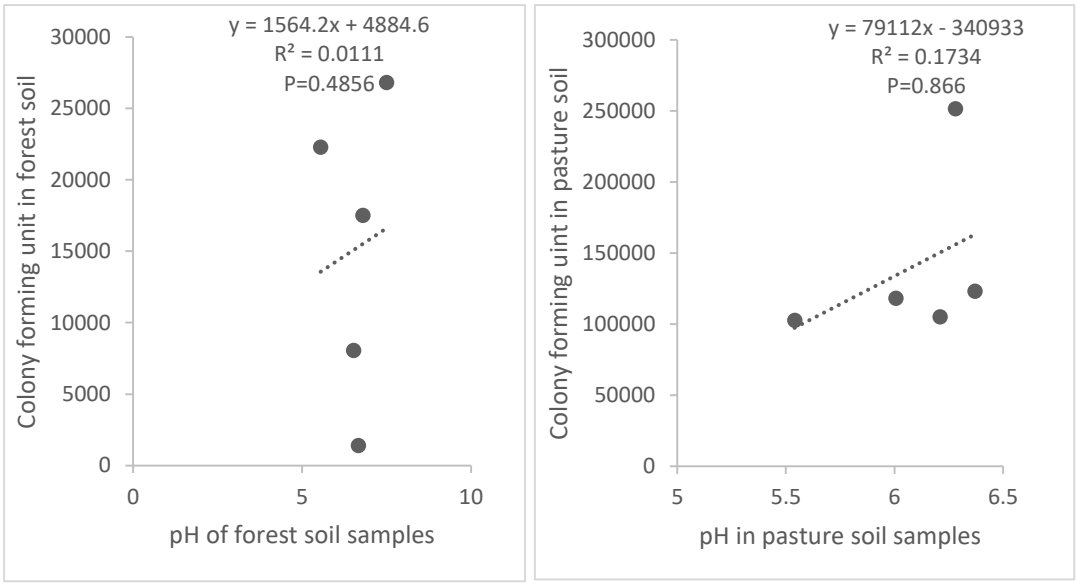
Correlation between	Correlation coefficients		
	Agriculture	Forest	Pasture
SOC, SR	-0.39	-0.05	-0.05
SOC, pH	-0.43	-0.44	0.39
SR, pH	-0.27	0.85	-0.21
SOC, CFU	0.2	-0.67	0.24
SR,CFU	-0.13	0.02	0.27
CFU, pH	0.07	0.41	0.1

Note: Pearson correlation coefficients relate the calculated SOC, MA, pH and CFU of each land use types

From statistical analysis, there was negative correlation coefficients among: SOC and soil respiration in all land use types; SOC and pH of agriculture and forest land use type; soil respiration and pH of agriculture land use types; SOC and CFU of forest land use types; soil respiration and CFU of agriculture land use types (Table 6). Moreover, positive correlation coefficients were found among: SOC and pH of pasture soil samples; soil respiration and pH of forest soil samples; SOC and CFU of agriculture and forest soil samples; soil respiration and CFU of forest and pasture soil samples; and CFU and pH in all land use types (Table 6). In all soil samples (agriculture, forest and pasture), basal respiration deceased with increase in soil organic carbon content and vice-versa.



a



b c

Figure 7: Correlation between Colony forming unit (CFU) and pH of a. agriculture land; b. forest land and c. pasture land

Colony forming unit (CFU) and soil pH had a positive correlation with each other in all three land use types studied. Figure 7 clearly showed with the increase in pH, colony-forming unit also increases in agriculture, pasture, and forest land use types.

4.8 Correlation among Simpson's and Shannon Diversity index with SOC, MA, CFU and pH

Table 7: Pearson's correlation coefficients between Simpson's (D) and Shannon diversity index (H) with soil organic carbon (SOC), soil respiration (SR), colony forming unit (CFU) and pH

Correlation between	Correlation coefficients
H, pH	-0.51
H, CFU	0.67
H, SR	-0.33
H, SOC	0.96
D, pH	-0.51
D, CFU	0.67
D, SR	-0.33
D, SOC	0.96

Note: positive values represents positive correlation and negative values represents negative correlation; P>0.05 which signified there was no strong evidence to support correlation among them

Above Table 7 showed the correlation coefficients between Simpson's and Shannon diversity index with soil organic carbon (SOC), Basal respiration (MA), colony forming unit (CFU) and pH of forest land use type. Simpson's (D) and Shannon diversity index (H) showed insignificant negative correlation with pH and basal respiration and significant positive correlation with colony forming unit and soil organic carbon (Table 7).

There was decrease in pH and CO_2 production (basal respiration) with the increase in species richness and species evenness and vice-versa. However, colony forming unit (CFU) and soil organic carbon (SOC) increases with the increase in species richness and species evenness of forest.

5. DISCUSSIONS

5.1 Impact of land use types on soil organic carbon (SOC)

Carbon input in soils through plant litter, root exudates, manure etc. and decomposition of soil organic matter, determine the soil carbon sequestration and soil respiration (Zhang *et al*., 2019). Soil organic carbon/total organic carbon concentration can be affected by many factors such as land use change, vegetation pattern etc. which consequently alters organic carbon input and decomposition rates (Zhang, Wang and Wang, 2014; Poeplau *et al*., 2011).

The above result showed that soil organic carbon (SOC) was highest in agricultural land as compared to forest land and pasture land. However, this result contradicts with the result of many other studies across the world. Xiangmin (2014) found that SOC was comparatively higher in forest followed by cropland and farmland. Ferriera and his co-workers (2014) stated that carbon losses occurs when native forest was converted to cropland.

Nevertheless, our results matched with the results of Poudel *et al*. (2019) where they found that SOC was highest in agricultural land followed by community forest and lease hold forest. Similarly, Wooliver and others (2022) reviewed and highlighted that SOC accumulation could be increased in agricultural or cropland by selecting ideal crop functional types, considering diversification options.

Highest SOC in agricultural land or cropland as compared to forest and pasture lands in our results could be due to the soil management practices applied by farmers such as applications of farm yard manure, compost and crop rotation that lead to carbon inputs in soil and increase in SOC accumulation (Dahal and Bajracharya, 2010). Furthermore, farmers in study area used leaf litters collected from nearby forests as a bedding for live-stocks which subsequently contributed to the carbon inputs in agriculture soils as it ultimately served as organic fertilizers to agriculture soils. Increase in quality and quantity of plant-derived carbon inputs to agricultural soils, not only encourage the growth and diversity of soil microbial communities but also increase the formation and storage of SOC (Zhang *et al*., 2021).

Also, fungal community/diversity increases the decomposition rate of soil nutrients, SOC that promotes in nutrients absorption and cycling (Kivlin *et al*., 2014) as the fungal

colony and colony forming unit was highest in agriculture soil samples as compared to forest and pasture soil samples. Likewise, low pH in agriculture soil samples as compared to forest and pasture soil samples could also be the reason of high soil organic carbon in agriculture soil samples as soil pH is also a chemical factor that influences soil organic carbon content significantly as it regulates soil nutrient availability, organic matter turnover and array of soil processes (Kemmitt *et al*., 2006). Similar to our study, some studies had demonstrated that with the increase in crop yields there was an increase in organic matter due to decaying of roots and this returning and improving SOC contents (Wiesmeier *et al*., 2015). Nevertheless in the study of Ou *et al*. (2017), with the increase in crop production, SOC content was negatively affected which could be due to the less carbon input as compared to carbon loss through soil organic matter decomposition and soil erosion. In agricultural land, crop residues might only represented a part of total carbon input into soils and other main contributors may be due to input of organic fertilizers (Song *et al*., 2011). Moreover, addition of unreasonable fertilizers in soil could lead to decrease in carbon input as it accelerates soil carbon decomposition in high crop yield area (Ou *et al*., 2017). All these findings suggest that chemical fertilizers input in soils cannot guarantee the sustainable development of agriculture.

5.2 Impact of land use types on basal respiration/ soil respiration

Land use change (LUC) not only changes carbon stock in soil but also affect soil respiration and nutrient availability. Agricultural soil had highest basal respiration as compared to forest and pasture soils in this study. This could be due to greater amount of crop residues and root exudates that provide additional carbon sources for soil microbes, enhancing soil respiration. Fan *et al*. (2015), monitored soil respiration across different land use types such as tea gardens, woodland and a vegetable field. Similar to this study, their results showed that tea garden and vegetable field had high annual cumulative respiration flux as compared to woodland. Also, Rhoades *et al*. (2000) found total soil carbon was lost from forest derived soil as compared to pasture and sugarcane vegetation (farmland).

According to Chen *et al*. (2022), SOC showed positive relation with basal microbial respiration. Unlike our results, their results had less soil respiration in cropland with human disturbance as it had lower SOC content. However, in our study SOC and soil respiration showed negative correlation (Table 6). Despite higher SOC in agriculture

land, soil respiration rate was still highest in agriculture land. This could be due to the intensive agriculture practices such as fertilization and irrigation which subsequently enhanced nutrient availability and moisture to the microbes which leads to faster decomposition and higher soil respiration rates.

Fan *et al.* (2015) discovered a significant correlation between the annual mean soil respiration rate and the concentrations of organic carbon and total nitrogen, which varied across different land use types. Their findings indicated that converting woodland to tea gardens (HP) and vegetable fields (VF) increased the annual cumulative respiration flux by 25.6% and 20.9%, respectively, with the highest organic carbon found in VF, then followed by HP. This result aligns with Wang *et al.* (2013), who also found that the annual mean respiration rate in woodland converted from grassland was largely dependent on organic carbon and total nitrogen content. Organic matter serves as the substrate for soil respiration, and environmental factors such as soil temperature and moisture are primarily influenced by the energy released from decomposing soil organic matter (Davidson and Janssens, 2006). Wang *et al*. (2013) reported that conversion of grassland to woodland would increase annual soil respiration by 3-22%. However, Zhang *et al*. (2013), observed that after converting paddy fields (agriculture) to bamboo stands (forest), cumulative emission of CO_2 decreased.

Conversely, Liu *et al*. (2011) found no significant difference in soil respiration efflux and organic carbon when evergreen forest was converted to bamboo forest. However, they did observe an increase in soil respiration efflux due to intensive management practices, alongside a decrease in organic carbon.

Hence, in this study higher basal respiration in agriculture soil samples could be due to the high intensive agriculture practices such as fertilization, irrigation, tilling and other cultivation activities that accelerates decomposition process and increase soil respiration rate.

5.3 Impact of land use types on colony forming unit (CFU) and soil fungal-population

Our results showed that CFU value was highest in agriculture land followed by forest land and pasture land. This could be due to high number of fungal colony formed in agriculture soil than in other two types of soil. Fungal colony was positively related with

SOC which was more in agriculture soil followed by forest and pasture soils. In the similar study carried out by Puangsombat *et al*. (2010) determined that soil fungal diversity was higher in pineapple field followed by *Dalbergia* forest and grassland.

Likewise, high number of fungal colony forming unit in agriculture soils might be due to pH level of soils and organic matter content in soils as well. Fungi generally prefer acidic medium (Dix and Webster, 1995) but there are some genera of fungi that grow well in neutral to slightly alkaline conditions (Warcup, 1951). High pH, lower organic matter and lower mineral P do not favors the micro-organisms species richness (Puangsombat *et al*., 2010). Some fungal taxa are unable to thrive if the soil pH falls outside a certain range as pH may directly affect fungal physiological constraint.

Hence, higher CFU (fungal population) in agriculture land as compared to forest and pasture land could be due to frequent addition of organic amendments such as manure, compost and crop residues. Also, the use of chemical or organic fertilizers in agriculture provides essential nutrients and suitable pH for the growth of fungal communities that led to higher CFU counts. In contrast, forest and pasture lands had less CFU counts which could be due to lower nutrient inputs as all the leaf litters from forest were collected and converted to manure for agriculture crops, which limited fungal population expansion in forest and pasture lands.

5.4 Soil pH and its relation with SOC, MA and fungal colony

Above results showed that pH level of agriculture soil samples were comparatively lower than that of forest and pasture soil samples. This might be due to the decay of organic matter that releases more anions and increase soil pH in forest soils. Soil microbes further break down the plant material for mineralization that may also add in the pH increment.

Similarly, Zhou *et al*. (2019) found that soil carbon and nitrogen are negatively correlated with soil pH demonstrating that relatively low pH benefits the accumulation of organic matter. In this study, pH was negatively correlated with SOC. Higher the SOC content lower the pH. Since, agriculture soil samples had highest SOC content, this could be the reason for lower pH in agriculture soil samples as compared to forest and pasture soil samples. In top soil, pH is generally lower due to rich in organic matter and its decomposition leads to the production of more organic acids (Hong *et al.*, 2019).

Similarly, with the increase in vegetable production/crop production, there will be an increase in root systems and with this rhizosphere micro-flora, root exudation and respiration will be more and consequently more carbonic and organic acids will be produced which could be reason for the decline in pH (Hinsinger *et al*., 2003) in agriculture land as compared to forest and pasture land.

5.5 Impact of plant diversity on different soil parameters in forest

Above results showed that Chapagaun forest had high species abundance and evenness. The results illustrated that importance value index (IVI) of Chapagaun forest were positively correlated with soil organic carbon and basal respiration but were negatively correlated with soil pH. Likewise, Simpson and Shannon diversity index were positively correlated with soil organic carbon and CFU but were negatively correlated with basal respiration and soil pH. This indicated that high plant diversity of forest favors the soil organic carbon concentration and basal respiration. Increase in SOC storage due to high plant diversity could be due to either higher primary production or slower decomposition of plant derived organic materials that leads to longer persistence (Schmidt *et al*., 2011; Jastrow *et al*., 2007).

Chen *et al*. (2017) observed 6,098 forest shrub land and grassland sites across China to find out if plant diversity enhances productivity and soil carbon storage. They found that climate favorability (i.e. high temperature and precipitation was positively correlated with species richness and below ground biomass which consequently had positive effects on SOC storage. SOC and pH was negatively correlated. In addition climate favorability (high temperature and precipitation) had negative effect on SOC storage but it was accompanied by the high species richness and below ground biomass.

Some experimental studies had suggested that with high plant diversity, SOC storage also increases by increased inputs of carbon and elevating diversity of soil microbial community and by reducing decomposition that suppresses carbon losses (Lange *et al*., 2015; Formara and Tilman, 2008). But in contrast, other studies had suggested that SOC storage was determined by climatic conditions, vegetation patterns and composition along with soil conditions (Jenny, 1980; Houghton, 2007; Crowther, 2016). For a certain level of precipitation, SOC storage shows positive correlation with precipitation but not with high temperature as soil respiration increases with temperature which leads to decline in SOC storage (Deyn *et al*., 2008; Post *et al*., 1982).

Plant diversity enhances SOC storage by stimulating litter input through increased productivity and also reduces SOC by stimulating microbial respiration. Chen *et al*. (2019) found that when 50% of global forests was converted plant species mixtures to monocultures, an average of 2.70 Petagram Carbon would release from soil annually and fossil fuel emissions by 30% annually. Hence, there is a high importance of preservation of plant diversity for the regulation of carbon sequestration in soil amid the climate change policy globally.

In addition, Lange *et al*. (2015) showed that basal respiration and carbon storage are enhanced by higher plant diversity as rhizosphere carbon inputs increases due to microbial community. Higher amounts of plants residue inputs in soil might accelerate and enhance decomposition and reduce carbon storage but in contrast, microbial accumulation could increase plant residue inputs over the time (Liang *et al*., 2011). Hence, their results demonstrated that higher plants diversity mediates higher root inputs and this governs metabolic activity of soil microbes which consequently enhance storage of soil carbon.

6. CONCLUSION

In conclusion, this study demonstrated that maximum soil organic carbon (SOC), basal respiration and colony forming unit (CFU) in agricultural land than in forest land and pasture land. Similarly, pH of agriculture land was found to be acidic as compared to forest and pasture land. Above results and discussions indicate that agricultural land to be greater carbon sink as compared to forest and pasture land in context of Nepal. This could be due to the fact that most of the litter fall from forest has been collected by local farmers for their livestock and other purposes which subsequently decreased carbon inputs through litter fall in forest whereas in agriculture land carbon inputs was increased through manure and fertilizers. However, forest land with AGTB (stand carbon stock) addition to soil carbon stock could make more in comparable to the SOC of agricultural and forest lands. Hence, forest is the only method to reduce carbon in atmosphere which is the current global challenge amid climate change. Nevertheless, our results has compelled to think agricultural land as sink of carbon like forest land if proper management practice is implemented on these land with higher production and the global climate change issue.

RECOMMENDATIONS

- Results of this study have compelled to think in country like Nepal, agricultural land could be a sink of carbon like forest land if proper management practice is implemented on these land with higher production and the global climate change issue.
- As similar to this study, if potential of land use types to sequester carbon could be determined all over the Nepal then it would give general trend of land use types and their overall potential as a carbon sink. Such type of study would eventually aid in the mitigation of carbon emission globally.
- Therefore, more study should be conducted and more emphasis must be given to determine and analyze the carbon content in different land use types of Nepal.

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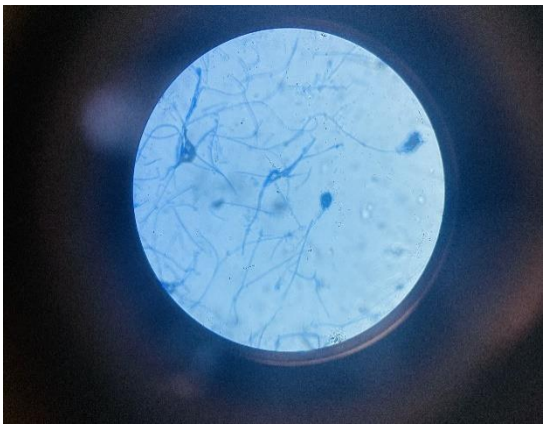
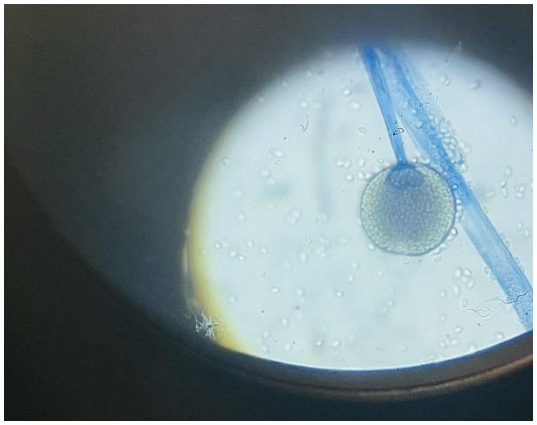
APPENDICES

Mucor sp.

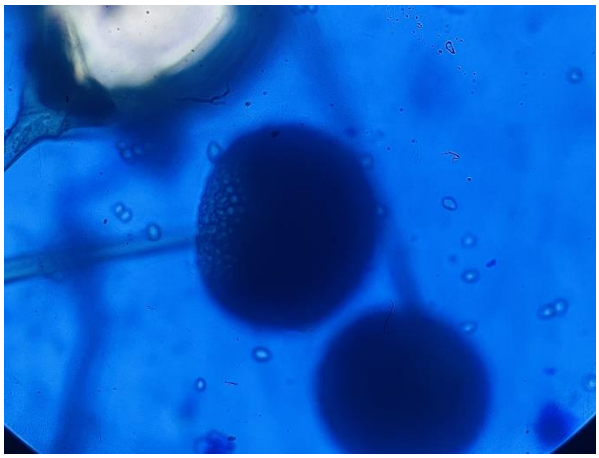


Microsporum sp.

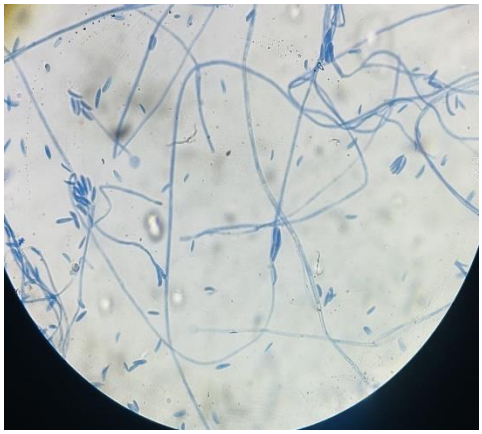


Mucor sp.

Glicladium sp.



Mucor sp.



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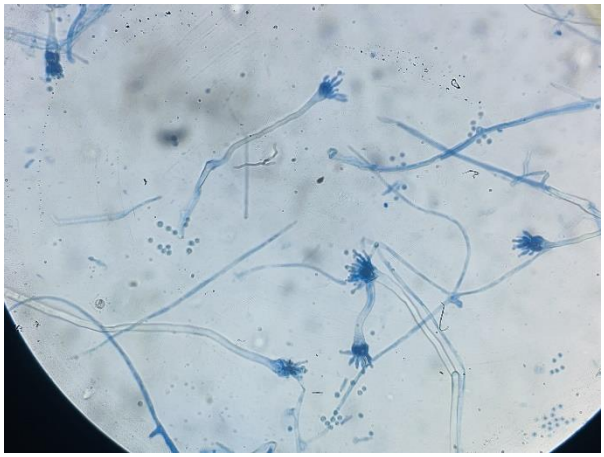
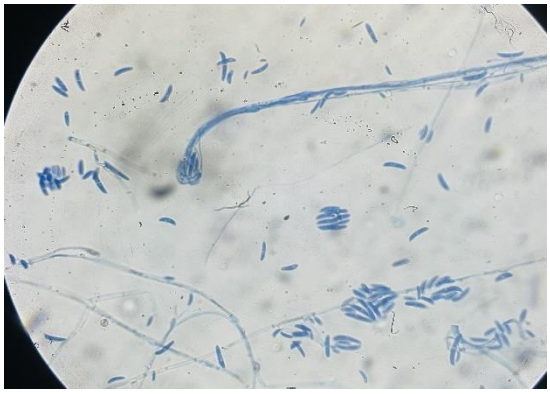


Penicillium sp.



Fusarium sp.

Cultured plates

Identification of fungi through microscope

Serial dilutions

Colony count

Observing colony color

Cultured petriplated

Fungal growth

Fungal growth

Colony count

Fungal growth

Fungal growth

Fungal growth

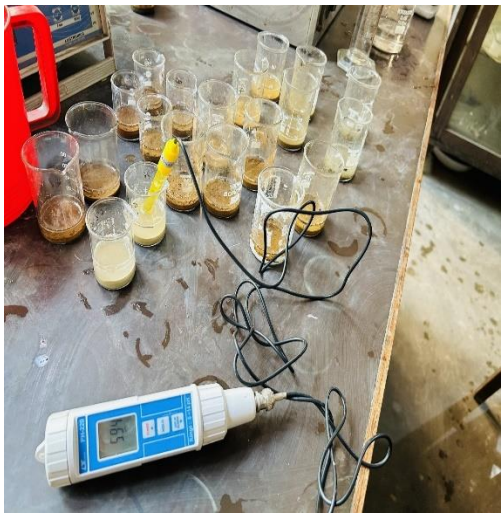
Petriplates ready to be incubated

Quadrate sampling

noting down plants name observed

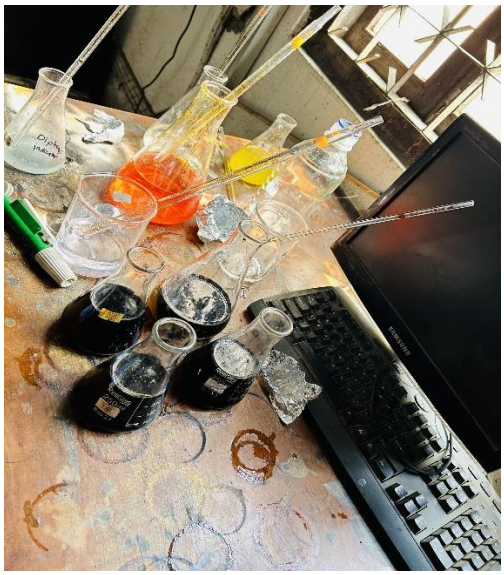
Preparation of media

Soil sample collection



Measuring pH

Measuring pH



Chemicals used for titration

Soil samples with chemicals to be titrated

Titration against Ferrous ammonium sulphate

Chemicals after titration

Urtuca sp.

Ziziphus incurva

Lonicera ligustrina

Oplismenus compositus

Reinwardtia indica