SOIL PROPERTIES AND SOIL MICROBIAL BIOMASS ALONG ELEVATION GRADIENT IN RAINFED AGROECOSYSTEM IN KASKI DISTRICT, NEPAL

A Dissertation Submitted for Partial Fulfilment of the Requirement for the Master's Degree in Science, Central Department of Botany, Tribhuvan University

Submitted by

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RECOMMENDATION

This is to certify that M.Sc. Dissertation entitled **" Soil properties and soil microbial biomass along elevation gradient in rainfed agroecosystem in Kaski district, Nepal** "has been carried out by **Ms. Kalpana Acharya** under my supervision. This work has been completed based on the candidate's original research work based on field visits and lab work. The work has not been submitted for any other academic degree. I recommend this dissertation work to be accepted as partial fulfillment for a Master's Degree in Botany at the Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

The M.Sc. Dissertation entitled " **Soil properties and soil microbial biomass along elevation gradient in rainfed agroecosystem in Kaski district, Nepal "** submitted by **Ms. Kalpana Acharya** has been accepted for the partial fulfillment of her Master's Degree in Botany (Applied Mycology and Plant Pathology Unit).

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ABSTRACT

Soil is important natural resources for the sustenance of life on the earth and also a crucial part of agriculture. The study was conducted to examine the soil physicochemical properties and microbial biomass carbon along elevation gradient in rainfed agroecosystem at Annapurna Rural Municipality Ward No.10, Kleu (1350) and Annapurna Rural Municipality Ward No.11(1650 - Jhinu danda, 1950 - Taulung and 2250 - Chhomorong) Kaski, Nepal. Altogether 60 composite soil samples were sampled from different elevation gradient. Soil samples were collected from depth layer 0-10cm, 10-20cm and 20-30cm by using soil corer. At each elevational point 5 plot of 10m ×10m was made with the distance of 15-50m between each plot. Each elevation gradient distance was apart 300m. Random sampling method was used to collect the samples. The result of the study shows that soil physical (soil temperature, soil textural fractions, moisture, and bulk density) chemical (pH, electrical conductivity, soil organic carbon, soil organic matter, soil organic carbon stock, total nitrogen, available phosphorous, available potassium) and biological (microbial biomass carbon) properties varied significantly with different elevation gradient and soil depths. The soil in the study region was mildly acidic to almost neutral. Loamy sand was the textural class observed. The bulk density, soil temperature and silt content were recorded greater in the lower elevation. The increasing trend in the Moisture, EC, SOC, SOM, SOC stock, TN, AP, AK, clay, sand and MBC along increasing the elevation gradient and decreasing trend in the soil depth 0-10cm to 10-20cm and 20-30cm whereas bulk density and pH was increase with increasing soil depth layers. The silt content and soil temperature were decreased with increasing the soil layer. All the nutrients, SOC and microbial biomass carbon were greater at elevation 2250masl. Soil quality index values were fair in all the elevation with depth, although the value was decreased with depth. At the depth 0-10cm, MBC has positive significant correlation with SOC, Soc stock, AK, and sand and negative significant correlation with temperature and silt. Similarly in the depth 10-20cm MBC has positive significant correlation with SOC, Soc stock, AP and clay and likewise in the depth 20-30cm, no significant correlation was found with SMBC.

Keywords: Rainfed land, physicochemical properties, microbial biomass carbon, soil quality index

ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AP	Available Phosphorous
AK	Available Potassium
BD	Bulk Density
C	Carbon
℃	Degree Celsius
cm	Centimeter
Conc.	Concentration
EC	Electrical Conductivity
FAO	Food and Agriculture Organization
FYM	Farmyard Manure
g.	Gram
ha	Hectare
K	Potassium
Kg	Kilogram
m	Meter
masl	Meter above sea level
mg	Milligram
ml	Millilitre
MOALD	Ministry of Agriculture and Livestock Development
N	Nitrogen
NARC	National Agricultural Research Council
OM	Organic Matter
P	Phosphorus
pH	Potential of hydrogen ion

ppm	Parts per million
SMB	Soil Microbial Biomass
SMBC	Soil Microbial Biomass Carbon
SPQ	Soil Physical Quality
SPSS	Statistical Package for Social Science
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SQI	Soil Quality Index
t C/ha	tonnes of carbon per hectare
TN	Total Nitrogen
USDA	United States Department of Agriculture
%	Percent

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CHAPTER – ONE

INTRODUCTION

Background

Soil is the diverse habitat on earth and contains the multiple assemblage of living organisms and it contribute to the maintenance and productivity of agro-ecosystems (Giller et al., 1997). According to FAO (2015) another definition of soil is a natural body made up of layers (soil horizons) that are made up of organic material, air, water and weathered mineral components. Soil is the result of the cumulative effects of temperature, terrain and creatures (including flora, fauna and humans) through time on parent materials (initial rocks and minerals). Soil is important natural resources for the sustenance of life on the earth. It is a critical part of agriculture and is the original source of the nutrients for the crop plants.

Soil quality and healthy soil determine the agriculture sustainability, environmental quality and climatic resilience (Karlen, 2004). Depending on the management inputs, soil quality changes and soils react differently. Physical, chemical and biological parameters are components of soil quality. Both innate and movable qualities are present in soil quality. According to Intergovernmental Technical Panel on Soil (ITPS), "soil health" refers to an ecosystem's ability to maintain its production, variety, and environmental services. In addition to producing better food and fiber, high quality soils also support the development of natural ecosystems and improve the quality of the air and water (Griffiths et al., 2010). Soil health and nutrient content are important sign of soil quality because they directly impact the productivity and health of plants (Jansen et al., 1995).

According to Hillel (1982) physical indicators are focused on permeability, water holding capacity, soil structures and bulk density. Chemical parameters include measurements of pH, salinity, organic matter, phosphorus concentrations, cation-exchange capacity, nutrient cycling and concentrations of elements that may be potential contaminants (heavy metals, radioactive compounds, etc.) or those that are necessary for plant growth and development (Kumar & Kumari, 2022). Microbiological activities that intervene in soil quality are dynamically driven (Abawi & Widmer, 2000; Doran & Parkin, 1994). Biological indicators of soil properties include mineralizable

nitrogen, respiration, organic matter in the soil and microbial biomass (total bacteria and fungus).

McKenzie et al. (2011) defined Soil physical quality (SPQ) as a soil's capacity to meet plant and ecosystem needs for aeration, strength and water throughout time as well as to be able to withstand and recover from events that could jeopardize that capacity. The quantity of organic matter in soils, as well as their texture, mineral constitution and porosity, all have an impact on bulk density. When soil is compacted, its bulk density increases because the soil particles are compressed closer together, reducing the amount of pore space between them. This increase in bulk density leads to a corresponding decrease in porosity, which is the volume of pore space in the soil relative to its total volume (Keller & Håkansson, 2010). It is regarded as a permanent characteristic of a soil under normal circumstances. According to United States Department of Agriculture (USDA) methodology, textures were classified as being coarse (sandy loam, sandy clay loam, loamy sand), medium (clay loam, loam, silty clay loam, silt, silt loam) and fine (clay, silt clay, sandy clay). Soil texture impacts soil water retention, leaching, erosion, nutrient storage, organic matter dynamics and carbon sequestration capacity. The capacity of soils to store carbon, water and nutrient ions is strongly influenced by soil texture, which also affects other hydrologic and biogeochemical processes in forest ecosystems (Jenny, 2012). Soil texture analysis is crucial for assessing soil quality for agricultural and forestry growth (Dahal et al., 2018).

Soil pH is a measure of the acidity or alkalinity of the soil, and it's an important chemical characteristic that affects the availability of nutrients to plants, the activity of soil microorganisms, and the overall health of the soil. Most plants grow best in soils with a pH between 6 and 7.5 (Jahn et al., 2006). It is an important soil attribute because it influences a variety of chemical and biological processes in the soil, such as nutrient availability, crop growth and development and microbial activity (Fernández & Hoeft, 2009). In addition, low pH in the surface soil inhibits the microbial activity and nitrogen nodulation, which in turn results in nitrogen deficiency in plants (Cienciala et al., 2016). Both soil physical and chemical properties are important for agriculture because they determine the soil's fertility and ability to support plant growth. Elevation change can also impact soil properties and agriculture. For example, as elevation increases, temperature and precipitation patterns may change, which can affect soil formation,

erosion, and nutrient cycling. Additionally, different elevations may have different soil types or geology, which can influence soil properties and fertility.

Soil organic carbon (SOC) is a critical component of healthy soils, both in natural ecosystems and in agricultural systems. It is the major constituent of soil organic matter and is formed from the decomposition of plant and animal residues (Manlay et al., 2007). It plays an important role in soil fertility and hydrology, contaminants control, and acts as a sink or source of terrestrial C, which influences the level of atmospheric CO_2 in the atmosphere. A rate of 1.5% per year results in an exponential rise in atmospheric carbon. The role of soil in reducing climate change and sequestering carbon is widely recognized (Tan & Lal, 2005) . As elevation increases, the temperature drops, slowing organic matter breakdown rates more than litter formation which leads to an accumulation of SOC (Choudhury et al., 2016a). In addition to playing a vital role in climate change mitigation, soil C is crucial for ensuring soil health and production in agricultural systems.

Soil organic matter (SOM) refers to the organic matter that are present in soil including, both living and non-living component. Additionally, there can be an increase or decrease in soil organic matter depending on number of variables, such as the climate, vegetation type, nutrient availability, disturbance and land use and management techniques (Barua & Haque, 2013). Additionally, physical soil characteristics like soil structure, particle size, and composition have a big effect on soil carbon (C). The rate at which soil organic carbon decomposes is also significantly impacted by soil particle size (Baldock & Skjemstad, 1999; Six & Jastrow, 2002).

Available Three nutrients in particular—nitrogen (N), phosphorus (P), and potassium (K)—have a significant impact on crop yield and the long-term viability of an agricultural system. The process of nitrogen accumulation is closely related to the accumulation of organic matter since the nitrogen in soil, especially that in the surface layer, occurs mostly in organic combination (Stevenson, 1965). A decreased pH in the soil's top layer inhibits microbial activity and nitrogen nodulation, which causes plants to lack sufficient nitrogen (Cienciala et al., 2016). The second-most important nutrient for most crops is phosphorus (P), which is necessary for agroecosystems and best crop production (Ziadi et al., 2013). It plays significant role for effective management of phosphorus in agricultural soils. Soil contains an average of 1.7% potassium (Martin &

Sparks, 1985). The soil's qualities, such as moisture content, aeration, temperature, tillage practices and K dynamics all affect plants' accessibility to K. The rate of K exchange varies amongst soil types, and ultimately, the uptake of K affects plant growth and yield. Thus the role of potassium in soils is prodigious (Mouhamad et al., 2016).

Soil microbial biomass is becoming more and more popular due to the significance of microorganisms in ecosystem functioning (Azam et al., 2003). Most biogeochemical processes in terrestrial ecosystems depend on the primary productivity of the soil microbial biomass as the active component of the soil organic pool, which affects soil nutrient content and, subsequently, organic matter decomposition (Franzluebbers et al., 1999; Haney et al., 2001). A crucial part of the soil's organic matter that controls how nutrients are transformed and stored is the biomass of soil microbes. Soil microbial biomass is a labile part of the soil organic fraction that contains 1 to 3% of the soil's total carbon and up to 5% of the soil's total nitrogen (Smith & Paul, 2017). It is made up of all soil organisms with a volume less than approximately $5 \times 10\text{^}3\mu m^3$. It is a significant labile reservoir of vital plant nutrients, such as phosphate, sulphate, and nitrogen. The changes in soil fertility may be reflected in changes in microbial biomass long before such changes are reflected in changes in the overall pool of soil organic matter (Brookes, 2001). So, soil microbial biomass is crucial component of soil quality assessment because of the size and activity of the microbial biomass in the availability of nutrients and the productivity of agroecosystems (Friedel et al., 1996).

Microbes in the soil are essential for regulating soil health and crop productivity. Microbes play a vital role in controlling soil fertility by altering soil characteristics both directly and indirectly. Many microorganisms, including bacteria, fungi, mosses and liverwort, could be found in soil. Three types of microbes—bacteria, fungi and actinomycetes—constitute the majority of the soil's microbial biomass. Microbes regulate the chemical and physical characteristics of soil and are a reliable sign of biological activity. All nutrient cycles and plant nutrients depend on microorganisms, which are a key component of soil. Microbial diversity and soil processes are negatively impacted by temperature variations, low water content, anthropogenic factors and grazing (Kumar & Verma, 2019). The biological nitrogen fixation mechanisms supported by microbes include a variety of biological transformations that help in the nutrient accumulation and utilization, support root and shoot growth, disease

management and enhance soil quality for crop cultivation. Soil microbes increase crop yield, provide nutrient-dense sustenance and recycle soil solutions. Therefore, they are crucial to the soil's fertility, nitrogen cycle and organic matter degradation (Shah et al., 2021).

Elevation is the main factor influencing the characteristics of the soil ecosystem. Variations in elevation have a significant impact on the characteristics and richness of the soil ecosystems. The physical characteristics of the soil, such as its porosity, density and texture, change with altitude. According to reports, soil's bulk density (BD) decreases as altitude rises, but its moisture content and water-holding capacity both increases. Sand, clay and silt make up a greater percentage of the soil texture as altitude increases (Jeyakumar et al., 2020). Many soil fertility traits, such as organic matter content, pH, CEC, phosphate sorption, and phosphorus availability, exhibit large elevational changes (Jobbágy & Jackson, 2000). The increase in soil organic matter with elevation is caused by a reduction in temperature as elevation rises. Low temperatures in high altitude soil reduce microbial and enzymatic activity, protecting the organic content from microbial decomposition.

Agricultural lands are major two types Khet (irrigated low land) and Bari (rain-fed upland). Rainfed ago-ecosystem is used to describe farming practices that rely on rainfall for water. It is one of the major ecosystems in Nepal and one of the most important components of agriculture system. Bari refer land with higher elevation than Khet fed terraces suitable primarily for maize, potato, millet etc. (Paudel & Thapa, 2001). Rainfed lands have historically been considered fragile, marginal, waste, problematical, threatened, low potential or less-favourable fields in the absence of irrigation, particularly those in the arid and semiarid agro-ecological zones of the world (Devendra, 2016). In Nepal, 65% of the total cultivable land area is used for rainfed agriculture (Kattel, 2022).

According to the FAO, In Nepal, the agricultural sector employs about 66% of the total population. It has a significant impact on the national economy and provides one-third of the country's GDP. Although Nepal's agriculture is diverse, it is mostly dominated by three primary cereals: rice, wheat and maize which together account for 30.92 percent of the nation's agricultural GDP (Adhikari, 2015). According to MOALD

(2020/21) In Nepal, in terms of production of cereals crops rice occupies 50.56%, maize occupies 26.96% and millet occupies 19.13%.

Maize is Nepal's second-most significant crop, behind rice in terms of area and production (MOALD, 2020/21). The main source of supplemental nutrition is maize, which can supply up to 30% of the animal's diet in terms of protein, 60% of its energy and 90% of its starch (Tiwari et al., 2013). About 56% of all agricultural land is used for growing corn and 50% of those fields are planted with hybrid varieties. 87% of the total maize used in the production of animal feed was imported each year by the feed industry from India (Timsina et al., 2016) .

According to the MOALD, Statistical information on Nepalese agriculture 2020/21, since the last ten years, 2012/13 the area of cultivation of maize 849,635 hectare, the production 1,999,010 metric tonnes and the yield 2.35Mt/Ha. In the year 2015/16, 13 the area of cultivation of maize 891,583 hectare, the production 2,231,517 metric tonnes and the yield 2.50 Mt/Ha. In the year 2018/19, the area of cultivation of maize 956,447 hectare, the production 2,713,635 metric tonnes and the yield 2.84 Mt/Ha and in 2020/21 the area of cultivation of maize 979,776 hectare, the production 2,9997,733 metric tonnes and the yield 3.06 Mt/Ha. Thus, the area of cultivation and the production of maize is increasing yearly, therefore the demand for maize is shifting from food to feed for livestock and poultry and it is also regarded as a means of ensuring food security in Nepal.

Ecosystem-based adaptation is defined as "sustainable management, conservation and restoration of ecosystems as part of an overall adaptation strategy that takes into account the numerous social, economic and cultural co-benefits for local communities" (Bhusal et al., 2022). There are several agricultural methods that are based on the management of ecosystems and environmental services. According to Harvey et al. (2017) Using agroforestry to protect soil from erosion, degradation and the impacts of floods, high temperatures and other climate impacts on livestock and crops are some examples of ecosystem-based adaptation practices. Other practices include crop rotation, intercropping and crop diversification within fields, as well as methods for conserving soil that will stop soil erosion and sustain soil fertility during periods of heavy rainfall (Tengö & Belfrage, 2004).

Overgrazing, deforestation, unpredictable and erosive rainfall, steep topography, poor land use, and inadequate soil management have all led to a global decline in soil quality, which has threatened agricultural output, economic growth, and a healthy environment. The main contributor to soil degradation in developing nations like Nepal is the overuse of chemical fertilizers and pesticides (Eswaran et al., 2019). Additionally, the degradation is also accelerated by socioeconomic and political factors like capital, infrastructure and land tenure (Denboba, 2005).

1.1 Justification of the study

The two most urgent issues affecting terrestrial ecosystems on a global scale are land degradation and biodiversity loss. Globally, land degradation has a negative impact on almost 1.5 billion people which currently affects about 23% of the world's terrestrial area (Stavi & Lal, 2015). Therefore, urgent action is required to stop further degradation and restore presently degraded areas in order to maintain ecosystem function and production, reduce the change of climate, protects biodiversity and ensure food supply and resource availability (Muñoz-Rojas, 2018). Agricultural yields are decreased by contaminated and deteriorated soil, which has an influence on farmer livelihoods and earnings. Any production system's productivity and sustainability depend not only on its management strategies but also on the state of the environment and the quality of the soil. A healthy soil supports and sustains strong agricultural productivity with minimal negative environmental effects and has the best physical, chemical and biological characteristics to meet these needs (Reynolds et al., 2009). Thus, it would seem to be the best sign of equitable land use. It is used to evaluate the overall condition of the soil, its response to management, and its resilience to anthropogenic and natural forces. It also aids in assessing changes in dynamic soil properties brought about by external influences. The status and responsiveness of the soil can be assessed using a variety of soil indicators that are derived from physiochemical properties. Hence it is desirable to view the use of soil indices for assessing the soil quality which will enable the provision of information on the current state of soil health and an early indication of it.

Kaski District has its own unique socio-cultural, biological, and geomorphologic features. Annapurna Rural Municipality lies in Kaski district which is tourism sector area. The land use pattern in agriculture is different and the study area was far from the

urban area. Although this area was once thought of granary, it is currently under great threat from land degradation and deforestation, poor land management practices. The area's soil quality is being degraded by the current methods of unmanaged farming, incorrect pesticide use, and unmanaged and illogical land management. As far as we are aware, studies on soil characteristics have not done in the rainfed land along the elevation gradient and there is a paucity of information regarding the soil parameters. Therefore, the goal of study is to initiated to assess the status of macro nutrients, SOC and other soil properties including microbial biomass along different elevation and soil depth. The information obtained will be helpful for local farmers, regional, national level and policymakers in creating better soil management plans to preserve or enhance the soil's quality and increase the productivity.

1.2 Research question

Research aimed to answer the following questions,

- What is the status of soil properties at different elevations in the Annapurna rural municipality ?
- Do the soil parameters vary with the different elevations and the depth layer?
- Do the soil physicochemical and microbial properties correlate with each other?

1.3 Objective

The primary objective of the study was to assess the soil properties and microbial biomass along elevational gradient of rain-fed land in Annapurna -10 and 11, Kaski Nepal.

1.4 Specific objectives

- To determine the soil physicochemical properties (soil moisture, temperature, bulk density and soil textures, pH, EC, SOC, SOM, Soc stock, TN, AP and AK) along elevation and depth.
- To determine the biological property.
- To analyzes the soil quality index (SQI).

1.5 Limitations of the study

The research was limited to maize cultivated land particularly rain fed / upland (Bari) agroecosystem. Only one season was considered for soil sample collection. The sample was collected at the mid of winter (January-February) season which is before the maize land preparation.

CHAPTER – TWO

LITERATURE REVIEW

2.1 Overview of soil quality and its indicator

Soil quality is the measure of a soil's ability to perform its natural functions within an ecosystem, including supporting plant growth, filtering and retaining water, cycling nutrients, storing carbon and providing habitat for soil organisms (Karlen et al., 1997). Soil health refers to the ability of soil to support the biological, chemical, and physical processes that are necessary for healthy plant growth and ecosystem function. A healthy soil is one that is able to sustain its biological activity, maintain its physical structure, and retain its nutrients and water-holding capacity, all of which are essential for supporting plant growth and other ecosystem services (Doran & Zeiss, 2000; Karlen et al., 2001). Soil quality and the soil health are interchangeable terms. The changing nature of soil quality may have an impact on the sustainability and productivity of land use. It is the result of processes that either degrade or conserve soil, and its development is influenced by the interplay between a soil's chemical, physical and biological constituents (Parr et al., 1992).

Soils are an important component of land systems, and they perform a wide range of functions that are vital for sustaining life on Earth. These soil activities assist in the provision of essential ecosystem services like the water and climate regulation, nutrient cycling, and carbon sequestration, all of which can be negatively impacted in devasted ecosystems. Due to the difficulty of directly measuring the bulk of soil ecosystem activities, they are frequently inferred from observable soil characteristics like soil quality measures, which can include a wide variety of soil physicochemical and biological properties (Muñoz-Rojas, 2018).

In Nepal, the evaluation of soil quality as such is almost lacking. Farmers, land managers and legislators have placed a strong emphasis on improving soil fertility and preventing erosion to increase agricultural production. The assessment of soil quality has been suggested as a helpful method for determining the viability of crop and soil management practices.

2.2 Soil physical parameter

Boix-Fayos et al. (2001) suggested bulk density (BD), texture, accessible water capacity (AWC), particle stability and crusting are significant soil physical qualities that are typically regarded as indicative indicators of soil physical quality.

A necessary component of the soil's three-phase system, which also consists of soil minerals (solids), moisture, and air, is soil moisture. The soil moisture content has a significant impact on the physical, chemical, mineralogical, mechanical, geotechnical, hydrological and biological aspects of the soils (SU et al., 2014). Land use had a significant impact on the differences in soil moisture in deeper layers, which were highly stable with seasonal changes. In contrast to other land uses, the moisture variation under agriculture, fallow land and shrubland decreased with depth. The soil moisture variation with depth was the same for farmland, fallow land and shrubland. Under the agricultural and fallow area, the soil moisture content increase with depth (Fu et al., 2003).

According to a Charan et al. (2013) the bulk density is influenced by the texture and structure of the soil, organic content, and the freezing and thawing processes. The mass (or weight) of the sample is divided by the bulk volume to determine the bulk density of a known-volume soil sample. According to Kakaire et al. (2015) soils with a lower bulk density are less compacted and can hold more water at field capacity. Conversely, soils with a higher bulk density can hold less water at field capacity. Osakwe & Igwe (2013) have shown that the increase in bulk density caused by the conversion of forest to cultivate land is a sign of the severity of soil degradation. Low soil porosity and soil compaction are both characterized by high bulk density. It might prevent roots from growing and obstruct air and water flow in the soil. According to Ahmad Dar & Somaiah (2015) bulk density raise significantly with increase in soil depth and drop with increase in altitude.

Increased soil BD results may be due to soil disturbances including frequent tillage, animal trampling, and erosion that removes SOM. Additionally, according to Jin et al. (2007), soil BD can reveal changes in soil structure and water-retention capacity under various tillage techniques. In addition to limiting root development and water flow, soil compaction exacerbates soil erosion.

At low altitudes temperatures rise, soil biological activity and decomposition rate both increase, which results in less carbon (C) accumulating in the soil. The best way to sustain a slow rate of soil organic matter decomposition is at higher altitudes with low temperatures (Ahmad Dar & Somaiah, 2015; Wei et al., 2013). Due to the apparent disparity in organic matter decomposition rates, upland soils had a much low bulk density than midland soils (Teferi et al., 2016).

The proportions of sand, silt, and clay in soil influence its texture. The ability of soil to hold water and nutrients, water and air flow, pore diameters and plant root growth are all impacted by soil texture (Huluka & Miller, 2014). Tadesse et al. (2016) suggested that cultivated land has high rates of clay accumulation. The abrupt changes in the distributions of sand and silt fractions and the associated variations in clay content within soil depth are a result of deposition and erosion, which resulted in the deposition of sediments with various particle sizes and/or parent materials.

According to Saeed et al. (2014) Silt demonstrated a substantial elevation-related correlation and variation, increasing at a rate of 0.997 correlation coefficient at $P<0.001$ significance. While the Sand and Clay have a negative connection that decrease with increase elevation, with correlation coefficients of -0.999 and -0.989 respectively, at a significance level of $P< 0.01$ for each.

2.3 Soil chemical parameter

Soil chemical properties include soil organic carbon (SOC), nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), pH, and conductivity, which play crucial roles for soil fertility and are determined after soil testing (McCauley et al., 2005).

The availability of the majority of the chemical components vital to plants is greatly influenced by the soil pH (whether it is acidic, alkaline, or neutral), which is the most critical characteristic (Amgain et al., 2020). Consequently, soil pH is referred to as the "master soil variable" because it affects a wide range of soil biological, chemical, and physical properties and processes that have an impact on plant development and biomass yield (Neina, 2019).

According to Tilahun (2007), All of the land use systems soils were determined to be slightly acidic. But the cultivated land had the most acidic soil. This may be the result

of ongoing farming and the application of nitrogenous fertilizers, which hastened the acidity of the soil. The decreased soil pH could be caused by a lack of basic metal ions or by increased microbial decomposition, which generates organic compounds and lowers soil pH.

Tasung & Ahmed (2017) found soil pH increased numerically as altitude increase and decrease numerically with depth. Zhang et al. (2019) suggested that the reason behind the decline in soil pH at a lower altitude may be caused by the prevalence of warm climates that encourage the accumulation of H+.

Soil electrical conductivity (EC) is related to the total cations or anions in the solution generally been associated with determining soil salinity and also the important indicator for soil quality and soil health (Smith & Doran, 1997). Measurements of soil EC can be impacted by the particular soil chemical characteristics, that is chemical pollution, salinity, porosity and integrity of chemical soil and cation exchange capacity (Disale et al., 2020). Plant growth and development will be impacted by EC levels above 0.15 mS/cm. For this reason, farmers must continue to rely more on organic fertigation while avoiding a total reliance on chemical inputs to maintain EC < 0.15 in soils (Tellen & Yerima, 2018a).

In terms of soil quality and productivity, soil organic matter is essential. It gives plants nutrients, improves soil texture, encourages water infiltration and retention, and supports soil flora and fauna in addition to retaining and cycling applied fertilizer (Johnston, 1986). According to Bationo et al. (2007) and Brady & Weil (2008), Forest soils and pastures have much higher levels of soil organic carbon than agricultural land. In a natural forest, no tillage is done to the soil, and all the organic matter that the vegetation produces is returned to it. In cultivated areas, a substantial portion of plant materials are removed for use as food by humans or animals, and only a small portion of these materials are returned to the earth. Additionally, soil tillage aerates the soil and fragments organic wastes, facilitating microbial breakdown. With continuous cultivation, the organic carbon content of the soil progressively decreases.

Bolstad & Vose (2001) and Garten et al. (1999) reported that in mountainous areas, soil carbon concentration rises with elevation. Consequently, increasing SOM content trends (%) as altitude rises maybe because of ongoing carbon emissions and decreasing

rate of carbon loss at various elevations. According to Wang et al. (2010), SOC concentration considerably varied across the vertical soil profile and decrease with increasing soil depth for all land uses.

One of the most significant carbons (C) reservoirs on Earth is the soil organic carbon (SOC) stock which is essential to the Earth's climate and plays a significant role in it. Due to the lack of bulk density in the soil, the SOC stock at the regional level is still unknown (Li et al., 2019). The variation in SOC stock may be attributable to SOC concentration or merely to the spatial variation in soil bulk density (Li et al., 2010). In the central-western Indian Himalayas, Sharma et al. (2010) observed that moisture and bulk density correlated negatively. According to the study, decrease SOC stocks in low-altitude forests than at high-altitude forests are caused by higher bulk density. SOC concentration and stock in all land uses were significantly impacted by altitudinal variation. SOC concentration and stock increase with elevation above baseline and peaked at higher altitudes (Choudhury et al., 2016b).

Every profile revealed that as depth is increased, the amount of nitrogen that is available decreases. With elevation, nitrogen levels have been increasing. In terms of the quantity and accessibility of nutrients necessary for plant growth, soil fertility illustrates the condition of various soils. Because crop cultivation is mostly restricted to the surface horizon (Rhizosphere), the available nitrogen was found to be at its highest in the upper layers and to decrease regularly with depth due to the trend of organic carbon decreasing with depth. At regular intervals, depleted nitrogen content is supplemented by addition of external fertilizers during crop cultivation. In comparison to mid and low altitude, high altitude was shown to have more nitrogen that is readily available. Because high altitude soils contain a lot of organic matter and carbon (Kumar & Naidu, 2012; Sireesha & Naidu, 2013). Animal waste (faeces and urine), which contains a large amount of nitrogen and continuous application of nitrogen-based fertilizer is thought to be the cause of the high total nitrogen (McNaughton et al., 1997; Tellen & Yerima, 2018a).

Available phosphorus is required for the essential plants to grow properly. It has been noted that both organic and inorganic materials containing phosphorus can be found in all terrestrial ecosystems. Yet, the primary supplies of phosphorus for plants are in organic forms (Gairola et al., 2012). According to Lalljee (1998), The existence of low

pH and high exchangeable acidity in the cultivated and grazing area soils appears to be the main cause of the poor available P status in these soils. On the other hand, the presence of high pH and OM, which were associated positively (r = 0.47*), could be the cause of the substantially greater accessible P in the forest soil.

As depth increases, the phosphorus content drops. The maximum P was seen in the surface horizons as depth increased. It might be caused by crop cultivation being restricted to the rhizosphere, the addition of exogenous sources of P to the depleted soil, such as fertilizers, and the availability of free iron oxide and exchangeable Al^{3+} in minor quantities (Singh & Mishra, 1996). The increase in phosphorus that was present in low altitude soils may have been caused by the ongoing use of phosphatic fertilizers, which led to the build-up of phosphorus in intensively cultivated low altitude soils (Sharma et al., 2008).

In comparison to community forest, pasture, protected forest and other land types, it was shown that agricultural land had substantially more readily available phosphorus. Increase in the concentration of available phosphorus was caused using fertilizers on agricultural land in anticipation of overproduction. It's possible that phosphorus fixation was responsible for the low levels of accessible phosphorus in the pasture and woodland soils (Moges et al., 2013).

Potassium is the third key nutrient for plants and is essential for several crucial metabolic processes (Havlin et al., 2010). The increasing in potassium availability from low to high altitude could be the growing likelihood of potassium-bearing minerals such feldspars, muscovite and biotite in high altitude. Similarly, crop intensities that are particularly high at low altitudes compared to high altitudes may lead to large losses of potassium that is accessible to plants and lower residual potassium levels in soils.

2.4 Soil biological parameter

A key factor that greatly affects soil quality and production is soil biology. Numerous tasks of the soil ecosystem are carried out by the biological component of the soil, including nitrogen fixation, xenobiotic degradation, nutrient cycling, breakdown of organic detritus and synthesis of humic substances (García-Ruiz et al., 2008). The most common biological soil indicators elements include soil microbial biomass and activity; soil enzyme activity, N mineralization rates and soil respiration; ratios of bacteria to

fungus and gram-negative to gram-positive bacteria, as well as the proportions of different functional groupings of soil flora (Shao et al., 2008).

Microbial biomass interacts with ecosystem production by controlling availability of nutrients, regulating soil carbon storage and increasing atmospheric CO_2 through respiration. The main factor controlling the movement of carbon and the cycling of nutritional components in ecological processes is microbial biomass. The huge size of the soil's microbial biomass indicates that it functions as a significant source of nutrients both during C immobilization (growth) and mineralization (decay). It is made up of several microorganisms, including bacteria, fungus, actinomycetes and protozoa. Despite this, fungi and bacteria are the dominate species in terms of biomass and metabolic activities (Anderson & Domsch, 1973; Anderson & Domsch, 1978). Microbial biomass is extremely sensitive, and it depends on a number of factors. Extreme climatic conditions, topographic conditions, soil types, and biotic availability all affect the microbial activities and abundance (Ingram & Fernandes, 2001; King et al., 2008).

Soil microbial biomass is significantly impacted by the decrease in temperature with elevation. High summer temperatures in a research by Blume et al. (2002) greater than 80% increase in microbial activity. According to Powlson et al. (1987), 18 years of straw integration in two Danish field studies (Studsgaard and Røn- have) led to increases 40–50% in biomass C and N, but only a 5% rise in total soil organic C and N, a statistically insignificant increase. In rotations with high residue-producing crops, higher microbial carbon values are frequently observed (Omay et al., 1997).

According to Chen et al. (2021), The amount of microbial biomass varied considerably with soil depth. In general, the surface layer's mean MBC, MBN and MBP values were much greater than those of the subsurface layer. MBC, MBN and MBP decreased by 37.4%, 32.8%, and 21.5% respectively, as soil depth increased.

2.5 Research gap

According to the reviewed literature, soil has been mostly taken from the forest, shrubland, abandoned land, irrigated land/lowland. Soil health and soil quality depends upon physical, chemical and biological properties. The associations of the soil properties vary with soil depth along elevation. Considering importance of soil of

rainfed ecosystem (Bari or Pakho Bari or upland) unexplored and largely ignored area of research, thus it has been proposed for the study. Thus, the importance soil health of Kaski District has been proposed for the study. The present research will give the information about soil health and biological activity of soil along elevational gradient in maize cultivated land in Kaski District, Annapurna Rural Municipality-10 and 11. This study helps to address the soil health status and to formulate the best land management practices for further soil quality improvement. It will also be able to improve the recording of the study area's soil quality status and open up new opportunities for research into the microbial biomass and activity of the soil.

CHAPTER – THREE

MATERIALS AND METHODS

3.1 Study area

The Gandaki Province includes the Kaski District, one of the seventy-seven districts that is part of Nepal. This district is in the geographic centre of the country. 28°20' N latitude and 84°00' E longitude are its coordinates. The Kaski district contains the Himalayan range's lowest point, which is 450 meters above sea level and its highest point, which is 8091 meters above sea level. The study area is located in Annapurna Rural Municipality which is surrounded by Machhapuchhare Rural Municipality, Magadi District, Manag District, Parbat District and Pokhara Metropolitan City are all located around Annapurna Rural Municipality, which has a total area of 417.74km^2. The meaning of Annapurna is the provider of sustenance. Agriculture and tourism (business of hotel) are the main occupation of this area.

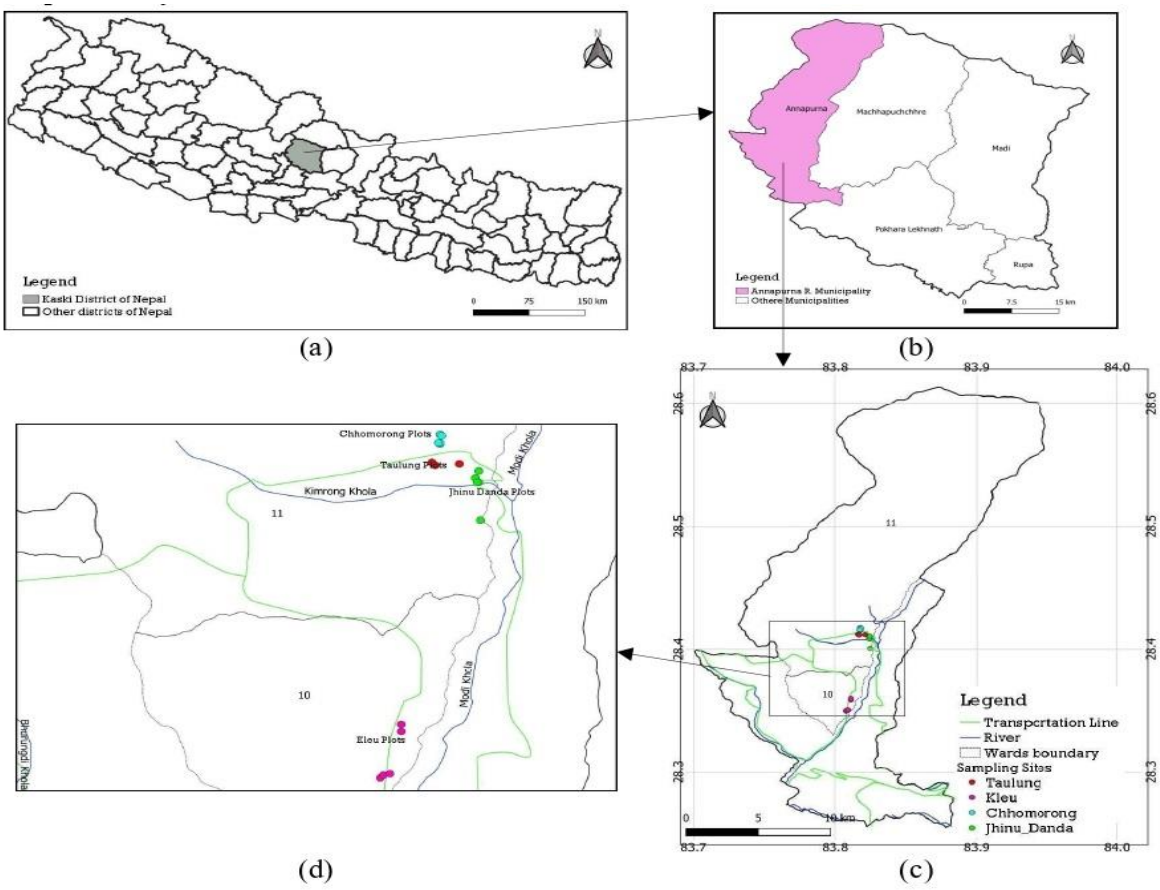

Figure 1: Map showing the study area and sampling sites

(a) Map of Nepal showing Kaski district
(b) Annapurna Rural Municipality
(c) Sampling sites
(d) Sampling plots

The study was conducted from Kleu to Chhomorong which lies between Annapurna rural municipality ward-10 Kleu (1350) and Annapurna rural municipality ward-11, Jhinu dada (1650), Taulung (1950) and Chhomorong (2250) meters above sea level (table 1). In the Figure1, map of Nepal showing the Annapurna R. Municipality with the sampling sites.

3.2 Climate

The study area is located in sub-tropical climatic zone. The climatic data were recorded from the nearest meteorological station, i.e., Lumle, weather station of the Department of Hydrology and Meteorology, Babar Mahal, Kathmandu. Temperature (maximum, minimum) and precipitation data were taken from 2012 to 2021. The minimal annual temperature ranges from 12.02° C (minimum) to 20°C (maximum). Based on the data, the extreme high temperature was in August (24 °C) and low in January (4 °C). Similarly, Data shows average annual rainfall of 438.40mm. The rainfall was highest in July (1540.16mm), whereas low rainfall occurs in November (4.55mm).

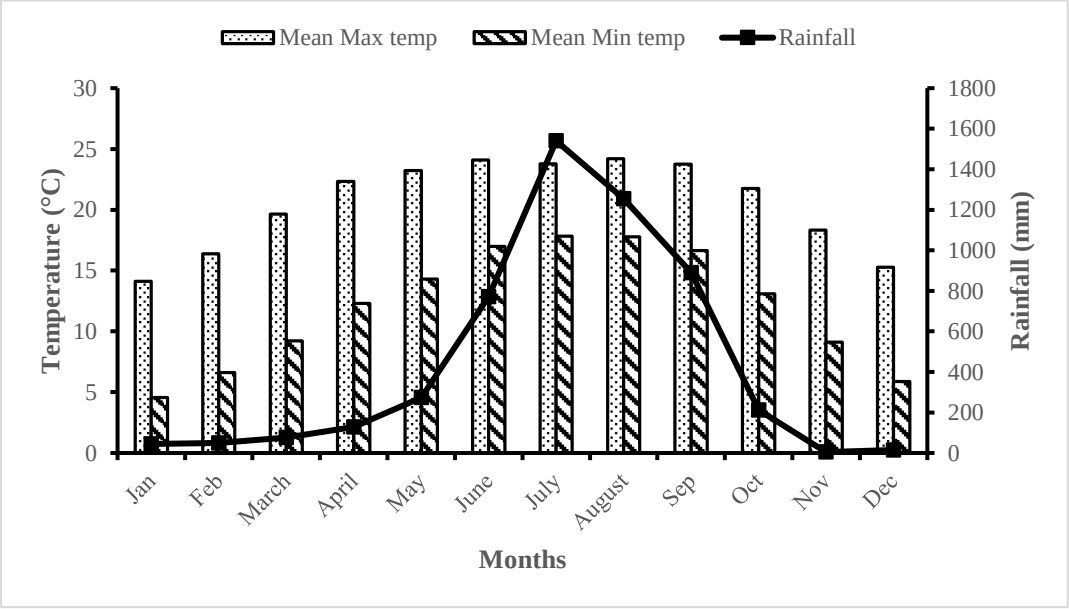

Figure 2: Average ten years (2012-2021) climatic data of study area
(Source: Department of Hydrology and Meteorology, Babar Mahal, Kathmandu)

3.3 Rainfed land/upland and its management practices in study site

On sloping Bari terrain (rainfed upland) in the middle hills, traditional crop such as maize, millet and potato is cultivated. The majority of farmers in Nepal's hills live their lives cultivating maize and their major crops is maize. It is farmed under rainfed conditions as a single crop from April to August, or later in the season, it is relayed with millet. Before maize cultivation farmers used to grow the grasses for livestock. They also cultivate vegetables (potato, bean, cauliflower, cabbage, Pea) in some field which is near the home and left barren which is far from the home until the maize cultivation. Maize with bean, pumpkin and cucumber are also cultivated at the same time. After harvesting of maize, they cultivate millets (Kodo) in the month of Sharawan. Some farmers used to leave fellow land for next year until the maize cultivation.

Tree like *Choerospondias axillaris, Ficus sp.* and shrub like *Rubus ellipticus, Urtica dioica, Artemesia indica* are the major found in the crop filed in the lower elevation. Chemical fertilizers like urea (8-10kg per ropani), DAP (Diammonium phosphate) (6-8kg per ropani), green manure and other organic fertilizers were used by farmers in lower elevation1350 masl. In elevation1650 masl, there were also used of fertilizers as, urea (6-7kg per ropani), DAP (3-4kg per ropani). The tillage practice was by conventional tillage, by ox- drawn and also by hand tractor.

Plants like *Rhodenderon sp, Alnus nepalensis, Himalayacalamus asper, Ficus sarmentosa, Choerospondias axillaris, Myrica esulenta* were found to be associated along with maize cultivated land. In the higher elevation, potatoes are cultivated in the month of November. Mustard is also cultivated in the same month of November. After the mustard and potatoes farmers used to cultivated maize and at same time bean and cucumber are also cultivated. There were mainly used of organic fertilizer as highly used of farm yard manure and they use only less amount of the chemical fertilizers in elevation 1950 masl and in elevation 2250 masl there was greater used of FYM, cow, buffalo urine and local compost which is comprised of animal waste, leaf litter and twigs collected from nearby forest and main is they don’t use chemical fertilizers. The farmyard preparation and application method were based on traditional practices, as the heap method in open place.

Tillage in maize land entails ox-drawn ploughing to a depth of approximately 15 cm, followed by manual tillage with a hand hoe, terrace side slicing, secondary tillage (clod-breaking, levelling, and smoothing of the soil surface) and secondary cultivation practice (ridging or earthing-up) of the soil for planting seed. In the month of February 2nd week, farmers used to keep farm yard manure about 50-80 Doko per ropani and land preparation and ploughing was done. Seed was sown in the March . The weeding was done twice before the harvesting, in the interval 30-45 days of seed sowing at the time of April 2nd week and first June . Flowering started in 3rd week of July. Fruiting and ripening in the last of July. Harvesting was done in the 2nd week of August. Seed of maize was stored by making jholi. Maize rust, leaf blight was the major disease seen in the field. Compost and mulching, cover crop, crop rotation and land preparation are the management practices for the soil conservation were done by the farmers.

3.4 Sampling locations

The soils samples were collected from 3 depths (0-10cm, 10-20cm and 20-30cm) along elevation gradient. There were four villages having rainfed agro-ecosystem located along the elevation start from 1354masl to 2259masl (Table 1). The elevations of the village Kleu ranged from 1354m to 1407 masl named as elevation 1350 masl. Likewise, the elevations of the village Jhinu Dada ranged from 1654 m to 1706 masl named as elevation 1650 masl. The elevations of the village Taulung ranged from 1902 m to 1996 masl named as elevation 1950 masl. The elevations of the village Chhomorong ranged from 2255 m to 2259 masl named as elevation 2250 masl.

Table 1 : Location details of the study sites

S.N.	Name of elevation	Plot code	Latitude	Longitude	Elevation (masl)	Aspect
1	Kleu	ML1P1	28°20'59"N	83°48'27"E	1357	NE
		ML1P2	28°21'32"N	83°48'40"E	1389	NE
		ML1P3	28°21'1"N	83°48'29"E	1362	NE
		ML1P4	28°21'37"N	83°48'40"E	1407	NE

		ML1P5	28°21'2"N	83°48'33"E	1354	NE
2	Jhinu Dada	ML2P1	28°24'30"N	83°49'28"E	1684	NE
		ML2P2	28°24'33"N	83°49'26"E	1654	NE
		ML2P3	28°24'30"N	83°49'27"E	1673	NE
		ML2P4	28°24'36"N	83°49'29"E	1708	NE
		ML2P5	28°24'38"N	83°49'28"E	1706	NE
3	Taulung	ML3P1	28°24'44"N	83°48'59"E	1996	NE
		ML3P2	28°24'44"N	83°48'59"E	1984	NE
		ML3P3	28°24'44"N	83°48'59"E	1979	NE
		ML3P4	28°24'43"N	83°49'16"E	1902	NE
		ML3P5	28°24'42"N	83°49'01"E	1972	NE
4	Chhomorong	ML4P1	28°24'57"N	83°49'4"E	2239	NE
		ML4P2	28°24'58"N	83°49'4"E	2255	NE
		ML4P3	28°25'4"N	83°49'4"E	2255	NE
		ML4P4	28°25'3"N	83°49'5"E	2256	NE
		ML4P5	28°24'58"N	83°49'3"E	2259	NE

NE: North East

3.5 Research design and soil sampling

First of all, general survey with local people staying nearby study site was taken so as to know the more information related to study and permission for the study was taken from related people. Soil sampling was conducted at the mid of winter (January – Feburay,2022). Soil samples was collected from rainfed maize cultivated land along elevational gradient from Kleu to Chhomorong. Soil was collected from a depth of 0–10 cm, 10–20 cm, and 20–30 cm below the soil surface using a soil corer. At each elevational point 5 quadrates of 10×10m were made with the distance of 15-50m between each quadrate (random sampling). Each elevation gradient distance was apart 300m. From each plot, altogether 3 composite soil samples were taken (from the depths of 0–10 cm, 10–20 cm, and 20–30 cm) and altogether 60 composite soil samples were taken from site. During the collection of soil samples, gravel materials, stem and root branches, litter, debris were excluded to minimize the variations. The soil was collected from the four corners, as first from the depth 0-10cm from all four side and mixed it and it was called composite soil sample and similarly in the depth 10-20cm and 20-30cm. About 1000 gm of well mixed sub sample was collected in tight plastic bag and labelled properly and were taken to the Laboratory for analysis. Undisturbed soil core sample (the soil not mixed with other) as collected from the centre and separately taken for the determination of bulk density. About 100g from each composite sample was kept in refrigerator at 4^{o}C for microbial biomass determination.

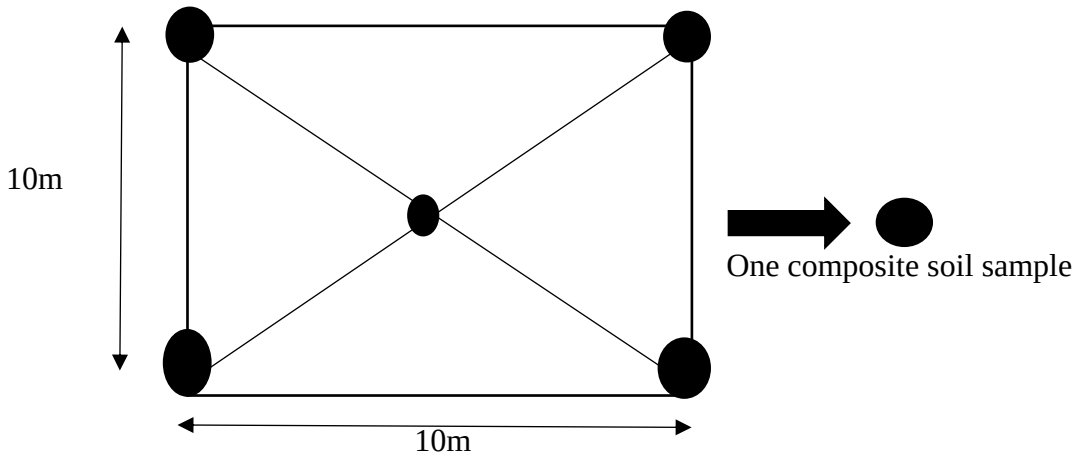

Figure 3: Sampling plot size and sampling design

3.6 Laboratory analysis

The soil samples were air dried for two weeks. Soil was crushed by using mortar pistil and passed through 2mm mesh size for the pH, electrical conductivity and texture and the again soil was sieve 0.2mm for organic carbon, total nitrogen, available phosphorus and available potassium. For microbial biomass carbon, the composite sample which was kept in 4°C was used. Soil samples were analysed at the Applied Mycology and Plant Pathology laboratory at the Central Department of Botany, Tribhuvan University, Kathmandu. Details procedures used for the analysis are presented as follows:

3.7 Physical, chemical and microbial soil properties analysis

Different physical, chemical and microbial properties of soil samples were analysed.

3.6.1 Soil temperature

A digital soil thermometer was used to measure the soil temperature in field. Firstly, hole was prepared by using soil corer at depth 0-10cm and thermometer was inserted into the hole and the readings of soil temperature were taken by waiting 5 minutes. Similarly, the same process in depth 10-20cm and 20- 30cm. Three readings were taken in each sampling plot.

3.6.2 Bulk density

Using the cylindrical core approach, bulk density was assessed as explained by Tellen & Yerima (2018b) and was used to measure soil bulk density. Soil corer having diameter of 3.5cm was used to collect the sample from the field. The cylinder containing an undisturbed soil was trimmed to the end with the help of knife and immediately packed to prevent the moisture loses. The fresh weight was taken after immediately taking the soil samples. The sample was brought to the lab and where it was dried in an oven for 24 hours at 105 °C. The oven-dried weight of soil was taken, and bulk density was determined using the formula.

$V = \pi r^2 h$ (cm^3)

Where, V = volume of core (cm^3)

$\pi = 3.14$

r = radius = diameter/2 (cm)

h = height (cm)

BD = Bulk density

$$BD\ (gcm^{-3}) = \frac{\text{Dry weight of soil (g)}}{\text{Volume of core } (cm^3)}$$

3.6.3 Determination of moisture content

The approach given by Brady et al. (2008) was used to determine the moisture content. From each soil sample, 100gm of fresh soil sample was kept inside a hot air oven at a constant temperature of 180^{o}C. Then, after 24 hours the dry weight of the soil sample was taken.

The percent of moisture content was calculated by using the following formula:

$$\%\ \text{of moisture content} = \frac{Wt\ of\ fresh\ soil - Wt\ of\ oven\ dried\ soil}{Wt\ of\ fresh\ soil} \times 100$$

3.6.4 Soil texture

The hydrometer method, which was used to determine the soil's texture, was described by Bouyoucos (1962). For this, 50 g of soil was weighted and taken in 250 mL beaker. An appropriate amount of distilled water was added which can cover the soil and then 10 mL of sodium hexa-metaphosphate solution was added and stirred properly with a glass rod. Sodium hexa-metaphosphate acts here as dispersing agent. The mixture was left over night. The next day, the solution of soil and sodium hexametaphosphate was shaken well and transferred to a jar, and distilled water was added to make up 1 L. The jar was shaken 10 times upside down closing the mouth of jar with hand palm. When well soil particles well dispersed the jar was placed in working table and hydrometer was immersed immediately after 40 second and 2 hours. The temperature of solution was recorded while taking the hydrometer reading. Using the formula below, the percentages of sand, silt, and clay were determined:

Calculation:

(Silt + Clay) %= Hydrometer reading at 40 second+ 0.3×(t-20) ^{o}C) × 2

Clay %= (Reading at 2 hours + 0.3 × (t-20) °C) × 2

Sand%=100- (Silt + clay) %

silt=% (silt + clay) - % clay

Where, t represents suspension's temperature

3.6.5 Soil pH

The pH of the soil was assessed using Fischer's digital pH meter (model HM-1003) and the method provided by Jackson (1967). Soil water suspension at the ratio of 1:2.5 was prepared by mixing 10gm of soil and 20mL distilled water. A glass rod was used to thoroughly mix the soil water suspension for about five minutes, after which it was left to stand for 30 minutes. The buffer solution with pH values of 4.0, 7.0, and 9.2 was used to calibrate the soil. The electrode was dipped into the beaker containing soil water suspension, and we waited until the pH reading on the pH meter stabilized and pH reading was noted.

3.6.6 Soil EC

Using a digital EC meter and a soil water mixture that was diluted 1:5 to determine the EC of the soil as described by USAD-NRCS (2011). For the mixture, 25 mL of distilled water and 5g of soil were added to a beaker and the soil water suspension was stirred with a glass rod for about 3 minutes. The EC meter was turned and allowed to warm up. Before measuring the soil's electrical conductivity, the EC meter was cleaned with distilled water and calibrated. For the measurement, electrode of the EC meter was dipped into the beaker containing soil water suspension and wait until EC reading on the EC meter stabilizes and EC reading was noted.

3.6.7 Soil organic carbon

The standard Walkley and Black approach, as reported by Gupta et al. (2007) was used to calculate the amount of soil organic carbon. This method is based on the principle that carbon is oxidized by the dichromate ion and excess dichromate ion is back titrated with ferrous ammonium ions, respectively.

Preparation of reagents:

1. Standard potassium dichromate (1N): 49.04 g of AR grade Potassium dichromate was mixed in distilled water and the volume was made up to 1 L.

2. Ferrous ammonium sulphate (0.5 N): 196 g of the hydrated crystalline salt were dissolved in 20 mL of concentrated sulfuric acid in 1 L of distilled water.

3. Diphenylamine indicator: 0.5 g of diphenylamine mixed in 20 mL of distilled water and 100 ml of concentrated sulphuric acid.

Procedure:

0.25 g soil was taken in 250 mL conical flask after passing through a fine sieve (0.25mm). With the use of a pipette, 5 mL of potassium dichromate ($K_2Cr_20_7$) were added to it. Then 10 mL of concentrated sulphuric acid was added to the mixture, swirled a little and kept for 30 minutes for digestion. After completion of 30 minutes, 100 mL of distilled water was added to it and 5 mL ortho-phosphoric acid was also added and 0.5mL of diphenyl indicator was also added to the mixture. After the addition of indicator, the mixture turns blue violet color. Lastly, 0.5 N ferrous ammonium sulphate was added to the mixture, titrating until the color changed from blue violet to green. Amount of ferrous ammonium sulphate consumed by the soil was noted. The same process was repeated for the blank solution without soil. Following equation was used to determine the soil organic carbon.

$$\text{Soil organic carbon (SOC \%)} = \frac{(\text{Blank reading - Titration reading}) \text{ N} \times 0.003 \times 100}{\text{Weight of soil(g)}}$$

Where N = Normality of ferrous ammonium sulphate

Total soil organic carbon was calculated by multiplying estimated organic carbon by factor 1.3 based on the assumption that 77 % recovery of organic matter in this procedure.

Total Organic Carbon (TOC)= Organic carbon estimated × 1.3

Soil organic matter (SOM) = TOC × 1.73

Soil Organic Carbon (SOC) stock was calculated by using the following formula, (Pearson, 2007; Subedi et al., 2010)

SOCStock (t C/ha) = $Soil\ OrganicCarbon$ (%) ×BD×d

Where,
SOC (t C/ha) = Soil organic carbon in ton per hectare

BD = Bulk Density (g/cm3) as determined by Blake (1965)

d = Soil layer thickness (cm)

3.6.8 Determination of total nitrogen (Kjeldahl's method)

The micro-Kjeldahl method was used to determine the soil sample's nitrogen level as described in soil and plant analysis manual (NARC, 1996 and Black, 1965). This method is based on the principle that the sample are digested by conc. sulphuric acid, digestion converts the any nitrogen present in the sample into ammonia, then ammonia trapping and quantification with standard acid.

Preparation of reagents:

1. Digestion mixture: 0.4 g of copper sulphate ($CuS0_4$) and 3.5 g of sodium sulphate ($NaSO_4$) was transferred.

2. Sodium hydroxide (NaOH): 400 g of sodium hydroxide dissolved in distilled water and the volume was made up to 1 L

3. Mixed indicator: 0.5 g of bromocresol green and 0.1 g of methyl red was dissolved in 100 mL of 95 % ethanol.

4. Boric Acid indicator (4%): 40 g of boric acid crystal was dissolved in 900 mL distilled water; it was heated and swirled until dissolved. Add 20 mL of the mixed indicator (Reagent 3). Adjust to a reddish-purple color with NaOH or HCl. This point indicates when 1 mL of tap water turns 1 mL of indicator solution a light green with a pH of around 5.0 and the volume of 1L was made with deionized water.

5. Hydrochloric acid (0.1N): 8.62 ml of HCI was dissolved in distilled water and the volume was made up to 1000 mL of volumetric flask.

Procedure:

1 g of soil sample, 0.4g copper sulphate and 3.5g potassium sulphate was kept in dry Kjeldahl digestion flask. After gently shaking the soil mixture, 6 ml of concentrated sulfuric acid were added. The flask was placed on the preheated heating mantle. The

digestion flask containing the mixture was left for the digestion until the temperature reaches 410°c in block digester decanters. During digestion color changes from green to brown milky color. The digestion flask was allowed to cool for 20–25 minutes after the digestion. After cooling of digestion flask 50 ml water was added to prevent the crystallization of solution. Transfer the solution into a 100 mL volumetric flask and make up the volume and stirred for few second for the proper mixing of digested material with water. Then aliquot was taken in distillation flask and 30 mL NaOH was added in aliquot and distilled it. After that NH_3 was collected in 10 mL 4% boric acid solution containing the 2 drops of mixed indicators in conical flask. Distillate with boric acid indicators was titrated with 0.01 N HCL. Similar process was repeated for the blank solution without soil after each batch of samples. The following formula was used to calculate the amount of nitrogen in the soil:

$$\text{Nitrogen \%} = \frac{7 \times N\,(T-B)}{S}$$

Where, N = Normality of HCl

T = Volume of HCL consumed with soil sample (mL)

B = Volume of HCL consumed with blank (mL)

S = Weight of soil

7 = Atomic number of nitrogen

3.6.9 Available phosphorous

Available phosphorus was determined by modified Olsen's method as described in soil and plant analysis manual (NARC, 1996 and OLSEN, 1982). This technique is based on the idea that when chloro-molybedic acid is present in an acidic medium, the phosphate ion forms a heteropoly complex molecule of phosphorus, and reduction gives the solution a blue color whose intensity may be detected in a spectrophotometer.

Preparation of reagents

1. Extracting Solution (0.5N $NaHCO_3$): Dissolved 42 g of CP grade $NaHCO_3$ in 1 L distilled water and pH was adjusted to 8.5 with 1M NaOH (4g NaOH per 100mL distilled water) solution.

2. H_2S0_4(5N): 35 mL of concentrated H_2S0_4 was diluted in 250 ml distilled water

3. Ammonium molybdate: 12 g of AR-grade ammonium molybdate was dissolved in 250 mL of distilled water, and 0.2908 g of antimony potassium tartrate was dissolved in 100 mL of distilled water. Both solutions were added to 1000 mL of 5N H_2SO_4 (141 mL of conc. H_2SO_4 per liter) was mixed thoroughly and volume was made up to 2 L.

4. Ascorbic acid: 1.056 g of ascorbic acid was dissolved in 200 mL of ammonium molybdate solution (Reagent 3)

5. 0.25% P-nitro phenol indicators: 0.25 g of indicator was mixed in 100 mL of distilled water.

6. Activated charcoal (Darco-G-60)

Procedure:

2.5 g of air-dried and sieved soil sample was taken in a 100 mL polythene bottle. A teaspoon of activated charcoal (Darc G-60) and 50 mL of 0.5 N $NaHCO_3$ extracting solution was added. After 30 minutes of shaking, the mixture was filtered using Whatman No. 42 filter sheets. Then 10 mL aliquot of filtrate was pipetted in 50 mL volumetric flask and acidified by 5N H_2S0_4. A p-nitro phenol indicator was added and after the addition of indicator, the color change from colorless to yellow. Again, 5 N H_2S0_4 was added until the yellow color to colorless. The sample solution was shacked gently after each addition of acid. After that, 8 mL of ascorbic acid and 40 mL of distilled water were added, and the mixture was thoroughly shaken. Maximum intensity of blue color was observed in 10 minutes and remain stable up to 24 hours. After 10 minutes, absorbance was measured using a spectrophotometer at 660nm. The value of phosphorus was determined by using the following formula:

Calculation:

Phosphorous (ppm) in soil =P (ppm) in solution × 50/10 × 50/2.5

P_2O_5 (kg/ha) = Phosphorous (ppm) in soil × 2.24 × 2.23

Where, 2.24 = Conversion factor for ppm in soil to kg/ha in soil

2.3 = Conversion factor for P to P_2O_5

3.6.10 Available potassium

Using the flame photometer method, potassium was measured in accordance with the soil and plant analysis manual developed by NARC (1996) and Thomas (1982). This technique is based on the idea that the extract, when atomized in a flame where the element's atoms are excited, emits radiation with a specific wavelength. The K atom emits radiation through filter papers, which fall on a photocell producing electrons, or electric current, which is measured on a galvanometer of a flame photometer. The amount of K present in the extract directly relates to the electric current generated.

Preparation of Reagents:

1. Ammonium acetate (1 N): 77.00 g of ammonium acetate was dissolved in 1L of distilled water

2. K standard (stock solution): 0.1905 g of dried KCI was mixed in 1L of volumetric flask

Procedure:

For this method, 2 g. of air-dried fine soil sample was taken in 100 mL beaker followed by addition of 20 mL of normal neutral ammonium acetate. It was shaken for 5 minutes in mechanical shaker and filtered through Whatman No. 42 filter paper. A standard curve was prepared of 0ppm, 0.5ppm, 2ppm, 4ppm, 6ppm and 10ppm after adjusting the full detection of the flame photometer10 ppm reading was noted with the soil samples. A flame photometer was used to measure the absorbance of the solution. Then the concentration was calculated with the help of a standard curve.

Calculation:

$$K_2O\ (kg/ha) = \frac{R \times 20 \times 1.2 \times 2 \times 1.12}{2}$$

Where, R= Absorbance of potassium soil extract from the standard curve

1.2= Conversion factor for K to K_2O

2 x 1.12= Conversion factor for ppm to kg/ha

20/2 = Dilution factor

3.6.11 Soil microbial biomass carbon

The fumigation extraction method, which includes exposing fresh soil to chloroform fumigation, which results in the cell wall lysing and denaturing and makes the cellular contents extractable in 0.5 M K_2SO_4, was used to measure the amount of microbial biomass. The procedure that is described here is based on that of Anderson & Ingram (1993) obtained from Estefan et al. (2013).

Preparation of Reagents:

1) Chloroform solution ($CHCl_3$), alcohol-free

2) Potassium sulfate pentahydrate solution (K_2SO_4), 0.5M: 87.13 g of K_2SO_4 was dissolved in distilled water, and the volume was made up to 1 L.

3) Potassium dichromate solution ($K_2Cr_2O_7$), 0.4N: 19.616 g of ($K_2Cr_2O_7$) were dissolved in distilled water, and the volume was made up to 1 L.

4) Ferrous ammonium sulfate solution [Fe $(NH_4)_2(SO_4)_2.6H_2O$], 0.2N: 78.4 g of ferrous ammonium sulfate was dissolved in distilled water containing 5 mL of concentrated H_2SO_4, and volume was made up to 1 L.

5) 1.10-Phenanthroline indicator: 14.85 g of 1.10-phenanthroline indicator and 6.95 g of ferrous sulfate ($FeSO_4.7H_2O$) were dissolved in distilled water and volume was made up to 1 L.

7) Sulfuric (H_2SO_4) - Orthophosphoric (H_3PO_4) acid mixture (2:1 ratio): 10mL of concentrated H_2SO_4 was mixed with 5mL of H_3PO_4 in each sample.

Preparation of soil extract:

In this procedure, 25 g of duplicate fresh soil samples were taken in a 100 mL beaker. The moisture content of the soil was determined from the soil sub-sample to express results on a dry-weight basis. Two desiccators were filled with the beakers containing the freshly sampled soil. Fumigated samples are in the first desiccator. One beaker of

100 mL capacity containing 40 mL chloroform (containing few pumice boiling granules) was placed into the middle of the desiccator. Apart from fumigation and evacuation, the non-fumigated control sample was handled similarly in the second desiccator, and the lids of the desiccators were sealed. The soil samples that had been fumigated were vacuumed up till the chloroform quickly boiled. Fumigated treatment was evacuated repeatedly using a vacuum pump (8-12 times) and desiccators were kept in dark areas for 72 hours at room temperature. Desiccators were opened after 72 hours, and soil samples (both fumigated and non-fumigated) were put into 250 mL Erlenmeyer flasks. On an orbital shaker, 100 mL of 0.5M K_2SO_4 were introduced to flasks and shaken for an hour. The soil suspensions were filtered using Whatman No. 42 filter paper after one hour.

Procedure:

The microbial biomass carbon was determined by using the fumigation/incubation technique (Anderson & Ingram, 1993). In this method, 8 mL of soil extract and 2 mL of 0.4 N $K_2Cr_2O_7$, solutions were taken into a 100 mL calibrated digestion tube. In addition, a 15 mL (2:1) H_2SO_4 :H_3PO_4 mixture and a few pumice boiling granules were added to digestion tubes before they were placed in the rack. The rack of digestion tubes was placed in the block digester and samples were digested in 150 °C for 30 minutes. The tubes were removed after 30 minutes and allowed to cool at ambient temperature. The digested samples were transferred with 25 mL of distilled water into a 250 mL Erlenmeyer flask. After that, the digested samples were given 2-4 drops of the 1.10 phenanthroline indicator, and they were titrated with 0.2 N ferrous ammonium sulfate until the color changed from bluish green to reddish-brown.

Calculations:

$$\text{Biomass-C (ppm)} = (B\text{-}V) \times N \times 0.003 \times \frac{100+\Theta}{Wt.} \times \frac{1000}{V1} \times 1000$$

Microbial Biomass C (ppm) = (C fumigated - C control)

Where:

V = Volume of 0.2 N $[Fe(NH4)_2(SO4)_2.6H_2O]$ titrated for the sample (mL)

B = Digested blank titration volume (mL)

N = Normality of $[Fe(NH4)_2(SO4)_2.6H_2O]$ solution

$0.003 = 3 \times 10^{-3}$, where 3 is equivalent weight of C

Wt. = Weight of oven-dry soil (g)

V1 = Volume of soil digest used for measurement (mL)

θ = Weight of water per oven-dry soil (g)

3.6.12 Determination of soil quality index

The method suggested by Bajracharya et al. (2007) was used to calculate the soil quality index based on these physical and chemical parameters.

$$SQI = [(a \times R_{STC}) + (b \times R_{pH}) + (c \times R_{OC}) + (d \times R_{NPK})]$$

where,

SQI = Soil Quality Index

R_{STC} = assigned ranking values for soil textural class

R_{pH} = assigned ranking values for soil pH

R_{OC} =assigned ranking values for soil organic carbon

R_N =assigned ranking values for nitrogen,

R_P =assigned ranking values phosphorus

R_K =assigned ranking values for potassium

And a=0.2 b=0.1 c=0.4 and d=0.3 are weighted values corresponding to each of the parameters.

Scoring method for SQI

Ratings for pH and nutrient values (OM, N, P_2O_5, and K_2O) are based on standards recommended by the Nepal Agricultural Research Council, Nepal (NARC, 2013).

Table 2 : Common soil parameters and ranking values for SQI in Nepal

Parameters	Ranking Values				
	0.2	**0.4**	**0.6**	**0.8**	**1**
Soil textural class	C, S	CL, SC, SiC	Si, LS	L, SiL, SL	SiCL, SC
Soil pH	<4	4-4.9	5-5.9	6-6.4	6.5-7.5
SOC%	<0.5	0.6-1	1.1-2	2.1-4	>4
Fertility (NPK)	Low	Mod. Low	Moderate	Mod. High	High
SQI	Very Low	Poor	Fair	Good	Best

Where, C- Clay S- Sand CL- Clay loam SC- Sandy Clay

SiC- Silty Clay Si- Silt LS- Loamy sand SiL- Silty loam

SL-Sandy loam L- Loam SiCL- Silty clay loam SCL- Sandy Clay loam

Table 3: Interpretation table for soil pH

p^H range	**Level**
<4.5	Highly acidic
4.5-5.5	Acidic
5.5-6.5	Slightly acidic
6.5-7.5	Neutral
>7.5	Alkaline

Table 4 : Interpretation table for soil fertility in Nepal

OM%		TN%		AP (Kg/ha)		AK (Kg/ha)	
Range	**Level**	**Range**	**Level**	**Range**	**Level**	**Range**	**Level**
>10	Very High	>0.4	Very High	>110	Very High	>500	Very High
10-May	High	0.2-0.4	High	55-110	High	280-500	High
2.5-5	Medium	0.1-0.2	Medium	30-55	Medium	110-280	Medium
1-2.5	Low	0.05-0.1	Low	30-Oct	Low	55-110	Low
<1	Very Low	<0.05	Very Low	<10	Very Low	<55	Very Low

OM= Organic matter; TN= Total nitrogen; AP= Available Phosphorous; AK=Available Potassium

3.8 Statistical analysis

The IBM Statistical Software Version 25 was used to analyze the data. One way analysis of variance (ANOVA) was used to compare variations in soil parameters. The different elevation gradient and soil depth were used as independent variables and the soil properties as dependent variables. To begin, tests for normality Kolmogorov and Smirnov were performed. Similarly, the relationship between the measured soil parameters was ascertained using Pearson's correlation coefficient.

CHAPTER – FOUR

RESULTS

4.1 Physical properties of soil along elevation and depth

4.1.1 Soil temperature

Temperature along all elevation gradient ranges from 10°C – 16.4˚C. The highest temperature was recorded at elevation 1350masl (16.4˚C) followed by elevation 1650 and elevation 1950 (13.4˚C) and elevation 2250 (11.4˚C) in the depth 0-10 cm. Similarly, in the depth 10-20 cm elevation 1350 (16.1˚C), elevation 1650 (12.6˚C), elevation 1950 (12.8˚C) and elevation 2250 (10.6˚C). Similar trend was observed in the depth 20-30 cm elevation 1350 (15.8˚C), elevation 1650 (12.4˚C), elevation 1950 (12.6˚C) and elevation 2250masl (10˚C).

The statistical analysis revealed a significant difference (p=0.000) in soil temperature along different elevation at depth 0-10 cm, 10-20 cm and 20-30 cm but there was no significant variation (p>0.05) with each elevation with the depths. The soil temperature decreases with increases the depths. The soil temperature also decreases with increasing the elevation gradient which is shown in the Figure 4.

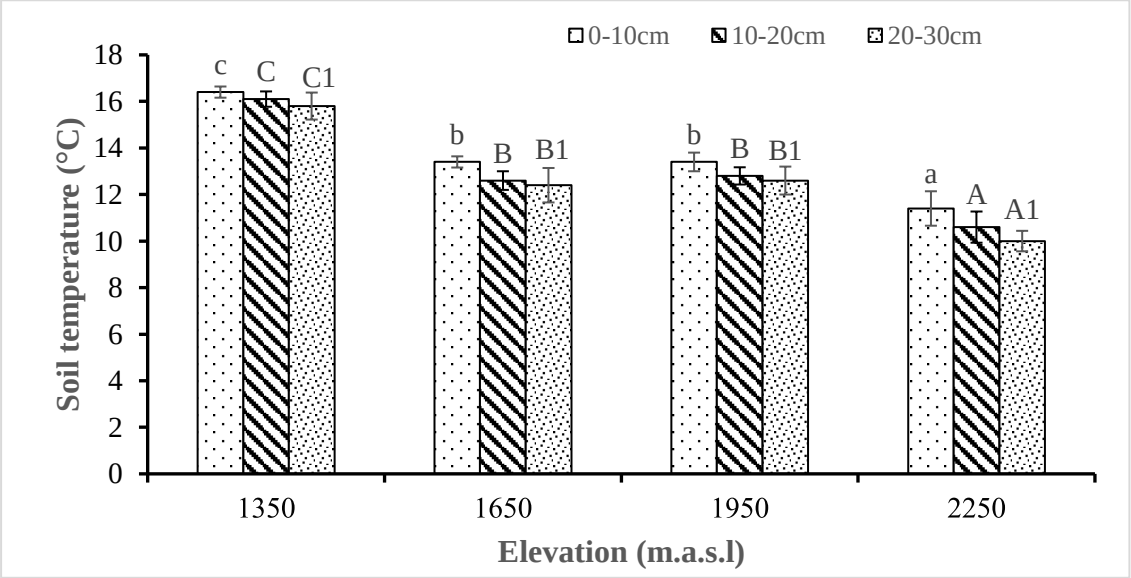

Figure 4: Soil temperature along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.1.2 Bulk density (BD)

The bulk density values at 0-10cm varied from 1.01g/cm^3 at elevation 1350m to 0.86g/cm^3 in elevation 2250masl. Likewise, at 10-20cm bulk density values varied 1.07g/cm^3 to 0.98g/cm^3.Similarly, at 20-30cm bulk density varied 1.13g/cm^3 to 1.04g/cm^3. The bulk density in elevation 1350 (1.01g/cm^3 at depth 0-10cm, 1.07g/cm^3 at depth 10-20cm, and 1.13g/cm^3 at depth 20-30cm) followed by elevation 1650 (0.96g/cm^3 at depth 0-10cm, 1.06g/cm^3 at depth 10-20cm, and 1.06g/cm^3 at depth 20-30cm) and in elevation 1950 (0.94g/cm^3 at depth 0-10cm, 0.98g/cm^3 at depth 10-20cm, and 1.07g/cm^3 at depth 20-30cm). Moreover, in higher elevation 2250 (0.86g/cm^3 at depth 0-10cm, 0.98g/cm^3 at depth 10-20cm and 1.04g/cm^3 at depth 20-30cm). In the three depths layer, elevation 1350 had highest bulk density followed by elevation 1650, elevation 1950 and elevation 2250masl.

The statistical analysis showed that there was no variation ($p>0.05$) in bulk density along different elevation at depth 0-10 cm, 10-20 cm and 20-30 cm and also there was not significant with each elevation with depth. The value of bulk density was increased with increasing the soil depths, however bulk density was decrease with increasing the elevation gradient as shown in the Figure 5.

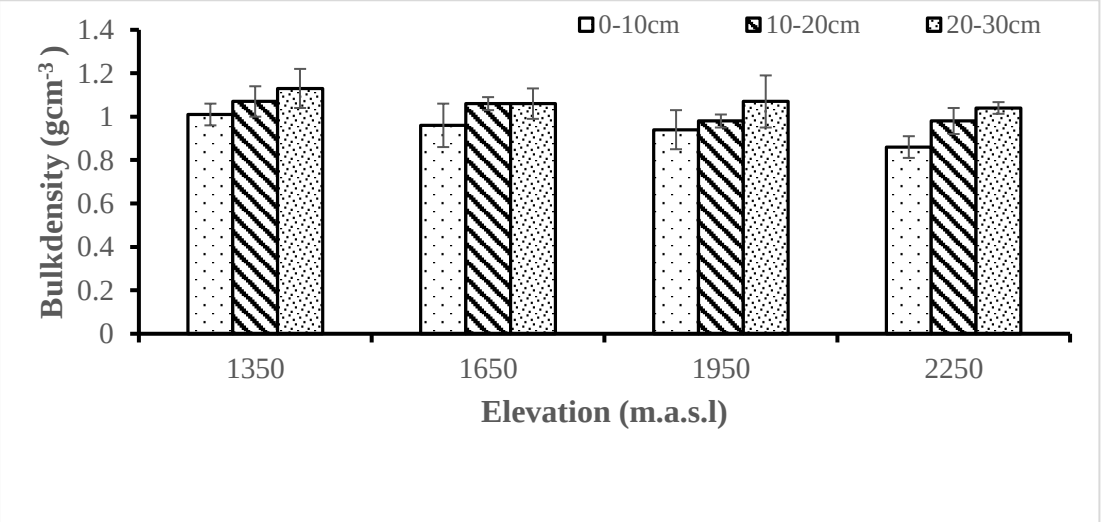

Figure 5: Soil bulk density along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.1.3 Soil moisture

In the soil depth of 0-10 cm, the average moisture content was the highest at the elevation 2250 masl (29.18%), followed by elevation 1950 (28.51%), elevation 1650 (27.78%), and elevation 1350 (21.33%). Similarly in the soil depth of 10-20 cm, the higher moisture content was observed in the 2250 (28.21%), followed by elevation 1950 (22.18%), elevation 1650 (21.51%), and elevation 1350 (18.90%). Similar trend varied at the soil depth 20-30 cm, at the elevation 2250 (27.03%), followed by elevation 1950 (19.75%), elevation 1650 (18.48%), and elevation 1350 masl (18.49%).

The analysis of variance revealed that the moisture content of the soil was not significantly affected by elevation. The differences in moisture content among the depths of soil was not significant. The moisture content of soil was decreased with the increasing soil depths in all elevation gradient. The moisture content of soil increases with increasing the elevation shown in the Figure 6.

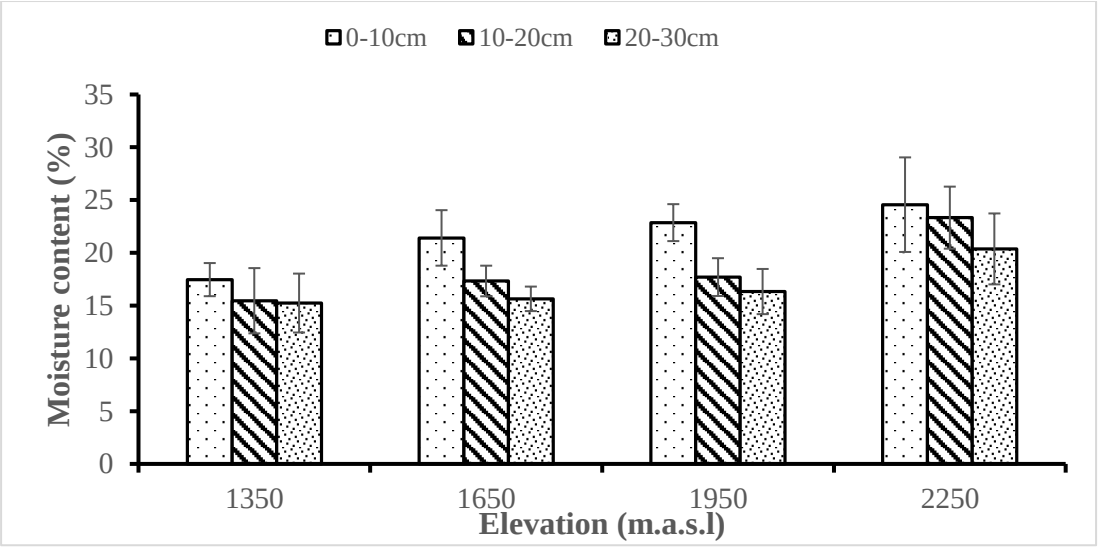

Figure 6: Soil moisture content along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.1.4 Soil texture

Sand, silt, and clay differ significantly along all elevation with soil depth ($p<0.05$) respectively. At the three soil depths layer, the highest sand percentage was recorded at the elevation 2250masl (86.08% at 0-10 cm, 86.48% at 10-20 cm and 86.34% at 20-30 cm) followed by elevation 1950 (84.54% at 0-10 cm, 84.68% at 10-20 cm and 84.68% at 20-30 cm), elevation 1650 (79.02% at 0-10 cm, 80.82% at 10-20 cm and 81.16% at 20-30 cm) and elevation 1350 (77.96% at 0-10 cm, 78.02% at 10-20 cm and 78.02% at 20-30 cm) respectively. Elevation 1650 was significantly varied with each depth layer ($p=0.002$), but other elevation was not differed with each depth. The percentage of sand in the study area was increased with increasing the elevation as presented in the Figure 7.

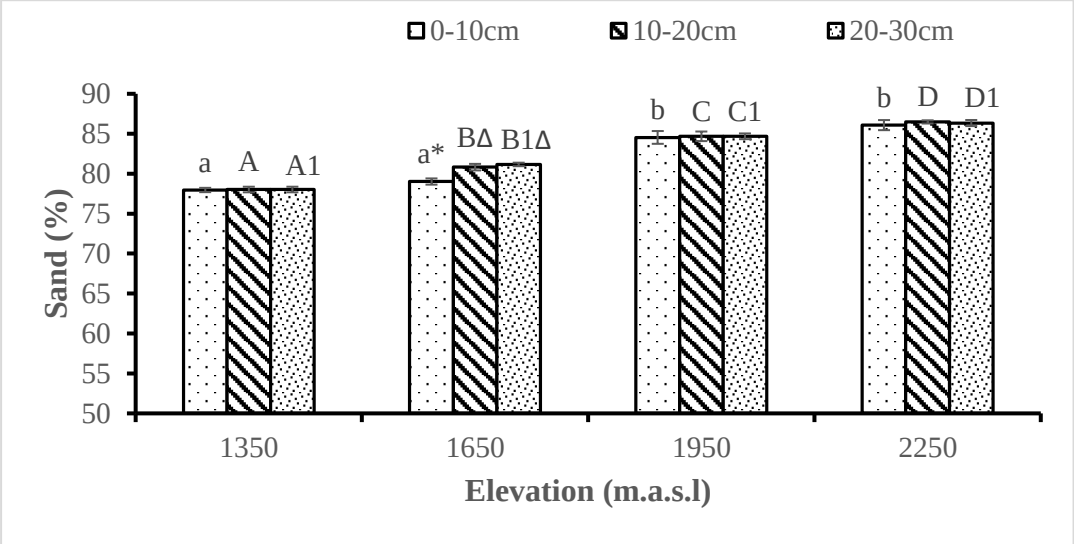

Figure 7: Sand particle along different elevation gradients in the soil depth layers
(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

Silt % was higher at the elevation 1350 masl with the depth layer (16.14% at 0-10 cm, 16.20 at 10-20 cm and 16.14% at 20-30 cm) followed by elevation 1650 (15.14% at 0-10 cm, 13.34% at 10-20 cm, 13.18% at 20-30cm, elevation 1950 (9.74% at 0-10 cm, 9.24% at 10-20 cm and 8.52% at 20-30 cm), elevation 2250 (7.66% at 0-10cm, 6.90% at 10-20cm and 7.10% at 20-30 cm). Elevation 1650 masl was significantly varied with each depth layer ($p=0.003$), but other elevation was not differed with each depth. The

silt % was higher in the lower elevation and lower in higher elevation, as increasing the elevation the silt % was decreased.

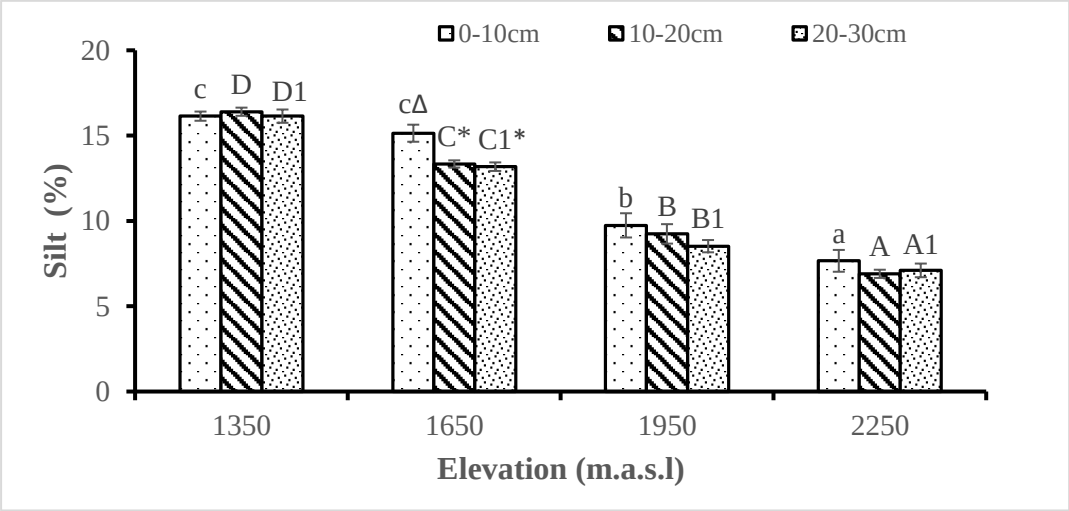

Figure 8: Silt particle along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

Clay % were not varied significantly with each elevation with each depth (P>0.05). Clay% were highest in the elevation 2250 masl (6.25% at 0-10 cm, 6.61% at10-20 cm and 6.56% 20-30 cm), followed by 1950 (5.71% at 0-10 cm, 6.08% at 10-20 cm and 6.79% 20-30 cm), elevation 1650 (5.83% at 0-10 cm, 5.83% at 10-20 cm and 5.65% at 20-30 cm) and elevation 1350 (5.89% at 0-10 cm, 5.77% at10-20 cm and 5.83% 20-30 cm).

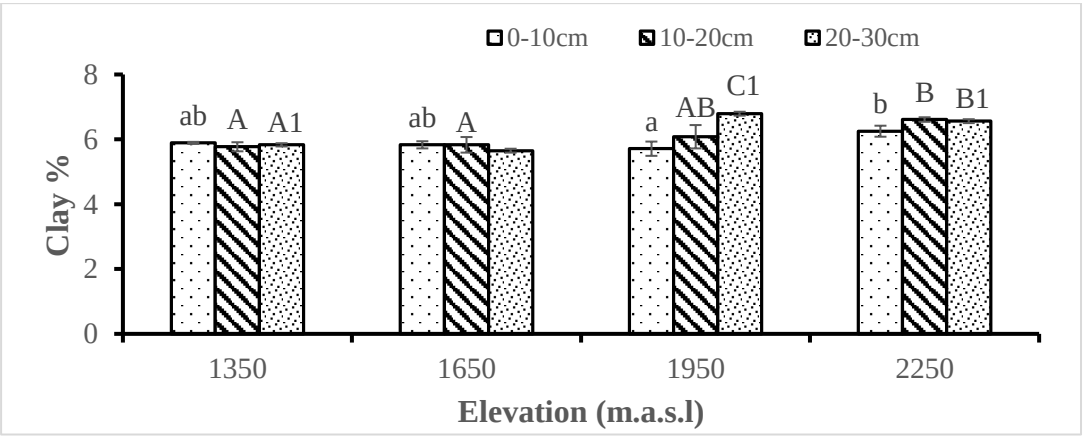

Figure 9: Clay particle along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

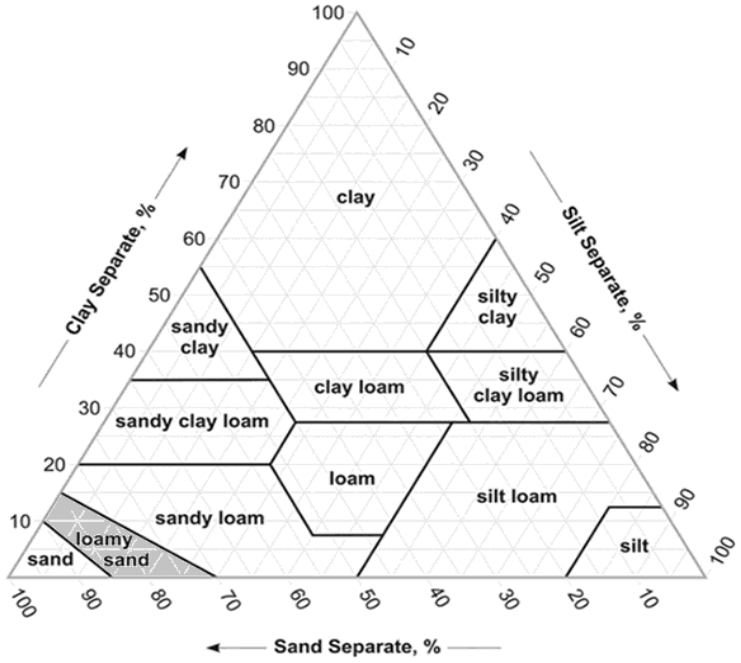

Figure 10: Soil textural triangle showing soil textural classes

4.2 Chemical properties of soil along elevation and depth

4.2.1 Soil pH

Regarding the soil pH, at depth 0-10cm, the highest pH was observed at elevation 2250 and elevation 1950 (7.18) followed by the elevation 1650 (7.02) and elevation 1350 (6.30). Likewise similar trend was followed at depth 10-20 cm highest pH was observed at elevation 2250 (7.30), followed by elevation 1950 (7.26), elevation 1650 (7.08) and elevation 1350 (6.34). And also, in depth 20-30 cm highest pH was observed in elevation 2250 (7.38), elevation 1950 (7.32), elevation 1650 (7.20) and elevation 1350 (6.38).

The analysis of variance showed that the mean pH value varied significantly ($p = 0.00$) over the various elevation gradients at all depths. There was no variation in pH among the depths at elevation 1350 ($p=0.942$), elevation 1650 ($p=0.332$), elevation 1950 ($p=0.759$) but there was slightly variation in elevation 2250 with depth. As the pH

slightly increase with increasing the depths and also increased with increasing elevation gradient as shown in the Figure 11.

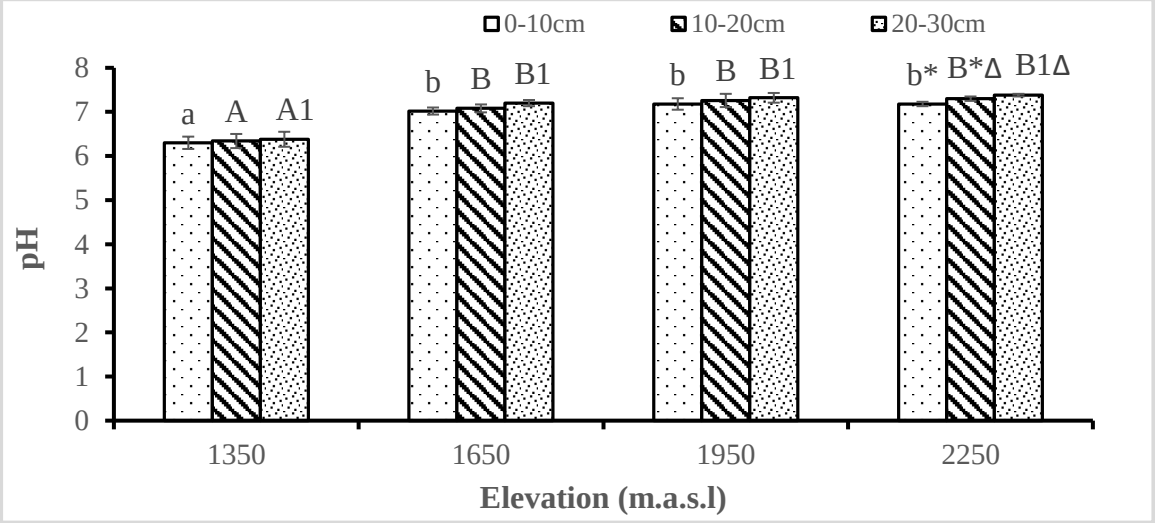

Figure 11: Soil pH along different elevation gradients in the soil depth layers
(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.2 Soil EC

The average mean value of soil EC along elevation gradient which was ranged from 76.6µS/cm to 149.8 µS/cm in the soil depth 0-10 cm. The average value of soil EC was the highest at the elevation 1950 masl (149.8 µS/cm), followed by elevation 2250 (149.4 µS/cm), elevation 1650 (126.4 µS/cm) and elevation 1350 (76.6 µS/cm) in the soil depth 0-10cm. Similarly, in the soil depths of 10–20 cm, the highest average mean value of soil EC was observed at an elevation of 2250 (129.2 µS/cm), followed by elevation 1950 (116.8 µS/cm), elevation 1650 (108.4 µS/cm), and elevation 1350 (65.8 µS/cm). Likewise in the depth 20-30cm, similar trend was followed as the highest value was observed at elevation 2250 (109.8 µS/cm), followed by elevation 1950 (108.2 µS/cm), elevation 1650 (95 µS/cm) and elevation 1350 masl (51.8 µS/cm).

Analysis of variance revealed that EC was significantly (p=0.026) affected by different elevation at soil depth 0-10 cm, whereas EC was significantly (p=0.016) affected by different elevation at soil depth 10-20 cm and also significantly varied (p=0.009) in the depth 20-30 cm. The difference in EC of soil among three depths was varied significantly in elevation 1350 (p=0.016) and whereas there was numerically different

but no significant difference in other elevation with depth. The soil EC decrease with increase depths and increase with increasing elevation as shown in the Figure 12.

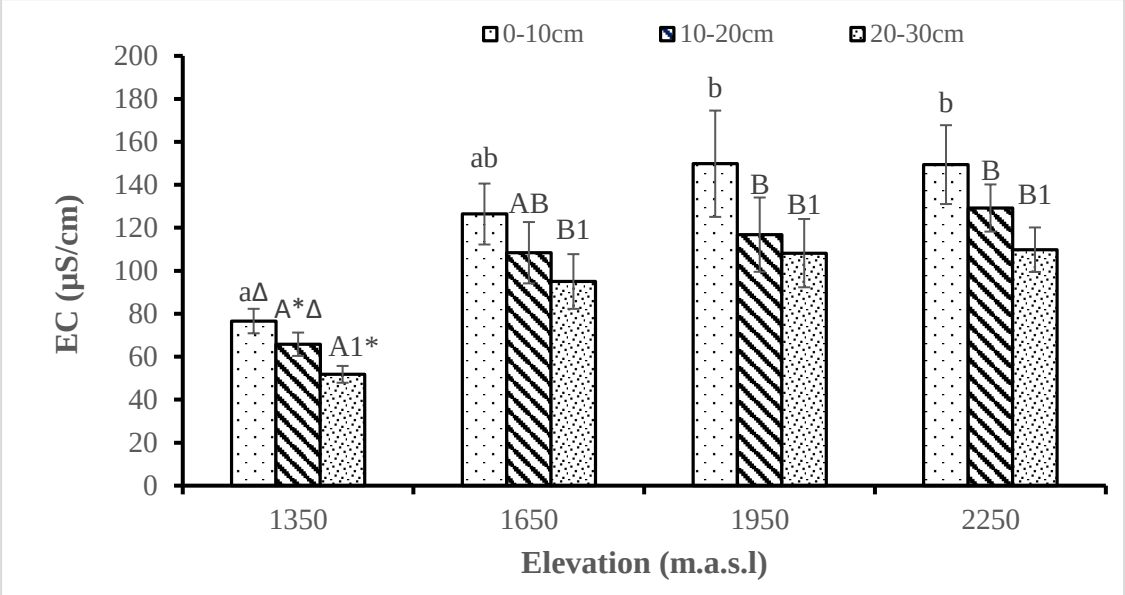

Figure 12: Soil EC (µS/cm) along different elevation gradients in the soil depth layers
(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.3 Soil organic carbon (SOC)

Organic carbon content of soil varies from 2.77% to 4.66% at depth 0-10 cm layer. In 10-20 cm depth SOC ranged from 2.47% to 3.97% and in 20-30 cm depth SOC ranged from 2.25% to 3.02%. The SOC content at elevation 2250 masl was the highest (4.66% at 0-10 cm, 3.97% at depth 10-20 cm and 2.90% at depth 20-30cm) followed by elevation 1950 (4.00% at 0-10cm, 3.48% at depth 10-20 cm and 3.02% at depth 20-30 cm) and at elevation 1650 (2.96% at 0-10cm, 2.61% at depth 10-20 cm and 2.30% at depth 20-30 cm) and the lowest at the elevation 1350 (2.77% at 0-10 cm, 2.47% at depth 10-20 cm and 2.25% at depth 20-30 cm). The significant effect of elevation ($p<0.05$) on organic carbon content of soil was recorded at 0-10 cm along the elevation and also significantly varied ($p=0.009$) at depth 10-20 cm along the elevation gradient. There was no significant variation in the depth 20-30 cm along elevation. Within in the depth of each elevation SOC was significantly differed in elevation 2250 masl ($p=0.003$).

The SOC was decrease with increasing the depths whereas SOC content was increased with increasing the elevation gradient as shown in the Figure 13.

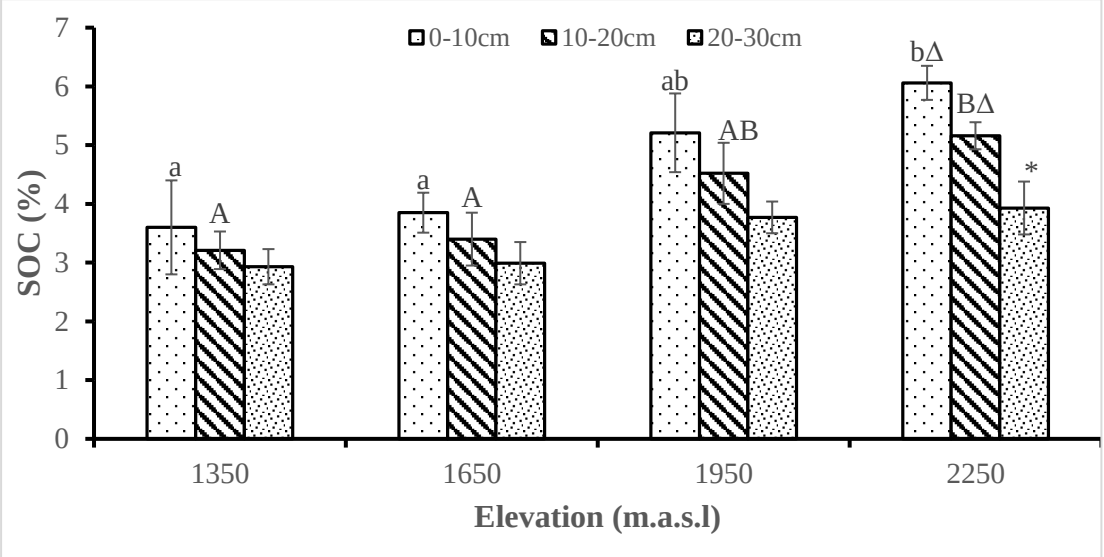

Figure 13: SOC (%) along different elevation gradients in the soil depth layers
(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.4 Soil organic matter (SOM)

Organic matter content of soil varies from 6.24% to 10.51% at depth 0-10 cm layer. In 10-20 cm depth SOM ranged from 5.54% to 8.90% and in 20-30cm depth SOM ranged from 5.08% to 6.80%. The SOM content at elevation 2250 was the highest (10.51% at 0-10cm, 8.90% at depth 10-20cm and 6.80% at depth 20-30cm) followed by elevation 1950 (9.02% at 0-10cm, 7.79% at depth 10-20cm and 6.53% at depth 20-30cm) and at elevation 1650 (6.67% at 0-10cm, 5.86% at depth 10-20cm and 5.18% at depth 20-30cm) and the lowest in the elevation 1350 (6.24% at 0-10cm, 5.54% at depth 10-20cm and 5.08% at depth 20-30cm).The significant effect of elevation ($p<0.05$) on organic carbon content of soil was recorded at 0-10 cm along the elevation and also significantly varied ($p=0.009$) at depth 10-20cm along the elevation gradient. There was no significant variation in the depth 20-30 cm along elevation. Within in the depth of each elevation SOM was significantly differed in elevation 2250 ($p=0.003$).

The SOM was decrease with increasing the depths whereas SOM content was increased with increasing the elevation gradient as shown in the Figure 14.

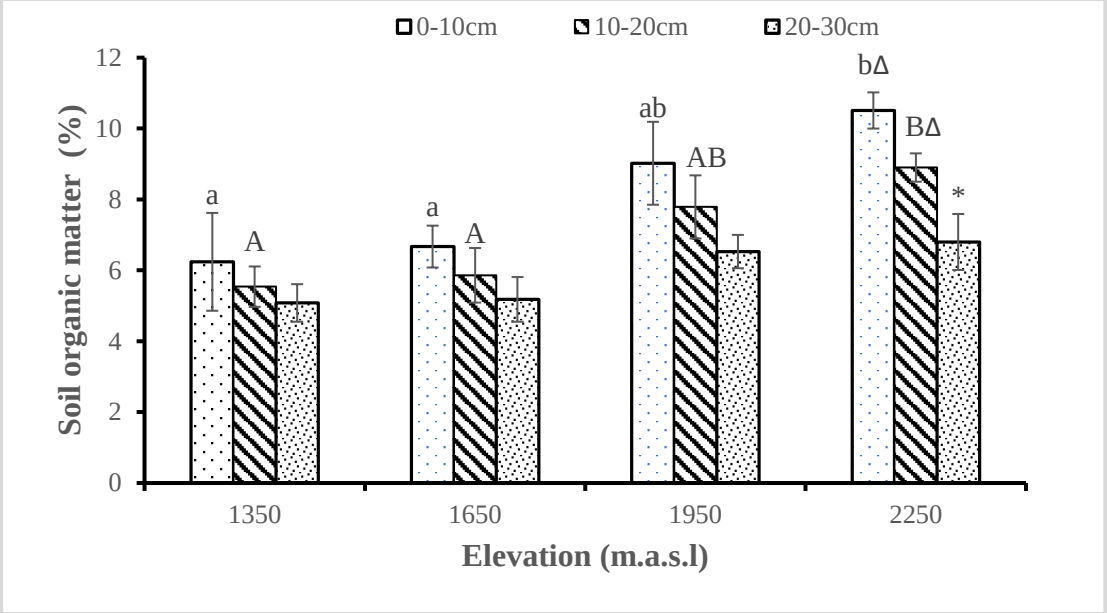

Figure 14: SOM (%) along different elevation gradients in the soil depth layers
(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.5 Soil organic carbon stock

The average value of SOC Stock was ranged from 27.97 t C/ha to 40.44 t C/ha. The highest SOC stock value was observed highest at the elevation 2250 masl (40.4 t C/ha) followed by elevation 1950 (37.26 t C/ha), elevation 1650 (29.01 t C/ha) and elevation 1350 (27.91 t C/ha) in soil depth 0-10cm. Similar trend was followed in soil depth layer 10-20cm where highest SOC stock was observed in elevation 2250 (38.77 t C/ha) and lowest at elevation 1350 (26.13 t C/ha). Likewise in the soil depth 20-30cm highest SOC stock was at elevation1950 (32.04 t C/ha), followed by elevation 2250 (31.68 t C/ha), elevation 1650 (24.42 t C/ha) and elevation 1350 (26.23 t C/ha). The highest SOC stock was at the elevation 2250 (40.4 t C/ha at depth 0-10cm, 38.77 t C/ha at depth 10-20cm and 31.68 t C/ha at depth 20-30cm) followed by elevation 1950 (37.26 t C/ha at depth 0-10cm, 34.43 t C/ha at depth 10-20cm and 32.04 t C/ha at depth 20-30cm) and at elevation 1650 (29.01 t C/ha at depth 0-10cm, 27.77 t C/ha at depth 10-20cm and 24.41 t C/ha at depth 20-30cm) and lowest at the elevation 1350 masl (27.91 t C/ha at depth 0-10cm, 26.13 t C/ha at depth 10-20cm and 26.23 t C/ha at depth 20-30cm).

There were no significant differences in the soil organic carbon stock along different elevation with depth and also there was no variation in each elevation with depth

(p>0.05). The SOC stock decreased with increasing depths. The SOC stock content along the elevation increased with increasing the elevation gradient as shown in the figure (15).

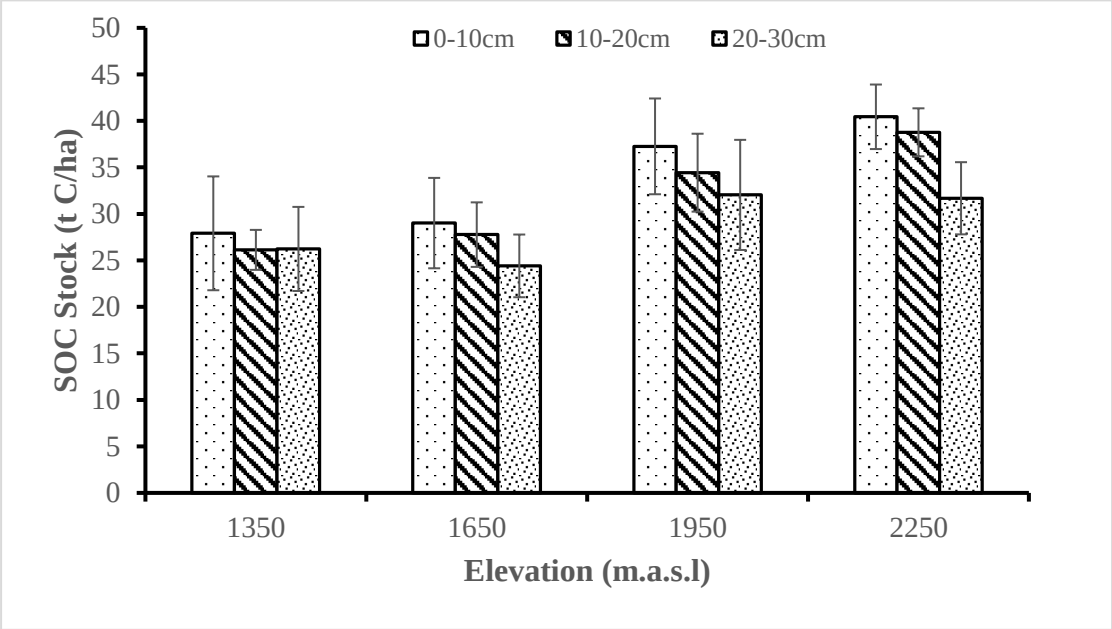

Figure 15: SOC stock (t C/ha) along different elevation gradients in the soil depth layers
(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.6 Total nitrogen (TN)

Total Nitrogen (TN) content of all elevation ranged from 0.11% to 0.206% (0-10cm) and 0.09 to 0.16% (10-20cm) and also 0.08 to 0.10 (20-30cm). Significantly higher N content was found in elevation 2250 masl (0.206% at depth 0-10cm, 0.16% at depth 10-20cm and 0.108% at depth 20-30cm) followed by elevation 1950 (0.202% at depth 0-10cm, 0.15% at depth 10-20cm and 0.108% at depth 20-30cm) and elevation 1650 (0.15% at depth 0-10cm, 0.13% at depth 10-20cm and 0.106% at depth 20-30cm). Contrarily, the lowest nitrogen was observed at elevation 1350masl (0.11% at depth 0-10cm, 0.09% at depth 10-20cm and 0.08% at depth 20-30cm) respectively. The trend of decreasing N content with increasing soil depths at all elevations and the trend of increasing N content with increasing the elevation gradient.

The total nitrogen content of soils was significantly affected by elevation at 0-10cm (p=0.043) and 10-20cm (p=0.020) and also 20-30cm (p=0.5) depth. The total nitrogen

content of each elevation with depth was highly significant variation in elevation 2250 (p=0.007) as shown in the Figure 16.

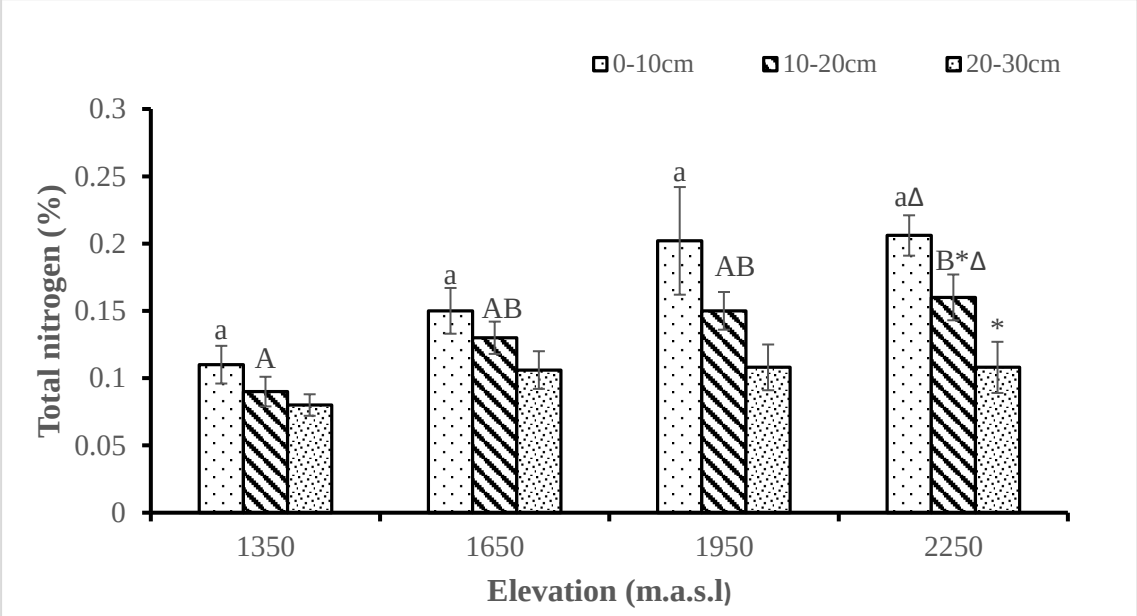

Figure 16: Total Nitrogen (%) along different elevation gradients in the soil depth layers (The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.7 Available phosphorus (AP)

The higher amount of available phosphorus (AP) was observed in the soil depth 0-10cm then the depth10-20cm and 20-30cm. The higher phosphorus content was found at elevation 2250masl (50.90 kg/ha at depth 0-10cm, 43.58 kg/ha at depth 10-20cm and 40.49 kg/ha at depth 20-30cm), followed by elevation 1950 (44.20 kg/ha at depth 0-10cm 41.73 kg/ha at depth 10-20cm and 39.46 kg/ha at depth 20-30cm) respectively. Similarly in the elevation 1650 (43.89 kg/ha at depth 0-10cm 41.42 kg/ha at depth 10-20cm and 39.25 kg/ha at depth 20-30cm) and among all the elevation, lower content of available phosphorus was in the elevation 1350 masl (43.07 kg/ha at depth 0-10cm 41.32 kg/ha at depth 10-20cm and 39.15 kg/ha at depth 20-30cm).

The statistical analysis shows that, available phosphorus was varied significantly with the elevation in depth 0-10cm (p=0.001) and not significant in along elevation with depth 10-20cm (p=0.381) and elevation with 20-30cm depth (p=0.796). Available phosphorus content in the soil showed great significant variability under depth with

each elevation, where elevation 1350 (p=0.05), elevation 1650 (p=0.021), elevation 1950 (p=0.010) and elevation 2250 (p=0.001). Available phosphorus content decreases with increasing depths and increase with increasing elevation as presented in the Figure 17.

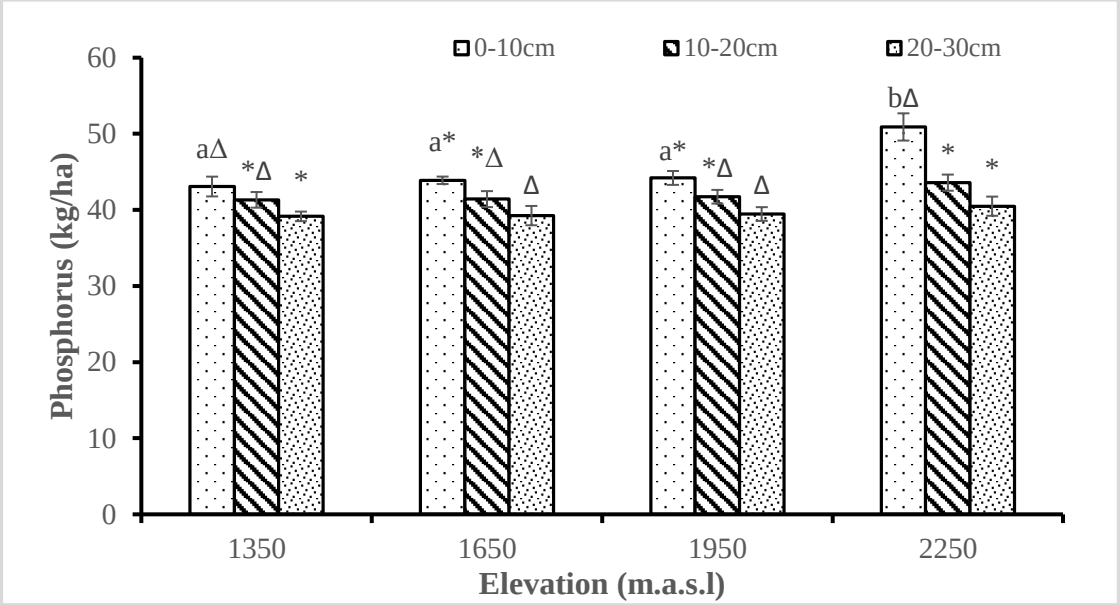

Figure 17: Available Phosphorus (kg/ha) along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.2.8 Available potassium (AK)

The highest available potassium was observed at elevation 2250 masl (823.60 kg/ha) followed by elevation1950 (534.91 kg/ha), elevation 1650 (529.53 kg/ha) and elevation 1350 (519.86kg/ha) in depth 0-10cm. Likewise same trend was followed in depth 10-20cm whereas, highest AP was content in elevation 2250 (684.90 kg/ha) and lowest AP content in elevation 1350 (456.96 kg/ha). Similarly same pattern in depth 20-30cm, where highest in elevation 2250 (619.85 kg/ha) and lowest in elevation 1350 (392.44 kg/ha). The highest AK content was observed in elevation 2250 (823.60 kg/ha at depth 0-10cm, 684.90 kg/ha at depth 10-20cm and 619.85 kg/ha at depth 20-30cm) followed by elevation 1950 (534.91 kg/ha at depth 0-10cm, 474.16 kg/ha at depth 10-20cm and 423.62 kg/ha at depth 20-30cm) and in elevation 1650 (529.53 kg/ha at depth 0-10cm, 469.86 kg/ha at depth 10-20cm and 397.28 kg/ha at depth 20-30cm). moreover, among

all the elevation, lower content of potassium in elevation 1350 (519.86kg/ha at depth 0-10cm, 456.96 kg/ha at depth 10-20cm and 392.44 kg/ha at depth 20-30cm).

The available potassium in the soil shows great variability along elevation gradient. The available potassium varied significantly along different elevations with a depth of 0-10 cm (p=0.05), 10-20 cm (p=0.008), and 20-30 cm (p=0.016). The available potassium with regards to each elevation with depth, there was insignificant difference (p>0.05) in elevation 1350, 1650 and 1950 but there was significant variation (p=0.007) in elevation 2250 with depth. The available potassium was decreased with increase depths and increase with increasing elevation gradient as shown in the figure (18).

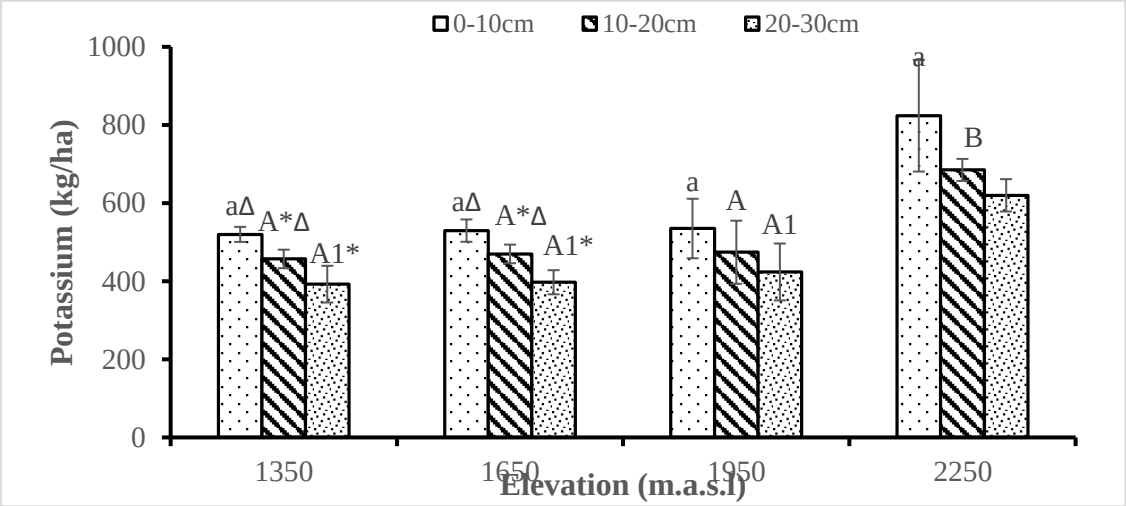

Figure 18: Available Potassium (kg/ha) along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.3 Biological properties of soil along elevation and depth

4.3.1 Soil microbial biomass carbon (SMBC)

The highest SMBC value varied from 478.24 mg/kg to 937.05 mg/kg at a depth of 0-10cm and 434.66 mg/kg to 703.77 mg/kg at depth 10-20cm and also at depth 20-30cm value ranged from 303.95 mg/kg to 455.52 mg/kg. Soil along elevation 2250masl had highest SMBC content (937.05 mg/kg at depth 0-10cm, 703.77 mg/kg at depth 10-20cm and 455.52 mg/kg at depth 20-30cm) followed by elevation 1950 (585.72 mg/kg at

depth 0-10cm, 446.10 mg/kg at depth 10-20cm and 429.99 mg/kg at depth 20-30cm). Similarly in elevation 1650 (560.07mg/kg at depth 0-10cm, 439.65 mg/kg at depth 10-20cm and 340.44 mg/kg at depth 20-30cm). Among all the elevation, lower SMBC content was in the elevation 1350 (478.24 mg/kg at depth 0-10cm, 434.66 mg/kg at depth 10-20cm and 303.95 mg/kg at depth 20-30cm).

On the basis of statistical analysis, it was found that, soil microbial biomass carbon was numerically varied with elevation and depth but not significantly varied along all elevation and also each elevation with depth ($p>0.05$). The SMBC was increase with increasing the elevation gradient but SMBC was decrease with increasing the soil depths as shown in the figure (19).

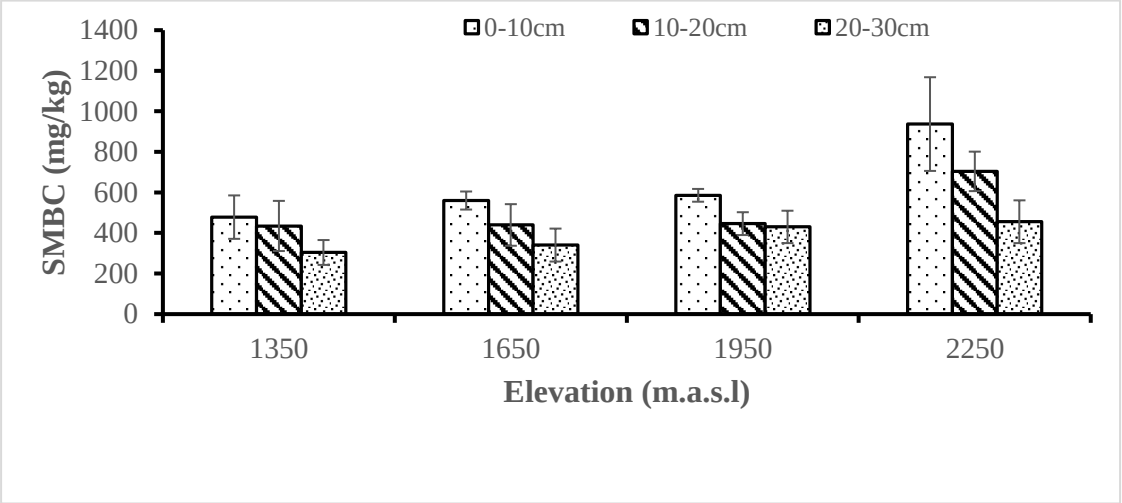

Figure 19: Microbial Biomass Carbon (mg/kg) along different elevation gradients in the soil depth layers

(The alphabets above error bar indicates significant difference among elevations at each soil depth and the symbols '*' and 'Δ' indicates significant difference among the depths at each elevation respectively).

4.4 Soil quality index (SQI)

The soil quality index provides information on soil fertility, production, and sustainability. The highest value of SQI was found in the depth 0-10cm, followed by depth 10-20cm and 20-30cm. SQI for elevation 2250, elevation 1950 and elevation 1350 has the highest scored (SQI=0.68) and elevation 1650 (SQI=0.64) at the depth 0-10cm. Similarly, SQI values of 0.64, 0.62, 0.60 and 0.54 were recorded for elevation 2250, elevation 1950, elevation 1650 and elevation 1350 respectively, in the 10-20cm

depth layer. Likewise, in the depth20-30cm highest scored was in the elevation 1950 (SQI=0.68) followed by elevation 2250 (SQI=0.64), elevation1650 (SQI=0.60) and elevation 1350 (SQI=0.54). Based on the SQI scores classes developed by NARC (2013), this result represents that the SQI value in the study area was fair.

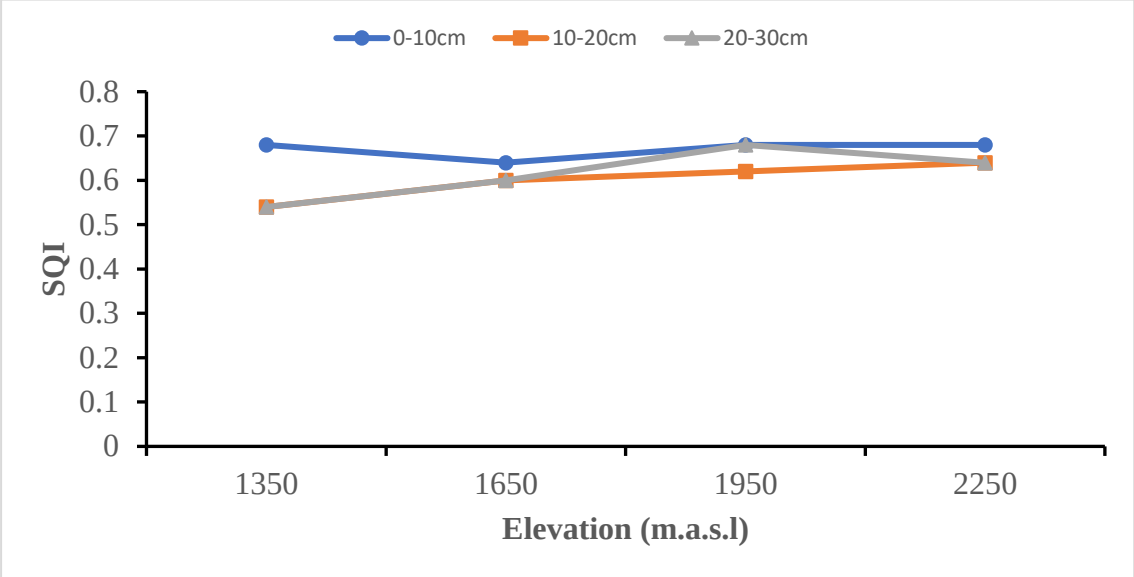

Figure 20: Soil quality index value representing in different elevation gradient and depths

4.5 Relationship between microbial biomass and soil physicochemical properties

The various parameter in this study were significantly correlated with each other (table in Annex). The majority of the soil physicochemical properties, temperature, carbon, nitrogen, microbial biomass carbon, clay, sand slit has the strong correlation. Correlation of pH, EC, SOC, SOM, TN, AP, AK, Sand, Clay, Silt and MBC at the depth 0-10cm have showed the negative significant correlation with temperature. Correlation of EC, moisture and sand have positive significant correlation with pH (r = 0.65, r = 0.52 and r = 0.63) and negative correlation with silt (r = -0.63). Correlation of bulk density and moisture have negative significant where r = -0.64. SOM, SOC stock, TN, AP, Sand and MBC have positive significant correlation with SOC and negative significant correlation with silt (r= -0.53). Sand has positive significant correlation with pH, EC, SOC, SOM, TN, AP and AK whereas, negative significant correlation with temperature. Silt has a negative significant correlation with temperature, pH, EC, SOC, SOM, TN, AP, AK and sand. MBC have a positive significant correlation with SOC,

SOM, SOC stock, AK and Sand whereas negative significant correlation with silt and temperature at depth 0-10cm which was shown in the (Annex 1).

At 10-20cm, pH, EC, SOC, SOM, TN, AK, Clay, Sand showed significant negative association with temperature and positive significant with silt. pH and EC have a significant positive correlation with SOC (r = 0.47 and r = 0.52) and a significant negative correlation with bulk density (r = -0.44). Total nitrogen has a significant positive correlation with pH, EC, SOC, SOM, and Soc stock, whereas it has a significant negative correlation with temperature and BD. Clay has a negative significant with temperature (r= -0.57) and positive significant correlation with EC, SOC, SOM, SOC stock, TN and AP. Sand have a significant correlation with temperature (r = -0.78) and positive significant correlation with pH, EC, SOC, SOM, SOC Stock, TN, AK and Clay. Silt has a positive significant correlation with temperature (r = 0.80) but pH, EC, SOC, SOM, SOC Stock, TN, AK, Clay and Sand. Likewise, MBC has significant positive correlation with SOC, SOM, SOC stock, AP and Clay (r = 0.51, 0.51, 0.45, 0.52, 0.44 and 0.55) respectively as shown in the (Annex 2).

At a depth of 20–30 cm, temperature has a negative significant correlation with pH, EC, and sand, and also a positive significant correlation with silt (r = 0.71). SOC Stock has a strongly significant positive correlation with BD, SOC, and SOM (r = 0.68, 0.87, and 0.87, respectively). SOC AND SOM have a significant positive correlation with TN (r = 0.63 and 0.62). Clay has a positive and significant correlation with pH, (r = 0.49), and (r = 0.47 in EC, SOC, and SOM). Sand has a significant positive correlation with pH, EC, SOC, SOM, AK, and Clay, but a negative correlation with temperature (r =-.0.74). Likewise, silt has positive significant with temperature (r = 0.71) and negative significant correlation with pH, EC, SOC, SOM, AK, Clay and Sand (r = -0.73, -0.64, -0.50, -0.50, -0.54, -0.84 and -0.99) respectively (Annex 3).

CHAPTER – FIVE

DISCUSSION

Soil temperature is the crucial variables affecting soil qualities and plant growth processes whereas it governs physical, chemical and biological activities that occur in the soil. Our results in the study area, represent that the soil temperature significantly differed with the elevation ($p<0.05$) but each elevation with depth, there were no appreciable variations in the soil's temperature ($p>0.05$). The mean value of soil temperature was varied from 11.4℃ to 16.4℃ in the depth (0-10cm) from 10.6℃ to 16.1℃ in depth (10-20cm) and 10.0℃ to 15.8℃ in depth (20-30cm). The high temperature was observed in the lower elevation and low temperature was observed in the higher elevation. The temperature was decreased with the increasing the soil depth. As the temperature was decrease or increased it depends on the different factors which influence soil temperature such as soil colour, mulching, moisture, organic matter, bulk density and vegetative cover (Onwuka & Mang, 2018). In the higher elevation, land was cover with different vegetation and the bulk density was low that might be the reason behind the decrease in the soil temperature. As the altitude increase the temperature is low it's the universal truth (Kumar et al., 2019) and this statement correspond with our result. The higher temperature in the lower elevation due to low content of moisture, it decreases with depth as organic matter also decrease with the increasing depth. The clay, sand content decreases and silt content increase as soil temperature increase due to the soil structure in physical properties (Inbar et al., 2014) however, this result consistent to our findings.

A measurement of compaction and soil health is soil bulk density and also essential soil physical characteristic of soil structure (Kakaire et al., 2015). The value of bulk density was not significantly varied along elevation and depth ($p>0.05$). The bulk density increased with increasing the soil depth. Same result was noted by Stockfisch et al. (1999) that compaction causes bulk density to rise with depth which is consistent with our findings. The decline in the amount of organic matter and the reduction in aggregation with depth are the main causes of the increase. The bulk density decreases with increasing the elevation gradient due increase in the organic matter content and low disturbance and that the soils are richer in soil organic carbon. These results concur

with similar studies by Kidanemariam et al. (2012) in which they reported greater pore space in soil results in lower BD than greater compactness and less pore space.

The percentage of water that is present in the soil as, soil moisture which helps to promotes plant development, chemical and biological processes in soil. The soil moisture was not significantly varied along elevation and depth ($p>0.05$). The percentage values of moisture content in the soil ranged from 21.33 % to 29.18 % in the soil depth of 0-10cm and from 18.90 % to 28.21 % in the soil depth of 10-20cm and in depth 20-30cm, 18.49 % to 27.03 %. The soil moisture was increased with increasing the elevation and decreased in the increasing depths. Due to higher OM content and higher clay content was in the higher elevation which increases the soil moisture (Tyagi et al., 2013) whereas, this result is consistent with our study. The increased bulk density implying increased compactness pore spaces and soil porosity are created in the soil, and these features are linked to a reduction in soil moisture. Comparatively, high temperature was in the lower elevation and low OM content that's why there was low moisture content (Chen et al., 2010).

Consequently, higher content of sand and lower content silt and clay was occurred in the all elevation. The highest content of sand and clay was in the elevation 2250 and silt in the elevation 1350. In this study, ANOVA showed that along all elevation with soil depths have significant effect on the sand, silt, and clay content. Along the elevation and depths, loamy sand is the dominant textural class in the study area (Figure 10 soil textural classes). Sand has a great role for root emergence, soil aeration and plant skeleton development. The percentage of sand content increased with increasing elevation and also increase with depth may result from the hilly terrain's geological structure, which favors a higher amount of rock inside the surface soils (Amgain et al., 2020) which consistent with our result. Sand, gravel and stones are present in varying concentrations in high altitude soils because they are immature and formed from weathered rocks (Ley et al., 2001). The influence of the parent materials and their capacity for weathering can be used to explain the high sand content (Brady et al., 2008). Silt proportion is quite inverse to the sand proportion according to the elevation gradient in mid hills the rainfed maize land because during summer rainfall, steeper surfaces are more susceptible to sheet erosion of parent material. In this study, higher silt but lower sand proportions were found at lower altitudes, which suggests the soil

contains quartz, feldspars, hornblendes and micas (Ley et al., 2001) which corresponds to our study. The clay content increase with increasing the depth, the higher clay content in the lower depth might be the continuous cropping and the movement of clay from upper to lower layer. Similar result found by Ketema & Yimer (2014) that the increase in soil depth and clay content were caused by the availability (growth and development) of root channels (macrospores), which favoured the movement of fine clay parts into the deeper soil layers. It is well knowledge that the lower slopes contain a lot of clay.

The different soil-forming variables are always in control of soil pH, which is a crucial regulator of soil (Fabian et al., 2014). There was a significant variation in soil pH along the elevation gradient with depth ($p=0.00$), with all depths. Soil pH was ranged from 6.30 to 7.018 in the depth layer (0-10 cm), from 6.34 to 7.30 in the depth layer (10-20 cm) and from 6.38 to 7.38 in the depth layer (20-30 cm). Soil pH was ranged from slightly acidic in the lower elevation 1350 and in other elevation soil pH obtained range was neutral according to the pH rating table established by NARC (2013). The neutral range is from 6.5 to 7.5. Soil pH was increased with soil depth, it can be assumed that leaching of alkaline metal ions (such as Ca, Na, K and Mg) from upper to lower layers may have contributed to the increase (Bhattarai & Mandal, 2016) where, it corresponds to our study. According to Reuter et al. (2008) the parent material's characteristics have a significant impact on the distribution of soil pH. According to their research, high pH soils primarily showed up in the calcareous nature of the parent materials, while low pH soils primarily developed from acidic materials. Soil pH increase numerically with increasing elevation. The decrease in soil pH at a lower elevation, may be caused by the presence of a warm climate that encourages the accumulation of H^+ (Zhang et al., 2019). The better productivity is linked to pH-high soils. This is because soils with a high organic matter content, continuous and high use of compost, animal dung and manure have a higher soil pH, which encourages base exchange and increases the availability of nutrients essential for plant growth (Demelash & Stahr, 2010) whereas, this result is consistent to our study. Moreover, use of acidifying fertilizers like urea on its own may have contributed to the pH's decline (Shrestha, 2009).

The EC content in the soil was greatly varied along elevation gradient with depth ($p<0.05$), 0-10 cm, 10-20 cm and 20-30 cm. The soil EC content in the study was increased with increasing the elevation gradient and decrease with increasing the soil

depth. Similar result was obtained by Othaman et al. (2020) which corresponds to our study as, higher soil EC values suggest higher salt or nutrient concentrations in the soil. The higher EC in high elevation due to soil organic matter content and continuous used of manure and also due to the addition of salts through fertilizer. Kaur & Bhat (2017) reported the decrease of soil EC in increasing depth, this may be caused by the ion salts' delayed movement toward the lower depth (Kaur & Bhat, 2017) which is consistent to our result. Compared to other land use schemes, EC had a larger under-cropped area. It results from the infiltration of soluble salts during irrigation (Somasundaram et al., 2013).

Soil organic carbon is the index of soil productivity. The SOC content is dependent upon the equilibrium of C input and decomposition rates (Fang et al., 2014). High grade of organic carbon were found in soils at higher altitudes, and generally, the amount of organic carbon increases as elevation increases similar result corresponds from (Minhas & NC, 1982; Sevgi & Tecimen, 2009). This is a result of the vegetation brought on by heavy rainfall and the slow rate of decomposition brought on by the low temperature, continuous organic manuring, which causes an accumulation of organic materials at high altitudes. However, the lower levels of organic carbon observed in soils at lower elevation may be the result of surface ploughing practices, in which the accumulation of organic carbon/matter would be minimal, particularly under continuous cropping. Similar results were earlier reported (Bhattarai & Mandal, 2016; Wani et al., 2016). The accumulation of plant debris on the soil surface and the reduced flow down the profile caused by the rapid rate of mineralization at higher temperatures and suitable level of soil moisture are the main causes of the steady decrease in organic carbon content with depth (Patangray et al., 2018).

The base of healthy and productive soils is organic matter because it enhances structures of the soil, water retention, porosity and infiltration, the ability of the soil to deliver nutrients and the ability of soil microflora and fauna to survive (Gurmu, 2019). According to the standard recommended by NARC (2013) and followed by Pandey et al. (2018) the rating of SOM contents <1, 1-2.5, 2.5-5, 5-10, and >10 are categorized as very low, low, medium, high and very high respectively. Regarding the organic matter's ranking content in the study area was high in range along all the elevation gradient. The OM content was high in the higher elevation and low in the lower

elevation. The application of farm yard manures and the implementation of best soil management practices may be the causes of the greatest OM content at elevation 2250 (10.51% at depth 0-10cm, 8.90% at depth 10-20cm and 6.80% at depth 20-30cm). These results concur with similar studies by Tadesse et al. (2016) in which they reported that, the comparatively high mean SOM land may be attributed to litter deposition and the decay of dead forage species roots, which enrich the soil with organic matter and cause sediment to build up close to the soil bunds. In Low elevation, organic matter loss may result from decomposition of pre-existing soil organic matter, runoff, as well as leaching as dissolved organic carbon (Han et al., 2010). Low organic matter at low elevations compare to the high elevation may be caused by the removal of agricultural residue during harvest due to multiple cropping and use of chemical fertilizers. High levels of tillage and harvesting in low areas destroy the surface soil structure, accelerating erosion (Kidanemariam et al., 2012). According to study, the percentage of organic matter was higher on the soil's upper surface and decreased as soil depth increased. This might be because of the assimilation of plant residue into the surface layer and the use of manure. Additionally, soil organic matter can increase or decrease depending on a number of factors, such as the climate, vegetation cover, availability nutrients, disruption and methods of managing and utilizing land (Six & Jastrow, 2002).

SOC stock in the soil does not show significantly variability with elevation ($p>0.05$). The mean value of SOC stock was high in higher elevation and low in the lower elevation in three depths layer. SOC stock was decrease with increasing depth. According to Li et al. (2010) variations in SOC stock could be related to SOC concentration or merely to geographic variations in soil bulk density. Lower SOC stocks at low elevation are a result of the higher bulk density. Similarly, there is a negative correlation between bulk density and SOC stock %. The lowest SOC stock in the lower elevation site, which is because of disturbed land use and increased soil disturbances brought on by its proximity to human habitations, caused by cattle and over grazing, reported by Lekhendra et al. (2015) corresponds to our study. Comparatively reduced organic matter inputs, slow breakdown of fine roots, increased soil disturbance, lower root biomass and loss of vegetation cover may all contribute to degraded lower SOC stocks. SOC stock was higher in elevation due to higher organic input from litter fall, debris and less soil disturbance can be linked to higher SOC stock. The result of the study is consistent with the findings of Ghimire et al. (2018).

The total nitrogen (TN) showed the similar pattern which was observed in soil organic carbon. The highest concentrations of total nitrogen were shown at the elevation 2250 (0.20% at 0-10cm, 0.16% at 10-20cm, and 0.10% at depth 20-30cm) it is significantly varied with each depth ($p=0.007$). According to the nutrient rating established NARC (2013) and followed by Pandey et al. (2018) TN value range was medium recorded in all elevation gradient. The TN content was increased in higher elevations which may be due to the larger levels of organic materials there (Charan et al., 2013; Kattel et al., 2022). Total nitrogen rises as organic matter accumulates. Since the rate of nitrogen mineralization is lower at high altitude due to higher TN content at a higher elevation, as altitude increases, temperature lowers and the related precipitation increases as concur to our result from Kattel et al. (2022). Total nitrogen decreasing consistently with depth was also observed and these findings were in line with the theory that they may be caused by the pattern of organic materials depletion with depth and increased mineralization of organic matter in upper layer, cultivation of crops mainly confined to surface horizon only and that depleted nitrogen content was supplemented by the external input of fertilizer. This result were similar with (Bhat et al., 2017; Singh & Rathore, 2013; Khanday et al., 2018).

The available phosphorus (AP) was significantly varied by each elevation with soil depths ($p<0.5$). The high mean value of AP was 50.90 kg/ha in the elevation 2250 and 43.07 kg/ha in the elevation1350 at the layer depth 0-10cm same trend followed in the depth 10-20cm and 20-30cm. The result was in line with the research, which showed that higher altitudes had more phosphorus accessible than lower altitudes (Luitel et al., 2020). Kidanemariam et al. (2012) the increased soil phosphorus concentration that is readily available at higher elevations may result from the soil's naturally higher phosphorus content. The AP was decreased with increasing the soil depth. With increasing depth, the highest P was found in the upper layer, and it dropped as depth increased. It may be caused by crop cultivation being restricted to the rhizosphere, the addition of exogenous sources of P to the depleted soil, such as fertilizers and the availability of free iron oxide and exchangeable Al3+ in less significant proportions (Singh & Mishra, 1996). These profiles' decreased phosphorus concentrations could be attributable to clay minerals, iron oxides and aluminium oxides fixing P (Luitel et al., 2020). The enhanced availability of phosphorus at the depth 0-10 cm may be because the content of organic materials is higher. Similar result were also reported (Devdas &

Srivastava, 2013; Kumar et al., 2014). According to the nutrient rating established NARC (2013), followed by Pandey et al. (2018) AP value range was medium recorded in all elevation gradient. A low to medium range of soils accessible P, however, may be primarily influenced by past fertilization, pH, organic matter content, texture and other soil management and agronomic methods (Verma et al., 2005). In the lower elevation there was highly used of DAP, the fertilizing impacts of DAP-a P-containing fertilizer and an enhanced rate of increased farm yard manure and compost were likely responsible for the improved practice soils' high levels of accessible P. However, these findings are somewhat at odds with the issue of phosphorus absorption in rain-fed soils. It was found that the agricultural land had substantially more readily available phosphorus than community forest, pasture, and protected forest. The application of fertilizers to agricultural land in anticipation of overproduction resulted in a rise in the concentration of available phosphorus. Phosphorus fixation may have contributed to the pasture and forest soils' low levels of accessible phosphorus, as suggested by Kalu et al. (2015).

The available potassium (AK) was highly affected by different elevation in three soil depths layer as 0-10cm, 10-20cm and 20-30cm ($p=0.05$, $p=0.008$ and $p=0.016$). Based on the nutrient rating developed by NARC (2013) and followed by Pandey et al. (2018) the amount of available potassium was high. It might be caused by the presence of potassium-rich clay minerals like illite, potassium supplements and manures. This concurs with Patil et al. (2015) findings. The trend of more potassium at higher elevations was confirmed (Luitel et al., 2020). As K predominantly occurs as soluble inorganic K from inorganic wastes and animal wastes were found to have a potassium content of about 0.22% of dry matter, it may be related to the increased addition of manures (Havlin et al., 2016). The AK content was higher in the depths of 0-10 cm followed by 10-20 cm and 20-30 cm depth. The burning of plant litter and relatively higher pH might be another reason for higher available K content. AK was decreased with increasing the soil depth. Similar findings were made by, who noted a declining tendency in the amount of potassium that was accessible as depth increased (Khanday et al., 2018). This may be caused by more severe weathering, the release of applicable K from organic debris and the use of external fertilizers (Bhat et al., 2017; Joshi et al., 2017).

Soil microbial biomass (MB) supports essential ecological processes as soil aggregation and nutrient cycling which contributes significantly to the organic matter in the soil (Kallenbach & Grandy, 2011). It is most important indicator of soil fertility. Soil microbial biomass carbon is a measure of the carbon contained within the living components of soil organic matter (fungi and bacteria). Soil Microbial Biomass Carbon (SMBC) was not significantly varied along the elevation gradient and also not varied along each elevation with depth ($p>0.05$). Soil microbial biomass carbon increased with increasing the elevation gradient and reduced with increasing soil depth. Similar outcome were also obtained (Adeboye et al., 2011) They show the significance of this soil layer's top 0–10 cm for microbial-mediated activities like nutrient cycling and decomposition. In the soil layer (0–10 cm), the contents of soil organic matter, such as C and N, remained higher, probably as a result of higher returns of litter in the form of fine root biomass and aerial plant remains. Maithani et al. (1998) reported increased microbial populations (fungi and bacteria) may be the cause of the larger build-up of microbial biomass C at the surface soil layer whereas, it concurs to our study. The large amounts of organic matter in the surface soil support a very strong and active soil microbial community (Arunachalam & Arunachalam, 2000). Due of the topsoil's high nutritional content, soil microbial biomass grew at the surface layer and declined as depth was raised. However, as recently reported by Maharjan et al. (2017) soil organic matter concentrations and SMBC decrease with deeper layers (10-20cm and 20–30 cm). This may be due to decreased plant residue inputs and due to variations in substrate quantity and quality (Van Leeuwen et al., 2017). In the higher elevation, high content of SMBC Due to the presence of a litter layer that retains soil moisture and encourages microbial activity, also the agriculture practices, there was highly and continuous used of farm yard manure then the lower elevation. Due to low resource availability, high disturbances and the effect of urbanization and the different farming techniques reduced mineralization rate, soil erosion, poor management, ploughing and soil tillage might be the reason for lower SMBC in lower elevation gradient in rainfed land. A similar tendency was reported by several research in various ecosystems (Bardgett et al., 2008; Soleimani et al., 2019).

The SQI measures the differences in soil quality between various land uses (Brejda & Moorman, 2001). The physicochemical properties of soil such as OM, soil pH, texture and NPK play major roles in making a significant difference in SQI under different

elevation. Figure 20 showed that the SQI value in the study was fair. The SQI on the cultivated land declined as a result of tilling and fertilizer application. The higher SQI rating have the best soil for growing plants sustainably as well as for the production of crops An integrated measure of environmental quality, food security and economic viability is being presented more and more often as the soil quality index (Lal, 2004). Thus, it would seem to be the best sign of sustainable land management. It is used to evaluate the general state of soil, management approach, or resilience to anthropogenic and natural factors, as well as changes in dynamic soil properties brought on by external influences (Herrick, 2000). By using the finest land management techniques for producing sustainable agriculture, it is vital to maintain the quality of the soil in research area. Hence, it is recommended to view the usage of soil indices for assessing the soil quality or fertility of a specific agricultural land as rational rather than absolute.

CHAPTER – SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The result of the present study showed that the soil properties respond to variation in the elevation gradient and soil depth. All the soil physicochemical properties and microbial biomass of the soil determine the characteristics of the all elevation at the soil depth layer at 0–10 cm, 10–20 cm, and 20–30 cm, respectively, and also show the differences in soil temperature, moisture, bulk density, pH, EC, SOC, SOM, SOC Stock, TN, AK, AP, Sand, Clay, Silt and SMBC. The soil in the study area was weakly acidic to neutral with a loamy sand texture. Along the different elevation gradient, higher elevation had the higher moisture content, EC, SOC, SOM, SOC stock, TN, AP, AK, Sand, Clay and SMBC and lower bulk density and slit content. However, the variation in soil properties along the elevation was lower in the depth 10-20cm and 20-30cm, whereas it indicates the soil properties decrease with depth except in the context of bulk density and temperature. However, this study showed a significant difference with soil depth. In comparison to other elevation, lower elevation has lower SOC%, total nitrogen, available phosphorus, and moisture content. This was due to more chemical fertilizer and less organic manure used. Moreover, soil quality index (SQI) was fair in the all the elevation with depth although the value was decreased with depth. At the depth 0-10cm, MBC has positive significant correlation with SOC, Soc stock, AK, and sand and negative significant correlation with temperature and silt. Similarly in the depth 10-20cm MBC has positive significant correlation with SOC, Soc stock, AP and clay and likewise in the depth 20-30cm, no significant correlation was found with SMBC. Therefore, it is important to pay attention to the soils through integrated nutrient management strategies and regular good soil monitoring for greater crop yield and sustainable agriculture.

6.2 Recommendations

Based on the results, the followings are the recommendations:

- Soil physicochemical characteristics should be constantly assessed in various management practices.
- To increase the soil quality in lower elevation, agricultural practices that encourage the build-up of soil organic matter through conservation tillage, improved FYM/compost preparation, and application should be done.
- Intensive use of chemical fertilizer should be stopped and integrated use of organic fertilizers should be practiced to enhance soil quality.
- Further research on soil microbial indices as microbial biomass nitrogen and microbial respiration along elevation gradient to determine the ecosystem processes.

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ANNEXURE

ANNEX 1: Pearson's correlation coefficient between all variables at 0-10 cm soil depth

Pearson's correlation coefficient between all variables at 0-10cm soil depth															
Variables	**Temperature**	**pH**	**EC**	**Moisture**	**BD**	**SOC**	**SOM**	**SOC Stock**	**TN**	**AP**	**AK**	**Clay**	**Sand**	**Silt**	**MBC**
Temperature	1														
pH	-.672**	1													
EC	-.663**	0.652**	1												
Moisture	-.359	.446*	.256	1											
BD	0.275	-0.471	-0.284	-0.648**	1										
SOC	-.625**	0.366	0.579**	0.061	-0.229	1									
SOM	-.625**	0.366	0.579*	0.061	-0.229	1.000**	1								
SOC Stock	-.476*	0.141	0.421	-0.3	0.286	.858**	.858**	1							
TN	-.520*	0.482	.703**	0.37	0.001	.724**	.724**	0.701**	1						
AP	-.469*	0.331	0.443	0.312	-0.36	.501*	.501*	0.287	.482*	1					
AK	-.444*	0.103	0.319	0.24	-0.114	0.261	0.261	0.183	0.138	.487*	1				
Clay	-0.198	0.167	-0.041	0.162	-.529*	0.23	0.23	-0.085	0.083	.534*	0.186	1			
Sand	-.698**	.638**	.505*	0.293	-0.279	0.528*	0.528*	0.362	.523*	.520*	.563**	0.229	1		
Silt	-.699**	-0.638**	-.490*	-0.3	0.317	-0.535*	-0.535*	-0.347	-0.518*	-0.553*	-.566**	-0.308	-0.997**	1	
MBC	-.713**	0.152	0.382	0.214	-0.039	.563**	.563*	.540*	0.182	0.265	.618**	-0.07	.478*	-.461*	1

*= significant at p=0.05;**=significant at p=0.01

ANNEX 2: Pearson's correlation coefficient between all variables at 10-20 cm soil depth

Pearson's correlation coefficient between all variables at 10-20 cm soil depth															
Variables	**Temperature**	**pH**	**EC**	**Moisture**	**BD**	**SOC**	**SOM**	**SOC Stock**	**TN**	**AP**	**AK**	**Clay**	**Sand**	**Silt**	**MBC**
Temperature	1														
pH	-.697**	1													
EC	-.787**	.644**	1												
Moisture	-.347	.447	.204	1											
BD	0.281	-0.177	-0.128	-0.287	1										
SOC	-.516*	.473*	.523*	0.318	-.446*	1									
SOM	-.515*	.472*	.523*	0.319	-.446*	1.000**	1								
SOC Stock	-0.428	.450*	.506*	0.155	-0.022	.895**	.895**	1							
TN	-.680**	.511*	.595**	0.203	-.536*	.824**	.824**	.637**	1						
AP	-0.231	0.156	0.236	0.154	-0.272	.490*	.490*	0.406	.535*	1					
AK	-.489*	0.122	0.045	0.157	-0.351	0.195	0.194	0.07	0.205	0.126	1				
Clay	-.576**	0.354	.728**	0.111	-0.057	.557*	.557*	.571**	.480*	0.449*	0.252	1			
Sand	-.787**	.686**	.560*	0.364	-0.342	.648**	.647**	.567**	.614**	0.328	.571**	.484*	1		
Silt	.809**	-.684**	-.620**	-0.352	0.324	-.677**	-.676**	-.605**	-.635**	-0.366	-.564**	-.588**	-.992**	1	
MBC	-0.315	-0.02	0.362	-0.064	-0.271	.511*	.512*	.452*	.522*	.449*	0.342	.559*	0.301	-0.357	1

*= significant at p=0.05;**=significant at p=0.01

ANNEX 3: Pearson's correlation coefficient between all variables at 20-30cm soil depth

Pearson's correlation coefficient between all variables at 20-30cm soil depth															
Variables	**Temperature**	**pH**	**EC**	**Moisture**	**BD**	**SOC**	**SOM**	**SOC Stock**	**TN**	**AP**	**AK**	**Clay**	**Sand**	**Silt**	**MBC**
Temperature	1														
pH	-.681**	1													
EC	-.802**	.666**	1												
Moisture	-0.203	0.164	-0.014	1											
BD	0.018	-0.113	0.128	-0.37	1										
SOC	-0.37	0.26	0.31	-0.111	0.261	1									
SOM	-0.37	0.262	0.308	-0.114	0.259	1.000**	1								
SOC Stock	-0.264	0.133	0.312	-0.269	.687**	.876**	.874**	1							
TN	-0.242	0.21	0.196	-0.158	-0.01	.630**	.628**	0.439	1						
AP	-0.223	0.203	0.197	-0.368	0.187	0.43	0.431	0.428	0.381	1					
AK	-0.314	0.202	0.104	0.303	-0.296	0.147	0.151	-0.069	0.198	0.114	1				
Clay	-0.396	.497*	0.479*	0.283	-0.12	.477*	.476*	0.309	0.172	0.089	0.361	1			
Sand	-.743**	.752**	.649**	0.304	-0.131	.498*	.498*	0.306	0.265	0.274	.559*	.798**	1		
Silt	.714**	-.736**	-.642*	-0.309	0.133	-.507*	-.507*	-0.313	-0.259	-0.256	-.546*	-.843**	-.997**	1	
MBC	-0.188	0.335	0.382	0.402	0.224	0.111	0.1	0.178	0.234	-0.057	0.213	0.411	0.384	-0.396	1

*=significant at p=0.05;**=significant at p=0.01

ANNEX 4 : Datasheet

Questionnaire for HH Survey

PART A: SOCIO-ECONOMIC

1) Farmers details:

Name…… Age…….. Occupation…….. Education……..

Altitude / Location…….. District…….Rural

Municipality/Municipality…….Ward no…….

Location: Latitude…… Longitude …….

Land information: Total land area……Maize cultivation area……

2) Information on maize:

Crop Name and variety	Area for cultivation (Ropani)	Type of Land	Source of origin	Seeds source / Seed required per ropani	Seed Storage Methods
Phaelo/Seto/Hybrid makai					

Crop Name and variety	Productivity (Kg /Pathi/ Muri) / per ropani	Associate crops	Causes of Preference	Land use after maize harvesting	Production satisfactory or not
Phaelo/Seto/Hybrid makai					

a) Land preparation/ Soil (Type and tilling or puddling)

……

b) Seed sowing (Time, requirement):

……

c) Fertilization (Type, amount, frequency)

……

d) Weeding (Time, method and Frequency):

……

e) Harvesting (Time after sowing)

……

f) Disease (major disease):

……

g) Phenology/Crop Calendar of Maize (Time-Months)

Maize variety	Land preparation	Seed sowing	Weeding	Flowering	Fruiting	Ripening	Harvesting

h) Is there any environmental problem which damages your maize crop? (Mention last ten years' experience)

i) Demand of maize is increasing or decreasing…….

3) Information on Soil:

a) Types of Soil Tillage: Conservation Tillage Method OR Conventional Tillage Method

b) Soil conservation technique

a) terracing b) no till farming d) contour plowing

c) Management Practices for soil conservation

a) Land Preparation b) Compost and Mulching c) Cover crop d) Crop rotation e) Contour farming f) all of above

d) What is a soil problem all farmers face?

a) soil hardening b) soil erosion c) soil spoiling d) soil pollution

PHOTOPLATES

Rainfed land at elevation 1350masl (Kleu)

Elevation 1650masl (Jhinu dada)

Elevation 1950masl (Taulung)

Elevation 2250masl (Chhomorong)

Collecting information from local people

Sample Left for Drying at Green House

Sieving soil

Measuring soil temperature in soil depth

Measuring the pH of soil samples

Measuring the EC of soil samples

Soil suspension for soil texture analysis

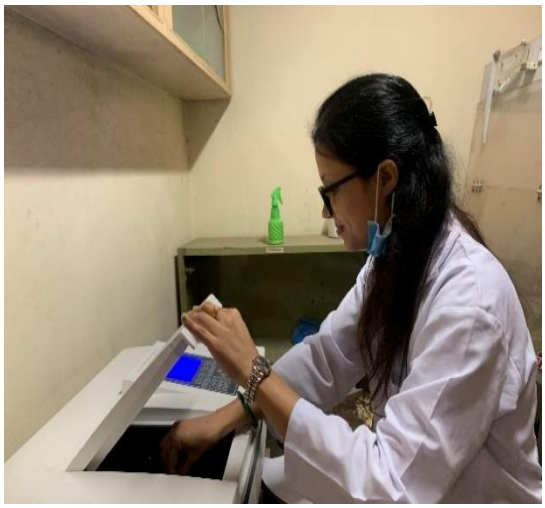
Adding chemical for SOC

Titration of SOC

Samples after digestion

Distillation process of TN

Soil aliquots extraction for phosphorus



Absorption reading for phosphorous analysis

Soil aliquots extraction for potassium

Taking readings in flame photometer

Use of vacuum with desiccator for SMBC

Filtering aliquots for MBC

Soil aliquots digestion

Determination of SMBC

“Supported by NAST”