Variation in Soil Physical, Chemical and Microbial Parameters under Different Land uses in Bagrot Valley, Gilgit, Pakistan

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Summary: Soil degradation due to unsustainable land use is a global problem and the biggest challenge for sustainability in mountain areas due to their ecological and socio-economic impacts. The study aims to evaluate the variation in the physical, chemical and microbial parameters of soil across various land uses in the Bagrot valley, Central Karakoram National Park (CKNP), Gilgit-Baltistan. Soil samples from 0-20 cm were collected from three land uses such as arable land, pasture, and adjacently located forest. The variables investigated were soil bulk density, total porosity, saturation percentage, sand, silt, clay, pH, electric conductivity, $CaCO_3$, organic matter, TN, available P, K, Fe, Mn, Cu and Zn and microbial parameters (16SrRNA & ITS copies number and fungi-to-bacterial ratio). A sigificant varriation in all parameters were found accross the land uses (ANOVA, $p < 0.01$). Similarly, the highest bulk density, sand, pH, EC, $CaCO_3$ were found in arable land, with the lowest values in forest. In contrast, soil under forest showed a higher total porosity, percent saturation, clay, OM, macro and micronutrients, microbial abundance and fungi-to-bacterial ratio than for other land uses. The differences in soil parameters across the land uses indicated detrimental impacts of agricultural activities on soil health. Soil pH and organic matter are the main controlling factors for microbial indicators as well as physical and chemical parameters. The results suggest that restoration of natural vegetation in degraded land and decrease in intensity of land use could improve soil properties in the study area, as well as other similar mountainous regions.

Key Words: Land degradation, Mountain soil, qPCR, Soil health, Soil organic matter.

Introduction

Soil degradation due to unsustainable land use and climate change is a global problem and the biggest challenge for sustainability in mountainous region [1]. It has also caused severe soil health deterioration in mountain areas. Thus, the effects of land use on soil properties and functions in mountain areas have recently received more attention for sustainable land use planning [2]. Evaluating variations in soil properties due to land use is crucial for addressing problems of ecosystem transformation and sustainable soil productivity. Studies have shown that a variation in soil properties were found among different land uses [3-7] and cultivation significantly effects the physical, chemical, and microbial properties of soils [8, 9]. Likewise, soil microbial properties are affected by different edaphic factors, such as, soil electric conductivity [10], soil pH [11] and soil nutritional status [12]. A distinct regional variability in soil properties has been observed even within the same land use systems. Similarly, land use and management can have significant effects on soil properties within a single region [13]. Therefore, further studies are needed under different land uses in various regions such as the Karakoram region, for which, thus far, there is very limited information.

In the Himalaya and Karakoram mountain ranges, the soil is vulnerable to climate change and land use activities. These soils are highly fragile, pedologically young and have developed slowly on steep slopes under harsh climatic conditions [2, 14]. Severe ecological and environmental degradation of soil has been observed in the Himalayas and Karakoram [15]. The Bagrot Valley (our study area) is in the southern part of the Karakoram Range, surrounded by the famous peaks of Rakaposhi (7,788 m) and Diran (7,266 m) in the north, while the Dubani (6,120 m) lies to the east. In general, due to the topographical setting and harsh climate of mountainous areas, there are limited natural resources in this region [16]. For millennia, inhabitants have adopted "Mixed Mountain Agriculture" and managed their resources in a sustainable way without over exploitation. However, because of rapid population growth, coupled with the increasing demands of commercial and domestic use of sub-humid forests, pasture, shelters and food, the utilization of natural land covers has increased at an alarming rate. These activities may increase the risk of soil erosion and mass movement after heavy and persistent rainfall. This results in a decline in the soil's health, which has direct and indirect effects on agricultural productivity.

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The aim of this work was to evaluate the variation in soil physical, chemical and microbial parameters across different land uses in Bagrot Valley. Understanding the effect of land uses on soil parameters is essential for efficient land use planning and resource management, and also for predicting the effects of future land use activities on soil ecosystems.

Experimental

Study Area

Bagrot Valley is in the southern part of the Karakoram Range, about 17 km to the northeast of Gilgit, Pakistan. The Karakoram Range lies in the center of the high mountain node; Hindukush to the west, Pamir and Tien Shah to the northwest, Kulun Shah to the northeast and Himalaya in the southeast. The total watershed area is 440 km^2, and is comprised of pasture (16.14%), forest (16.43%), and arable land (3.35%); ice, glacier, rock cover 64.08% of the area [17]. Geologically, the area consists of the Chalt Volcanic Group, which has an especially high content of SiO_2, MgO, Fe_2O_3 and CaO [18]. Depending on the topography, there is an extreme variation in the climate of these mountains, where as, the monsoonal influence is negligible in the region [19]. Agriculture, pastoralism and forestry form the backbone of the inhabitants' livelihood.

Soil Sampling and Processing

A random sampling method was used for the study. At each site, five random 40 m long transects were selected. Each sampling point (30 x 30 cm quadrat) was located at the edge of a 5 m wide strip along the transect, and ten soil sub-samples were collected in a zigzag type pattern and made into a composite sample; in this way, five composite samples were taken from each site. Samples were collected from the 0-20 cm layer using a stainless steel corer. One undisturbed core sample was also collected from each site for bulk density by using a 100cm^3 core ring. Roots, stones, and debris were removed before sampling. The samples were packed in separate polyethylene bags and labeled. The samples were brought to the laboratory and each sample was divided into two parts. One portion of the sample was air dried. The dried samples were then lightly ground, sieved through a 2-mm mesh and used for analysis of physico-chemical parameters. Samples destined for microbial analysis were kept in a freezer at - 4°C. Two grams of each sample were preserved in 9 ml Lifeguard Soil Preservation Solution (MO BIO Laboratories Inc., Carlsbad, CA, USA), and transported frozen to the University of Oregon, USA, for molecular analysis.

Laboratory Analysis

Soil samples were used for analyses of physical and chemical parameters (bulk density, porosity, particle distribution, texture, pH, EC, CaCO3, organic matter, TN, available P & K, Fe, Mn, and Cu and Zn) and microbial parameters (16Sr & ITS copies number and Fungi-to- bacterial ratio).

Physical and Chemical Analysis

Blake and Hartge's method was used for determination of bulk density [22]. Total porosity was calculated by using a formula given by Danielson and Sutherland [21]. Saturation percentage was measured by using a Keen Raczkowaski brass cup [22]. A Bouyoucos hydrometer method was used for analysis of particle size, and soil textural class was determined by using a US textual triangle [23]. Soil pH in 1: 2.5 (soil: water) was recorded by the Rowel method [24].) Rhoades' method was used for the determination of EC_e in the soil saturation extract [25]. $CaCO_3$ in the soil was determined by the U.S. Salinity Laboratory Saff method [26]. A modification of the Walkely and Black method was used to determine the soil's organic matter in fine ground soil [27]. Total N was determined by the Kjeldahl's digestion and titration method [28]. AB-DTPA extractable phosphorus, potassium, iron, manganese, copper and zinc were determined in the soil by the standard method [29]. Phosphorus was determined by an ammonium molybdate color complex measuring absorbance on 880 nm wavelength using a spectrophotometer (Perkin Elmer, Lamda 35). The concentration of K was determined by flame photometry, while Cu, Fe, Zn and Mn were determined by an atomic absorption spectrophotometer (Perkin Elmer, 2380).

Microbial Analysis

Extraction and Quantification of DNA

DNA was extracted from Lifeguard-preserved (described above) soil samples. Briefly, the soil-Lifeguard solution was vortexed to mix. From each sample 3.5 ml of soil solution was centrifuged to remove the liquid supernatant. DNA was extracted from the soil following removal of the supernatant using the Power Soil DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), as per manufacturer's instructions. The concentration of DNA was measured using Qubit with the Quant-iT PicoGreen kit (Molecular Probes/Invitrogen, Carlsbad, CA, USA).

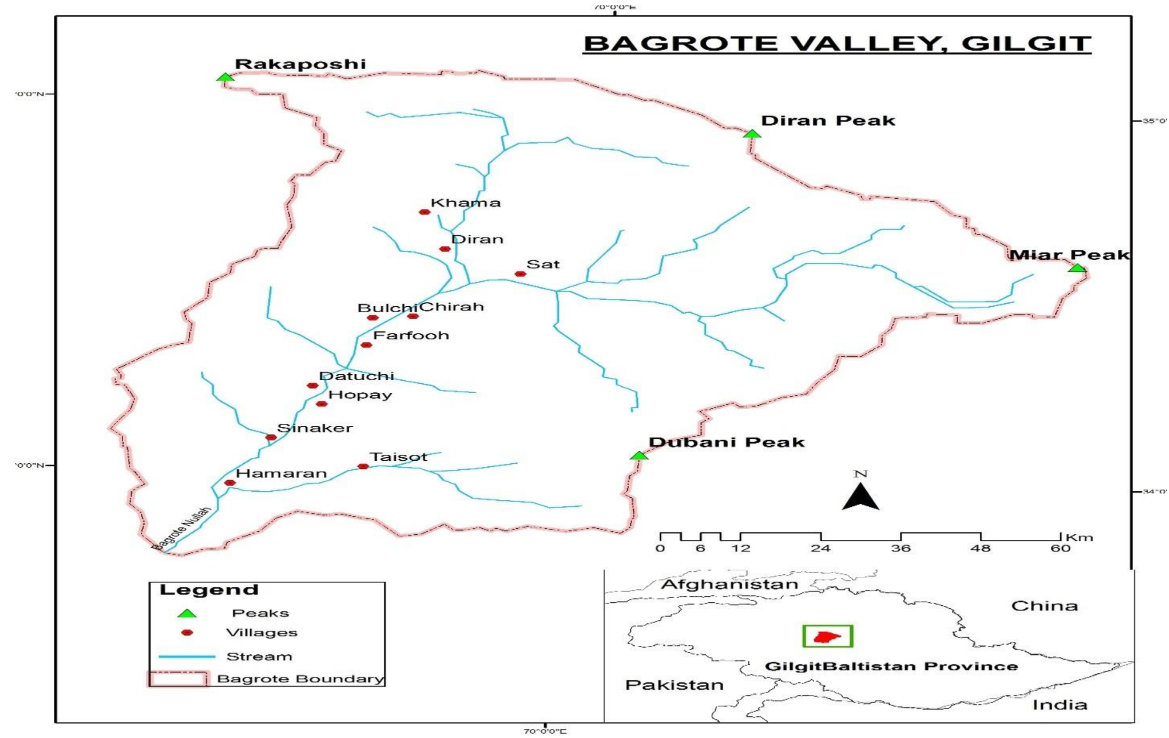


Fig. 1: Bagrot valley, Central Karakoram National Park, Gilgit-Baltistan.

Quantitative Real Time PCR

Triplicates of each soil sample were performed in 20 µl containing 5 µl of 2X SYBR Green supermix with ROX as an internal reference (Bio-Rad, Hercules, CA, USA), 0.2 µl of each primers and 1.0 µl of total soil DNA (5 ng). Bacteria was amplified by 16S rRNA gene specific primer 515F and 806R [30]. Similarly, fungi was amplified with primers ITS1F and ITS2 [31]. Quantitative PCR was performed on an ABI7300 real time PCR instrument (Applied Bio-systems, Calrsbad, CA, USA). The amplification conditions were as follows: 5 min at 95°C, followed by 50 cycles of 20s at 94°C, 20s at 55°C, and 30s at 72°C, while for fungi 5min. at 95°C, followed by 30 cycles at 95°C, 30s at 64°C, and 30s at 72°C. To quantify the copy number, a serial dilution of bacteria and fungi were prepared to generate a standard curve. The linear correlation coefficient for the standard curve was 0.998 or higher. The gene copy number was calculated by calibration curve.

Data Analysis

An analysis of variance (ANOVA) was used to determine the effects of land use on soil parameters. A Least significant difference (LSD) comparison test was carried out to separate statistically different means ($p \leq 0.05$). Correlation analysis was carried out to determine the relationship among various soil parameters. Statistical Package for Social Sciences (SPSS) version 19, 2010, and Microsoft Excel were used for all analysis [32].

Results and Discussion

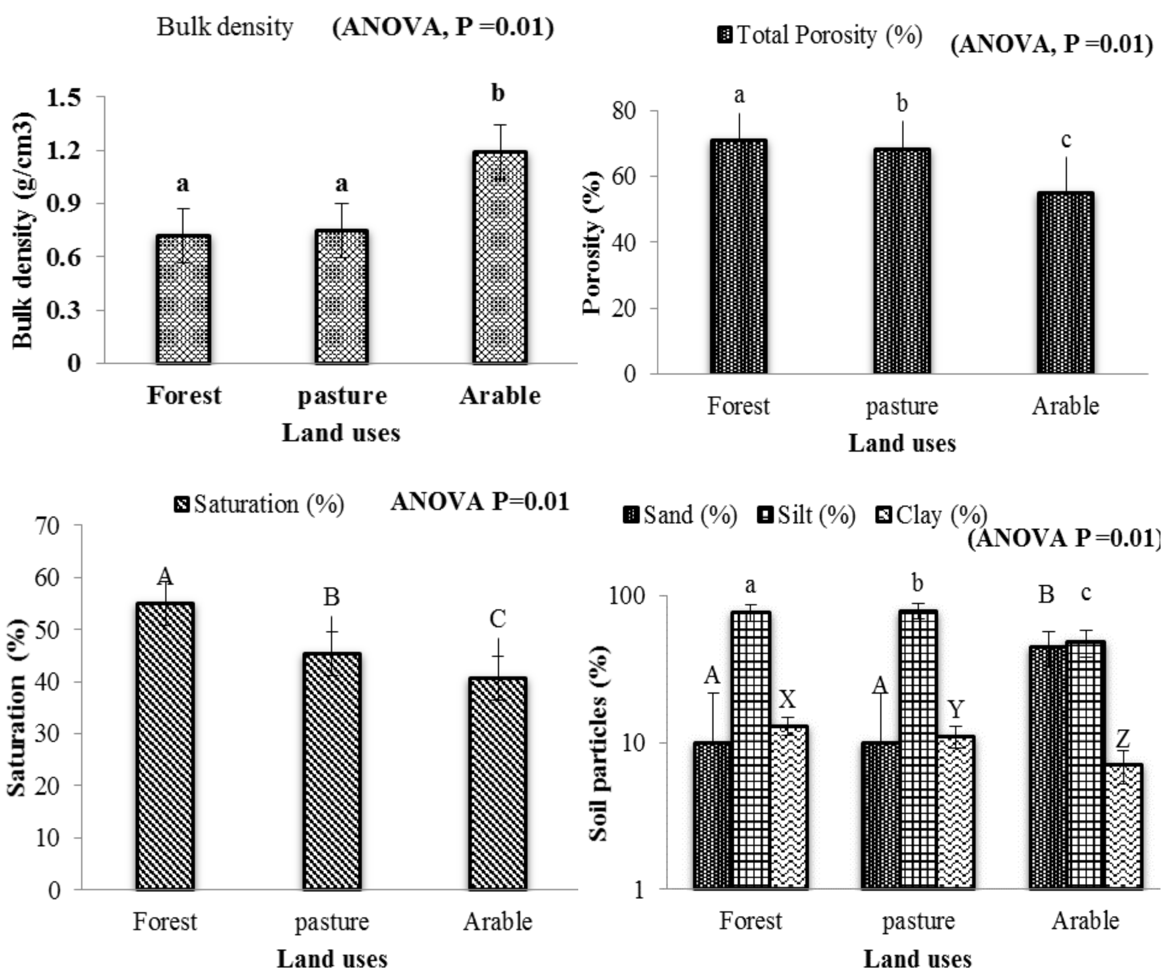
Effect of Land Uses on Soil Physical Parameters

Analysis of variance (ANOVA Fig. 2) revealed that land use had a significant effect on bulk density, total porosity, saturation percentages, sand, silt and clay at $p < 0.01$. Correlation analysis showed that bulk density, sand content had negative relationship, while total porosity, percent satuaration, silt and clay had a positive relationship with organic matter (Table-1). The mean bulk density in arable land (1.19 g cm^{-3}) was significantly higher than pasture (0.75 g cm^{-3}), and was the lowest in forest (0.72 g cm^{-3}) (Fig. 2). Soil bulk density determines the soil compactness, functionality and physical conditions of the soil for microorganisms [33]. The low and high bulk density indicates the poor physical environment of the soil. The high bulk density in arable soil could be due to intensive tillage, which promotes the loss of organic matter. Furthermore, continuous use of machinery during cultivation may also cause a decline in soil aggregate size resulting in compaction causing a higher bulk density. The low bulk density in forest and pasture could be due to high organic matter and less frequent manipulation of the soil. Bulk density typically has a negative relationship with organic matter [3, 34]. Total porosity and saturation percentage showed similar patterns, but in the opposite direction of bulk density. The soil under arable land had a lower total porosity (55.05%) than pasture (68.28%), with the highest values in forest (70.75%) (Fig. 2). Similarly, forest had a significantly higher saturation percentage (55.0%) than pasture (45.33 %), with the lowest values in arable land (40.63%) (Fig. 2). Soil porosity influences soil aeration, availability of water for plants and field capacity [35]. It is closely related to root growth and soil enzyme activities [36]. The normal range of total porosity in soils is between 30 - 70% [37]. The high total porosity and saturation percentage in forest and pasture in the present study could be due to high organic matter accumulation. Others have reported that the decrease of porosity and saturation percentage and increase of bulk density can result from removal of organic matter [3, 38]. The lower porosity and saturation percentage in arable land could be due to compaction and breakdown of soil aggregates [3, 38]. Porosity has a negative relation with bulk density and a direct relation with organic matter [39].

Table-1: Relationship between soil physical parameters and organic matter.

Variables	BD	TP	SP	Sand	Silt	Clay
OM	-0.894^{**}	0.926^{**}	0.980^{**}	-0.873^{**}	0.836^{**}	0.936^{**}

Note: **, ***, ^{ns} indicates correlation is significant at $p < 0.05$, $p < 0.01$. Abbreviations BD (Bulk density; TP (total Porosity and SP (saturation percentage.



Values with different letters are least significant difference among three land uses (LSD at $p < 0.05$)

Fig. 2: Effects of land use on physical parameters of soil.

Sand content was significantly higher in arable land (45.0%) than pasture (10.50%), and was lowest in forest (10.0%) (Fig. 2). Pasture had a significantly higher silt content (79.0%) than forest (77.0%), with the lowest values in arable land (48.0%). On the other hand, clay content was significantly higher in forest (13.0%) than pasture (11.0%), with the lowest values in arable land (7.0 %) (Fig. 2). Soil texture is the proportion of soil sand, silt and clay, which controls water infiltration and retention, aeration, nutrients absorption and microbial activity [40]. In this study, low clay and high sand contents in arable land could be due to preferential removal of clay by accelerated erosion (water). Others researchers have also reported the selective lost and removal of the clay fraction from the soil due to erosion [38, 41]. A higher proportion of clay in forest and pasture could be due to covered vegetation, which protects the soil against erosion. The high silt content in forest and pasture could be due to colluvium deposition due to gravity acting on the steep slopes. Overall variation in soil particles may be due to land scape processes.

Effect of Land Use on Soil Chemical Parameters

Analysis of variance (ANOVA Fig. 2) revealed that land use had a significant effect on soil pH, EC $CaCO_3$, organic matter, TN, available P, K Fe, Mn, Cu and Zn ($p < 0.01$). Forest had a significantly lower pH (6.4) than pasture (7.1), with the highest in arable land (7.5) (Fig. 2). Soil pH determines the degree of acidity or alkalinity of the soil environment [42]. Many chemical and biological processes of soil include acidification, calcareousness, salinization, nutrient availability and toxicity, and cycling and microbial activity is controlled by soil pH [43]. The slightly acidic pH in forest soil could be due to high organic matter, which by oxidation produces organic acids in the soil solution. Furthermore, plants uptake basic cations as nutrients for growth and development. Fey reported that organic matter can have a negative relationship with pH [44]. The slightly alkaline pH in arable land may be due to the farming activities [39].

Electric conductivity in arable land (1.33 dSm^{1}) was significantly higher than pasture (0.60 dSm^{-1}), with the lowest value in forest (0.52 dSm^{-1}) (Fig. 3). The results indicate that arable soil is more saline than pasture and forest. EC reflects soil salinity, nutrients cycling, microbial activity and is a measure of the decline of the soil structure in alkaline soil [45]. Low EC in forest and pasture may be due to the accumulation of salts in the root zone because of greater water detention on the surface and infiltration into the soil. The forested landscape is likely to have less leaching, owing to the high evaporative demand of trees and the greater canopy interception of rainfall [46]. A higher value of electrical conductivity in arable soil could be due to salts in the soil and uses of chemical fertilizer [47]. Similarly, forest had significantly lower $CaCO_3$ (0.09%) than pasture (1.0 %), with the highest values in arable land (2.67%) (Fig. 3). $CaCO_3$ reduces soil acidity, increases availability of phosphorus and increase nitrogen fixation, mineralization and nitrification [48]. The low $CaCO_3$ in forest and pasture could be due to high organic matter, and acidity. $CaCO_3$ is dissolved in acidic medium [49] and organic matter can have a negative relationship with $CaCO_3$ [50].

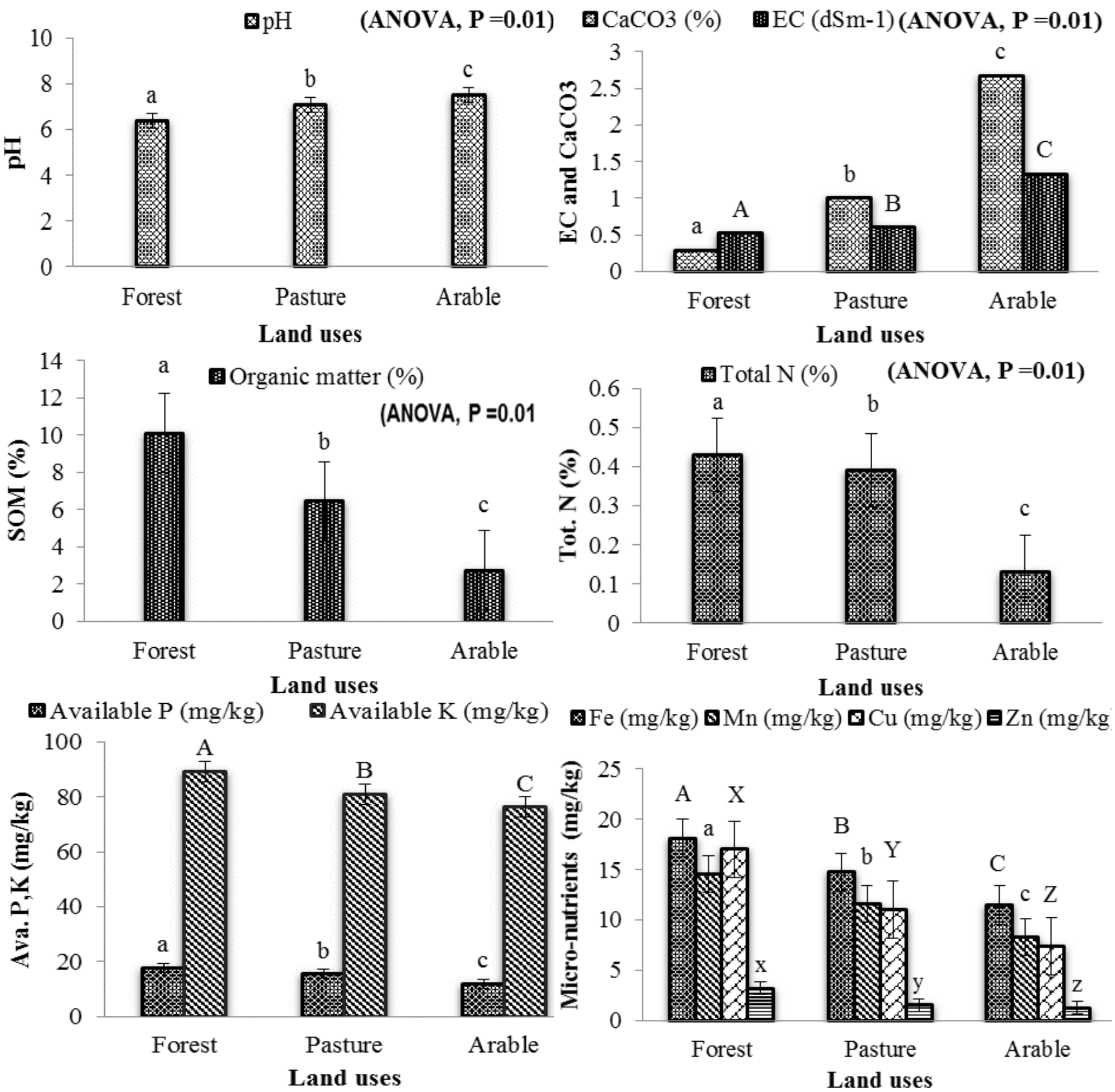
Soil organic matter in forest (10.10%) was significantly higher than pasture (6.45%), with the lowest values in arable land (2.75%) (Fig. 3). SOM helps to control soil erosion, increase in infiltration and porosity of clay soil, improve soil structure and stability, cycling of plant nutrients, providing energy to microbes and fauna, and increasing their activity [51]. In this study, soil organic matter was found to be adequate in all land uses. However, somewhat low organic matter in arable soil may be due to continuous ploughing and cultivation, which accelerate the rate of organic matter decomposition and mineralization. Further, the removal of biomass during harvesting and periodic tillage, which breaks up macro aggregates, results in increased soil erosion and run off. Cultivation can reduce organic matter [52]. Higher accumulation of organic matter in forest could be due to the greater biomass input by vegetative material, which is the main source of OM. Organic matter generally has a positive relationship with plant density [53].

The total nitrogen in forest (0.43%) was significantly higher than pasture (0.39%), with the lowest value in arable land (0.13 %). (Fig. 3). Similarly, forest had significantly higher available phosphorus (17.73 mg kg^{-1}) than pasture (15.50 mg kg^{-1}), with the lowest value in arable land (12.0 mg kg^{-1}). The same trend was observed for available potassium with highest values in forest (89.13 mg kg^{-1}), followed by pasture (81.0 mg kg^{-1}), and the lowest in arable land (76.20 mg kg^{-1}) (Fig. 3). The greatest variation in P and K were observed between forest and arable land, with a small variation between pasture and arable land. Correlation analysis showed that all parameters had a positive relationship with soil organic matter and a weak negative relationship with pH (Table-2). Macronutrients, such as N, P and K, are the key indicators of soil health providing information about available plants nutrients but can impact other components of the environment through losses [54]. Hence they reflect soil productivity and environmental quality [43]. In the present study all the macronutrients were noted to be adequately available for the three land uses. However, the significantly higher amounts of macronutrients in forest and pasture land could be attributed to high organic matter contents and more biological activity leading to nutrient cycling. Organic matter is the primary supply source of N, P and K [55]. Lower macronutrients in arable land might be due to the low organic matter, and extractive effect of cultivation. Another possible cause of low macronutrients in arable land is that phosphate and potassium based fertilizer is not used in the area. Other researcher has reported that fertilizer application can increase available P and K [56].

Table-2: Correlation of soil organic matter and pH with the soil nutrients.

Variables	TN	Ava P	Ava K	Ava Fe	Ava Mn	Ava Cu	Ava Zn
OM	0.927^{**}	0.871^{**}	0.972^{**}	0.899^{**}	1.0^{**}	0.877^{**}	0.809^{**}
pH	-0.203^{ns}	-0.431^{ns}	-0.637^{*}	-0.578^{*}	-0.528^{ns}	-0.646^{*}	-0.736^{*}

*, **. Indicate correlation is significant at the $p < 0.05$ and $p < 0.01$ level. Abbreviation; OM, TN, Ava. P & K, Fe, Mn, Cu and Zn (organic matter, total nitrogen, available phosphorus and Potassium Iron, Manganese, Copper and Zinc



Values with different letters are least significant difference across land uses (LSD at $p < 0.05$)

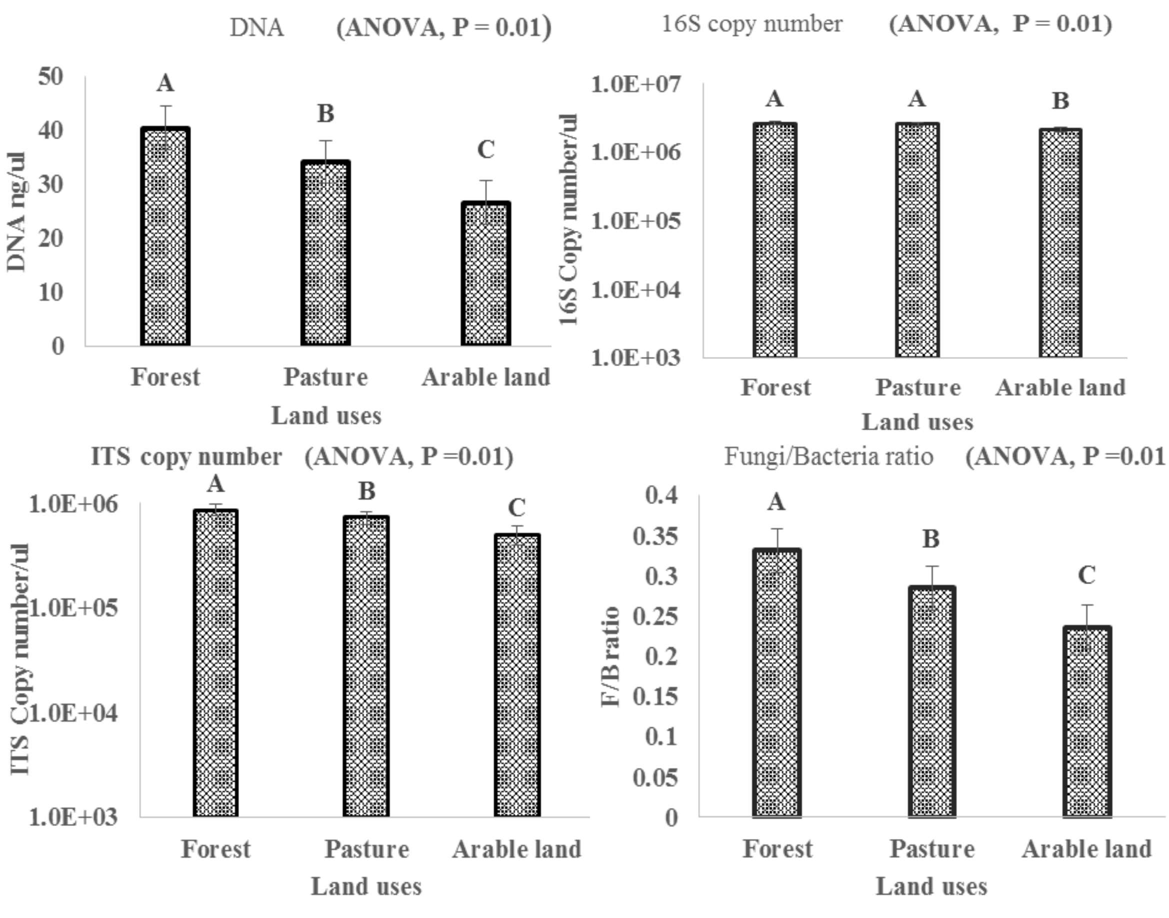
Fig. 3: Effects of land use on chemical parameters of soil.

The concentration of available Fe was significantly higher in forest (18.13 mg kg^{-1}) than pasture (14.73 mg kg^{-1}) and arable land (11.50 mg kg^{-1}) (Fig. 3). Similarly, forest had a significantly higher concentration of available Mn (14.55 mg kg^{-1}) than pasture (11.55mg kg^{-1}), with the lowest concentration in arable land (8.28 mg kg^{-1}) (Fig. 3). The mean available Cu concentration was significantly lower in arable land (7.39 mg kg^{-1}) than pasture (11.04 mg kg^{-1}) and forest (17.03 mg kg^{-1}), while the same trend was observed for available Zn [forest (3.23 mg kg^{-}1), pasture (1.55 mg kg^{-1}), and arable land (1.27 mg kg^{-1})] (Fig. 3). The greatest variation in all four micronutrients was observed between forest and arable land, with smaller variations between arable land and pasture. Correlation analysis showed that all available micronutrients had a positive relationship with soil organic matter and a weak negative relationship with pH (Table-2). Micro-nutrient uptake by plants in insufficient quantities [37] and a deficiency of any micronutrient in the soil can inhibit plants growth [40]. In this study all micronutrients were found to be within the normal range. However, the relatively high concentration of micronutrients in forest and pasture thought to be due to high organic matter and the slight acidic nature of soil. Other researchers have reported that available micronutrients had a positive relationship with SOM and negative relationship with pH [48], while the solubility and availability of micronutrient cations were increased under acidic conditions [39].

Table-3: Correlation of soil microbial parameters with soil pH and organic matter.

Pearson Correlation	16SrRNA copy number	INTS copy number	Fungi/Bacteria ratio	OM
pH	0.329*	- 0.915**	- 0.926**	-0.968
Organic matter	0.537*	0.984**	0.786**	1

Note: * and ** indicates correlation is significance at $p < 0.05$ and $p < 0.01$.



Values with different letters are least significant difference among three land uses (LSD at $p < 0.05$)

Fig. 4: Effects of land use on soil microbial parameters.

Effect of Land Uses on Soil Microbial Parameters

Quantitative PCR results showed that land use had a significant effect on soil 16S and ITS copy number as well as the fungi-to-bacterial ratio (*i.e.* the ITS-to-16S ratio) (Fig. 4). Furthermore, fungal abundance and fungi-to-bacterial ratio showed a strong negative relationship with soil pH and positive relationship with organic matter (Table-3). Bacterial abundance showed a weak positive relationship with organic matter and showed no relation with pH (Table-7). Arable land had significantly lower 16S copy number (2.13 x 10^6) than pasture (2.57 x 10^6) and forest (2.60 x 10^6) (Fig. 4). However, no significant variation in 16S copy number was found between forest and pasture ($p = 0.01$). Likewise, forest soils had significantly higher ITS copy number (8.52 x 10^5) than pasture (7.30 x 10^5), and the lowest was observed in arable land (5.03 x 10^5) (Fig. 4). A similar trend was observed in the fungal to bacteria ratio (Fig. 4). Microbes in soil give a quick response to natural or anthropogenic activities and are, therefore, a more sensitive indicator of soil health [57]. Soil microbes produced measureable changes in physico- chemical parameters, thus can be used as early warning for soil degradation [58]. In the present study, forest soil had a higher abundance of fungi and higher fungi-to-bacteria ratio than pasture and arable land. Similar results have been reported by other researchers [59, 60]. The higher fungal abundance and fungi-to-bacteria ratios in forest was presumably due to its higher organic matter content, elevated nutrient status, and lower pH compared to pasture and arable land. Fungal abundance and fungi-to-bacteria ratios tend to increase under acidic conditions [61] and high organic matter [61].

The relatively low abundance of fungi and fungi-to-bacteria ratio in arable land may be due to intensive agricultural practices (tillage, fertilization and irrigation), low organic matter and higher pH. Tillage influences the soil's physical and chemical properties and soil environment [62, 63], disrupts soil fungal hyphae [62] and lowers fungal abundance [64]. Soil management and application of mineral fertilizers has been shown to decrease the F/B biomass ratio [65] and decrease the fungal to bacterial ratio [66]. Similar to fungi, the highest abundance of bacteria was expected in forest in the present study, due to high organic matter and other nutrients. However, only slightly higher bacterial abundance was observed in forest than pasture, which was statistically non-significant. This unexpected pattern may be due to the acidic and non-saline nature of forest soil. The higher nutrient and organic matter effects may be overridden by acidity. Regression analysis showed that soil pH had a stronger positive relation with bacterial abundance than with soil organic matter. Other researchers have also suggested that lower bacterial abundance in forest may be due to lower pH [59, 60], and that bacterial abundance is higher under alkaline and saline conditions as well as anaerobic (waterlogged) soils [61]. On the other hand, low bacterial abundance in arable land was likely due to low soil organic matter and nutrient status [67].

Conclusions

Significant effects of land use on soil physical, chemical and biological properties were observed in Bagrot Valley. Our study indicates that intensive tillage with the use of machinery and fertilizers during cultivation on arable land can increase the soil's compaction, erosion, alkalinity, salinity, calcareousness, and decrease soil aeration, water holding capacity, organic matter content, available nutrients and microbial abundance, as compared to soil in pasture and forest land. On the other hand, in forest and pasture, vegetation coverage can produce greater biomass production, protect the soil against erosion and increase acidity. Our study further suggests that soil organic matter has a strong relationship with soil properties, as it improves soil health. Furthermore, soil chemistry seems to be the main controlling factor for microbial indicators, as it increases fungal abundance and fungi-to-bacterial ratio. The study suggests that restoration of natural vegetation and sustainable land use (such as practices that reduce soil disturbance and that do not deplete soil organic matter and nutrients) can reduce soil erosion and improve soil health in the study area, as well as other mountain regions.

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