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Carbon and Nitrogen Sourcing in High Elevation Landscapes of Mustang in Central Nepal

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Abstract: Mustang valley in the central Himalaya of Nepal is a unique landscape formed by massive soil mass during a glacial period, which is attributed to a mix of vegetations and long agricultural history. Soil nutrients and their sourcing is highly important to understand the vegetation assemblage and land productivity in this arid zone. Twenty soil samples (from 0 to 20 cm depth) were collected from three landscape positions in Mustang district: valley, ridge, and midslope. We explored nutrient sourcing using natural abundance carbon ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) employing isotope ratio mass spectrophotometry. The results showed that the total soil carbon (TC) and total nitrogen (TN) ranged from 0.3 to 10.5% and 0.3 to 0.7%, respectively. Similarly, the CN ratio ranged from 0.75 to 15.6, whereas soil pH ranged from 6.5 to 7.5. Valley soil showed higher values of TN, CN, and soil pH than the ridge and midslope soils. The valleys had more positive $\delta^{15}\text{N}$ signatures than ridge and midslope, which indicates higher inorganic and organic N fertilizer inputs in the valley bottom than in the midslope and ridge. This suggests that a higher nutrient content in the valley bottom likely results from agro-inputs management and the transport of nutrients from the ridge and midslope. Soil pH and CN ratio were a non-limiting factor of nutrient availability in the study regions. These findings are crucial in understanding the nutrient dynamics and management in relation to vegetation and agricultural farming in this unique topography of the Trans-Himalayan zone of Mustang in central Nepal.

Keywords: carbon; isotopic signature; Mustang; natural abundance; nitrogen; nutrient sourcing



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1. Introduction

Nepal exhibits unique topographic features with a great variation in climate and biodiversity observed in every five kilometers across the longitude [1]. The origin of the Nepal Himalaya started from the Miocene period (50 million years ago), throughout which a constant weathering of soil parent materials occurred [2,3]. Mustang geology is believed to have originated around the Plio-Pleistocenous age and is well known as a Thakkhola formation [4]. The evolution of the Thakkhola formation aligns with major Himalayan uplift events that are set on unique geomorphic and climate patterns of Mustang compared to other parts of Nepal [4].

Mustang is located in the high mountains, where weathering is mainly constrained by climate. Overall, the climate of Mustang is characterized by low temperatures and dry seasons with high wind speed. Specifically, the northern part of Mustang represents the rain shadow area of Nepal [5], locally referred to as the Trans-Himalayan zone. The

southern part is relatively more humid than the northern part and is covered with forest area which is only 3.3% (12,324 ha) of total landmass [6]. Low temperature and scant precipitation decelerate the weathering process [7] and result in fragile, weak aggregates, and shallow soil mass (i.e., skeletal soil) in Mustang [8]. High wind speed, however, accelerates the physical weathering of rocks and minerals. Therefore, Mustang exhibits unique topographic and climatic features, and its soil behaves differently compared to other parts of Nepal.

The altitude of Mustang district ranges from 2010 to 8167 m above sea level (masl). It is covered by 57.7% barren land, 30.3% grassland, 5.6% forest and bushes, 2.7% sand and cliffs, 2.1% water bodies, and 1.6% cultivated areas [8]. The geomorphology of Mustang is composed of high peaks, ridges, midslopes and valley bottoms attributing to different landscape positions [5]. The following three landscape positions are found in the hillslope. The ridge is the peak of the hill of the sloped land, midslope is in the middle part between the ridge and the valley bottom, and the basal part of the hill is valley bottom which is generally flat land located near the river channels. These landscape positions are characterized by their own specific micro-climate, micro-relief, aspect, and soil type. Generally, the valley bottoms are relatively warmer, moist, fertile, and have a lower slope than the midslope and ridge.

Most of the cultivated area in Mustang is occupied with apple (*Malus domestica*) orchards, one of the major income sources of Mustang residents. It covers around 72% of the district's total fruit production and is mainly dominant in the lower part of Mustang [9]. Besides apples, crops such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), buckwheat (*Fagopyrum esculentum*), barley (*Hordeum vulgare*), naked barley (*Hordeum vulgare* ssp. *Vulgare*), pea (*Pisum sativum*), mustard (*Brassica* sp.), potato (*Solanum tuberosum*), vegetables, and other temperate fruits (apricot and walnut) are grown in Mustang. Generally, orchards are planted in the ridge and midslope areas and crops/vegetables are grown in the valley bottoms. Most parts of the central and southern Mustang and a few villages of the northern Mustang harvest two crops each year.

Carbon (C) and nitrogen (N) are two fundamental nutrients that serve as key soil fertility indicators [10]. The availability of nutrients for crops is governed by soil pH [11] and the Carbon–Nitrogen (CN) ratio [12]. The stable isotopes (natural abundance) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) and the CN ratio have been increasingly used to identify organic matter origin, mixing, and transformations in soil and their cycling in the atmosphere [13–16]. A lower CN ratio indicates a higher mineralization of organic matter and vice versa [17]. The signature of $\delta^{13}\text{C}$ differs with different vegetation assemblage; higher in C_4 vegetation (-9 to -17‰) and lower in C_3 vegetation (-23 to -30‰) [18]. Measuring the natural abundance of $\delta^{13}\text{C}$ provides information about the sourcing and migratory nature of soil organic carbon (SOC) in soil, while the natural abundance of $\delta^{15}\text{N}$ tells us about the biological tracking of the nutrients [16,19]. The isotopic signature of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the CN ratio can be used to track the processes and mechanisms related to organic matter origin, formation, and turnover [17,20].

The availability of plant nutrients in higher elevation soil is mostly linked to the vegetation types and their photosynthetic pathways. As the elevation gradient changes, a shift in the isotopic signature of C and N occurs, and the change in vegetation types governs these dynamics [21]. For instance, the natural abundance of C ($\delta^{13}\text{C}$) increases in the foliage of plants along with the increase in elevation [21] but there is a large variation in soil's $\delta^{13}\text{C}$ values [22,23]. Plant leaves or litters are one of the major contributors to soil organic matter formation through microbial decomposition [24,25]. However, the mixing and fractionation of stable isotopes in the soil during the decomposition process results in a larger variation of the isotopic composition [26]. The elevation and the rate of litterfall in natural ecosystems strongly influence the SOC sourcing and mixing [21]. Similarly, the natural abundance of nitrogen ($\delta^{15}\text{N}$) isotope is enriched in an intensively managed environment. The relationship between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with soil nutrients provides

important information about the sourcing of nutrients in the higher elevation soils and such information is very limited in the mountainous country of Nepal.

Plant nutrients in the cultivated soils of Mustang are typically low [27], which is attributed to a low mineralization rate, low mobility, and low exchange potential. With an increase in elevation, the availability of plant nutrients are limited due to reduced mineralization rates [21,26]. Nutrient availability in the higher elevation is mostly constrained by the climate, vegetation type, and input management [21,23]. In Mustang, animal husbandry is closely linked with agriculture [28]. There is a significant transfer of biomass from the forest and rangeland to the cropland as fodder and roughages for livestock, ultimately ending in cropland as manure [29]. In addition, as livestock graze, there is also a reciprocal exchange of nutrients from crop residues back to the rangeland and forest through their excrement. This favours the nutrient source mixing in between cropping land and nearby native vegetation coupled with erosion-led nutrient transport [30]. The soils in the agricultural land of the Mustang region are poorly investigated and the existing plant nutrients status is not well known. Furthermore, soil test-based plant nutrient management is barely practised at the local level. The identification of the source, status, dynamism, and retention of those nutrients in the upper elevation soils is critically important to know the managerial aspects of the nutrients in a sustainable manner. We aimed to explore the TC, TN, CN ratio, and soil pH along the transect of different landscape positions (ridge, midslope, and valley) where cropping or orchard plantation is common in Mustang with surrounding natural vegetation. We further analysed the isotopic signature of the natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to identify the source of C and N where the nutrients source mixing is common in higher elevations of Mustang, Nepal.

2. Materials and Methods

2.1. Location and Climate

Mustang is one of the mountainous districts in central Nepal. It is located in the rain shadow of the world's 7th and 10th highest mountains (Dhaulagiri and Annapurna standing 8168 and 8137 masl, respectively) and receives on an average <400 mm annual rain with relatively higher rainfall in the southern part of the district. It presents a diversity of climates ranging from tundra, arid types in the higher elevations above 4500 masl, to alpine and cold temperate in 3000 to 4500 masl and 2000 to 3000 masl, respectively [31]. Furthermore, it is a deeply incised valley of the Kali Gandaki river with an arid valley bottom and characteristic diurnal wind system. It is divided into upper Mustang (above 3800 m) and lower Mustang (below 3800 m), the two divisions differing from each other with respect to the prevailing climatic conditions.

2.2. Soil Sampling Point Determination

We selected 20 sampling points along the Kaligandaki corridor from the southern (Tukuche) to northern part (Koral) of the Mustang district, considering a vertical transect to capture the best possible landscape positions (Figure 1 and Table 1). Out of 20 points, we collected four samples from the midslope, five samples from the ridge, and eleven samples from the valley in October 2011. Difficulties in accessing the varied topographic or landscape positions resulted in uneven sampling points. The details of sampling points are given in Table 1.

Table 1. Detailed information on sampling points in the Mustang district of Nepal.

Sampling Points	Latitude	Longitude	Elevation, masl	Location	Micro-Relief	Site Characteristics	Nearby Dominant Vegetation
1	28.71225	83.64908	2628	Tukuche	Midslope	Orchard	Juniper, Pine
2	28.83692	83.78242	2837	Kagbeni	Valley	Cropped land	Juniper
3	28.80392	83.77322	2852	Between Kagbeni and Lupra	Valley	Cropped land	Juniper
4	28.80389	83.77322	2852	Between Kagbeni and Lupra	Valley	Cropped land	Juniper
5	28.92494	83.82758	2963	Tsungsang	Valley	Cropped land	Juniper shrub, grasses
6	28.80244	83.79028	2997	Lupra	Midslope	Orchard	Juniper shrub, grasses
7	28.80211	83.78958	3017	Lupra	Midslope	Apple orchard	Juniper shrub, grasses
8	28.88406	83.80836	3092	Thangbe	Valley	Cropped land	Juniper shrub
9	28.96161	83.80847	3447	East of Samar	Valley	Cropped land	Pine, Juniper
10	28.81758	83.84944	3524	Jharkot	Ridge	Orchard	-
11	28.94964	83.80181	3560	South of Samar	Midslope	Orchard	Pine, Juniper
12	29.06139	83.87169	3579	Ghami	Valley	Cropped land	Planted Populas
13	28.96169	83.80142	3606	Samar	Ridge	Orchard	Pine, Juniper
14	28.99114	83.83819	3778	Syanboche	Valley	Cropped land	-
15	29.18361	83.95714	3823	Lomanthang	Valley	Cropped land	Planted Populas
16	29.18272	83.95711	3825	Lomanthang	Valley	Cropped land	Planted Populas
17	29.25469	83.96025	4027	South of Chonup, North of Lomanthang	Valley	Cropped land	Grasses
18	29.30347	83.96836	4612	North of Chonup	Ridge	Orchard	Juniper
19	29.30347	83.96836	4612	North of Chonup	Ridge	Orchard	Juniper and grasses
20	29.30347	83.96836	4612	North of Chonup	Ridge	Orchard	Grasses

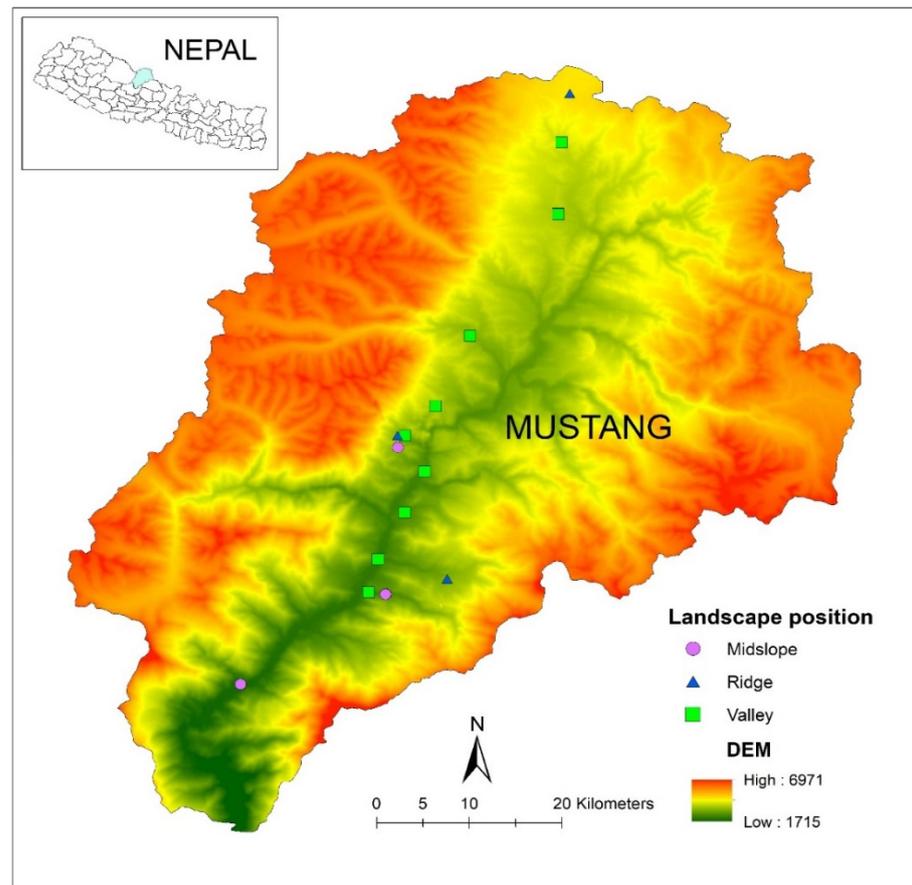


Figure 1. Distribution of soil sampling points showing in the digital elevation model (DEM) map across the transects in the study area, the Mustang district of Nepal.

2.3. Soil Sampling and Laboratory Analysis

We collected soil samples with the help of auger from 0 to 20 cm depth from selected points. A composite sample was taken, which was then spread in a sample box. Roots, undecomposed plant debris, and gravel were removed in the field. Upon returning to the lab, the soil was air dried, ground in mortar and pestle to break aggregates, and sieved to a 2 mm mesh size, which was then subjected to lab analysis.

The total soil carbon (TC) and total nitrogen (TN) were analysed using the dry combustion method for which soil was ground to 0.5 mm in size. The soil pH was determined in a 1:5 soil to water ratio with a digital soil pH meter. The isotopic signature of carbon ($\delta^{13}\text{C}$) and nitrogen (^{15}N) were obtained from isotopic ratio mass spectrometry (Thermo Fisher Scientific, Analyzer: FLAS 2000-Conflo IV-Delta V Advantage) for which soil was ground to 0.1 mm in size. The soil sample was replicated twice to determine each of the carbon and nitrogen signatures and the average value of the replicates was reported. Soil standards and reference samples were placed after every 12 samples. The standard error of soil standards/reference sample was $<0.23\%$.

2.4. Data Analysis

Initially, data were entered in MS Excel and then imported to R studio for descriptive analysis. Standard least square analysis of variance (ANOVA) models were used to investigate the effects of landscape position on soil C and N. The data were tested for ANOVA assumptions prior to the analysis, and they met these assumptions. We employed log and square root transformations to fit data into a normal distribution curve before subjecting them to ANOVA. Tukey means separation tests were used for post-hoc comparisons of the soil parameters amongst landscape positions. A correlation between the variables was

calculated at 5% level of significance. Graphs of variables (TC, TN, CN ratio, pH, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$) were prepared in R-studio [32] using ggplot2 package [33]. The relationship between different variables were performed in ggpairs function which is an extension of the ggplot2 package [33]. The statistical significance was determined as $p < 0.05$, unless otherwise noted. Analyses were conducted using 15.0.0 JMP SAS software. To elucidate the relationship between the geographical coordinates and the soil properties, a multiple linear regression model was built where soil properties were kept as a dependent factor and geographical coordinates as independent factors.

3. Results

The TC was significantly higher in the valley (6.2%) than in the ridge (2.8%) while the midslope had the intermediate values (4.7%) (Figure 2). The maximum and minimum TC in the valley was 10.5 and 4.0%, the midslope was 5.8 and 2.4%, and the ridge was 6.6 and 0.3%, respectively. Although there was no significant difference in the TN contents between these slope locations, the average TN content showed a decreasing trend from the valley (0.8%) to the midslope (0.6%) and ridge (0.5%). The maximum and minimum TN in the valley was 0.7 and 0.3%, the midslope was 0.9 and 0.4%, and the ridge was 0.7 and 0.3%, respectively.

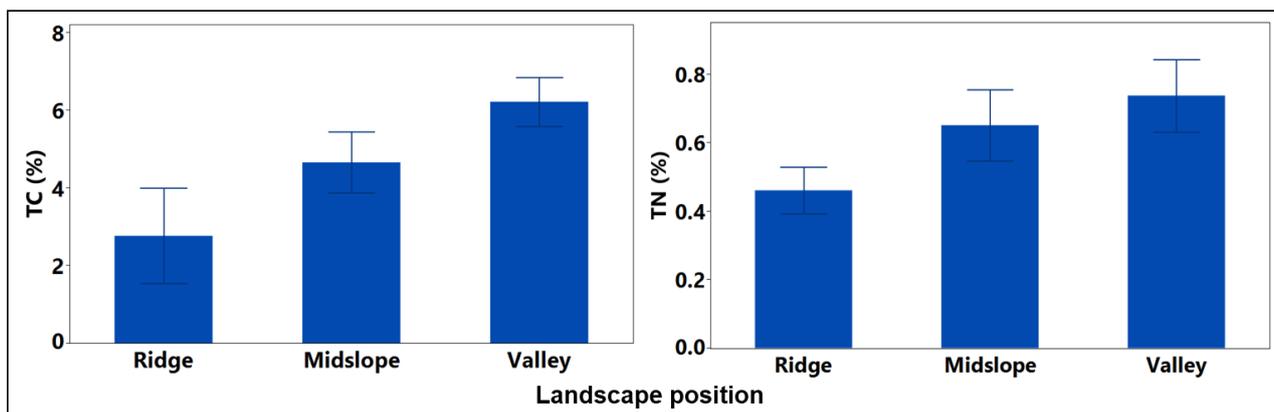


Figure 2. Total soil carbon (TC) and total nitrogen (TN) variation (mean \pm SE) at different landscape positions in Mustang district of Nepal. Significant differences between the landscape positions are shown with differing letters.

The $\delta^{15}\text{N}$ values in soil were significantly different across the landscape positions (Figure 3). On average, the valley soil contained the positive $\delta^{15}\text{N}$ values, whereas the midslope and ridge soils contained negative $\delta^{15}\text{N}$ values. However, the range of $\delta^{15}\text{N}$ signature showed both positive and negative values at all landscape positions. The maximum and minimum natural abundance of $\delta^{15}\text{N}$ in the valley was +7.0 and -15.2‰ , the midslope was +7.3 and -16.1‰ , and the ridge was +5.4 and -14.9‰ , respectively. The soil $\delta^{13}\text{C}$ value did not differ significantly between the landscape positions, although the average value showed a slightly increasing trend from the valley to the ridge (Figure 3). The maximum and minimum natural abundance of $\delta^{13}\text{C}$ in the valley was -6.6 and -23.6‰ , the midslope was -7.6 and -19.4‰ , and the ridge was +1.1 and -22.0‰ , respectively.

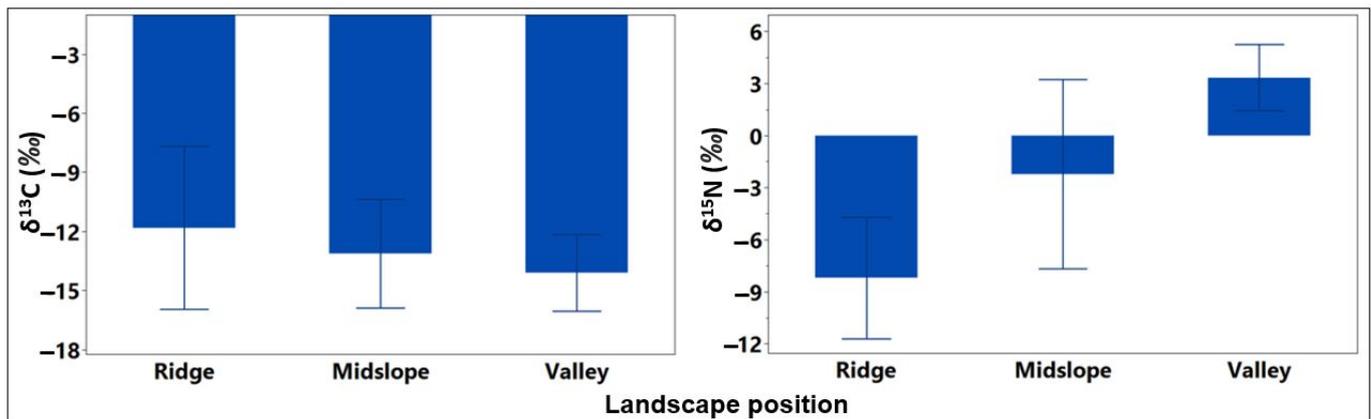


Figure 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation (mean \pm SE) in soil at different landscape positions in Mustang district of Nepal. Significant differences between the landscape positions are shown with differing letters.

The CN ratio did not differ significantly between the landscape positions, although there was an indication of a higher CN ratio in the valley than in the midslope and the ridge (Figure 4). A significantly higher soil pH was observed in the valley than in the ridge, while the midslope had intermediate values (Figure 4). The maximum and minimum CN ratio in the valley was 12.0 and 6.0, the midslope was 10.0 and 6.0, and the ridge was 16.0 and 1.0, respectively. Similarly, in the valley and midslope, we found the same soil pH range (7.0 to 7.5) and in the ridge soil the pH range was 7.0 to 6.5.

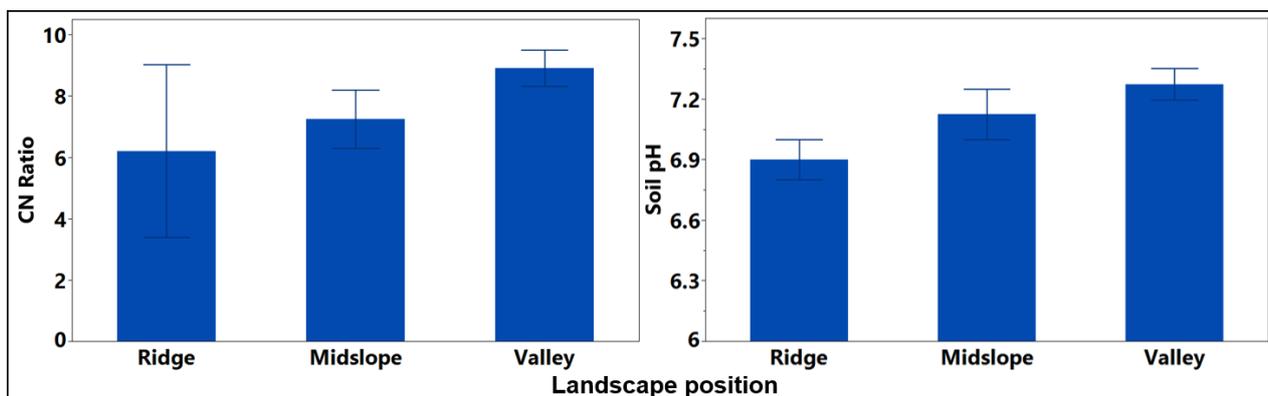


Figure 4. The CN ratio and soil pH variation (mean \pm SE) at different landscape positions in Mustang district of Nepal. Significant differences between the landscape positions are shown with differing letters.

Both a positive and a negative correlation was observed between the soil parameters (Figure 5). A significant positive correlation was found between the TC and TN ($r = 0.7$, $p < 0.001$); the TC and CN ratio ($r = 0.6$, $p < 0.001$); the TC and $\delta^{15}\text{N}$ ($r = 0.7$, $p < 0.001$); the TN and $\delta^{15}\text{N}$ ($r = 0.5$, $p = 0.019$); and a significant negative correlation was found between the TN and the $\delta^{13}\text{C}$ ($r = -0.6$, $p = 0.004$). A detailed correlation matrix including all variables is provided in Appendix A Figure A1. We did not find any significant correlation between geographical co-ordinates (latitude, longitude, and elevation) and soil parameters (Appendix A Figure A1). Furthermore, there was no significant effect of latitude, longitude, and elevation on soil properties as suggested by multiple linear regression models (results not shown), which is in compliance with correlation results.

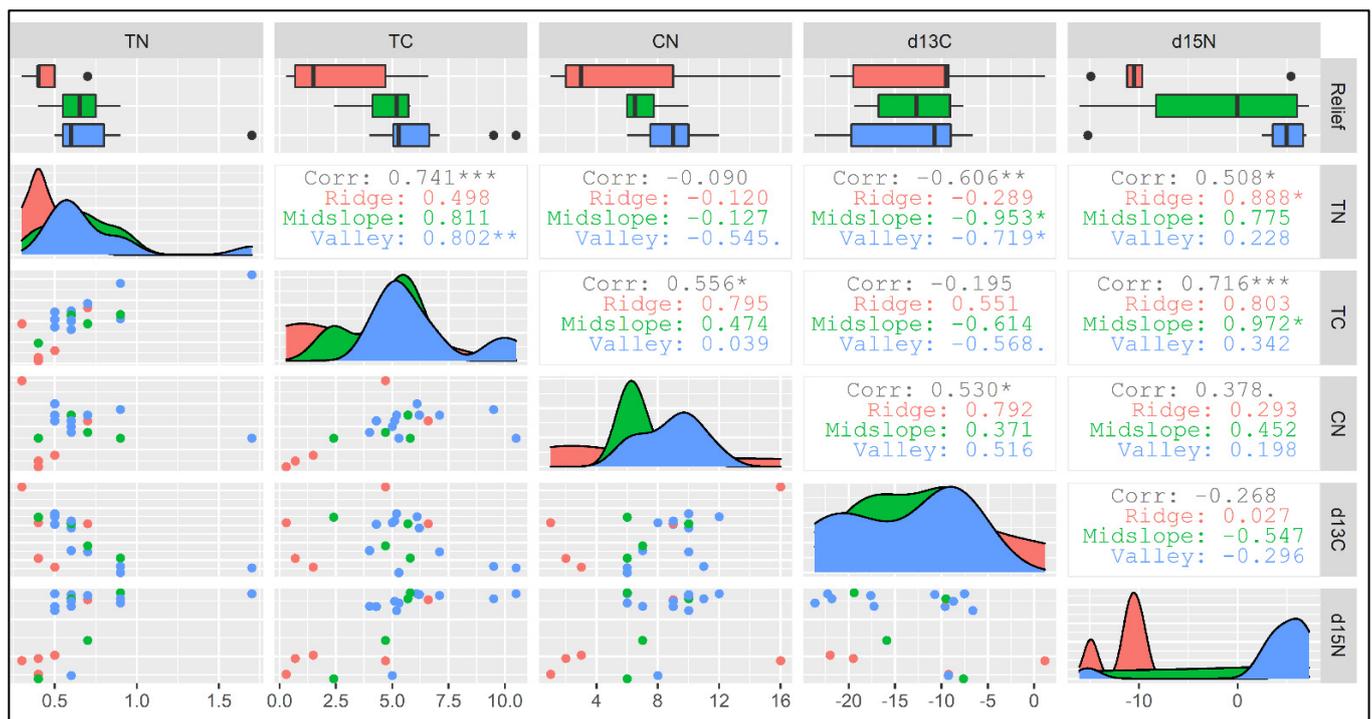


Figure 5. A correlation matrix between the selected variables, scatter points on the lower panel, and correlation values and their 5% significance level on the upper panel. *** p -value < 0.001; ** p -value < 0.01, * p -value < 0.05.

4. Discussion

Our current study showed that the valley bottom had a higher TC and TN concentration compared to the midslope and ridge. There is a progressive increment in the TC and TN concentration from the ridge to the valley bottom. This implies that the valley bottom is more fertile than the ridge and the midslope. The transport of the nutrients from the upper slope (ridge and midslope) towards the lower slope (valley bottom) due to soil erosion may have resulted higher concentration of the TC and TN [34] in the valley bottom. The deposition of sediment in the valley bottom and nutrient transport from the upper slope and forest litter [30] coupled with heavy textured soil [27] resulted in higher TC and TN. Lü et al. [35] reviewed the nutrient transport process associated with rainfall-runoff events and reported their results in terms of factors, forms, carriers, and sources of nutrient transport. Lü et al. [35] concluded that during the erosion process water is a carrier of soil nutrients in the soluble form. The dissolved organic C and available form of N resulting from litter decomposition from the forest is the primary source of nutrient transport, along with eroding water and sediments from the upper slope to the lower slope [36,37]. The lower slope, where the deposition of sediments occurs, are generally high in soil carbon and nitrogen compared to the eroding upper slope [38–40]. Thus, in the current study area, this erosion-led nutrient transport is dominant where the nutrients from the upper slope of the ridge and the midslope are deposited in the valley bottom.

The low CN ratio in the soil that we observed in the current study might be due to presence of inorganic carbon (natural carbonates) or inorganic nitrogen (input management) or both. The presence of inorganic forms of C and N alters the CN ratio [41]. The ratio of total organic C to N is the indicator of the decomposition rate of organic matter with an inverse relationship [42]. The CN ratio at any landscape position does not affect the nutrient availability [43] in the Mustang soil. Similarly, the average soil pH of the study area was 6.9 ± 0.5 units, which is the most favourable range for the nutrient availability. Many of the nutrients essential for plants are available in the pH range of 6.5 to 7.5 [44]. There, the CN ratio and soil pH are found to be non-limiting factors for nutrient availability in the study area.

The range of $\delta^{13}\text{C}$ value from -23.6 to $+1.1\text{‰}$ in the current study indicates the presence of both inorganic and organic C in soil. The C content of few soil samples, particularly from the ridge, was dominated by inorganic C (i.e., carbonate mineral). Plant photosynthesis discriminates against the heavier C isotope, and the degree of discrimination mainly varies with the type of photosynthetic pathways (C_3 , C_4 , CAM). The $\delta^{13}\text{C}$ values of C_3 and C_4 plants generally lie between -20 to -40‰ , and -9 to -17‰ , respectively, while $\delta^{13}\text{C}$ values in CAM typically lie in between -10 to -20‰ [45]. Garziona et al. [46] reported that C_3 plants, dominantly trees, shrubs, and cool-growing-season grasses in the region produce soil respiration with $\delta^{13}\text{C}$ values of about -22 to -32‰ , whereas C_4 plants, dominantly warm-growing-season grasses, produce soil respiration with $\delta^{13}\text{C}$ values between -10 and -15‰ . The average observed range of soil carbonate formed in equilibrium with C_3 -respired CO_2 is $\delta^{13}\text{C} = -13$ to -9‰ , whereas soil carbonates formed in the presence of C_4 plants have $\delta^{13}\text{C} = +1$ to $+3\text{‰}$ [47].

Galy et al. [48] reported the presence of $\delta^{13}\text{C}$ value of -0.4 to $+1.9\text{‰}$ in the carbonates of the bedload sediment around the Lomangthang and Kagbeni regions of the Mustang district. The presence of carbonates in the Tethyan sedimentary series, which also includes the Mustang district, comprises of Paleozoic–Mesozoic carbonates and clastic sediments that have $\delta^{13}\text{C}$ values ranging from -2.5 to 0‰ [49]. The $\delta^{13}\text{C}$ value in Paleosol carbonates of the Thakkhola formation is between -5.6‰ and $+3.5\text{‰}$ with a mixed C_3 and C_4 plant species, but predominantly C_4 species [46]. An arid environment like Mustang is likely to have higher $\delta^{13}\text{C}$ values resulting from a low respiration rate or the dominance of C_4 plants. Garziona et al. [46] collected different species of grass in between 3000 and 4000 masl and found $\delta^{13}\text{C}$ values from -12.3 to -12.8‰ in these grass species. They concluded that the higher value of $\delta^{13}\text{C}$ in the valley floor of the Thakkhola formation deposition is from paleosol carbonates with the presence of both C_3 and C_4 vegetation. However, the presence of only C_3 vegetation in the lower elevations (Tetang formation), yielded $\delta^{13}\text{C}$ values from -21.9 to -26.5‰ . Szpak et al. [50] demonstrated that foliar ^{13}C values increased with a site's altitude, which is in agreement with our data trend of greater soil ^{13}C enrichment in the ridge compared to the valley. Therefore, the sourcing of soil C can be attributed to the mixture of soil organic matter and paleosol carbonates with the increasing influence of organic matter (mostly from C_3 vegetation) as we move from the ridge towards the valley floor of the Mustang district.

The natural abundance of $\delta^{15}\text{N}$ in soil represents an integrated signal of the ecosystem's N processes that help constrain N budgets, identify sources, and their fates. The range of $\delta^{15}\text{N}$ values in our study sites is between -16.1 to $+7.0\text{‰}$. Since Mustang is located in an arid climate, the $\delta^{15}\text{N}$ enrichment in soil and plants is expected. The loss of ^{14}N and enrichment of $\delta^{15}\text{N}$ values in soil and vegetation samples in dry regions have been reported previously [51,52]. Zhou et al. [53] reported increasing $\delta^{15}\text{N}$ values with decreasing rainfall in the Qinghai–Tibetan Plateau, a region similar to our site. The authors suggested that the precipitation and temperatures that influence the CN content and ratio are also the primary factors determining the patterns of soil $\delta^{15}\text{N}$ on a regional scale [53]. In a landscape scale similar to our site, variability in $\delta^{15}\text{N}$ was positively related to moisture availability, soil fertility, and vegetation cover [54]. Szpak et al. [50] demonstrated that foliar $\delta^{15}\text{N}$ values decreased with an increase in altitude, similar to our trend observed in soil $\delta^{15}\text{N}$ values. The higher value of $\delta^{15}\text{N}$ in valleys in our sites could also be associated with the use of organic fertilizer ($\delta^{15}\text{N}$ value of around 2 to 30‰) and synthetic N fertilizer ($\delta^{15}\text{N}$ value of around -4 to 4‰) [55]. Regmi et al. [56] reported the use of synthetic N (23 to 280 kg urea ha^{-1}) and farmyard manure (2.1 to 5.3 t ha^{-1}) in apple orchards of the Mustang district. Hence, the enrichment of $\delta^{15}\text{N}$ in the Mustang district and the variations we observed between various sites might come from the agro-inputs or enrichment due to micro and macro scale topographic and climatic variations in these sites.

Furthermore, the higher positive correlation of the TC with the TN, $\delta^{15}\text{N}$, and CN ratio; $\delta^{13}\text{C}$ with CN ratio; TN with $\delta^{15}\text{N}$; and the higher negative correlation of the TN with $\delta^{13}\text{C}$ (Figure 5) indicates the interdependence of sourcing between the TC and the TN. This

indicates that the source mixing between soil organic matter is C₄ and C₃ vegetation. The nutrient source mixing is further augmented by the transfer of forest litter as manure to farmland by the farmers of the Mustang valley [34]. The use of fresh animal manure in the valley bottom might result in higher $\delta^{15}\text{N}$ [57]. There is a mixing of isotopically distinctive carbon and nitrogen in the study area as a weak and negative correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Figure 5) [58]. Hence, the isotopic techniques are useful in organic matter source identification, their mixing, and stability in the soil.

5. Conclusions

Our study discloses the sourcing and availability of C and N in higher elevation soils of the Mustang District, Nepal using stable isotope techniques, CN ratio, and soil pH. The findings suggest that the landscape positions strongly influence the nutrient sourcing and mixing in higher elevation soils. As our data is limited to cover the broader geographic range, we do not find a relation of C and N with longitude, latitude, and elevation. C and N sourcing are specific to different landscape positions in Mustang. The ridge and midslopes are dominant with the litter decomposition, either of the forest trees species or of fruit orchards. Valley slopes are mostly dominant with the fresh organic and inorganic substrates through agro-input management by farmers, along with the source mixing of C and N transfer associated with erosion-led nutrient transport from the ridge and midslopes. Hence, we emphasize that the cultivated croplands in the valley or orchards in the midslope and the ridge should be managed according to the nutrient sourcing in the region for sustainable land management. It is important to consider landscape position, broad geographic co-ordinates, and micro-relief to study C and N sourcing in future studies. Further research is necessary to study the micro-climate, decomposition rate constant, and the microbial diversity to understand the cycling of C and N in the higher landscapes.

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Appendix A



Figure A1. A correlation matrix showing the correlation between all variables used in the study, scatter points on the lower panel and correlation value and their 5% significance level on the upper panel. *** p -value < 0.001; ** p -value < 0.01, * p -value < 0.05.

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