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Soil microplastic accumulation from plastic mulching in vegetable cropping systems across three agro-ecological regions in Nepal

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Abstract

Plastic mulching is widely used in agriculture globally, and its use in Nepal is on an increasing trend for vegetable production. Limited studies have been done on the accumulation of microplastics in the soil across different agro-ecological regions. This research aimed to find out the relationship of microplastic contamination with mulching practice across three locations (Kathmandu, Chitwan, Makwanpur) representing three agro-ecological regions. Soil samples (n=126) were collected from plastic-mulched fields, non-mulched fields, and forest soil at two depths (0-10 cm and 10-20 cm). Microplastics from soil were extracted by density separation and identified using a stereomicroscope, and microplastic was characterized by using Fourier Transformed Infrared Spectrophotometer. A greater concentration of microplastics was found in plastic mulched fields (17.27 ± 10.12 particles per 50 gm of soil), followed by non-mulched fields (13.68 ± 7.42 particles per 50 g soil) and forest soil. Microplastic counts were significantly higher in farm soils than forest soils, and higher in mulched than non-mulched soils. Soil depth had no significant effect suggesting relatively even distribution of microplastic particles along depth (0-20 cm). Kathmandu recorded the highest microplastic count followed by Chitwan and Makwanpur. Fragments and films were the dominant particle shapes in agricultural soils, while fibers predominated in forest soil. The size distribution of microplastic was mainly within 100 to 500 µm. A significant positive correlation was found between microplastic and soil pH, and a non-significant correlation between microplastic and organic matter content. This study provides evidence of microplastic contamination as emerging pollutant in Nepalese agricultural soils across land uses that require attention and action of plastic management.

Keywords: Agricultural plastics, Emerging pollutants, Plastic pollution, Soil contamination, FTIR spectroscopy

Highlights

- Agricultural soils contained more microplastic particles than forest soils
- Microplastic levels were significantly higher under plastic mulching conditions
- Soil depth did not significantly affect microplastic abundance
- Soil pH was positively associated with microplastic abundance

1. Introduction

Plastic materials have become ubiquitous across global industries, including agriculture due to their versatility, durability and cost effectiveness (Shah and Wu, 2020). Plastics in agricultural sectors are used extensively in seedling trays, polybags, tunnels, irrigation components, packaging, and notably, plastic mulch films (Lamont and Orzolek, 2004). Among these applications, plastic mulching is now an essential part of modern vegetable production as a mulching and poly-tunnel material (Hussain and Hamid, 2003). It provides cost effective solution to challenges such as weed competition, soil erosion, water shortage, and climatic variability by regulating root-zone temperature, conserving moisture, and suppressing weeds (Ghouse, 2020). However, the widespread adoption of plastic mulch has raised growing environmental concerns, particularly regarding the accumulation and distribution of microplastics in agricultural soils.

Microplastics are tiny pieces of plastic smaller than 5mm in size (Arthur et al., 2009). The shape, size, and polymer composition of microplastics influence their mobility, degradation and interactions within soil systems. Because of their small size, microplastics can easily move around and accumulate in different places causing risk to both aquatic and terrestrial ecosystems (Dahal et al., 2025; Dawadii et al., 2026; Li et al., 2023). Agriculture soils are highly vulnerable to microplastic contamination as they are mostly exposed to human activities which serve as key entry points for microplastic (Horton et al., 2017). Potential sources include plastic mulch, littering, irrigation with wastewater, use of soil amendments like compost and fertilizer application (Bläsing and Amelung, 2018; Upreti et al., 2025; Weithmann et al., 2018). The long term applications of plastic mulching can result in large number of plastic debris remaining in the soil (Upreti et al., 2025)that can negatively impact soil health and food security (Zhang et al., 2021). Few researches have shown that microplastics can influence plant growth and change physical, chemical and biological property of soil (Wang et al., 2022). They can persist in the soil for very long periods and may potentially cause various ecological impacts. In addition, microplastics may be uptaken by the plants and transferred into the food chain potentially impacting food quality and safety for human consumptions. Despite increasing awareness, the mechanism involved in release, transport and fate of microplastic in soil ecosystem are still not well understood (de Souza Machado et al., 2019).

Despite growing interest in agricultural microplastics, research focusing on comparison in different agri-ecological regions remains sparse. Nepal's mountainous terrain with complex topography, diverse microclimates, and varied farming practices is suitable natural platform to study ecological impacts. Furthermore, the vertical migration of microplastics within the soil profile and relationship between microplastic distribution and soil properties including pH and organic matter remains poorly unexplored. It is important to understand these gaps for mitigating microplastic pollution and advancing sustainable agricultural practices.

In this study, we determined the concentration, vertical distribution and morphological characterization of microplastics in soils (n=126) managed with and without plastic mulch in three agro-ecological regions in Nepal. Additionally, we assessed how microplastic accumulation interacted with soil pH and organic matter, while examining the influence of environmental factors across distinct agro-ecological conditions. This work provides crucial baseline evidence on microplastic contamination in Nepali agricultural soils, offering actionable insights for soil quality management and informing future environmental impact research.

2. Materials and Methods

2.1 Description of study area

The research was conducted in Kathmandu District (Tarakeshwor and Kirtipur municipalities), Chitwan District (Kalika and Bharatpur municipalities), and Makwanpur District (Thaha municipality), all located within Bagmati Province, Nepal (Figure 1; Table S27). These districts such as Kathmandu, Chitwan, and Makwanpur are major vegetable production zones representing three agro-ecological conditions Valley, Lower plain and mid-Hills, respectively with unique environment. Makwanpur (mid-Hills) lies in altitude variation of 166 masl to 2584 masl. Chitwan (Lower plain) experience a warmer climate with the altitude variation of 150 to 200 masl and Kathmandu (Valley) lies at an elevation of 1393 masl (National Statistics Office, 2025)

The three agro-ecological regions exhibited distinct meteorological conditions during the study period with difference in mean wind speed (Kathmandu: 5.57 m s^{-1}; Chitwan: 4.79 m s^{-1}; Makwanpur: 4.16 m s^{-1}) and total precipitation (Kathmandu: 2170.6 mm; Makwanpur: 1761.7 mm; Chitwan: 1178.4 mm). These regions also showed clear difference in mean temperature with

Chitwan having the highest mean temperature (23.5 °C) followed by Kathmandu (17.9 °C) and Makwanpur with lowest mean temperature (15.7 °C) (Table S28). These variations in climate, altitude, and precipitation influence agricultural practices, soil properties, and biodiversity across the study regions and make it suitable for evaluation microplastic distribution under different land management systems and soil depths.

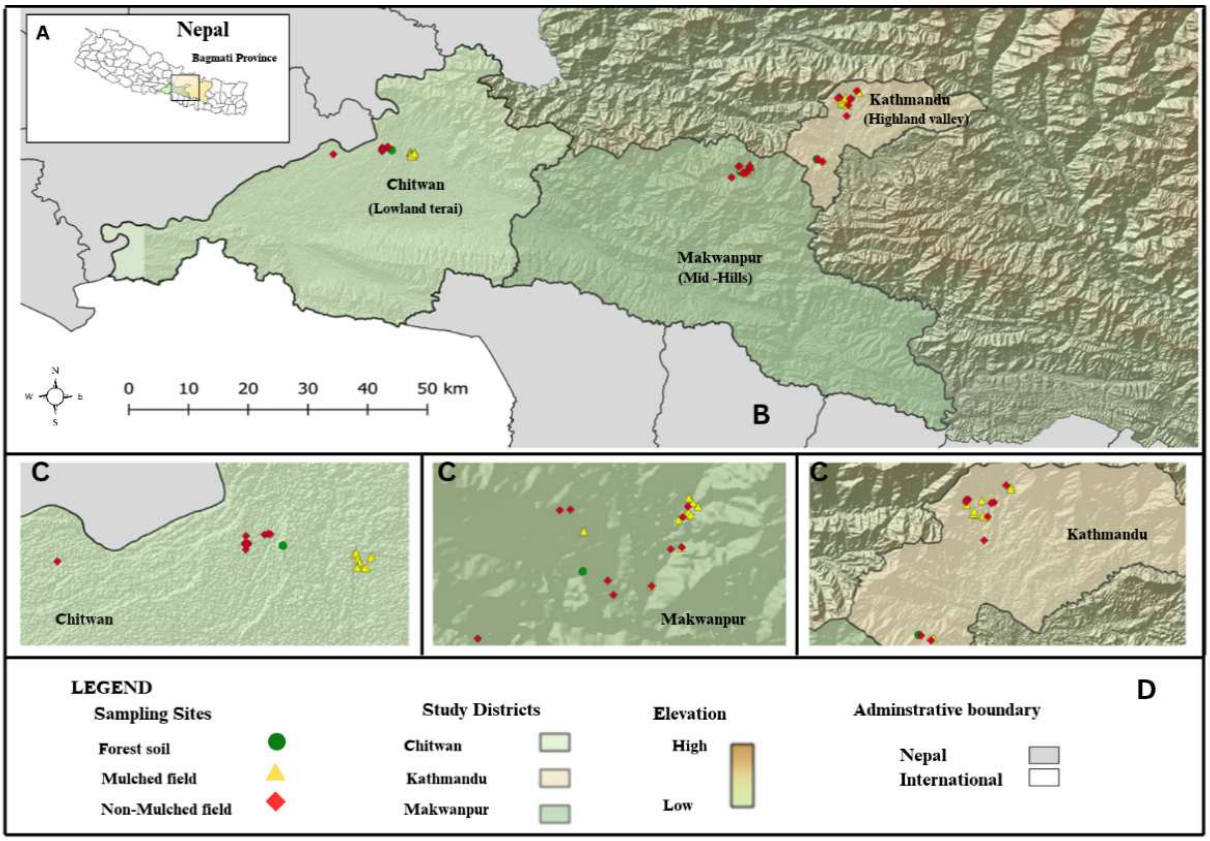


Figure 1: Location of the study area and soil sampling sites across three contrasting agro-ecological zones in Central Nepal: Chitwan (Lowland Terai), Makawanpur (Mid-Hills), and Kathmandu (Highland Valley). (a) Map of Nepal highlighting the target districts; (b) Regional topographic overview depicting elevation gradients across the three zones; (c) Distribution of soil sampling points in plastic-mulched and un-mulched vegetable farms.

2.2 Soil sample collection

The sites in all three districts were selected based on topography, land-use history, and proximity to potential contamination sources. Prior to sampling, a preliminary field visit and discussions with farmers were conducted to know about field management history including irrigation sources, fertilizer applications, duration of plastic mulch used, farm waste management practices.

A total of 126 soil samples were collected using a purposive stratified sampling strategy (42 samples per location). At each location, soil samples were collected from plastic-mulched fields (n= 20), non-mulched fields (n= 20), and adjacent forest soils (n= 2) serving as a low-anthropogenic controls. Mulched fields were restricted to those under continuous plastic mulch cover for at least one year, whereas non-mulched fields had no prior history of plastic mulch use. To ensure spatial independence, vegetable fields were separated by a minimum distance of 100m, while control forest sites were located at least 200 m away from roads and settlements.

To evaluate vertical microplastic migration, samples were collected at two depths: topsoil (0 – 10 cm; n=10) and subsoil (10 – 20 cm; n=10) across both agriculture and control sites. Soil cores were retrieved using stainless steel and aluminum probes following systematic spatial patterns (W, X, Z, and V configurations) tailored to field dimensions. Individual sub-samples per plot were homogenized into a composite sample, transferred to a 300 ml glass jars to avoid contamination and sealed immediately. The soil probe was cleaned thoroughly with water after every sampling to reduce any cross contamination between the consecutive sampling points.

2.3 Laboratory analysis of soil samples

The soil samples were oven-dried at 40°C to a constant weight and passed through a 5 mm stainless-steel sieve to remove large debris, gravels, and stones. Coarse organic matter (e.g., plant roots) was manually removed using forceps. Soil dry weight was recorded using a digital weighing balance. A 50 g sieved dry soil was dispersed in a 250 ml beaker with 50 ml of 4 % sodium hexametaphosphate solution (Sisco Research Laboratories) following EN ISO 17892-4/2017 (Thomas et al., 2020). The suspension was thoroughly shaken and left to settle for 24 hours.

Following dispersion, microplastics were isolated using density separation(Thomas et al., 2020) using a saturated sodium chloride solution (2.0 g/cm^3) (Thermo Fisher Scientific). Sodium chloride was chosen for its cost-effectiveness, eco-friendliness, and suitable density for floating low-density plastics of 0.9 – 1.6 g/cm^3 and leaving heavier mineral components like silica to sink (Zhang et al., 2023). Saturated solution of sodium chloride (100 mL) was added to the soil suspension at a 1:2 volume ratio, agitated using a magnetic stirrer for 30 minutes, and allowed to settle undisturbed for 24 hr. The floating microplastic rich supernatant was carefully decanted into a clean glass beaker.

We used Fenton's reagent to digest residual soil organic matter. The decanted supernatant was treated with 20 mL of 2% ferrous sulfate heptahydrate (Qualikems Lifesciences) catalyzed with 6 mL/L concentrated sulfuric acid and 20 mL of 30 % Hydrogen peroxide (Thermo Fisher Scientific) in a 1:1 ratio (Hurley et al., 2018). The mixture was left for 72 hours at room temperature to ensure complete digestion of labile organic fractions. The digested solution was then filtered through a glass fiber filter paper (1.2 µm pore size and 47 mm diameter from Cytiva) via vacuum filteration, and filters were air dried at room temperature in a covered glass petri dish.

The filter papers were visually inspected under a stereomicroscope (Amscope, USA; 30X magnification) coupled with a digital camera (OMAX A3503S). Suspected plastic fragments were subjected to a hot-needle test was to confirm synthetic polymers. Photographs were taken and were processed using Image J software to quantify particle abundance and determine their dimensions.

The chemical characterization of the particles was performed using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) (IRPrestige-21, SHIMADZU) at the Department of Plant Resources, Government of Nepal, Kathmandu. Spectra were recorded at a spectral resolution of 4 cm^{-1} with 20 scans per sample from 4500–500 cm^{-1}. Functional groups were manually identified via infrared absorption tables and matched against standard reference spectra libraries to confirm the polymer types.

To ensure quality assurance and control, all chemicals and reagents used were pre-cleaned by filtering them using glass fiber filter paper with a pore size of 1.2 µm and a diameter of 47 mm. All glass and metal apparatus (no plastic wares used) were thoroughly washed and rinsed with 50% ethanol followed by filtered distilled water to minimize secondary contamination and kept covered with aluminum foil when not in use. Laboratory procedural blanks (n=14) were processed simultaneously alongside soil samples. Microplastic particles recovered from the blank was subtracted from the soil sample counts (Table S24). Method recovery efficiency was evaluated by spiking pristine plastic mulch fragments into control forest soil samples in triplicate (n=10 fragments per assay) (Upreti et al., 2025). Spiked samples were processed following the exact extraction, density separation, digestion, and filtration protocol. The mean recovery rate was 70% (7 ± 1 particles recovered). Microscopic examination revealed no visible thermal, chemical, or

mechanical modifications to the recovered plastic fragments (e.g., size, shape, surface texture), confirming the non-destructive nature of the extraction process.

Soil pH was measured in a 1:2.5 soil-to-water suspension using a digital pH meter (Lutron). Soil organic matter (SOM) was measured using classic Walkey-Black wet oxidation method (Walkey and Black, 1934).

2.4 Environmental Data Collection

Climatic variables were obtained from open access meteorological dataset to study environmental conditions at each sampling locations. Site specific data for temperature (°C), precipitation (mm), relative humidity (%), wind direction (m), wind speed (m s^{-1}), for Kathmandu, Chitwan, and Makwanpur districts were extracted via Open Meteo API. Data covering the study period were extracted and summarized using descriptive statistics (Table S28). These environmental characteristics were used to provide supporting context for interpreting the observed differences in microplastic abundance among the sampling locations.

2.5 Data Analysis

All statistical analysis were carried out in R (Version 4.4.1; R Core Team, 2024) using the Studio integrated development environment (Version 2024.12.0+467). Microplastic count data were analyzed using a Generalized Linear Modeling (GLM) framework. At first, a Poisson GLM was fitted to evaluate the effects of location, land management system, soil depth, and their interaction term. However, the model diagnostics showed over dispersion (variance>mean), violating the Poisson distribution assumption. Consequently, a Negative Binomial GLM was selected for the final analysis to appropriately handle overdispersal count data and improve overall model fit. Descriptive statistics were calculated for microplastic concentration (particles per 50 gm of soil) across farm soils (n=120), forest reference soil (n=6), mulching status, soil depth and agro-ecological zones (Kathmandu, Chitwan, Makwanpur; n=40 each). Agriculture farm soil and forest soil differences were tested using a Negative Binomial GLM, corroborated with a Wilcoxon rank-sum test. For agriculture soil separate Negative Binomial GLMs assessed the effects of mulching status, soil depth, their interaction, district, and district × depth interaction, with significance evaluated via type II likelihood ratio tests and effect sizes reported as incidence rate ratios (95%

CI). Tukey-adjusted pairwise comparisons were used for multi-level or significant interaction terms. Associations between microplastic count and soil organic matter and pH were evaluated using Spearman's rank correlation. Significance was set at $\alpha = 0.05$.

3. Results and discussion

3.1 Microplastic abundance

Microplastic were detected in all analyzed soil samples (Figure 2) The mean microplastic concentration across all farm soils excluding forest samples (n=120) was 15.48 ± 9.01 particles per 50 g soil with a median of 14.5 particles per 50 g soil and a range of 2–48 particles per 50 g soil (Table S1).

Forest soil showed a relatively lower microplastic concentration compared to agriculture soil with mean of 4.50 ± 2.07 particles per 50 gm of soil and median of 4.5 particles/50gm of soil and with an observed range of 2 to 8 microplastic particles per 50 gm of soil sample (Table S1). A negative binomial generalized linear model (GLM) confirmed that agriculture soil had significantly higher amount of microplastic as compared to forest soils (likelihood ratio test: $\chi^2 = 15.92$, df = 1, $p < 0.001$). Agriculture soils had an incidence race ratio (IRR) of 3.44 (95% CI: 1.94- 6.06) indicating that microplastics were approximately 3.4 times greater in agriculture soil as compared to the forest soil (Table S2). This difference was further supported by Mann- Whitney U test ($U = 58$, $p < 0.001$; Table S3). These results suggest a strong association between agriculture management and soil microplastic accumulation. The minimum presence of microplastic in the forest soil is likely attributed to the reduced anthropogenic activity. Similar results from strawberry farm were reported by our group in our previous publication (Upreti et al., 2025). The notable distinction between microplastic level in forest soil and agriculture soils highlights how human land management practices contribute to microplastic accumulation in soil. As forest soils were largely unaffected by tillage practices, plastic mulching, inputs from agrochemicals they provide a reference baseline for assessing the contribution of agricultural activities to soil microplastic contamination. This finding is in line with earlier studies, which found that forests and other undisturbed ecosystems act as relative sinks with lower accumulations of microplastic as agriculture soil (Bläsing and Amelung, 2018; Upreti et al., 2025). However, given the small number of forest samples used in this study (n=6) forest soils were used only as a baseline reference

for comparing with agriculture soil and the observed difference should be treated as indicative rather than conclusive.

Mulched vs non-mulched soil: Across all three districts plastic mulched soils (n=60) showed a higher mean microplastic concentration (17.27 ± 10.12 particles per 50 gm of soil) as compared to non-mulched field (13.68 ± 7.42 particles per 50 g soil, n=60) (Table S1). The median microplastic concentration in mulched field was 16.0 particles per 50gm of soil (range 2-48) and in non-mulched field was 13.5 particles per 50 gm of soil (range 2-36). This difference was statistically significant according to the negative binomial GLM ($\chi^2 = 5.03$, df = 1, $p = 0.025$; IRR = 1.26, 95% CI: 1.03–1.55) with plastic mulched soils showing approximately 26% more microplastic particles than non-mulched soils (Table S4). The presence of higher level of microplastic particles in mulched soils indicates that plastic mulch use is an important source of microplastic contamination in agricultural soils. The higher level of microplastics might be related to degradation of plastic mulch during its use as they are frequently exposed to sunlight, rainfall, and temperature fluctuations which can weaken the plastic and promote its fragmentation into tiny particles. During the preliminary field survey farmers also reported that rainfall and hailstorms frequently damaged plastic mulch particularly in mid hills and highland valley. They also reported that mulch films are often torn and punctured with sharp agriculture tools during the field operations. Repeated damage from climatic conditions and farm activities leads to removal and replacement of plastic mulch each year. Consequently, small fragments of plastic mulch might remain in the soil during the removal process and contribute to accumulation of microplastics overtime. This pattern is consistent with previous studies showing that elevated microplastic concentration in the plastic mulched field is largely due to degradation and fragmentation of plastic mulch which is caused by photooxidation (Upreti et al., 2025; Zhang et al., 2023). They also support the view that agriculture soils are particularly vulnerable to microplastics pollution due to their continuously exposure to human activities which serve as key entry points for microplastics (Horton et al., 2017).

Topsoil vs subsoil: A slightly higher mean microplastic concentration was observed in soil at 0-10 cm depth (n=60, 16.6 ± 9.9 particles per 50gm of soil) as compared to 10-20 cm depth (n=60, 14.4 ± 7.9 particles per 50 gm of soil). The median concentrations were 15 (range 2-48) and 14 (2-34) particles per 50 gm of soil respectively (Table S1). Although results showed the presence of higher level of microplastics in 0-10 cm depth as compared to 10-20 cm depth, but this difference

was not statistically significant (negative binomial GLM, $\chi^2 = 1.76$, df = 1, $p = 0.185$; IRR = 0.87, 95% CI: 0.71-1.07) (Table S5). A similar result was observed in forest soil samples with mean particles of 5.33 ± 2.52 (n=3) at 0-10 cm depth and 3.67 ± 1.53 (n=3) at 10-20 cm depth. Given the limited number of forest soil samples (n=3 per depth) this forest data serve mainly as a baseline reference. The slightly higher microplastic particles observed in the upper soil layer (0-10 cm depth) suggest that plastic mulch films were one of the factors that contributed to surface level accumulation of microplastics as mulching introduced microplastics directly into the upper soil layer through degradation of plastic films (Zhang et al., 2023). The lack of significant different indicated that factors beyond mulching such as soil mixing or downward migration might influence microplastics distribution with depth. Additionally, this may partly result from relatively narrow depth ranged samples (0-20 cm depth), which might not be able to capture depth related stratification that may be evident in greater depth. Study conducted by (Elbasiouny et al., 2022) also suggested the present of microplastic in both upper and lower levels of soil with the topsoil offering conditions conducive to their degradation or breakdown. The previous studies have also suggested vertical movement of microplastics through biopores, ploughing or cracking and horizontal was due to soil organism or agricultural practices such as harvesting (Rillig et al., 2017). Alternatively frequent tillage and soil disturbing agricultural practices performed by farmers might promote the distribution of microplastics particles throughout the cultivated soil layer. The farmer also reported frequent tillage operations performed during vegetable cultivation in both mulched and non-mulched soil during preliminary field survey. A similar depth wise pattern was reported by Baniya et al. (2026) who observed no significant difference in microplastic concentrations between topsoil and subsoil in farmland, suggesting that regular tillage operations might promote the redistribution of microplastics throughout the soil profile. This pattern is consistent with our findings.

To examine the potential interaction between mulching practice and soil depth a negative bionomial GLM with mulching × depth interaction was applied to the agriculture soil samples (n=120) (Table S6, S7, S8) The highest mean microplastics concentrations were observed in mulched soil at 0-10 cm depth (18.8 ± 11.2 particles per 50 g of soil, n=30) followed by mulched soil at depth 10-20 cm (15.8 ± 8.9, n=30). In non-mulched soil mean microplastic concentration was 14.3 ± 8.1 particles per 50gm of soil at 0-10 cm depth (n=30) and 13.0 ± 6.8 in 10-10 cm depth

(n=30). However, the interaction between mulching practice and soil depth was not significant (χ^2 = 0.15, df = 1, p = 0.701). This non-significant difference suggest that the effect of soil depth on microplastics concentration was consistent across mulched and non-mulched soils. Similarly post-hoc pairwise comparison (Tukey adjusted) between four subgroups (mulched soil: 0-10 cm, 10-20 cm; non-mulched soil: 0-10 cm, 10-20cm) revealed no statistically significant difference (Table S9). These results also suggested that microplastics were similarly distributed across soil depths regardless of soil management practice.

Three agro-ecological zones: Microplastic concentration varied across different agro-ecological zones (Makwanpur: Midhills, Kathmandu: Highland valley, Chitwan: Lowland Terai) with the highest mean microplastics particles observed in Kathmandu (20.3± 8.8 particles per 50 gm soil, median= 19.5, range=6-45, n=40) followed by Chitwan (16.6 ± 7.7 particles per 50 gm soil, median= 16, range= 8-48, n=40) and Makwanpur (9.5 ± 7.0 particles per 50 gm soil, median= 7.5, range=2-36, n=40) (Table S1). A negative binomial generalized linear model (GLM) (Table S10) confirmed highly significant overall effect of agro-ecological zones (χ^2 = 47.83, df = 2, $p < 0.001$). The results from Tukey-adjusted pairwise comparison (Table S11, S12) showed that Makwanpur had significantly lower microplastic abundance than both Kathmandu (ratio = 2.15, $p < 0.001$) and Chitwan (ratio = 1.76, $p < 0.001$), while Kathmandu and Chitwan did not differ significantly from one another (ratio = 1.22, p = 0.150). To assess whether the effect of soil depth varied among agro-ecological zones the interaction effect was evaluated (Table S13, S14, S15, S16). The interaction between district and soil depth was non-significant (χ^2 = 0.74, df = 2, p = 0.690) which indicated that difference in microplastics concentration among districts were consistent across the two sampled soil depths. At both depths, Makwanpur had significantly lower microplastics concentrations than Kathmandu and Chitwan (all $p \leq 0.006$), while Kathmandu and Chitwan did not differ significantly at either 0–10 cm (p = 0.176) or 10–20 cm (p = 0.677). Within individual districts, microplastic concentrations also did not differ significantly between topsoil and subsoil (Kathmandu: p = 0.223; Chitwan: p = 0.781; Makwanpur: p = 0.179) (Table S17, S18, S19).

Agro-ecological regions emerged as the most influential factor associated with soil microplastics abundance with Makwanpur soil containing roughly half of the microplastic concentration of Kathmandu and Chitwan. This large and consistent difference across both soil depths suggested that agro-ecological location and broader regional factors might play a greater role in determining

microplastics in soil rather than plastic mulching practice alone. Difference in urbanization, road density, irrigation sources, intensive agriculture practices, plastic waste management in agriculture field, extent and duration of plastic mulch use, atmospheric deposition might collectively contribute to spatial variation in soil microplastics level. Preliminary farmer survey data indicated variation in farm management practices including the duration of plastic mulch use, method of plastic waste disposal which might partly explain the contrasting levels of microplastics abundance. These findings are consistent with other studies showing that the level of microplastic contamination varied widely depending on local conditions such as distance from urban areas, industrial activity and market intensity (Yu et al., 2025). Similar zone to zone variation has been reported within the agriculture soil sampled from different land use within the same country (Sharmin et al., 2024). Previous study done in Nepal also showed human activity appeared to be a stronger driver of microplastics contamination. Soil study conducted in Sagarmatha National Park found lower microplastic concentrations in farmland compared to settlement area, attributing farmland contribution was mainly due to air pollution and irrigation water from nearby village (Baniya et al., 2026). Himalayan stream water near settlements also showed higher microplastic levels than water near glacial sources (Han et al., 2023). This might explain our pattern as Kathmandu (highland valley) being Nepal most urbanized district (population density: 5169 person/km^2) and Chitwan (Lowland terai) being a major commercial farming district (population density: 325 person/km^2) likely receive more contamination via irrigation, air pollution, and waste as compared to rural district Makwanpur (Mid-Hills, population density: 192 person/km^2) (Government of Nepal, Department of Information and Broadcasting, 2025). The three agro-ecological regions exhibited distinct meteorological conditions during the study period. Such climatic differences might influence the fate and distribution of microplastics through processes such as wind mediated transport and deposition, runoff due to precipitation, climate dependent plastic degradation. However, the present study did not directly test the relationship between meteorological conditions and microplastic abundance so their contributions to the observed agro-ecological level differences remained uncertain. Further studies incorporating site specific climatic and environmental variables into statistical models could help in clarifying their contribution to microplastics distribution across agricultural landscapes.

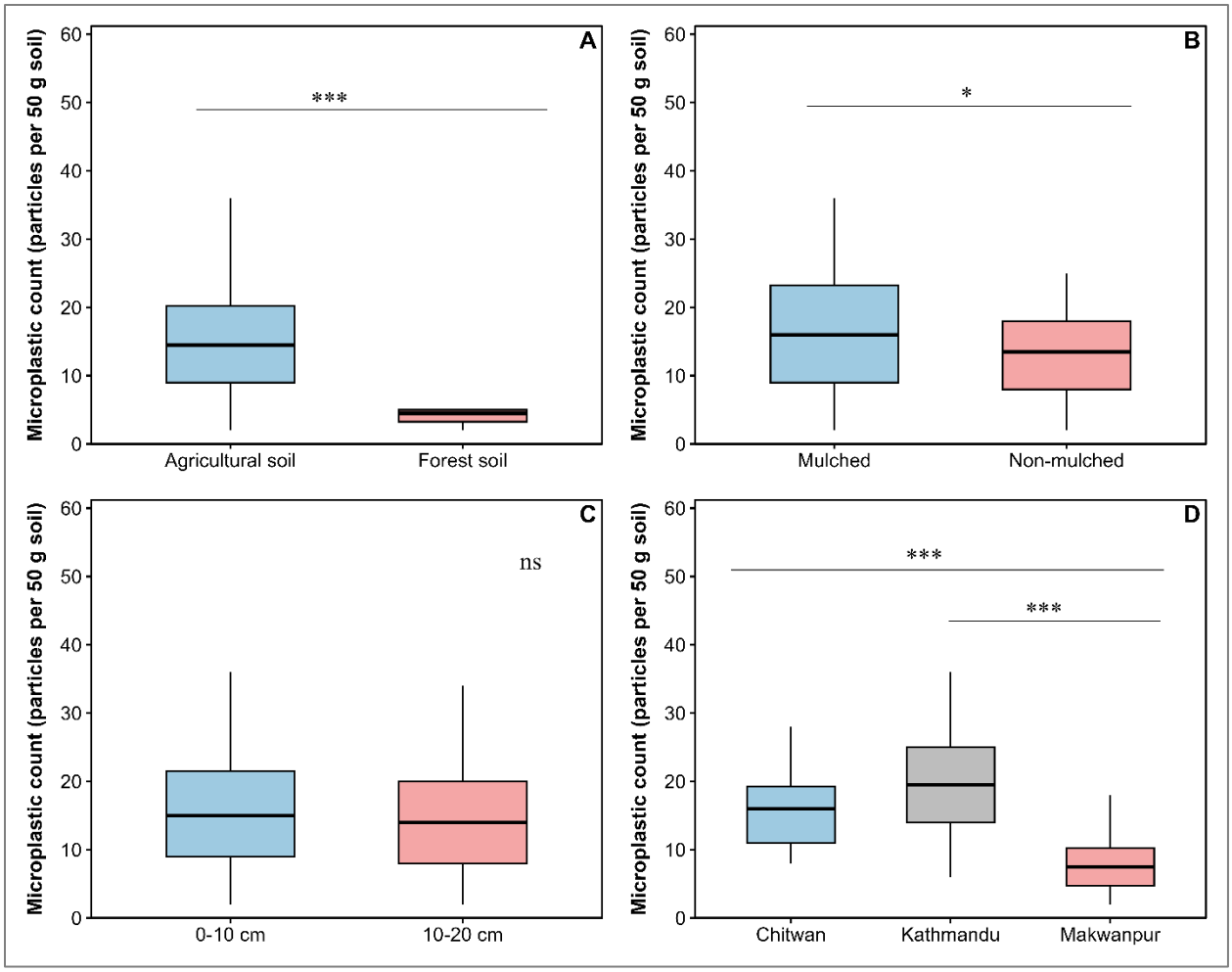


*Figure 2: Box plots describing microplastics abundance in (A) farm samples (n=120) and forest samples (n=6); (B) mulched (n=60) and non-mulched farm samples (n=60); (C) topsoil (n=60) and subsoil farm samples (n=60); and farm samples in different districts (n=40 each); Horizontal lines with asterisks indicate significant differences between the connected groups (*p < 0.05, ***p < 0.001), and ns indicates no significant difference.*

3.2 Morphology and size variation in microplastics

Microscopic analysis identified three different shapes of microplastic namely fibers, fragments and films (Figure S1). The figure 3 showed the relative composition of microplastic shapes (fiber, fragments, film) under different mulching practice, soil depths and agro-ecological regions. The shape composition differed among forest soil, non-mulched and mulched soil (Fig 3A, Table S25). The dominant microplastic shape in mulched soil was fragments (59%) followed by fibers (33%) and film (8%). The non-mulched soil also showed similar pattern and contained (61%) fragments followed by fibers (30%) and films (10%). Forests soils were dominated by fibers (59%) followed by fragments (37%) and films (4%). This result likely reflected the distinct pathway through which

microplastics inputs to forest vs agriculture system. Forest soil which was largely free of direct plastic amendments might accumulate fibers predominantly through atmospheric deposition, litter fall from the surrounding environments. These results are consistent with previous studies which identified atmospheric deposition as a major fiber source in undisturbed terrestrial systems like forest soil (Zhang et al., 2020). In contrast to the forest soil plastic mulched and non-mulched soil marked a shift towards dominance of fragments. This trend strongly implicated that agricultural mulching practices are a key contributor to fragment type microplastics as the degradation of plastic mulch films likely generated these particles. It is in line with mechanical and photo oxidative degradation of plastic mulch films under agriculture field conditions(Qi et al., 2020). The dominance of fragments particles in non-mulch field suggested other pathway of enter of microplastics other than plastic mulch film. The results from preliminary soil survey from farmers also reported burning and disposal of plastic directly into the field as a common method of plastic waste disposal. These plastic debris might undergo degradation through burning, weathering, or mechanical breakdown, contributing to the microplastic load in the soil. They also reported the use of plastic packaging materials, plastics nets, plastic seed trays, and plastic for constructing tunnels along with the use of fertilizers and manures. Littering and street runoff as one of the causes of possible sources of plastic in soil had been reported by (Bläsing and Amelung, 2018). The shape distribution was relatively similar between two soil depths with fragment remained the dominant shape at both soil depths (0-10cm, 10-20 cm) followed by fiber and film (Fig 3 B). This similarity suggested that the relative composition of microplastics shapes was consistent between both the soil depths. There was noticeable variation among microplastic shapes among different agro-ecological regions. Kathmandu had the highest proportion of fragments (72%) followed by fibers (20%) and film (8%). Chitwan had relatively similar proportion of fibers (45%) and fragments (48%). Makwanpur had the highest proportion of films (13%) among all the three regions. These differences indicated that the microplastic shape composition varied geographically potentially reflecting difference in agricultural practice, plastic use, environmental conditions and other source of plastic inputs.

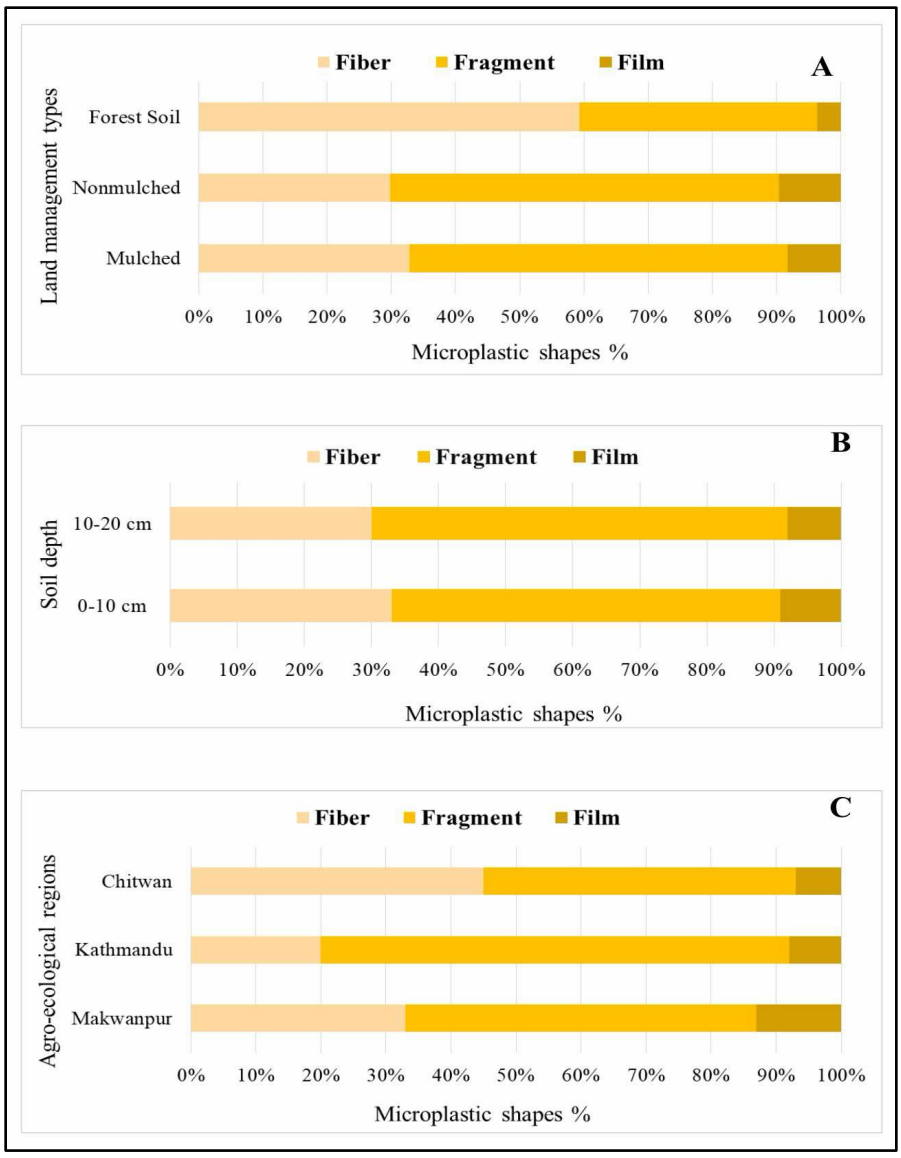
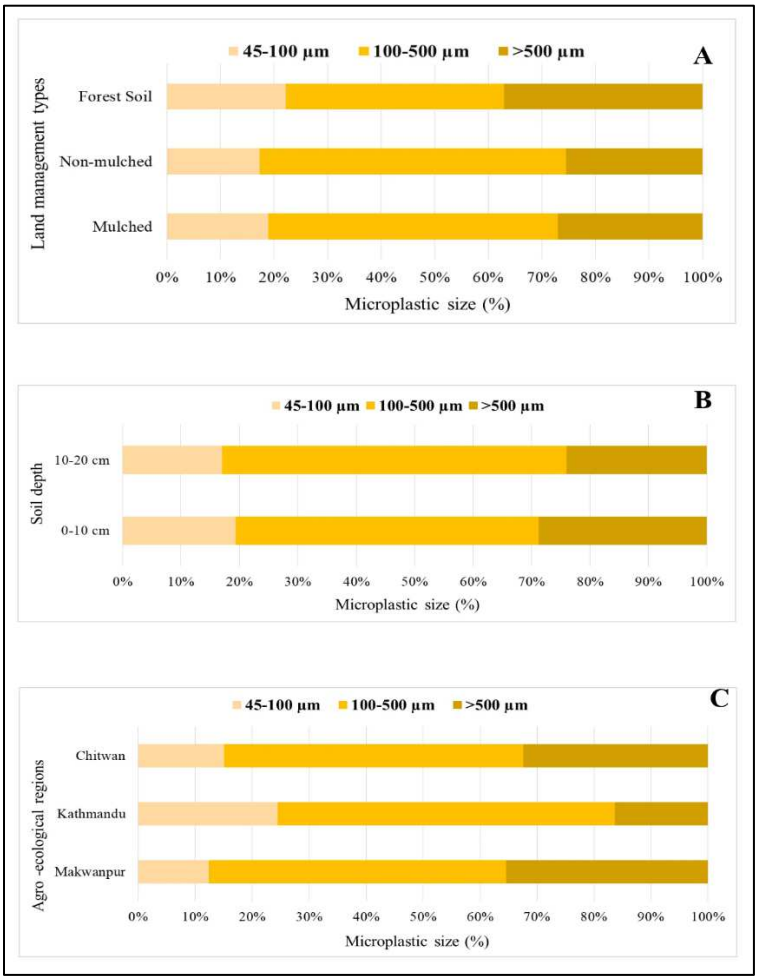


Figure 3: Relative proportion of different shapes of microplastic found in (A) mulched, non-mulched samples, and forest samples; (B) topsoil and subsoil; (C) different districts

The size of microplastic in this study ranged between 45 µm and 5000 µm (Table S26). Our method did not detect microplastics particles smaller than 45 µm. Figure 4 showed the percent distribution of microplastics particles based on their size in soil samples collected from non-mulched and mulched field in Kathmandu, Makwanpur and Chitwan. The largest proportion of microplastic fell under the size of 100-500 µm across land use types, soil depth and agro-ecological regions. Plastic mulched soil was dominated with microplastic of size 100-500 µm followed by >500 µm. Similar size-based pattern was observed in non-mulched soil and forest soil samples. The smallest particles size (45–100 µm) generally represented the lowest proportion. These results indicated that most

451 microplastics recovered in this study were of medium to large size within our detectable range.
452 Forest soil showed a relatively greater share of larger particles (>500 µm, 37%) compared to
453 mulched and non-mulched soils, which were more concentrated in the 100–500 µm range. There
454 was minimal variation across soil depth. Among the agro-ecological regions Kathmandu showed
455 a comparatively higher proportion of small particles as compared to Chitwan and Makwanpur.
456 Overall, the size distribution pattern reflects the general characteristics of microplastic
457 contamination in our study.



458

459 *Figure 4: Relative proportion of different size of microplastic found in (A) mulched, non-*
460 *mulched, and forest samples; (B) topsoil and subsoil; (C) different districts*

461 **3.3 Chemical characterization of microplastic**

462 Figure 5 showed FTIR spectra of eight selected microplastic particles from P1 to P8. The spectrum
463 of the standard plastic mulch in which P1 had a strong peak typically found in Polyethylene.

Similarly, the visual comparison between P2 and P3 gave similar results. They may belonged to same polymer type which was Polyethylene as they had identical spectra. P5, P6 and P8 exhibited spectral features potentially associated polystyrene and P4 and P7 showed peaks that may be associated with Polyamide. The reference spectral peaks for the selected particles are presented in Table 1.

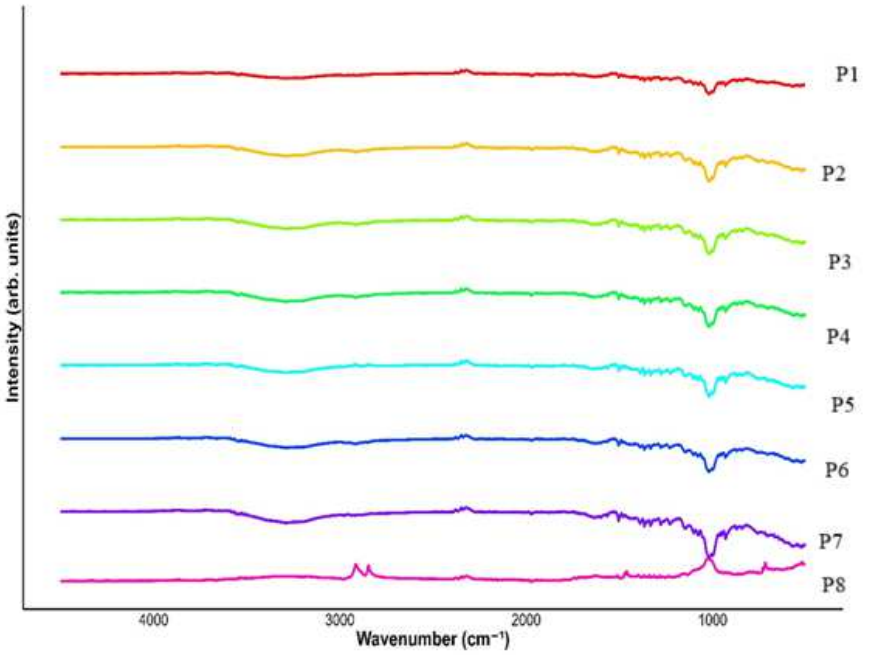


Figure 5: *FTIR spectrum of the representative microplastics sample. Particle P1 was the standard plastic mulch while particle P2-P8 represented the selected microplastic particles.*

The similarity between the spectra of the standard polyethylene mulch and soil derived particles P2 and P3 suggested that PE based agriculture plastics might contribute to the microplastics contamination observed in the study soils. This was consistent with our results showing presence of significant higher microplastics in mulched soil. The elevated microplastics concentration in the plastic mulched field was largely due to degradation and fragmentation of plastic mulch which was caused by photooxidation (Zhang et al., 2023). However, the detection of PS and PA indicated that plastic mulch was not the only source of microplastics in the soil. These polymer might enter the soil through other agricultural materials, plastic waste, organic amendments, wastewater and atmospheric deposition (Chinyo et al., 2026). Farmers also reported using plastic packaging materials, plastic nets, plastic seed trays, and plastic for constructing tunnels along with use of

fertilizers and manures which might be the potential source of PS and PA type of polymer. Blasing and Amelung (2018) also discussed soil amendments like compost and sewage sludge and irrigation water to a major source of microplastics pollution in soil. These FTIR reports suggested a multiple source of origin of microplastics including agriculture plastics and other environmental sources. However, the FTIR analysis was performed only on selected particles, so the results provided only qualitative evidence of possible polymer diversity based on comparisons of their spectral peaks with reference spectra. These findings indicate potential polymer types and should not be considered representative of the overall polymer composition of all collected microplastic particles.

Table 1: Comparison of observed and reference spectral peaks for polymer identification

Particle	Observed spectral peak	Polymer type	Reference spectral peak
P1	2914.44 cm^{-1},2846.93 cm^{-1}, 717.52 cm^{-}	Polyethylene	3000-2800 cm^{-1} (Xu et al., 2020)
P2	2922.16 cm^{-1}, 2852.72 cm^{-1}	Polyethylene	
P3	2914.44 cm^{-1},2846.93 cm^{-1}	Polyethylene	
P4	1516.05 cm^{-1}, 1288.45 cm^{-1}, 1242.16 cm^{-1}	Polyamides	1240 cm^{-1}, 1525 cm^{-1} (Mahltig, 2021)
P5	1533.41 cm^{-1}, 2324.22 810.10 cm^{-1}	Polystyrene	1500-800 cm^{-1} (Xu et al., 2020)
P6	1516.05 cm^{-1} 806.25 cm^{-1}	Polystyrene	1500-800 cm^{-1} (Xu et al., 2020)
P7	1533.41 cm^{-1}, 1288.45 cm^{-1}, 1244.09 cm^{-1}, 881.47 cm^{-1}, 817.82 cm^{-1}	Polyamides	1240 cm^{-1}, 1525 cm^{-1} (Mahltig, 2021)

P8	1525.69 cm^{-1}, 1489.05 cm^{-1}	Polystyrene	1500-800 cm^{-1} (Xu et al., 2020)

3.4 Impact of soil properties on microplastic count

Spearman's rank correlation showed a weak but significant positive relationship between microplastics counts and soil pH ($\rho = 0.332$, $p < 0.001$) (Table S20). Soil Organic matter showed almost no relationship with microplastic count ($\rho = 0.049$, $p = 0.597$). The results from Negative Binomial GLM supported this pattern (Table S21). When tested alone soil pH had a significant positive effect on microplastic abundance ($\beta = 0.217$, SE = 0.056, $z = 3.90$, $p < 0.001$; IRR =1.24, 95% CI: 1.11-1.38; Table S22). Soil Organic matter content alone had no significant effect ($\beta = 0.030$, SE = 0.057, $z = 0.54$, $p = 0.590$; IRR = 1.03, 95% CI: 0.92–1.15; Table S23). To check whether these effects hold up together soil pH and soil organic matter content were entered into the same model. Soil pH remained a significant positive predictor ($\beta = 0.217$, SE = 0.056, $z = 3.90$, $p < 0.001$; IRR = 1.24, 95% CI: 1.11–1.39) while Soil OM stayed non-significant ($\beta = -0.032$, SE = 0.056, $z = -0.57$, $p = 0.570$; IRR = 0.97, 95% CI: 0.87–1.08). This showed that the effect of Soil pH did not depend on soil organic matter. The positive trend implied that more alkaline soils (higher pH) tended to retain greater microplastic concentrations. One possible explanation could be linked to soil chemistry and microbial activity. Microplastics also affected soil chemical properties including soil pH, electrical conductivity (EC) and nutrient cycling leading to reduced soil fertility (Qi et al., 2020; Scheurer and Bigalke, 2018). Higher pH soils might be associated with specific land use practices such as agricultural fields treated with lime or fertilizers which could both raise pH and introduce microplastics through plastic mulch, packaging, or irrigation water. The weak positive correlation between organic matter content and microplastic count suggested that there was almost no linear relationship between the two variables.

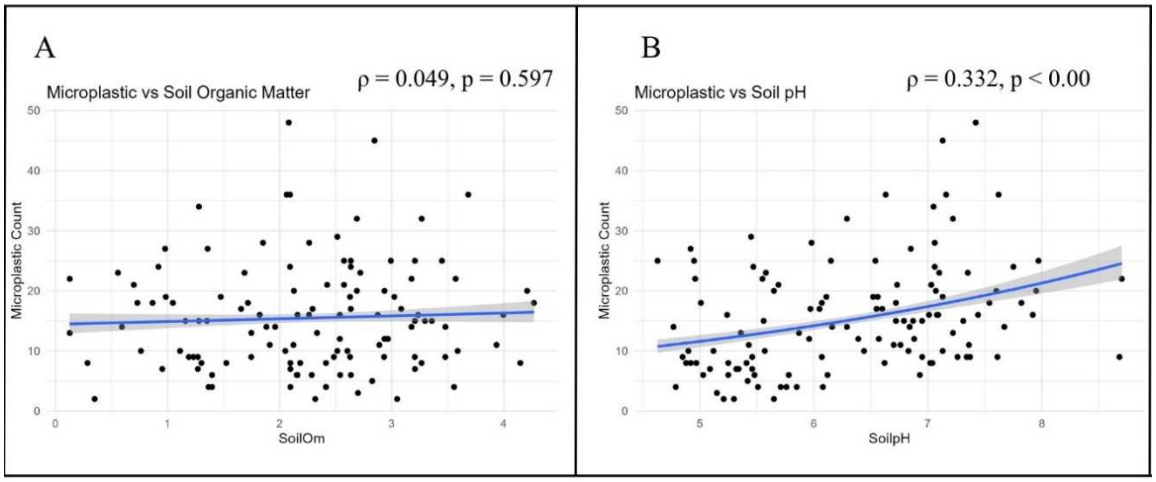


Figure 5: Correlation analysis between microplastic count with soil organic matter content (A) and soil pH (B)

4. Conclusion

This study showed that plastic mulching practice in agriculture is a driver of soil microplastics contamination across different agro-ecological zones of Nepal. Microplastics concentration was significantly higher in farm soils than in forest soils and further elevated under plastic mulched conditions. Depth showing no significant effect suggested that microplastics were relatively distributed along both soil depth rather than accumulating in the surface layer. Microplastics abundance varied significantly across agro-ecological zones with Kathmandu and Chitwan showing relatively higher level of microplastic in the soil while the lowest level was recorded in Makwanpur. Fragments were the predominated microplastic shape present in the mulched soil indicating that degradation of plastic mulch as a primary source whereas the presence of fibers in non-mulched and forest soil pointed out to additional inputs such as atmospheric deposition and surface runoff. Soil pH and organic matter content showed limited but notable relationships with microplastics abundance. The observed positive relationship between microplastics and soil pH suggested that alkaline soil condition might influence the retention of microplastic in soil, although the underlying mechanisms need further investigation. These results highlighted the complex nature of microplastics accumulations in the soil. Microplastics accumulation was not uniform across plastic mulched and non-mulched soil. The study emphasized the need for more extensive research to explore various factors that contribute to microplastic pollution in soil. Understanding the interaction of microplastic particle with physical, chemical and biological property of soil

along with external factors that caused microplastic input in soil is necessary for developing a detail overview of microplastic pollution.

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Bhaba Prasad Tripathi: Supervision, Writing – review and editing

Basant Giri: Conceptualization, Project administration, Resources, Supervision, Writing – review and editing

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