

# Litter carbon and nitrogen dynamics along an elevation gradient in the Darjeeling Himalaya

\*<sup>1</sup>Samjetsabam Bharati Devi, <sup>2</sup>Suratna Sur Shan Sher Sherpa, <sup>3</sup>Kishor Sharma

<sup>1</sup>Ph.D. Scholar, <sup>2</sup>IFS, <sup>3</sup>Ph.D. Scholar,

<sup>1</sup>Department of Ecology and Environmental Sciences, School of Life Sciences, Pondicherry University, Kalapet, Puducherry-605014, India

<sup>2</sup>Divisional Forest Officer, Kurseong Forest Division, West Bengal Forest Department, Government of West Bengal, Dow-Hill, Kurseong, Darjeeling, West Bengal-734203, India

<sup>3</sup>Department of Zoology, School of Life Sciences, Sikkim University, 6<sup>th</sup> Mile, Samdur, P.O. Tadong, Gangtok, Sikkim-737102, India

## ABSTRACT:

High altitude forest and soil have large carbon and nitrogen pools. In this study an attempt has been made to understand the role of litter in carbon storage in different forests type occurring along the elevation. Both carbon and nitrogen play an important role in nutrient cycling as well as an indicator of the global climate change. Carbon stock have been widely studied however, however available nitrogen stock studies is negligible. The litter carbon stock in Indian forests have increase from 131 to 136 (million tonnes) during 2015 to 2017 (ISFR, 2017). Many studies have been conducted in temperate and tropical forests, however the fundamental aim of this paper is to assess litter carbon and nitrogen at different forest type including tropical deciduous forest, temperate forests, and sub alpine forests present in three protected areas, Mahananda Wildlife Sanctuary, Neora Valley National Park, and Singalila National Park, respectively of Darjeeling Himalaya. Singalila National Park has the highest carbon (%) and C:N ratio whereas Neora Valley National Park has highest Nitrogen (%) in litter. Litter Carbon (%) increased from year 1 ( $40.45 \pm 0.95$ ) to year 2 ( $40.94 \pm 0.96$ ), Nitrogen (%) increased from year 1 ( $0.96 \pm 0.01$ ) to year 2 ( $1.01 \pm 0.01$ ), however, C:N ratio decreased from Year 1 to year 2. Litter Carbon (%) and C:N ratio increased with elevation whereas Nitrogen (%), declined with elevation in Darjeeling Himalaya.

**KEYWORD:** Litter, Eastern Himalayas, Protected areas, Carbon (%), Nitrogen (%), C:N ratio, Altitude.

## I. INTRODUCTION

The atmospheric carbon dioxide content has risen from 315 ppm in the year 1958 to 408 ppm in 2018 (NOAA, 2018). Carbon sequestration is one of the solutions, which could potentially help in mitigation of global climate change. Taking in consideration of elevation gradient is a reliable tool to study the temperature sensitivity of litter decay (Malhi et al., 2010). The soil can act as a sink or source depending on input and decomposition of litter. Litter decomposition and its rate have huge impact in accumulation of nutrient in the upper surface layer as well as limitation of nutrients to the primary producers (Melillo, 1982). Around 35% of the global carbon sink is contributed by litter, soil, deadwood and harvested wood and they constitute 60% of the global stock (Pan et al., 2011). C:N ratio serve as a good indicator of quality of organic matter however they are most likely to be wrongly interpreted if the two controlling variables are wrongly calculated (Batjes, 1996). Soil can store twice as more carbon than the atmosphere and for a longer period of time. The soil carbon depends on how the net primary productivity and decomposition rate changes relative to each other (Liski, 1999). The increase in carbon concentration in the atmosphere will lead to accumulation of more aboveground litter, which in turn will enhanced soil respiration. The litterfall represents a major pathway through which carbon and nutrients exchange happening between the soil and vegetation. Litter fall are more likely to affect the below ground processes (Sayer et al, 2011). The interaction between carbon and nitrogen cycle will control the response of forest against future climate change (Zak et al, 2003). The world forests have carbon pool of 1500-1800 PgC, out of this 37% is present in low latitude forests, 14% in middle latitude forest and 49% is in high latitude forest (Dixon et al., 1994). The carbon stored in soil increases from Tropical, temperate to boreal. Carbon sequestration has to be enhanced by delaying the rate of decomposition, which will lead to conservation of carbon in the humic form. Nitrogen plays a very important role in humification as well as stabilization (Prescott, 2010). The greater availability of litter means more food substrate for micro-organisms, which may lead to more respiration further enhancing the overall global warming effect. Therefore it is necessary to understand litter quantity and its component of a specific forest type with precision. Litter is defined as organic horizon (all the leaves, twigs, small branches, fruits, flowers, roots and bark) on the mineral soil surface (IPCC, 2006). Three general components that determine decomposition processes including physical parameters such as climate and mineralogy of the parent material, the quality of the decomposing resources, and organisms (Swift et al., 1979). The litter carbon may be different in same forest as well as between forest types. The turnover time of litter carbon is more or less about three years which makes the soil more of a source then sink, this is one of the constraint of litter carbon (Schlesinger et al., 2001). In a study conducted in Western

Himalayas, the litter carbon ranges 1.1 to 1.4 Mg/ ha which accounts for about 8% of total carbon (Dar et al., 2015). West Bengal has total geographical area (TGA) of 88,752 km<sup>2</sup> and the total Carbon stock of forests is 163.201 million tonnes which is 2.3% of total carbon of the country (ISFR, 2017). The study of litter carbon is important as it contributes to carbon stock of terrestrial as well as soil. The world forest has a carbon stock of 861±66 PgC, Highest carbon stock is in soil with 383±30PgC (44%) and least carbon stock is in litter with 43±3 PgC, contributing 5% of the total carbon pool (Pan et al., 2011). In the present study we hypothesized that (1) litter Carbon (%) increases along elevation, (2) litter nitrogen (%) is highest in temperature forest, and (3) temperature and precipitation are the main driving force in controlling carbon and nitrogen dynamics along the elevation in Darjeeling Himalaya.

## II. MATERIALS AND METHODS

### 2.1 Study area and study sites

The study was carried out in three protected areas of Darjeeling Himalayas. Mahananda Wildlife Sanctuary (MWLS), Neora Valley National Park (NVNP), Singalila National Park (SNP) situated at different elevation range (Fig. 1). The study sites are: the Eastern Himalayan Sal Forest (3C/C1a(i)) in the MWLS, the East Himalayan Montane Wet Temperate Forest (11B/C1c) in the NVNP and East Himalayan Sub-alpine Birch/Fir Forest (14/C2) in the SNP. Each of these study sites has a unique assemblage of species thus having distinctive forest type. MWLS (Panchanai) is located in the eastern lowland Himalayas. It covers an area of 159 km<sup>2</sup>. The Sanctuary is dominated by Sal species (*Shorea robusta*). Neora Valley National Park (Rachila) is located in the Kalimpong Sub-Division of Darjeeling district, it covers 88 km<sup>2</sup> and highest point of this national park is Rachila Danda. Neora Valley National Park has a wide altitudinal range of 183 m - 3200 m. Singalila National Park (Phalut) covers an area of 80 km<sup>2</sup> and it is located in Singalila range. The Singalila range (Phalut) forms the eastern part of the Great Himalayas, and is in the tri-junction of Nepal, West Bengal and Sikkim. It forms the north-western boundary of Darjeeling district and altitude ranges from 2400 m to 3650 m. The objective of the study was to assess annual carbon and nitrogen dynamics along the elevation gradient of Darjeeling Himalaya. Soils are shallow to deep, pale brown to dark brown in colour and coarse to moderately fine textured, acidic, high in organic carbon and low in CEC and base saturation (Ray and Mukhopadhyay 2012). Most of the soils of this region are developed due to fluvial action or by lithological disintegration (Majumdar et al. 2014). The rock formations belong to the Darjeeling gneiss, daling series, schist sand shales, gondwana and the tertiary system (Banerjee, 2014). Darjeeling has 1370 km<sup>2</sup> of forests in dense and moderately dense category which is 43.51% of the TGA of 3149 km<sup>2</sup> (ISFR 2017). The three study sites have distinct climatic pattern (Fig. 2).

### 2.2 Study design and data collection

Each of the three study sites were divided into different elevational transects and 20x 20 m quadrats were laid and investigated ranging from 154m to 3450 m. A total number of 153 samples were collected, 51 from each respective protected areas for both year 1 (2014) and year 2 (2015). Thus we collected 306 samples in the span of two years. The sampling was designed in a way in which we used 1m\*1m wooden frame with a same dimension mesh fitted inside it and kept under the dominant tree of each forest type. The litter collected in the mesh was kept in tagged bags. The tagged bags were weighed in the field. The bags were subjected to immediate oven drying in the laboratory to ensure minimal loss. The litter bags were oven dried at 65° C 24 hrs until dried thoroughly, it is later removed from the oven and kept at the room temperature for about 24 hrs to equilibrate (Murphy et al, 1998). The sample was weighed and ground in powder form by using a grinder and unwanted particles were removed by passing it through 2 mm sieve. The carbon and the nitrogen content of the litter were determined by Vario Elemental CHNS analyzer (dry method). Finally, C:N ratio was calculated by using the obtained nitrogen (%) and carbon (%) values.

### 2.3 Data analysis

The statistical analysis was carried out to assess the variation in litter carbon (%), Nitrogen (%) and Carbon:Nitrogen ratio (C:N ratio) across the three study sites for both year 1 and year 2. The statistical significance in the difference in values was checked by ANOVA between the study sites and year. Multiple comparisons Carbon (%), Nitrogen (%), and C:N ratio was made by using Tukey's HSD. The difference among the two years was also evaluated. The analysis was carried out in IBM SPSS Software version 22.

## III. RESULTS

### 3.1 Litter carbon (%) dynamics in three protected areas of Darjeeling

For litter, carbon (%) increased from Year 1(40.45±0.95) to Year 2 (40.94±0.96) and for both the years, showed significant difference among the three protected areas (ANOVA, Year 1:  $F_{2, 150}=202.983, p=0.000$ ; Year 2  $F_{2, 150}=220.220, p=0.000$ ) which increased with elevation (MWLS<NVNP<SNP), and the highest value in SNP was greater than NVNP and MWLS by 1.3 and 1.9 times, respectively (Fig. 3; Table 1). The multiple comparisons of carbon (%), based on Tukey's HSD for both the years, showed significant difference among all the pair of protected areas for both the years (Table 2).

### 3.2 Litter nitrogen (%) dynamics in three protected areas of Darjeeling

For litter, Nitrogen (%) increased from Year 1 ( $0.96 \pm 0.01$ ) to Year 2 ( $1.01 \pm 0.01$ ) and for both the years, differed significantly among the three protected areas (ANOVA, Year 1:  $F_{2,150} = 81.531$ ;  $p = 0.000$ ; Year 2:  $F_{2,150} = 74.328$ ,  $p = 0.000$ ) and showed opposite trend to that of carbon (%), as it tend to decline with increasing elevation (NVNP > MWLS > SNP) though the highest value was observed in NVNP (Fig. 3; Table 1). The multiple comparisons of nitrogen (%), based on Tukey's HSD for both the years, showed significant difference among all the pair of protected areas (Table 2).

### 3.3 Litter C:N ratio dynamics in three protected areas of Darjeeling

For litter, C:N ratio declined from Year 1 ( $43.24 \pm 1.25$ ) to Year 2 ( $41.57 \pm 1.18$ ) and for both the years, differed significantly among the three protected areas (ANOVA, Year 1:  $F_{2,150} = 624.077$ ,  $p = 0.000$ ; Year 2:  $F_{2,150} = 657.371$ ;  $p = 0.000$ ) which increased with elevation (MWLS < NVNP < SNP), and the highest value in SNP was greater than NVNP and MWLS by 1.6 and 2.2 times, respectively (Fig.3; Table 1). The multiple comparisons of C:N ratio based on Tukey's HSD for both the years, showed significant difference among all the pair of protected areas (Table 2).

## IV. DISCUSSIONS

The carbon and nitrogen contributed by litter differed in the different forest types of Darjeeling Himalaya. The East Himalaya tropical sal forests (Sal dominated forest type) found in the lower elevation have substantially lower carbon content in litter as compared to temperate forest and sub-alpine forests found in mid and high elevation. The highest value of litter Nitrogen (%) in temperate forests, followed by tropical forests and lowest in sub-alpine forests. Along the elevation gradient, temperature and precipitation act as main drivers in litter decomposition and thus also on carbon accumulation. The three study sites showed distinct climatic pattern in the present study.

The increase in litter Carbon (%) along the elevation gradient in the present study conforms to the previous reports from the Eastern Himalaya (Tashi et al. 2016). In the present study, litter Carbon (%) was lowest in tropical sal forests of MWLS ( $28.21 \pm 0.48$ ) which is less than previous report by ( $41.9 \pm 4.8$ ) from the tropical forests of Eastern Himalaya (Tashi et al. 2016). The litter Carbon (%) observed in the East Himalaya temperate forests of NVNP ( $41.17 \pm 0.59$ ) was comparable though less than the previous report from the wet temperate broad leaved forests ( $44.8 \pm 2.8$ ) and cool temperate broad leaved forests ( $46.4 \pm 3$ ) of Eastern Himalaya (Tashi et al. 2016). However, the litter Carbon (%) observed in East Himalaya sub alpine forests of SNP ( $52.86 \pm 0.69$ ) was greater than the previous report from this type of forests ( $47.8 \pm 1.1$ ) of Eastern Himalaya (Tashi et al. 2016).

The highest value of litter Nitrogen (%) in temperate forests, followed by tropical forests and lowest in sub-alpine forests observed in the present study conforms to the previous reports from the Eastern Himalaya (Tashi et al. 2016). The litter Nitrogen (%) observed in the East Himalaya tropical sal forests of MWLS ( $1.01 \pm 0.01$ ) is less than previous report by ( $1.2 \pm 0.2$ ) from the tropical forests of Eastern Himalaya (Tashi et al. 2016). The litter Nitrogen (%) observed in the Eastern Himalaya temperate forests of NVNP ( $1.09 \pm 0.01$ ) is substantially less than the previous report from the wet temperate broad leaved forests ( $1.8 \pm 0.1$ ) and cool temperate broad leaved forests ( $1.5 \pm 0.2$ ) of Eastern Himalaya (Tashi et al. 2016). However, the litter Carbon (%) observed in East Himalaya sub alpine forests of SNP ( $0.86 \pm 0.01$ ) is comparable to the previous report from this type of forests ( $0.9 \pm 0.2$ ) of Eastern Himalaya (Tashi et al. 2016).

The Organic carbon present in the litter is labile as around 60-80% of the total carbon will be released back into the atmosphere and the remaining small portion either turns into microbial biomass or forms humic substances after many transformations (Gonzalez et al., 2004). The organic carbon is a balance product of loss and gain of carbon, of which gains are determined by vegetation type and loss by oxidation of existing organic matter (Han et al., 2010) as well as by decomposition. Study of litter carbon contribution will help in understanding the role of soil in carbon sequestration in future long term. The present study is an attempt to understand the role of litter in carbon and nitrogen cycling in the forest soil making it more of carbon sink then becoming a carbon source. The C:N ratio increases from lower elevation forest to higher elevation due to decrease in temperature along the elevation and due to the presence of mineralized nitrogen in the soil (Houghton et al., 1998) and it may also be due less litter decomposition at higher altitude with limited presence of nutrients. A meta- analysis of 30 papers analyzed by Gartner and Cardon (2014) concludes that the decomposition of mixed species forests are higher than forests with single species. Litter carbon is controlled by the quality of the litter, plant species composition has impact on litter carbon and nitrogen along with climatic factors. At the higher altitude due the presence of the optimal conditions temperature and moisture, substantial amount of litter is humified due to this reason, the %C increases from lower to higher altitude. Forests along with the soil have huge potential of carbon storage, is often under evaluated. The carbon and the nitrogen dynamics are susceptible to changes in increased in carbon dioxide in atmosphere and subsequent rise in temperature. The study is an attempt to fill the caveat of data regarding litter carbon in Eastern Himalayas. Eastern Himalayas, part of global biodiversity hotspot of Himalaya is a mountainous ecosystem and host an array of endemic flora and fauna, and most

vulnerable to climate change and tectonic activities. Therefore, understanding the nature of carbon stock and nitrogen stock along the elevation gradient will not only help in mitigation but also in adaptation strategies.

## V. ACKNOWLEDGEMENT

We would like to thank West Bengal Forest Department specially the PCCF and DFO, Darjeeling Silviculture (Hills) division for the permission to carry out this research work and all the supports. We also acknowledge the support from Dr. Binod Sharma, Raju Pradhan, Nayan Thapa, Sailendra Dewan, Prashant Ghose, Sharma ji, Paras Mani Pradhan at different stage of the study. Fellowships granted by University Grant Commission, Government of India to SBD (Non-NET) and KS (UGC-JRF) are gratefully acknowledged. SBD would like to thank Prof. K.V. Deviprasad for supervision and guidance during the study period.

## REFERENCES

- [1] Banerjee, S.K. 2014. Forest soil carbon stock along an altitudinal gradient in Darjeeling Himalayan Region. *Indian Forester*, 140(8):775-779.
- [2] Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the World. *European Journal of Soil Science*, 47(2):151-163.
- [3] Bohra, C.S., Tewari, S.K. and Bhatt, M.D. 2014. Trends in soil carbon stockpile of three major forests along an altitudinal gradient in Indian central Himalayas. *Ecoprint*, 21:7-13.
- [4] Dar, J.A. and Sundarapandian, S. 2015. Variation of biomass and carbon pools with forest type in temperate forests of Kashmir Himalaya. *Environment monitoring and assessment*, 187(2):55. doi: 10.1007/s10661-015-4299-7
- [5] Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C. and Wisniewski, J. 1994. Carbon pool and flux of global forest ecosystems. *Science*, 263:185-190.
- [6] Fick, S.E. and Hijmans, R.J. 2017. Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37: 4302-4315.
- [7] Gartner, T.B. and Cardon, Z.G. 2004. Decomposition dynamics in mixed-species leaf litter. *Oikos*, 104: 230-246.
- [8] Han, X., Atsushi T., Mitsuru T. and Shiqing, L. 2009. Effects of land-cover type topography on soil organic carbon storage on Northern Loess plateau, China. *Acta Agriculturae Scandinavica*, 60(4): 326-334.
- [9] Houghton, R.A., Davidson, E.A. and Woodwell, G.M. 1998. Missing sinks, feedbacks, and understanding the role of terrestrial ecosystems in the global carbon balance. *Global Biogeochemical Cycles*, 12(1):25-34.
- [10] IPCC. 2006. CHAPTER 1: Introduction (principles for developing the guidelines). 2006 IPCC Guidelines for National Greenhouse Gas Inventories, 11(S3), S79–S80. [https://doi.org/10.1111/j.1440-1843.2006.00937\\_1.x](https://doi.org/10.1111/j.1440-1843.2006.00937_1.x)
- [11] ISFR. 2017. India State of Forest Report 2017. Forest Survey of India, Dehradun.
- [12] Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P., Kurtz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, D., Piao, S., Rautiainen, A., Sitch, S. and Haye, D. 2011. A large and persistent carbon sink in the world's forests. *Science*, 333:988
- [13] Prescott, C.E. 2010. Litter decomposition what controls it and how can we alter it to sequester more carbon in forest soils? *Biochemistry*, 101:133-149.
- [14] Liski, J., Ilvesniemi, H., Makela A. and Westman C. J. 1999. CO<sub>2</sub> emissions from soil in response to climatic warming are overestimated - the decomposition of old soil organic matter is tolerant of temperature. *Ambio*, 28:171-174.
- [15] Majumdar, K., Ray D.P., Chakraborty, S. and Pandit, T. 2014. Change of Nutrient Status of Hilly Soil in Darjeeling District within Five Years. *International Journal of Bioresource Science* 1(1): 25-30.
- [16] Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P. and Saatchi, S. 2010. Introduction: elevational gradients in the tropics: laboratories for ecosystem ecology and global change research. *Global Change Biology*, 16:3171-3175.

- [17] Melillo, J.M., Aber, J.D. and Muratore, J.F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, 63(3):621-626.
- [18] Murphy, K.L., Klopatek, J.M. and Kolpatek, C. 1998. The effects of litter quality and climate on decomposition along an elevation gradient. *Ecological Applications*, 8(4):1061-1071.
- [19] NOAA. 2018. Trends in Atmospheric Carbon Dioxide. Earth System Research Laboratory, National Oceanographic and Atmospheric Administration. <https://www.esrl.noaa.gov/gmd/ccgg/trends/>
- [20] Ray S.K. and Mukhopadhyay, D. 2012. A study on physicochemical properties of soils under different tea growing regions of West Bengal (India). *International Journal of Agriculture Sciences*, 4(8): 325-329.
- [21] Sayer, E.J., Heard, M.S., Grant, H.K., Marthews, T.R., Tanner, E.V.J. 2011. Soil carbon release enhanced by increased tropical forest litterfall. *Nature Climate Change*, 1, 304–307.
- [22] Schlesinger, W.H. and Lichter, J. 2001. Limited carbon storage in soil and litter of experimental forest plots under increase atmospheric CO<sub>2</sub>. *Nature*, 411:466-469.
- [23] Simon, A., Dhendup, K., Rai, P.B. and Gratzer, G. 2018. Soil carbon stocks along elevational gradients in Eastern Himalayan mountain forests. *Geoderma Regional*, 12:28-38.
- [24] Sheikh, M.A., Kumar, M. and Bussmann, W.R. 2009. Altitudinal variation in soil organic carbon stock in coniferous subtropical and broadleaf temperate forests in Garhwal Himalaya. *Carbon Balance and Management*, 4:6. doi: <https://doi.org/10.1186/1750-0680-4-6>
- [25] Swift, M.J., Heal, O.W., Anderson, J.M., 1979. *Decomposition in Terrestrial Ecosystems*. Blackwell Scientific Publications, Oxford, UK.
- [26] Tashi, S., Singh, B., Keitel, C. and Adams, M. 2016. Soil carbon and nitrogen stocks in forests along an altitudinal gradient in the Eastern Himalayas and a meta-analysis of global data. *Global Change Biology*, 22(6): 2255-2268.
- [27] Zak, D.R., Holmes, W.E., Finzi, A.C., Norby, R.J. and Schlesinger, W.H. 2003. Soil nitrogen cycling under elevated CO<sub>2</sub>: A synthesis of forest FACE experiments. *Ecological Applications*, 13(6):1508-1514.

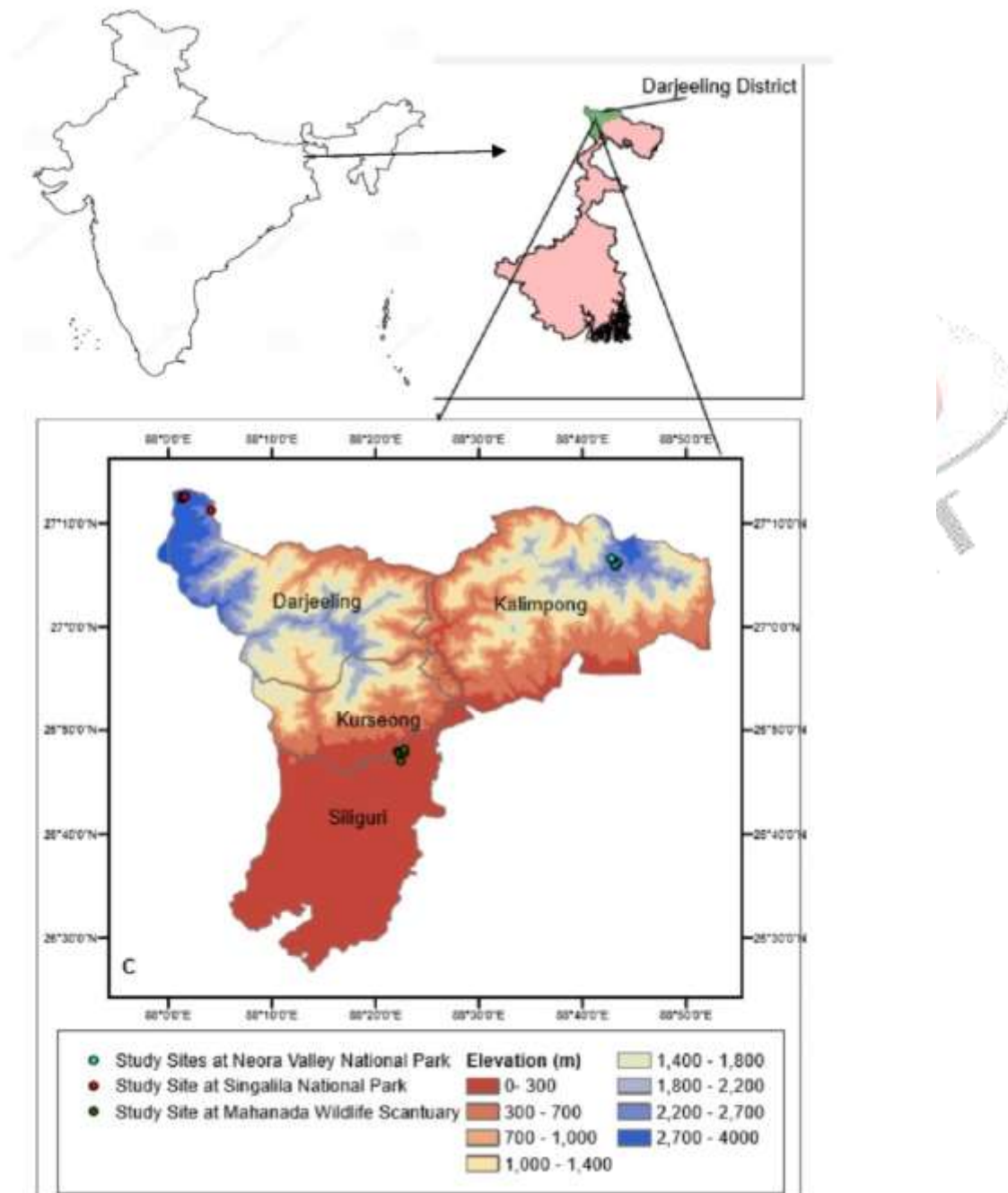
**Table 1:** Litter % Carbon, % Nitrogen (%) and C:N ratio in the three protected areas: Mahananda Wildlife Sanctuary (MWLS), Neora Valley National Park (NVNP) and Singalila National Park (SNP) in Darjeeling Himalaya, India.

|              | Sample     | Year         | MWLS              | NVNP              | SNP               | Total             |
|--------------|------------|--------------|-------------------|-------------------|-------------------|-------------------|
|              |            |              | 51                | 51                | 51                | 153               |
| Carbon (%)   | 51         | 1            | 28.12±0.68        | 41.08±0.85        | 52.63±1.02        | 40.45±0.95        |
|              | 51         | 2            | 28.3±0.68         | 41.26±0.84        | 53.25±0.98        | 40.94±0.96        |
|              | <b>102</b> | <b>Total</b> | <b>28.21±0.48</b> | <b>41.17±0.59</b> | <b>52.86±0.69</b> | <b>40.75±0.67</b> |
| Nitrogen (%) | 51         | 1            | 0.99±0.01         | 1.06±0.01         | 0.84±0.02         | 0.96±0.01         |
|              | 51         | 2            | 1.03±0.01         | 1.12±0.01         | 0.89±0.02         | 1.01±0.01         |
|              | <b>102</b> | <b>Total</b> | <b>1.01±0.01</b>  | <b>1.09±0.01</b>  | <b>0.86±0.01</b>  | <b>0.99±0.01</b>  |
| C:N ratio    | 51         | 1            | 28.47±0.60        | 38.74±0.69        | 63.28±0.84        | 43.24±1.25        |
|              | 51         | 2            | 27.51±0.60        | 36.93±0.66        | 60.27±0.69        | 41.57±1.18        |
|              | <b>102</b> | <b>Total</b> | <b>27.99±0.42</b> | <b>37.83±0.48</b> | <b>61.63±0.55</b> | <b>42.48±0.86</b> |

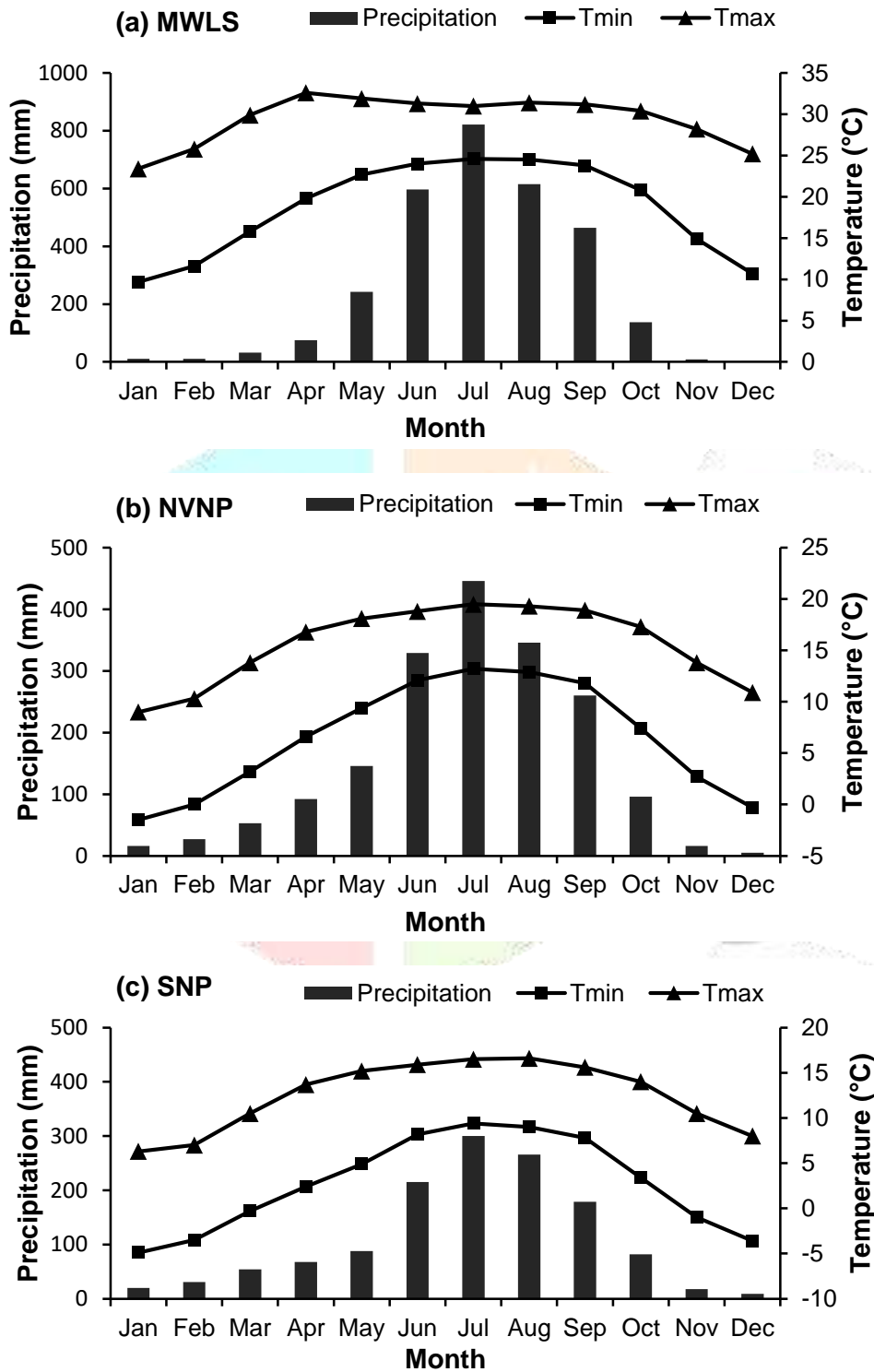
**Table 2:** ANOVA and multiple comparisons based on Tukey's HSD for litter Carbon (%), Nitrogen (%) and C:N ratio for two years among the three protected areas: Mahananda Wildlife Sanctuary (MWLS), Neora Valley National Park (NVNP) and Singalila National Park (SNP) in Darjeeling Himalaya, India. \*\*\*: p<0.001

|            | Year | ANOVA                      |          | Multiple comparison based on Tukey's HSD |             |             |
|------------|------|----------------------------|----------|--|-------------|-------------|
|            |      | <i>F</i> <sub>2, 150</sub> | <i>P</i> | MWLS vs NVNP                             | MWLS vs SNP | NVNP vs SNP |
| Carbon (%) | 1    | 202.983                    | 0.000    | 12.957***                                | 24.503***   | 11.546***   |

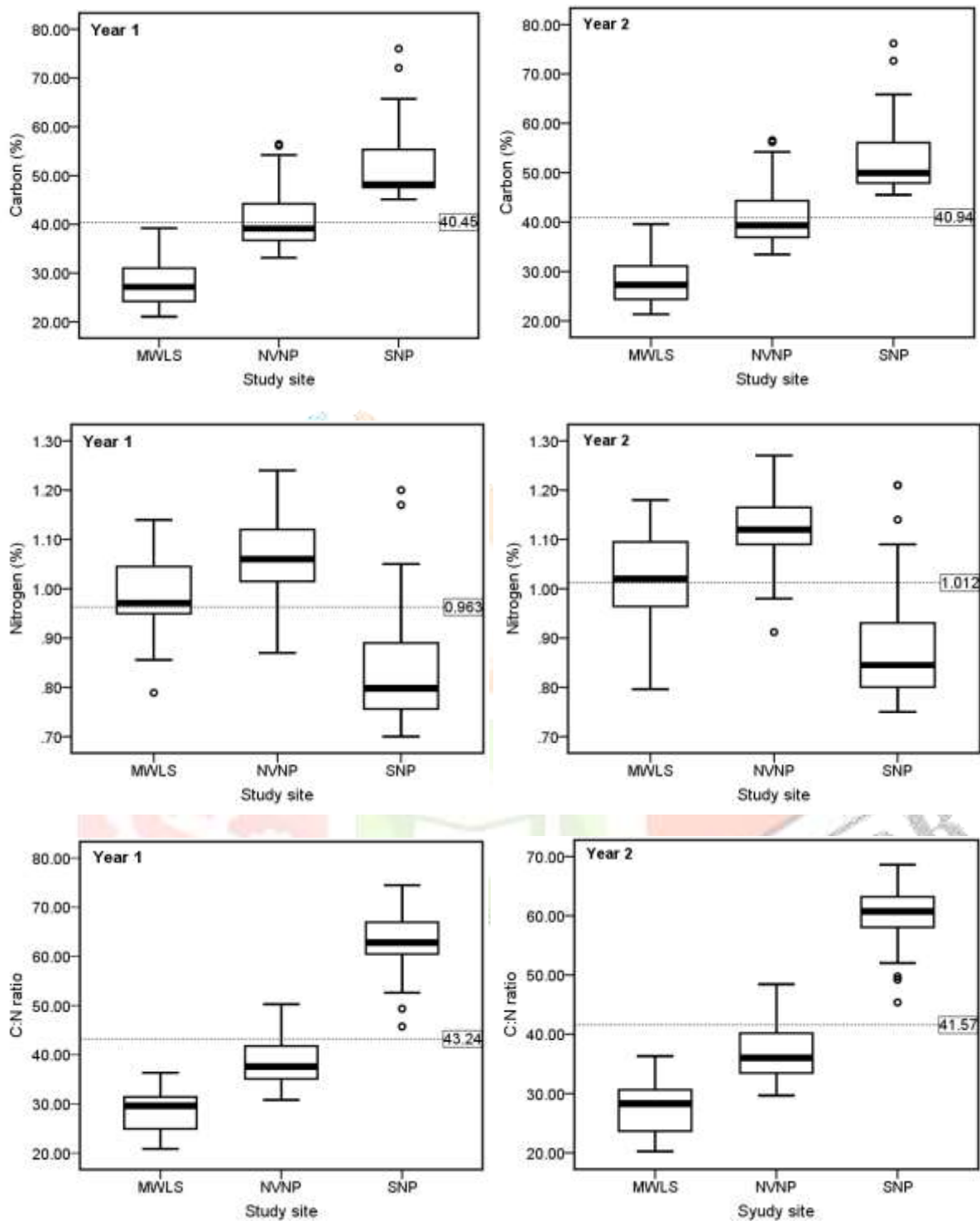
|              |   |         |       |           |           |           |
|--------------|---|---------|-------|-----------|-----------|-----------|
|              | 2 | 220.220 | 0.000 | 2.963***  | 4.957***  | 1.995***  |
| Nitrogen (%) | 1 | 81.531  | 0.000 | -0.074*** | 0.152***  | 0.226***  |
|              | 2 | 74.328  | 0.000 | -0.087*** | 0.143***  | 0.230***  |
| C:N ratio    | 1 | 624.077 | 0.000 | 10.268*** | 34.811*** | 24.543*** |
|              | 2 | 675.371 | 0.000 | 9.419***  | 2.757***  | 3.338***  |



**Figure 1:** Map showing the location of the four study sites in three protected areas: Mahananda wildlife sanctuary (MWLS); Neora valley national park (NVNP) and Singalila national park (SNP) of Darjeeling Himalaya, India.



**Figure 2:** Mean monthly maximum and minimum temperature and precipitation in the three study sites in (a) Mahananda wildlife sanctuary-MWLS, (b) Neora valley national park- NVNP and (c) Singalila national park –SNP, Darjeeling Himalaya, India. Source: WorldClim Version 2 database (Fick and Hijmans, 2017).



**Figure 3:** Annual changes in litter Carbon (%), Nitrogen (%) and C:N ratio in the three protected areas: Mahananda wildlife sanctuary (MWLS), Neora valley national park (NVNP) and Singalila national park (SNP) of Darjeeling Himalaya, India.