International Journal of Agriculture Innovations and Research
Volume 7, Issue 5, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 14/12/2018 | Accepted on: 02/01/2019 | Published: 26/03/2019

Microbial Biomass Carbon and Nitrogen Dynamics of A Pure Pine Stand and an Enriched Pine Stand

P. GHOSH
G.B. Pant National Institute of Himalayan Environment & Sustainable Development, Kosi – Katarmal, Almora – 263643, Uttarakhand, Telephone: +91. 05962. 241014, Fax No. +91.05962. 241150.
Corresponding author email id: paroghosh@rediffmail.com

Abstract **– A study was conducted in pure and enriched Pine stand to access the impact of enriched plantation on the dynamics of microbial biomass across the season. The experiment was laid out in a completely randomized block design with pure Pine stand and enriched Pine stand as treatments with three replicates. Amongst the treatments the enriched Pine stand maintained a higher microbial biomass carbon and nitrogen compared to pure Pine stand. The repeated measures analyses showed soil moisture, microbial biomass nitrogen and pH were significantly affected due to enrichment of Pine stand. Soil moisture, microbial biomass carbon, nitrogen and organic carbon changed significantly during the growing season. The microbial biomass in soil declined during vigorous growth period across all the treatments with a maximum decline recorded for enriched Pine stand indicating microbial biomass formed an important source of nitrogen during the growth period. Our study demonstrated that the practice of interspacing pure Pine stand with saplings of broad leaved tree species is beneficial in terms of amount of microbial biomass carbon and nitrogen required for plant growth. Enrichment of pure Pine stand is a good practice and should be encouraged for higher multipurpose benefits. More priority should be given to improve the soil health and utility of composition of Pine forests to improve their nutrient use efficiency and make them more productive.**

Keywords **– Central Himalaya, Enriched Pine Stand, Microbial Biomass Carbon and Nitrogen, Pure Pine Stand.**

I. INTRODUCTION

The soil microbial biomass carbon is used to characterize the activity of soil organic carbon. (Harris 2003). In terms of energy cycle and nutrient transfer soil microbial biomass carbon and nitrogen is extremely sensitive and reflects small alterations in soil organic matter much earlier than total carbon and nitrogen changes (Zhang et al. 2005). It also reflects the kind of energy that can be utilized directly by microorganisms. Therefore the study of microbial biomass carbon and nitrogen is of great significance for vegetation restoration and enrichment plantations. Since soil microbial biomass can act as a significant source or sink for soil nutrients and influence how much nitrogen is retained within soil organic matter (Insam et al. 1989, Brookes 1995) it is important to understand the patterns of microbial abundance, turnover and nutrient sequestration in forest ecosystems/ plantations. Deng et al. 2016, reported that afforestation resulted in alterations in soil microbial biomass which were associated with declines in basal microbial respiration, net nitrogen mineralization and nitrification which likely maintained higher soil carbon and nitrogen storage and stability. An understanding of microbial processes is important for the management of plantation systems, particularly those that rely on organic inputs of nutrients (Smith and Paul 1990). Enrichment plantation is being adopted extensively to render the pure Pine stand more productive and beneficial for stakeholders. To our knowledge, however, there is scanty information on the microbial biomass carbon and nitrogen dynamics in organically managed mixed Pine plantations of central Himalaya. Such information is needed, because continued emphasis on sustainable development has generated renewed interest in evaluating the effect of different soil management systems on carbon and nitrogen dynamics in soils. Since the amount of soil microbial biomass can be quickly affected by changes in ecosystem processes, temporal patterns on soil microbial biomass amount can also be a sensitive indicator of ecosystem health and function. The potential use of information on the effect of different plantation systems on the amount of microbial biomass carbon and nitrogen in assessing soil health of this region is obvious.

The objectives of this study were to compare the effect of pure and enriched Pine stand on the amount of soil microbial biomass carbon and nitrogen and to determine how the amount of microbial biomass carbon and nitrogen varied across the growing season.

II. MATERIALS AND METHODS

Study Site

The study site was situated in the Nanda Van experimental station of the institute (Gobind Ballabh Pant National Institute of Himalayan Environment and Sustainable Development), located 29^0 36' N latitude and 79 ^{0} 37' E longitude and 1250 meters above mean sea level. The region has a warm temperate climate with typical monsoonal character with an annual rainfall of 1233mm (Singh et al. 2000). The soil is an *Inceptisol*, sandy loam in texture and has a neutral reaction. In general the soil is well drained and moderately fertile (Singh et al. 2000).

Experimental Design

The experimental plots (5m x 3m) were laid out in a completely randomized block design in a pure Pine stand, and enriched Pine stand as treatments with three replicates. Pits were dug in between Pine trees and farmyard manure was surface - applied and lightly incorporated (upto 10 cm) in the soil. The farmyard manure consisted of dung, animal urine, bedding leaves and feed leftovers. The percentage nutrient composition of the farm-yard manure

International Journal of Agriculture Innovations and Research
Volume 7, Issue 5, ISSN (Online) 2319-1473

applied was (mean ± SE) 33.17 ± 2.33 C, 0.83 ± 0.20 N, 0.26 ± 0.09 P. Saplings of broad leaved tree species (Table 1) were planted in the rainy season of August 2014 and 2015.

Table 1. Species details planted at Nanda Van restoration site.

S. No.	Name of species (Local)	Scientific name & Family name of species	Number of sapling	Age of sapling (Years)
1.	Banj	*Quercus leucotrichophora* Fagaceae	200	2.5
2.	Bottle brush	*Callistemon citrinus* Myrtaceae	100	2
3.	Phaniyat Oak	*Quercus galuca* Fagaceae	100	3
4.	Shisham	*Dalbergia sissoo* Fabaceae	30	2
5.	Utis	*Alnus nepalensis* Betulaceae	100	2.5
6.	Maple	*Acer pseudoplatanus* Sapindaceae	25	2
7.	Reetha	*Sapindus saponaria* Sapindaceae	50	2.5
8.	Shatut	*Morus macroura* Moraceae	100	3
9.	Tejpat	*Cinnamomum tamala* Lauraceae	50	4
10.	Bains	*Salix alba* Salicaceae	50	1.5
11.	Total no. of saplings		830	

Soil Sampling

Triplicates of three soil samples were collected randomly from each treatment plot from the upper 10cm soil layer and mixed to form a composite sample to account for spatial variation in the field. Soil monoliths (10 x 10x 10 cm) were removed and stored in polyethylene bags and brought to the laboratory. Soil samples were taken at regular intervals of 30 days after planting. The soils were spread on paper sheets and visible roots and fragments of organic debris were removed and sieved (2 – mm mesh). Each composite soil sample was divided into two parts. The soil in field moist condition was used for determination of physico-chemical biological parameters.

Soil Analyses

Preparation and chemical analyses of the soil samples were performed by well known standard methods described in detail elsewhere (Ghosh and Kashyap 2003; Ghosh and Dhyani, 2004 a). Particle size analysis was done by (Anderson and Ingram 1993). Bulk density was determined by using a soil corer and measuring the weight of dry soil of a unit volume to a depth of 10 cm. Water holding capacity was determined according to (Piper 1944). Organic carbon as given by (Walkley 1947). Total nitrogen was analyzed by microkjeldahl digestion (Jackson 1958).

Soil pH (1: 2, soil: water) was measured using a pH meter equipped with glass electrode. Gravimetric soil moisture content was measured with freshly pulled out soil according to (Buresh 1991). The organic carbon and nitrogen contents of the soil were measured after fumigation with ethanol – free $CHCl_3$ for 24 hours at 25 ^{0} C in the dark (Brookes et al. 1985, Vance et al. 1987). After evacuation of $CHCl_3$, the moist soil samples (20g) were extracted with 50 ml of 0.5 M K_2SO_4 for 30 minutes. The suspensions were then filtered through Whatman No. 42 filter paper and stored at – 4 ^{0} C for seven to fourteen days prior to analysis. Subsamples of unfumigated control soils were extracted the same way and at the same time as the fumigated samples. Between sampling and extraction control subsamples were stored at 4^0 C in sealed containers to prevent moisture loss. Organic carbon was measured in 10 ml aliquotes of the K_2SO_4 extracts after digestion with $K_2Cr_2O_7$ / H_2SO_4 and back – titration with ferrous ammonium sulphate. Biomass carbon was calculated using the equation

$$\text{Biomass C} = 2.64\ E_C$$

Where E_C = (organic C from fumigated soil) – (organic C from unfumigated soil) (Vance et al. 1987). Total nitrogen in the extracts was measured by micro – Kjeldahl digestion. Aliqoutes (20 ml) of extract were digested with H_2SO_4 for three hours after the addition of 0.4 ml of 0.2M $CuSO_4$ to promote organic matter breakdown. Biomass nitrogen was calculated using the equation:

$$\text{Biomass N} = 2.22\ E_N$$

Where E_N = (total N from fumigated soil) – (total N from unfumigated soil) (Jenkinson 1988). The values 2.64 and 2.22 are correction factors for microbial carbon and nitrogen for aerobic soils and represent the incomplete recovery of biomass carbon and nitrogen constituents respectively, extracted from soil after fumigation (Vance et al. 1987). The microbial quotient (microbial biomass carbon / organic carbon and microbial biomass nitrogen / total nitrogen) was calculated by expressing microbial biomass carbon and nitrogen as a percentage of soil organic carbon and total nitrogen respectively.

Statistical Data Analysis

GLM (general linear model) repeated measures analysis was performed using SPSS software (SPSS 2002) on the major soil parameters with successive sampling dates as the repeated measure.

International Journal of Agriculture Innovations and Research
Volume 7, Issue 5, ISSN (Online) 2319-1473

III. RESULTS

Physico - Chemical Properties of Soil

Physico – chemical features of soils are summarized in Table 2. The soil was sandy loam with the bulk density ranging from 0.88 – 0.92 g cm $^{-3}$ and slightly acidic with pH ranging from 6.6 to 6.7. It was characterized with low levels of organic carbon (0.88 – 1.07 %), total nitrogen (0.09 – 0.10 %). The Water holding capacity ranged from 30.7 – 36.8 % (Table 2). The organic carbon and nitrogen values were higher in soils under enriched Pine plantation in comparison to pure Pine plantation. The soil moisture content of top 10cm soil layer ranged from 25.3 – 33.4 % (Table 2). There were significant differences in soil moisture due to plantation type, season and plantation type x season interaction (Table 3). The C:N ratio in soil ranged from (9.7 to 10.7) Table 2. The organic carbon content was negatively correlated to soil moisture (r^2 = - 0.31, $P <$ 0.01).

Table 2. Soil characteristics of plots planted to pure and enriched Pine, seasonal averages ± 1SE, n = 18.

Parameters	Treatments	
	Pure Pine	Enriched Pine
Soil texture		
Sand (%)	65.3 ± .26	63.3 ± .72
Silt (%)	30.1 ± 1.10	27.5 ± 2.56
Clay (%)	4.6 ± .04	9.2 ± 5.18
Soil moisture (%)	33.4 ± .36	25.3 ± .43
Bulk density (g cm $^{-3}$)	0.88 ± 0.02	0.92 ± .05
pH	6.7 ± 0.24	6.6 ± 0.21
WHC (%)	36.8 ± 0.78	30.7 ± 1.46
Total N (%)	0.09 ± 0.01	0.10 ± .02
Organic C (%)	0.88 ± 0.22	1.07 ± 0.21
Organic matter (%)	1.56 ± 0.29	1.84 ± 0.37
Organic C / Total N	9.7	10.7
Microbial Biomass C (µg g^{-1})	173.6 ± 24.0	190.3 ± 21.3
Microbial biomass N (µg g^{-1})	17.8 ± 2.1	19.6 ± 1.4
Microbial C/Organic C	1.97	1.77
Microbial N / Total N	1.97	1.96

Microbial Biomass

Throughout the season maximum microbial carbon values were recorded in the enriched Pine stand (Table 2, Figure1). Both microbial biomass carbon and nitrogen decreased during the vigorous growth of the saplings in rainy season and increased slightly in winter season (Figure 1and 2). The rainy season decrease in biomass carbon was 68.81% in pure pine soil, and 42.3% in enriched pine soil (Figure 1). Across the season maximum microbial nitrogen was recorded in the rainy season (Figure 2). The seasonal decrease in biomass nitrogen was 37.2% in pure pine stand and 35.4% in enriched pine stand (Figure 2). There were significant differences in microbial biomass carbon due to season and plantation type x sampling time interaction (Table 3). Microbial biomass nitrogen in soil was significantly different due to plantation type, sampling time and plantation type x sampling time interaction (Table 3). Microbial biomass carbon was correlated with soil microbial biomass N (r^2 = 0.41, $P <$ 0.01). Microbial biomass carbon and nitrogen ratio ranged from 9.7 to 10.2 (Table 2). The microbial biomass carbon was 1.78 to 1.97 % of organic carbon. Percentage of soil nitrogen reflected in microbial nitrogen pool (microbial biomass N: total N (%)) were 1.87 to 2.03 % (Table 2). Thus the microbial quotient was always greater than one (Table 2).

Table 3. F – ratios and their significance levels for two way ANOVA with repeated measures for soil pH, microbial C, N, organic C and soil moisture for the pure pine and enriched Pine, where sampling time was treated as a repeated measure. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. n = 90.

Parameters	Source of variation		
	Between subject	Within subject	
	Plantation type	Time	Time x Plantation Type
pH	3.74 *	0.71 ns	1.49 ns
Microbial C	0.42 ns	18.63 ***	4.58 ***
Microbial N	8.67 ***	10.89 ***	2.03 *
Organic C	0.83 ns	3.47 **	1.37 ns
Soil moisture	12.31 ***	14.09 ***	15.26 ***

International Journal of Agriculture Innovations and Research
Volume 7, Issue 5, ISSN (Online) 2319-1473

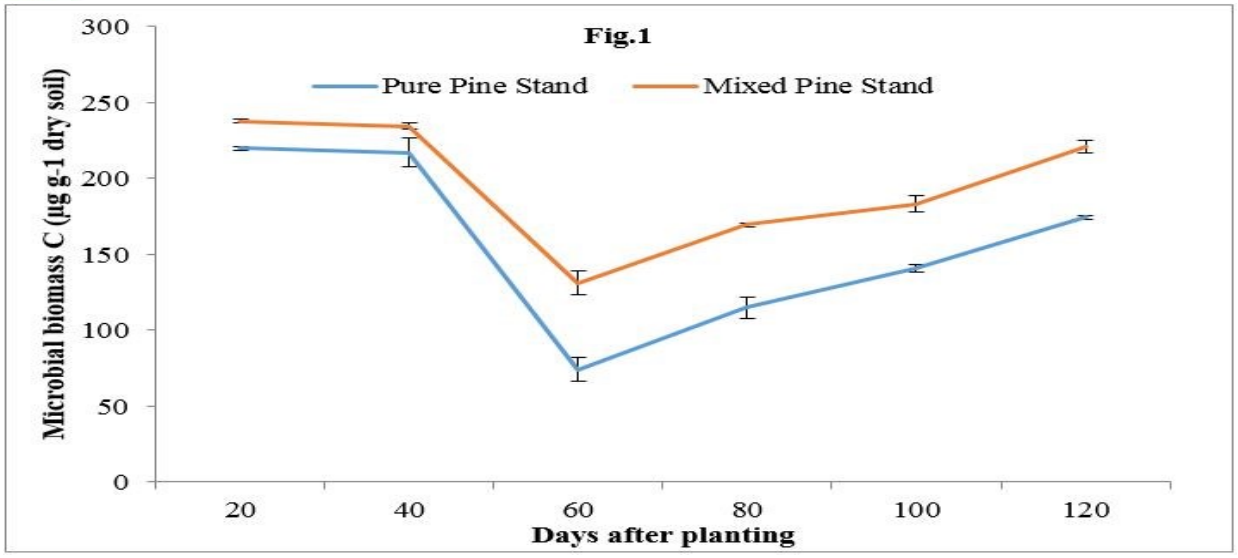

Fig. 1. Temporal changes in microbial biomass carbon in the pure Pine and enriched Pine stand. Error bars represent standard error, n = 18 for each treatment.

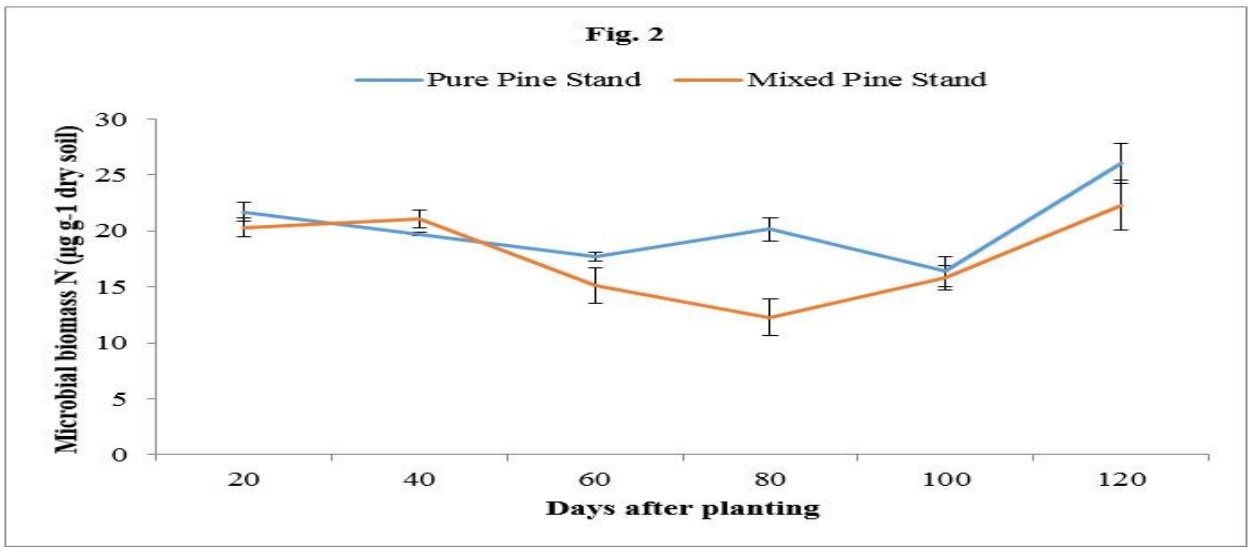

Fig. 2. Temporal changes in microbial biomass nitrogen in the pure Pine and enriched Pine stand. Error bars represent standard error, n = 18 for each treatment.

IV. Discussion

In the present study results show that mixed plantation affected the microbial biomass carbon and nitrogen. The microbial biomass carbon and nitrogen values were higher in enriched pine compared to pure pine plantation. The enriched plantation showed relatively higher microbial biomass carbon and nitrogen content compared to pure Pine plots suggesting that they supplied more readily decomposable organic matter. In addition the growth of heterogenous saplings lead to an increased root biomass and root exudates due to greater growth. Our results demonstrate that enrichment of pure Pine stand enhanced biological activity of soils. This is because each plant litter provides carbon, nitrogen and other elements in different amounts and available forms, the amount and type of residue left in soils by different saplings affect microbial population and activity. Studies have shown that soils under mixed plantations have significantly higher levels of microbial biomass carbon and nitrogen (Moore et al. 2000) than soil under mono-culture. Studies by Li et al. 2007 on planted and Chinese fir forests in Fujian province and Liu and Wang, 2010 on temperate forests also indicated that soil microbial biomass carbon increased with restoration. The soil microbial quotient is a sensitive indicator of the quality of soil carbon and nitrogen pool and could reflect more effectively the effect of re-vegetation on the behaviour of soil carbon compared to soil microbial biomass. A previous research (Singh et al. 1989) pointed that higher soil microbial quotient indicated carbon accumulation in the soil and the microbial number and population structure in the soil changed with re-vegetation, which would slow down the conversion of non active organic carbon to active carbon and ultimately increase the soil organic carbon. The soil microbial quotient increased with progress of re-vegetation, indicating that the soil carbon element was effective and carbon sequestration capacity was increased and thus soil organic carbon

gradually accumulated in the enriched plantation plots. Tree species differ in quality of leaf litter (e.g. C: N ratio), so microbes receive organic matter of varying quality across stands of different tree species (Pastor and Post 1986). Soil microbes associated with different tree species often have variable amount of microbial biomass (Bauhus et al. 1998).

The lowest organic carbon and nitrogen values were obtained from soils of pure Pine plots whereas higher values were recorded in soils under enriched mixed systems proving that compared to sole plantations mixed plantations produce and conserve soil organic matter (Deng and Tabatabai 2000). In comparison to pure plantation the enriched plantation might have altered the interaction between microbes and the soil organic substrate by affecting substrate availability in and around them. Our study reflects that the nature and the quantity of microorganisms present in the soil may be different in the pure and enriched plantation.

Across the season, significant variations in the levels of microbial biomass carbon and nitrogen were recorded which may be due to variation in quantity and quality of decomposable substrates during and between saplings or in the composition and physiological state of the soil biological communities and in their responses to fumigation by chloroform in the microbial biomass carbon and nitrogen assay. There was a flush in the soil microbial biomass at the beginning of the plantation season. This can be attributed to application of farm yard manure (Paul and Beauchamp 1995). There was a gradual fall in the microbial biomass during vigorous growth stage of saplings, similar observations were reported by Ritz and Robinson (1988) in spring barley. During the active growth phase of the sapling uptake of nutrients by plants is increased resulting in intensive competition for nutrients and limits the quantities of nutrients available for microbial immobilization (Jones and Woodmansee 1979). At the later seedling stage, plant nutrient requirement is decreased and more of it is available for microbial biomass proliferation. A wider microbial C: N ratio was observed in the present study indicating a high proportion of fungi compared to bacteria and actinomycetes (Campbell et al. 1991). For the present study the microbial biomass C: organic C and microbial biomass N: organic N ratio was almost constant for both pure pine and enriched Pine stand indicating that the microbial communities in the enriched pine plots are at par with the pure pine plots in substrate use efficiency.

A good co-evolutionary relationship existed between the soil microbial biomass and other soil properties in enrichment plantation as indicated by the ANOVA table.

Advantages of successful enrichment systems have been related to minimizing inter specific competition for light, water and nutrients and indicated greater biological efficiency of saplings grown in association and was probably due to temporal and spatial complementarity effect (Singh and Arya 1999). Enrichment of pure Pine stand is a beneficial and good practice and should be encouraged for higher multipurpose benefits. More priority should be given to improve the soil health and utility of composition of Pine forests to improve their nutrient use efficiency and make them more productive.

ACKNOWLEDGEMENTS

We thank all members of Scientific Advisory Committee and the Director, GBPNIHESD.

REFERENCES

[1] Anderson, JM and Ingram, J. S. I: 1993 Tropical Soil Biology and Fertility: Second edition, A Handbook of Methods. Centre for Agriculture and Biosciences. International Publishing, Wallingford, United Kingdom. 221pages.

[2] Bauhus, J. Paré. D. and Côté, L. 1998. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30: 1077 - 1089.

[3] Brookes, P.C. 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils 19: 269 - 279.

[4] Brookes, P.C.; Landman, A.; Pruden, G. and Jenkinson, D. S.1985.Chloroform fumigation and release of soil N: a rapid direct extraction method to measure microbial biomass N in soil. Soil Biology and Biochemistry 17: 837 – 842.

[5] Buresh, R. J.1991. Extraction of ammonium, nitrate and nitrite from soil. In: IFDC / International Rice Research Institute Collaborative Project, 1991, pp. 1 – 3.

[6] Campbell, C. A.; Biederbeck, V. O.; Zenter, R.; P. and Lafond, G. P.1991. Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin Black Chernozem. Canadian Journal of Soil Science 71: 363 – 376.

[7] Deng, Q.; Chen, X.; Hui, D.; Zhang, Q.; Li, M. and Zhang, Q. 2016. Soil microbial community and its interaction with soil carbon and nitrogen dynamics following afforestation in central China. Science of the Total Environment 541:230-237.

[8] Deng, S.P. and Tabatabai, M.A. 2000. Effect of cropping systems on nitrogen mineralization in soils. Biology and Fertility of Soils 31: 211 – 218.

[9] Ghosh, P. and Dhyani, P. P. 2004. Baranaaja – traditional mixed cropping system of the central Himalaya. Outlook on Agriculture 33: 261 - 266.

[10] Ghosh, P. and Kashyap, A.K. 2003 Effect of rice cultivars on rate of N – mineralization, nitrification and nitrifier population size in irrigated rice ecosystem. Applied Soil Ecology 24: 27 – 4.1

[11] Harris, J.A. 2003. Measurement of the soil microbial community for estimating the success of restoration. European journal of Soil Science. 54: 801-808.

[12] Insam, H.; Parkinson, D. and Domsch, K.H. 1989. The influence of microclimate on soil microbial biomass level. Soil Biology and Biochemistry 21: 211-221.

[13] Jackson, M.L. 1958. Soil Chemical Analysis. Prentice-Hall Inc. Englewood, Cliffs, New Jersey. 197 – 201.

[14] Jenkinson, D.S. 1988. Determination of microbial biomass carbon and nitrogen in soil. In: Wilson, J.R. (Ed.), Advances in nitrogen cycling in agricultural ecosystems. Centre for Agriculture Biosciences International, Wallingford. 368 – 385.

[15] Jones, M.B. and Woodmansee, R.G. 1979. Biogeochemical cycling in annual grassland. Botanical Review 45: 111 – 114.

[16] Li, L.; Zang, Y. and Wang, L. 2007. Analysis of vertical distribution and correlation of soil microbial biomass of different forest lands. Journal of Central South University of Forestry and Technology. 27:52-57.

[17] Liu, S. and Wang, C. 2010. Temporal and spatial patterns of soil microbial carbon and nitrogen in five types of temperate forests. Journal of Ecology. 30: 3135-3143.

[18] Moore, J.M.; Klose, S. and Tabatabai, M.A. 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biology and Fertility of Soils 31: 200 – 210.

[19] Pastor, J. and Post, W.M. 1986. Influence of climate, soil moisture and succession on forest carbon and nitrogen cycles. Biogeochemistry 2: 3 - 27.

[20] Paul, J.W. and Beauchamp, E.G. 1995. Availability of manure slurry ammonium for corn using ^{15} N- labelled $(NH_4)_2SO_4$ Canadian Journal of Soil Science 75: 35 – 42.

[21] Piper, C. S. 1944. Soil and Plant Analysis. Interscience, New York, 368 pp.

[22] Ritz, K. and Robinson, D. 1988. Temporal variations in soil microbial biomass Carbon and Nitrogen under a spring barley crop. Soil Biology and Biochemistry 20: 625 – 630.

[23] Singh, J.S.; Raghubanshi, A.S. and Singh, R.S. 1989. Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. Nature. 338: 499-500.

[24] Singh, R. D.; Ghosh, B. N.; Prakash, V.; Bisht, J. K. and Gupta, H. S. 2000. Soil management for agricultural production in North – West Himalayas. Vivekananda Parvatiya Krishi Anusandhan Sansthan (Indian Council of Agricultural Research) Technical Bulletin 17. Almora 263643, India.

[25] Singh, R. V. and Arya, M. P. S. 1999. Nitrogen requirement of finger millet (*Eleusine coracana*) and pulse intercropping system. Indian Journal of Agronomy 44: 47 – 50.

[26] Smith, J.L. and Paul, E. A. 1990. The significance of soil microbial biomass estimations. Soil Biochemistry 6: 357-396.

[27] SPSS. 2002. SPSS / PC SPSS for the IBM PC /XT /AT. Version 11.5, SPSS Inc., Chicago, Illinois.

[28] Vance, E.D., Brookes, P.C. and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass Carbon. Soil Biology and Biochemistry 19: 703 – 707.

[29] Walkley, A.A. 1947. Critical examination of a rapid method for determining organic carbon in soils – effects of variations in digestion conditions and of inorganic soil constituents. Soil Science 63: 251 – 264.

[30] Zhang, J. S., Guo, J.F. and Chen, G.S. 2005. Concentrations and seasonal dynamics of dissolved organic carbon in forest floors of two plantations (*Castanopsis kawakamii* and *Cunninghamia lanceolata*) in subtropical China. Journal of Forestry Research. 16: 205-208.