Turk J Agric For
32 (2008) 281-288

The Effect of Different Land Uses on Soil Microbial Biomass Carbon and Nitrogen in Bartın Province

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Received: 07.11.2007

Abstract: The microbial biomass of soil is being increasingly recognized as a sensitive indicator of soil quality. Its knowledge is fundamental for sustainable environmental management. This study aimed to determine the impact of different land uses (forest, pasture, and agricultural lands) on soil microbial biomass carbon and nitrogen using the chloroform fumigation extraction (CFE) method. This study also aimed to determine interrelationships between microbial biomass C (C_{mic}) and N (N_{mic}) and the physico-chemical characteristics of the soil. For this purpose, a total of 45 soil samples were taken from 3 different land uses located in the Ağdacı Village in Bartın. Additional core samples were collected from each sample site to determine other physico-chemical characteristics of the soils. The average microbial biomass C were found as 1028.29 μg g^{-1}, 898.47 μg g^{-1}, and 485.10 μg g^{-1}, respectively, for forest, pasture, and agricultural soils. As with microbial biomass C, the average microbial biomass N was found as 129.99 μg g^{-1}, 100.90 μg g^{-1}, and 42.60 μg g^{-1}, respectively, for forest, pasture, and agricultural soils. One-Way ANOVA showed a significant difference in microbial biomass C and N among the study areas. Microbial biomass C and N were shown to be significantly correlated to the physico-chemical properties of the soil, such as organic C, total N, clay, and pH. Present study clearly shows that land use has a significant effect on microbial biomass C and N in soil by altering natural soil characteristics under the same ecological conditions.

Key Words: Microbial biomass C, microbial biomass N, land use type, soil

Bartın Yöresinde Farklı Arazi Kullanım Şekillerinin Toprağın Mikrobiyal Biyokütle Karbon ve Azot İçeriğine Etkisi

Özet: Toprak mikrobiyal biyokütlesi toprak kalitesinin hassas bir göstergesi olarak değerlendirilmektedir. Bu çalışmada, farklı arazi kullanım şekilleri (orman, mera ve tarım) altındaki üst toprakların (0–5 cm) mikrobiyal biyokütle C ve N içerikleri kloroform fumigasyon ekstraksiyon metodu kullanılarak araştırılmıştır. Aynı zamanda incelenen topraklardaki mikrobiyal biyokütle C ve N ile diğer bazı fiziksel ve kimyasal toprak özellikleri arasındaki ilişkilerin de ortaya konulması amaçlanmıştır. Bu amaçla, Bartın İli Ağdacı Köyü civarında yer alan üç farklı arazi kullanım şekillerinden çalışma alanını temsil edecek şekilde rasgele toplam 45 adet toprak örneği alınmıştır. Ayrıca toprakların fiziksel ve kimyasal özelliklerini belirlemek için her örnek alandan bozulmamış toprak örnekleri alınmıştır. Toprakların ortalama mikrobiyal biyokütle C içerikleri, orman alanında 1028.29 μg g^{-1}, mera alanında 898.47 μg g^{-1}, ve tarım alanında 485.10 μg g^{-1}, olarak bulunmuştur. Ortalama mikrobiyal biyokütle N içerikleri ise orman alanında 129.99 μg g^{-1}, mera alanında 100.90 μg g^{-1}, ve tarım alanında 42.60 μg g^{-1} olarak ölçülmüştür. Basit varyans analizi sonuçlarına göre; farklı arazi kullanım şekilleri mikrobiyal biyokütle C ve N içeriklerinde önemli farklılıklara yol açmıştır. Mikrobiyal biyokütle C ve N ile organik C, toplam N, % kil ve pH v.b. fiziksel ve kimyasal toprak özellikleri arasında istatistikî anlamda ilişki bulunmuştur. Yapılan bu araştırma, aynı yetişme ortamı koşulları altında, arazi kullanım şekillerinin toprak özelliklerini değiştirdiğini ve böylece toprakların mikrobiyal biyokütle C ve N içeriklerini etkilediğini göstermiştir.

Anahtar Sözcükler: Mikrobiyal biyokütle C, mikrobiyal biyokütle N, arazi kullanım şekli, toprak

Introduction

Soil organic matter is an important component of soil quality and productivity; however, its measurement alone does not adequately reflect changes in soil quality and nutrient status (Franzluebbers et al., 1995; Bezdicek et al., 1996). Measurements of biologically active fractions of organic matter, such as microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), and potential C and N mineralization,

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could better reflect changes in soil quality and productivity that alter nutrient dynamics. This reflection is based upon rapidly changing capacity of both C and N forms (Saffigna et al., 1989; Bremner and Van Kessel, 1992). These fractions can also provide an assessment of soil organic matter changes induced by management practices, such as forest, tilling, and cropping (Campbell et al., 1989).

The importance of microorganisms in ecosystem functioning has led to an increased interest in determining soil microbial biomass (Azam et al., 2003). The soil microbial biomass is the active component of the soil organic pool, which is responsible for organic matter decomposition affecting soil nutrient content and, consequently, primary productivity in most biogeochemical processes in terrestrial ecosystems (Franzluebbers et al., 1999; Gregorich et al., 2000; Haney et al., 2001). Therefore, measuring microbial biomass is a valuable tool for understanding and predicting long-term effects on changes in land use and associated soil conditions (Sharma et al., 2004).

The absence of available data indicating the effects of land use change on soil microbial C and N have led us to assess the impact of these changes for forest, pasture, and agricultural lands in the western Black Sea region of Turkey. The second objective of this study was to establish relationships between microbial biomass C (C_{mic}) and N (N_{mic}) and the physico-chemical characteristics of the soil, such as texture, organic C, N_{total}, $CaCO_3$ and pH under the same ecological conditions.

Materials and Methods

Study area

The study area is located in the vicinity of the Bartın province in Northwest Turkey (41°38´ N, 32°20´ E). Three different land use types are considered for this study (forest, pasture, and agricultural lands) which more or less exhibit the same ecological conditions. The elevation of the forest land is approximately 100 m and the average slope is 55%, with a northeast aspect. The vegetation of the forest land consists of *Fagus orientalis* Lipsky, *Quercus petraea* (Mattuschka) Liebl., *Carpinus betulus* L., *Tilia tomentosa* Moench., *Rhododendron ponticum* L. *subsp. ponticum*, *Ilex colchica* Poj., *Crataegus curvisepala* Lindman, *Ruscus aculeatus* L. The elevation of the pasture land is approximately 90 m and the average slope is 40%, with a north-northeast aspect. The vegetation of the pasture land consists of *Dactylis glomerata* L., *Bellis perennis* L., *Euphorbia* sp., *Geranium* sp., *Vicia sativa* L., *Trifolium* sp., *Pteridium aquilinum* L., *Rubus* sp. and *Rosa canina* L. The elevation of the agriculture land is approximately 70 m and the average slope is 20%, with a north-northwest aspect. The major annual crops alternately cultivated in the agriculture land include maize (*Zea mays* L.) and wheat (*Triticum vulgare* L.). At the end of the harvest periods, a common vetch (*Vicia sativa* L.) occurs as an herbaceous plant within the area. In the agricultural area, before sowing, DAP (diammonium phosphate) fertilizer is used and, after sowing, CAN (calcium ammonium nitrate) fertilizer is used once a year. Principal geological formation of the research area consists of limestone rock. The climate in this region is humid mesothermal characterized by warm summers. According to the climatological data gathered over the past 30 years, the annual mean temperature in this province is 12.6 °C. The mean temperatures of the hottest months, July and August, are 22.4 and 21.9 °C, respectively. Annual mean precipitation in the region is 1087 mm and annual relative humidity is 80%.

Soil sampling

This experiment was conducted in a completely randomized design. Undisturbed soil core samples from mineral top soil (0-5 cm) were collected using stainless steel rings (10 cm diameter, 5 cm height) from 15 different points of each land use types. Stones, plant and root debris were removed. Soil samples were passed through a 2 mm sieve and stored at 4 °C before microbial analysis. Subsamples of soils were air-dried and grounded to pass through a 2 mm sieve for physical and chemical analysis.

Physical and chemical properties of soils

Physical and chemical properties of soils were determined by standard methods: soil particle size distribution by the hydrometer method (Bouyoucos, 1962), pH in 1:2.5 soil/water suspension by pH-meter (Rowell, 1994), EC in 1:5 soil/water suspension by an electrical conductivity meter (Rhoades, 1982), soil moisture by the gravimetric method, soil organic matter by the Walkley-Black wet oxidation method (Walkley and Black, 1934), total nitrogen by the Kjeldahl method (Bremner and Mulvaney, 1982), and $CaCO_3$ content by the Scheibler calcimeter method (Allison and Moodie,

1965). The bulk density of soils (g cm^{-3}) was calculated using mass and volume (Blake, 1965). The particle density of soils (g cm^{-3}) was measured using the Pycnometer method and pore space was calculated using the bulk and particle densities (Brady, 1990).

Microbial biomass C (C_{mic})

Soil microbial biomass C was estimated by extracting 30 g of oven dry equivalents from field-moist mineral soil samples in 0.5 M K_2SO_4 (1:4 w/v), known as the chloroform-fumigation-extraction method, described by Brookes et al. (1985) and Vance et al. (1987a). Duplicate subsamples from each type of soil were placed in 50 ml glass beakers. Samples designated for fumigation were placed in vacuum desiccators. Ethanol-free $CHCl_3$ containing boiling chips were placed in a 50 ml beaker in the center of the desiccator. Paper towels, moistened with deionized water, were also placed in each desiccator to help maintain the water content of soils during fumigation. The desiccators were sealed, placed in a laboratory hood, and evacuated, allowing the chloroform to boil for approximately 30 s. Samples were fumigated for 24 h in the dark at 25 °C. After the chloroform was removed, soils were transferred to a 250 ml bottle where 120 ml of 0.5 M K_2SO_4 was then added. At the same time, unfumigated soil samples were placed in the bottles and were treated in the same way, where they serve as controls. Bottles were shaken for 30 min on a reciprocating shaker and supernatants were filtered through a Whatman no. 42 filter. Filtrates were kept for up to 1 week at 4 °C.

Microbial biomass C was measured in 8 ml aliquots of K_2SO_4 extracts after oxidation with 0.4 N $K_2Cr_2O_7$ at 150 °C for 30 min and back-titration with ferrous ammonium sulphate. Microbial biomass C was calculated by measuring the difference in extractable organic C between the fumigated and unfumigated soils, which are simply formulated as Equation 1 (Vance et al., 1987a):

$$\text{Biomass C} = 2.64 \times E_C \quad (1)$$

where E_C refers to the difference in extractable organic C between the fumigated and unfumigated treatments, 2.64 is the proportionality factor for biomass C released by fumigation extraction.

Microbial biomass N (N_{mic})

The Kjeldahl digestion-distillation-titration method was used to determine the total N in the K_2SO_4. With 10 ml of 95% H_2SO_4, 15 ml of extract was digested after the addition of 0.4 ml of 0.2 M $CuSO_4$ to promote organic matter breakdown. This mixture was digested at 380 °C for 3 h until all of the organic compounds were decomposed. The solution was brought to a volume of 250 ml with deionized water. A 50 ml subsample was steam-distilled in a strong alkaline solution (10 M NaOH) and the distillate was collected in a boric acid-mixed indicator solution; the solution was then back-titrated (Anderson and Ingram, 1993). N was calculated using the following Equation 2 (Brookes et al., 1985):

$$\text{Biomass N} = F_N / 0.54 \quad (2)$$

where F_N = (total N from fumigated soil) - (total N from unfumigated soil).

Statistical analyses

Statistical analyses were carried out using SPSS 11.00 package program. The effect of land uses on soil properties was determined by 1-way analysis of variance. A 95% confidence limit ($P < 0.05$) was chosen to indicate differences between samples. Student Newman Keuls (S-N-K) were calculated when samples were significantly different. Data were analyzed by correlation analysis to evaluate relationships between different soil parameters.

Results

Mean values of major physical and chemical properties of forest, pasture, and agricultural soils are presented in Table 1. According to 1-Way ANOVA test, the determined bulk density, particle density, pore space, percentage of clay, pH, organic C (C_{org}), and total N (N_{total}) differ significantly by land use types (Table 1).

In this study, the mean values for microbial biomass C were 1028.29 µg g^{-1}, 898.47 µg g^{-1}, and 485.10 µg g^{-1} in the forest, pasture and agricultural soils, respectively. Accordingly, the mean microbial biomass N values under the forest, pasture and agricultural soils were 129.99 µg g^{-1}, 100.90 µg g^{-1}, and 42.60 µg g^{-1}, respectively. The results of the soil microbial biomass analysis also indicate that the forest soil contains the highest microbial biomass C and N. One-way ANOVA test implies that there is a significant difference among the soil microbial biomass C and N contents of the 3 land uses. It may be clearly understood that land use type has a significant impact on the soil microbial biomass C and N (Table 2). The variations in the microbial biomass C and N among the

Table 1. Physical and chemical properties of soils in the different land uses.

Soil properties	Forest	Pasture	Agriculture
Bulk density (g cm^{-3})	0.97 (± 0.15)[a]*	1.16 (± 0.08)[b]	0.98 (± 0.08)[a]
Particle density (g cm^{-3})	2.52 (± 0.03)[a]	2.60 (± 0.06)[b]	2.72 (± 0.02)[c]
Pore space (%)	61.58 (± 5.81)[a]	55.07(± 3.98)[b]	64.06(± 3.30)[a]
Sand (%)	23.17 (± 6.20)[a]	22.64 (± 3.99)[a]	15.7 (± 3.03)[a]
Clay (%)	37.27 (± 18.59)[a]	43.68 (± 9.95)[a]	61.84 (± 4.96)[b]
pH (H_2O)	5.20 (± 0.53)[a]	6.62 (± 0.90)[b]	7.84 (± 0.31)[c]
$CaCO_3$ (%)	0.03 (± 0.07)[a]	2.44 (± 0.08)[a]	5.93 (± 0.03)[a]
Electrical conductivity (dS m^{-1})	0.08 (± 0.02)[a]	0.12 (± 0.08)[a]	0.17 (± 0.03)[a]
Organic C (%)	4.14 (± 0.22)[a]	2.69 (± 0.60)[b]	1.19 (± 0.27)[c]
Total N (%)	0.32 (± 0.02)[a]	0.27 (± 0.05)[b]	0.15 (± 0.02)[c]

*Values are the mean of 15 samples and respective standard deviations are in brackets. Values in the same row that are followed by the different letter indicate significant ($P < 0.05$) differences between means.

Table 2. Microbial biomass C and N values of soils in different land uses.

Land uses	Microbial Biomass C (μg g^{-1})			Microbial Biomass N (μg g^{-1})		
	Min.	Max.	Mean	Min.	Max.	Mean
Forest	588.09	1309.54	1028.29 ± 192.50[a]	61.19	229.87	129.99 ± 45.25[a]
Pasture	428.95	1418.24	898.47 ± 228.64[b]	39.36	236.22	100.90 ± 46.10[b]
Agriculture	97.18	682.04	485.10 ± 105.54[c]	18.00	84.81	42.60 ± 14.80[c]

Values are Min., Max. and Mean ± SD (n = 15). Different letters (a, b, c) show that there is difference among the mean values at the significance level $P < 0.05$.

land use types indicate the differences in microbial activities.

Pearson's correlation coefficients between C_{mic}, N_{mic}, organic C, total N, pH, bulk density, particle density, pore space, and clay were calculated (Table 3). The highest positive correlations were between C_{mic} and N_{mic}, C_{mic} and organic C, N_{mic} and organic C, C_{mic} and total N, organic C and total N. On the other hand, the lowest negative correlations were between organic C and pH, C_{mic} and clay, organic C and clay, bulk density and pore space.

Discussion

Microbial biomass C values obtained in the present study well coincide with those reported (61-2000 μg g^{-1}) by Vance et al. (1987b) and (102-2073 μg g^{-1}) by Hernot and Robertson (1994) for various temperate and tropical forest soils. Microbial biomass C values found by the other studies range from 279 to 910 μg g^{-1} for stands of different age, soil type, and species composition (Bauhus et al., 1998), and from 219 to 864 μg g^{-1} for different land uses (forest, agroforestry, agriculture, and wasteland) according to Sharma et al. (2004). Microbial biomass C values range from 166 to 1539 μg g^{-1} for pasture land (Tracy and Frank, 1998), from 726.70 to 1529.14 μg g^{-1} for wasteland (Kara and Bolat, 2007), and are at mean values of 1684 μg g^{-1} for Sacred Grove forest, 806.1 μg g^{-1} for grassland (Arunachalam et al., 1999), 248 μg g^{-1} for agricultural soil, and 1326 μg g^{-1} for forest soil (Hu et al., 1997).

The microbial biomass N values are in accordance with the results of the previous works, reported by Sharma et al. (2004) for different land uses (forest, agroforestry, agriculture, and wasteland) (30-142 μg g^{-1}), and by Diaz-

Table 3. Correlation matrix (r-values) for physical, chemical, and microbiological characteristics of soils in different land uses.

Variables	A	B	C	D	E	F	G	H	I
C_{mic} (µg g^{-1})	1	0.713**	0.719**	0.678**	-0.589**	0.119	-0.747**	-0.319	-0.608**
N_{mic} (µg g^{-1})		1	0.668**	0.586**	-0.590**	0.099	-0.640**	-0.380*	-0.475**
Organic C (%)			1	0.910**	-0.905**	-0.026	-0.899**	-0.363*	-0.710**
Total N (%)				1	-0.817**	0.091	-0.799**	-0.431**	-0.656**
pH (H_2O)					1	0.148	0.809**	0.280	0.649**
Bulk density (g cm^{-3})						1	-0.039	-0.792**	0.069
Particle density (g cm^{-3})							1	0.412**	0.762**
Pore space (%)								1	0.450**
Clay (%)									1

A: C_{mic} (µg g^{-1}), B: N_{mic} (µg g^{-1}), C: Organic C (%), D: Total N (%), E: pH (H_2O), F: Bulk density (g cm^{-3}), G: Particle density (g cm^{-3}), H: Pore space (%), I: Clay (%), Pearson's correlation coefficient, n = 15, * $P < 0.05$, ** $P < 0.01$.

Ravina et al. (1988) for soils of broad leaved deciduous forest (132-240 µg g^{-1}) and evergreen forest (42-242 µg g^{-1}). Also included are microbial biomass N stated by Martikainen and Palojarvi (1990) for coniferous forest soils (52-125 µg g^{-1}), by Tracy and Frank (1998) for pasture soil (50-463 µg g^{-1}), by Garcia and Rice (1994) for pasture soil (mean 116 µg g^{-1}), and by Cleveland et al. (2003) for forest (mean 251.3 µg g^{-1}) and pasture soils (153.9 µg g^{-1}). These differences in the microbial biomass C and N may be due to the climatic conditions, differences in ground cover vegetation, the number of roots, soil types and properties, types of land use and management, as well as variations in sampling times (Anderson and Domsch, 1989; Priha, 1999; Murrieta et al., 2007).

Both the forest and pasture soils have significantly greater organic C content and total N content in the study area when compared with agricultural soil (Table 1). In support of this finding, the highest microbial biomass C and N values were found in forest soils with highest organic C and N content. It is well known that soil organic C strongly affects the amount and activity of soil microbial biomass (Diaz-Ravina et al., 1988; Jenkinson, 1988).

There are positive and significant relations between the soil microbial biomass C and soil organic C (Figure 1A); and soil microbial biomass N and total N (Figure 1B). Our results are consistent with previously reported studies (Arunachalam and Arunachalam, 2000; Sharma et al., 2004; Wright et al., 2005). The relatively dense structure of plants and a greater accumulation of litter and fine roots in the understorey of forest and pasture may favor the growth of microbial populations and the accumulation of C in microbial biomass.

In this study, there is a significant positive relationship between soil organic C and total N (Figure 1C). Similarly, previous studies state that if soil organic C increases, the total N increase (Manu et al., 1991; Li et al., 2007). The dynamics of N in mineral soil is closely linked to C, because most N exists in organic compounds and heterotrophic microbial biomass, which utilize organic C for energy. As a result, the microbial biomass N showed a significant positive correlation with microbial biomass C (Figure 1D). The result coincides with previous studies (Klose and Tabatabai, 1999; Arunachalam and Arunachalam, 2000; Arunachalam and Arunachalam, 2002; Sharma et al., 2004; Wright et al., 2005).

The pH of soils in forest, pasture, and agricultural land were moderately acidic (pH 5.20), lightly acidic (pH 6.62) and lightly alkaline (pH 7.84), respectively. Relatively high values of microbial biomass C and N in the forest and pasture soils, compared to agricultural soil, was likely due to pH, because it showed a negative correlation with microbial biomass C and microbial biomass N (Table 3). The results of this study reveal that distinct plant community composition associated with 3

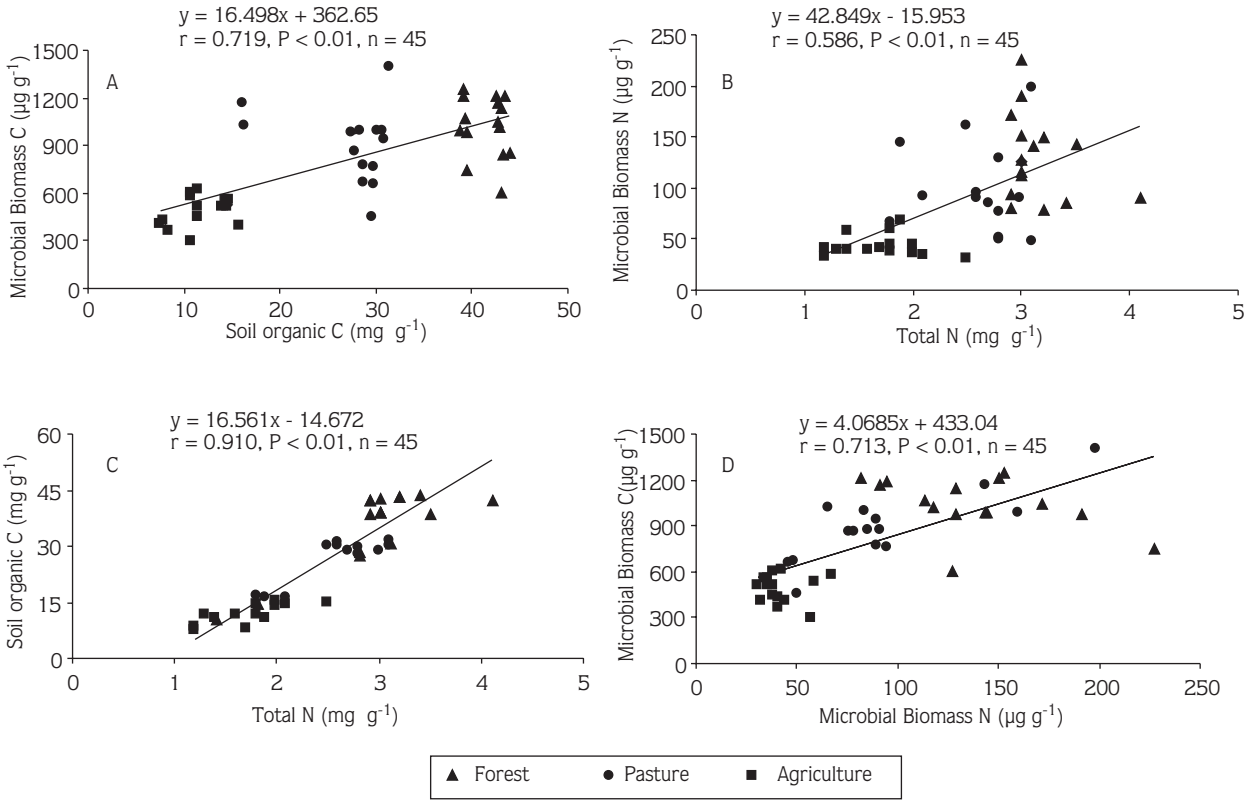

Figure 1. Relation between the microbial biomass C and soil organic C (A), the microbial biomass N and total N (B), the soil organic C and total N (C), the microbial biomass C and N (D) determined for soils under different land uses.

land use types, reflecting changes in soil pH and microbial biomass. Previous work has shown variability in microbial biomass that can be caused by alterations in soil pH (Wardle, 1992). Some authors suggest that maximum activities of soil microbial biomass occur at pH values of about 6.5 (Tabatabai, 1994; Acosta-Martínez and Tabatabai, 2000).

The clay content of soil is known to play a role in the determining microbial biomass and activity as well as influencing the composition of microbial community (McCulley and Burke 2004). Soils with high clay content lead to more stabilization of soil organic C and higher microbial biomass (Schimel et al., 1994). In contrast, our results indicate that C_{mic} ($r = -0.608$, $P < 0.01$) and N_{mic} ($r = -0.475$, $P < 0.01$) were negatively correlated with clay content of soils. Most likely this is due to the variability in the controlling factors of microbial biomass, such as soil organic matter, management practices, and plant species composition, that may have masked the impact of clay content on soil microbial biomass.

Conclusion

Results from the present study demonstrate that management practices and certain types of vegetation exert a profound influence on microbial biomass C and N. Different plant species affect soil microbial processes, which are dependent upon their litter quality and quantity and also upon below-ground biomass supporting microbial activities. The substrate and nutrient limitation of microbial biomass and their central role in the soil nutrient cycling facilitate the use of microbial biomass as an indicator for soil health of land use types. Our data suggest that forest soil may be healthier when compared to other land use soils. In other words, the soil health of land use types is in the order of forest, pasture, and agriculture.

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