

Research Paper

Humic fractions of forest, pasture and maize crop soils resulting from microbial activity

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Abstract

Humic substances result from the degradation of biopolymers of organic residues in the soil due to microbial activity. The objective of this study was to evaluate the influence of three different ecosystems: forest, pasture and maize crop on the formation of soil humic substances relating to their biological and chemical attributes. Microbial biomass carbon (MBC), microbial respiratory activity, nitrification potential, total organic carbon, soluble carbon, humic and fulvic acid fractions and the rate and degree of humification were determined. Organic carbon and soluble carbon contents decreased in the order: forest > pasture > maize; humic and fulvic acids decreased in the order forest > pasture=maize. The MBC and respiratory activity were not influenced by the ecosystems; however, the nitrification potential was higher in the forest than in other soils. The rate and degree of humification were higher in maize soil indicating greater humification of organic matter in this system. All attributes studied decreased significantly with increasing soil depth, with the exception of the rate and degree of humification. Significant and positive correlations were found between humic and fulvic acids contents with MBC, microbial respiration and nitrification potential, suggesting the microbial influence on the differential formation of humic substances of the different ecosystems.

Key words: humification, microbial biomass carbon, potential nitrification, respiratory activity.

Introduction

In natural ecosystems such as forests, there is a balance between the release of nutrients from litter and uptake by plants, so that the organic carbon content of the soil remains stable over time (Li *et al.*, 2005). Many forest areas have been converted in agricultural areas to attend to the growing demand for foods by the population. In Brazil, maize and soybean constitute 84% of agricultural production (IBGE, 2012). However, depending on land uses, specifically pastures and annual crops, changes can occur in the physical, chemical and biological characteristics of the soil (Boley *et al.*, 2009).

Recent studies reported that the content and quality of soil organic matter (SOM) and the biochemical activity are changed when natural ecosystems such as forest are re-

placed by different land uses such as pasture and annual crops (Cardelli *et al.*, 2012). In the degradation process of SOM, the organic carbon is the main component to be studied, with two main fractions: non-humic substances (proteins, carbohydrates, resins and lignins) that correspond to 10-15% of total carbon, and humic substances (humic acids, fulvic acids and humin) (Wolf *et al.*, 2005). It is believed that humic substances are the main indices of soil fertility influencing crop productivity (Ufimtseva and Kalganov, 2011). An increase in the concentration of humic acid by 11-28%, has been reported after the incorporation into the soil of various cover crops (Arlauskiene *et al.*, 2010). Consequently, it is expected that the content of humic acids will change during the conversion of ecosystems. Therefore, it is important to know the SOM mineralization, especially when the natural ecosystem is altered.

The humification of SOM can be understood as a process of synthesis and/or resynthesis of organic compounds that are added to soil and it depends on various factors such as climate, amount and quality of embedded plant material and soil management. According to Wu and Ma (2002), based on the predominant ecosystem management, different types of humic substances will be produced. The degree of humification has been shown to be an index that can respond appreciably to the addition of organic matter to the soil (Canali *et al.*, 2004). Therefore, any factor that influences the activity of microorganisms can modify the chemical properties of the soil.

Differences in the content of organic matter tend to vary according to the use of the soil. For example, the soil under annual oats had 26-42% lower total organic carbon and total nitrogen in comparison with native pasture (Li *et al.*, 2007). However, among the various factors that influence these processes, microbial biomass and mineralization of C and N were suggested as potential indicators to assess changes in soil quality (Alvear *et al.*, 2005; Oorts *et al.*, 2007), but they have not been sufficiently evaluated in ecosystems of forests, pasture and annual crops, especially at the conditions studied. The biochemical attributes of soil are valuable measures to assess changes resulting from microbial activity of soil from different agricultural ecosystems (Bending *et al.*, 2004).

The aim of this study was to evaluate the influence of three ecosystems (forest, pasture and maize) on the contents of soil humic substances and their relationship with the biological (microbial biomass carbon, microbial respiration and nitrification potential) and chemical attributes (total organic carbon and soluble carbon).

Materials and Methods

Study site and sampling methods

The study was carried out in the UNESP/Jaboticabal (21°15'5" S and 48°17'09" W), at an altitude of 595 m and with Aw climate, characterized as humid tropical with rainy summers and dry winters according to the Köppen

classification. The average annual rainfall was 1412 mm and temperature 22 °C. The selected areas were forest, pasture and annual crops. The pasture area of 21 hectares, planted with *Brachiaria decumbens* for 15 years, contained 30 Nelore cattle under a rotational grazing system. The annual crop area of 20 ha was rotated with soybean and corn for the last three years and was fertilized with 450 kg of NPK formulation (5-15-10) ha⁻¹. The soil of these areas (Table 1) was Oxisol. Soil sampling was carried out at the end of the maize period, *i.e.*, in August 2008, in three areas: forest, pasture and annual crops. In each area, four sub-areas with about 100 m² each were randomly selected. In each sub-area, twenty points were randomly selected. Thus, the experimental grid consisted of a total of 240 sub-sampling points. In each point, sub-samples were collected at 0-10, 10-20 and 20-40 cm deep with a Dutch auger. The sub-samples from each point and deep were pooled (composite samples), transported to the laboratory within five hours and sieved at 2 mm. Part of the sample, stored at 4 °C for up to 30 days, was used for biological analysis and part was air dried and used for chemical analysis.

Analytical procedures

The microbial respiration was determined after soil incubation at 30 °C (Rezende *et al.*, 2004). The microbial biomass was determined by fumigation-extraction (Vance *et al.*, 1987); the nitrification potential was determined by incubating the samples at 30 °C (Schmidt and Belser, 1994) and evaluating the NO₃⁻ produced, according to the procedure of Keeney and Nelson (1982). The total organic carbon content was determined after soil digestion with potassium dichromate (Sims and Haby, 1971); the SC content was determined after extraction with deionized water (Davidson *et al.*, 1987). The humic substances were extracted using NaOH, dispensing the humin fraction (Benites *et al.*, 2003). Fulvic acids and humic acids were separated after bringing the pH of the extract to 1.0 with 20% H₂SO₄. The humification rate (HR) and degree of humification (DH) were calculated as reported by Ciavatta *et al.*

Table 1 - Chemical composition of the forest, pasture and maize soils.

Soil	Deep (cm)	pH	SOM (%)	P resin (mg dm ⁻³)	K (mmol _c dm ⁻³)	Ca (mmol _c dm ⁻³)	Mg (mmol _c dm ⁻³)	H+Al (mmol _c dm ⁻³)	SB (mmol _c dm ⁻³)	T (mmol _c dm ⁻³)	V (%)
Forest	0-10	6.0	6.0	16	5.3	116	42	20	163.3	183.3	89
	10-20	5.7	2.8	6	5.2	61	32	25	98.2	123.2	80
	20-40	5.6	2.6	4	5.0	49	30	28	84.0	112.0	75
Pasture	0-10	5.5	3.8	19	3.6	45	23	31	71.6	102.6	70
	10-20	5.4	2.3	8	2.9	39	12	31	53.9	84.9	63
	20-40	5.4	1.7	6	2.4	31	10	28	43.4	71.4	61
Maize	0-10	5.6	2.5	28	6.0	45	21	28	72.0	100.0	72
	10-20	5.1	2.0	19	3.8	27	14	34	44.8	78.8	57
	20-40	4.8	1.8	14	2.9	18	8	42	28.9	70.9	41

(1990): $HR (\%) = 100 \times (C_{HA} + C_{FA})/TOC$; $DH (\%) = 100 \times (C_{HA} + C_{FA})/SC$.

Statistical analysis

Analyses of variance were performed using a split-plot design (three replications) with ecosystems (forest, pasture and annual crops) as the main factor and soil depth (0-10, 10-20 and 20-40 cm deep) sampling as a sub-factor, with four replicates. Data were analyzed using the SAS statistical package. The difference between treatment means was determined by Tukey's test at a 5% significance level. Pearson's correlation coefficient (r) was used to examine the relationship between two parameters at the 1% significance level.

Results and Discussion

The results of this study confirm those of Zinn *et al.* (2005) and Boley *et al.* (2009), suggesting that the conversion of forest to pasture and maize crop significantly influences the content of total organic carbon (TOC) and soluble carbon (SC). The TOC and the SC content from forest soil decreased significantly ($p < 0.05$), from 36% to 53% and from 40% to 61% compared to pasture and maize, respec-

tively, in the top soil layer, in the following order: forest > pasture > maize (Table 2). Below 10 cm depth, the soil contents of TOC and SC did not differ significantly between the ecosystems, except for SC in the 10-20 cm deep. Several changes of TOC contents occur when forest is cultivated. The decrease of 36% in the TOC content of pasture in relation to forest soil at 0-10 cm was similar to that reported by Glaser *et al.* (2000), who found a 30% decrease due to organic matter and soil humus loss. In addition, the concentration of C decreased by 37% (on average) after conversion of forest to pasture and banana plantation, due the reduction of C incorporation with land-use change (Powers, 2004). However, Saviozzi *et al.* (2001) found a 61% reduction in the content of organic carbon after conversion of forest to agriculture, although they did find a 37% increase after conversion from forest to pasture. Likewise, Boley *et al.* (2009) observed that the quantities of soil TOC were not influenced by conversion of forest to pasture in Costa Rica. The SC to TOC ratio (calculated from the data of Table 2) ranged from 0.27 to 0.76 (mean 0.50); the highest values were observed on the soil surface and values decreased with soil depth. The mean ratio observed in our study was similar to that found by Bonifacio *et al.* (2008) in

Table 2 - Total organic carbon, soluble carbon, humic acid and fulvic acid contents of the forest, pasture and maize soils.

Deep (cm)	Forest	Pasture	Maize	F
Total organic carbon (mg C g ⁻¹)				
0-10	24.32 ^{Aa}	15.46 ^{Ba}	11.42 ^{Ca}	44.78**
10-20	12.92 ^{Ab}	11.63 ^{Ab}	9.85 ^{Aa}	2.44 ^{ns}
20-40	10.82 ^{Ab}	7.75 ^{Ac}	9.27 ^{Aa}	2.42 ^{ns}
F test	53.26**	14.99**	1.24 ^{ns}	
Soluble carbon (mg C g ⁻¹)				
0-10	18.39 ^{Aa}	10.95 ^{Ba}	7.12 ^{Ca}	38.74**
10-20	8.01 ^{Ab}	4.15 ^{Bb}	4.71 ^{Bab}	5.14**
20-40	2.87 ^{Ac}	3.32 ^{Ab}	2.48 ^{Ab}	0.21 ^{ns}
F test	70.40**	19.78**	6.07**	
Humic acid (mg C g ⁻¹)				
0-10	3.45 ^{Aa}	2.34 ^{Ba}	1.82 ^{Ba}	11.51**
10-20	2.33 ^{Ab}	1.93 ^{Aab}	1.79 ^{Aa}	1.29 ^{ns}
20-40	2.07 ^{Ab}	1.31 ^{Ab}	1.65 ^{Aa}	2.38 ^{ns}
F test	11.28**	5.56*	0.17 ^{ns}	
Fulvic acid (mg C g ⁻¹)				
0-10	1.70 ^{Aa}	1.33 ^{Ba}	1.25 ^{Ba}	14.75**
10-20	1.14 ^{Ab}	0.96 ^{Ab}	1.09 ^{Aa}	2.48 ^{ns}
20-40	0.86 ^{Ac}	0.88 ^{Ab}	1.05 ^{Aa}	2.89 ^{ns}
F test	49.24**	16.31**	2.91 ^{ns}	

Lower case letters on the same column and upper case letters on the same row show no significant differences. F test: (*) 0.05; (**) $p < 0.01$ and ns, no significant.

forest of Central Europe, which ranged from 0.54 to 0.56. This result suggests that 50% of the carbon is in assimilable form or more susceptible to microbial decomposition.

The lack of a significant difference in the TOC content between maize soil layers may be related to soil disturbance leading to greater homogeneity, which did not occur in the other ecosystems. The decrease in TOC content in the lower layers of forest and pasture soils may be due to the deposition of organic matter on the soil surface (Bianchi *et al.*, 2008).

The determination of the humic acid (HA) and fulvic acid (FA) contents can provide an estimate of the effect of the different ecosystems studied on the process of humification of SOM. The HA and FA contents were highest in forest soil and decreased by 32% and 22% in pasture soil and by 47% and 27% in maize soil, respectively, in the 20-40 cm soil layer ($p < 0.05$). However, no significant difference was found between the contents of the 10-20 and 20-40 cm layers in the three ecosystems (Table 2). The decrease in the HA and FA contents in the surface layer of pasture and maize, in relation to forest soil, was much lower than that reported by Islam and Weil (2000), between 30% and 79% of HA and between 77% and 156% of FA, respectively. However, Portugal *et al.* (2008) found reductions of 26% HA and 49-61% FA in forest soil compared to citrus and pasture, respectively. Therefore, different results can be found depending on the region and environmental conditions of the study. Because the forest soil has no human interference, it is presumed to provide a favourable environment for microbial activity and synthesis of humic substances, unlike the pasture and maize soils. This can be explained by the similar trend observed in the variation of TOC, SC, HA and FA, *i.e.* higher concentrations in forest soil than in the other soils. A significant correlation was observed (Table 3) between HA and TOC ($r = 0.90^{**}$) and between FA and TOC ($r = 0.87^{**}$), similar to the results reported by Islam and Weil (18). This relationship indicates that the humification depends on SOM contents, as previ-

ously reported by González *et al.* (2003) and observed in the forest soil.

Among the physical factors, temperature and soil moisture can affect humification (Bonifacio *et al.*, 2008). In this study, a higher moisture content was found in the forest soil compared to other ecosystems (data not shown), which may have favoured the microbial activity and the formation of humus. Moreover, the accumulation of plant residues in the forest can outweigh the agricultural crops. In a pasture system, there is deposition of manure beyond that of plant residues. However, both the organic matter of the pasture and the residues of the maize crop deposited were not enough to boost the TOC and SC contents and thus, the accumulation of humic and fulvic acids was always significantly lower than that found in forest soil. Similar responses were found by Bonifacio *et al.* (2008) who found no change in the extractable C as well as the content of fulvic acids.

Both HA and FA contents decreased ($p < 0.05$) with increasing depth of forest soil; only in the last layer of the pasture soil was found significant results in relation to others soil layers; no differences were found in the amounts of humic acids between the layers of maize soil (Table 2). Similar responses were previously reported by Portugal *et al.* (2008), who observed a reduction in the HA and FA contents with depth in forest, citrus grove and pasture soils, and therefore in permanent systems.

Although the humification rate (HR) varied from 34% to 95% and the degree of humification (DH) from 25% to 29%, no significant differences were observed between the values found in the ecosystems studied (Table 4). A similar response in orange fertilized with composts and organic fertilizers was reported by Canali *et al.* (2004), who concluded that the composition and microbial activity were not affected by the addition of organic material. The lack of significant differences in the HR and DH values indicates that humification does not depend on the type of vegetation and the same proportion of organic matter is humified, even

Table 3 - Correlation between microbial and chemical attributes of the forest, pasture and maize soils.

Variables	CO ₂	TOC	SC	MBC	HA	FA	NP	GH
TOC	0.53**	-	-	-	-	-	-	-
SC	0.54**	0.92**	-	-	-	-	-	-
MBC	0.49**	0.44**	0.49**	-	-	-	-	-
Humic acid	0.35*	0.90**	0.81**	0.37*	-	-	-	-
Fulvic acid	0.54**	0.87**	0.91**	0.42**	0.77**	-	-	-
NP	0.64**	0.76**	0.76**	0.54**	0.66**	0.71**	-	-
HR	0.55**	-0.64**	-0.55**	-0.38*	-0.31 ^{ns}	-0.40*	0.54**	-
DH	0.52**	-0.61**	-0.75**	-0.65**	-0.50**	-0.73**	0.60**	0.42*

Significant *, $p < 0.05$; **, $p < 0.01$ and ns, no significant. CO₂, respiratory activity; TOC; total organic C; SC, soluble C; MBC, Microbial biomass C; HA, humic acid; FA, fulvic acid; NP, nitrification potential; HR, humification rate; DH, degree of humification.

Table 4 - Humification rate and degree of humification of the forest, pasture and maize soils

	Humification rate (%)	Degree of humification (%)
Soil (S)		
Forest	34.21 ^a	25.44 ^a
Pasture	60.34 ^a	26.00 ^a
Maize	94.96 ^a	28.77 ^a
Deep (P)		
0-10	48.14 ^c	24.09 ^b
10-20	70.53 ^b	27.39 ^a
20-40	70.84 ^a	28.71 ^a
F test (S)	2.99 ^{ns}	4.28 ^{ns}
F test (D)	26.66 ^{**}	8.25 ^{**}
F test (S x D)	2.35 ^{ns}	0.97 ^{ns}
C.V (S)	10.17	11.16
C.V (D)	5.63	10.73

Letters on the same column show no significant differences. F test: (**)
 $p < 0.01$ and ns, no significant.

if different amounts of material have been deposited in the soil (Bonifacio *et al.*, 2008). However, contrasting responses have been reported by the authors. For example, Gonzalez *et al.* (2003) reported, based on the values of HR, that humification is more intense in the presence of organic residues. The interpretation of HR and DH based on the C/N ratio of the soil was also contradictory. While Leng *et al.* (2009) reported significantly similar HR values in forest soils for different values of C/N, Marinari *et al.* (2007) found that a low C/N ratio favoured higher HR and DH in organic compared to conventional soil.

Both HR and DH increased significantly ($p < 0.05$) from the surface layer to the other soil layers (Table 4), confirming the findings of Favoreto *et al.* (2008). We assume that the majority of TOC and SC contents are in the form of humic substances, generating compounds more resistant to microbial attack. The negative and significant correlation between TOC and SC with HR and DH confirm this hypothesis (Table 3).

The microbial biomass carbon (MBC) contents were 58% and 59% higher in pasture and forest soils, respectively, than in maize soil, but no significant difference was observed (Table 5). Although in different proportions, reductions in the contents of MBC have been reported by several authors when comparing forests and pastures soils with agricultural crop. Riffaldi *et al.* (2003) reported that the MBC from pasture decreased by 30% to 34% when compared with crops, and Rangel and Silva (2007) found a reduction of 40% and 67% in the MBC of forest soil in relation to pasture and maize crop soils, respectively. The contents of the MBC were correlated with TOC ($r = 0.44^*$) and SC ($r = 0.49^*$) (Table 3). A significant and positive correlation between MBC and TOC was also reported by Islam

Table 5 - Microbial biomass carbon, respiratory activity and nitrification potential of the forest, pasture and maize soils.

	Microbial biomass C ($\mu\text{g C g}^{-1}$ soil)	Respiratory activity ($\text{mg CO}_2 \text{ g}^{-1}$ soil)	Nitrification potential ($\mu\text{g N-NO}_3\text{-g}^{-1}$ soil)
Soil (S)			
Forest	429.25 ^a	75.47 ^a	96.43 ^a
Pasture	425.22 ^a	82.50 ^a	89.43 ^a
Maize	269.79 ^a	77.79 ^a	79.09 ^b
Deep (D)			
0-10	527.88 ^a	139.39 ^a	113.07 ^a
10-20	359.61 ^b	63.00 ^b	83.73 ^b
20-40	236.76 ^b	33.36 ^b	68.14 ^b
F test (S)	3.44 ^{ns}	3.69 ^{ns}	18.62 ^{**}
F test (D)	12.14 ^{**}	163.24 ^{**}	20.15 ^{**}
F test (SxD)	2.48 ^{ns}	9.90 ^{**}	1.72 ^{ns}
C.V (S)	3.72	27.63	7.92
C.V (D)	2.36	21.02	19.93

Letters on the same column show no significant differences. F test: (**)
 $p < 0.01$ and ns, no significant.

and Weil (2000), suggesting the influence of organic matter on the soil microbial biomass.

No effect of the ecosystems studied was observed on soil microbial respiration (Table 5). The evolution of CO_2 in forest and pasture soils was similar to that observed in maize soil and this was also confirmed by Islam and Weil (2000) in forest, pasture and cultivated soils. In a previous study, Gonzalez *et al.* (2003) also showed the influence of TOC on respiratory activity ($r = 0.87^{**}$). Similarly, a significant and positive correlation between respiratory activity and the content of TOC ($r = 0.53^{**}$) and SC ($r = 0.54^{**}$) was found in our study (Table 3) showing the influence of organic matter content on CO_2 evolution.

A significant increase in the nitrification potential of forest and pasture soils was observed, 22% and 13% respectively, when compared to maize ($p < 0.05$) (Table 5). Neill *et al.* (1995) found the same content of C and N in forest and pasture soils, although higher and nitrification has been verified in forest soil. The N mineralization is related to the C/N ratio, so predominant N mineralization was observed in natural forest soil in relation to plantation forest (Xu *et al.*, 2012). According to Canali *et al.* (2004), although similar amounts of nitrogen were applied in all soils, significantly higher mineralization was found in soil fertilized with organic matter than in the control, suggesting that the form of N must also influence nitrification. It can be concluded that, as well as the amount of N, the organic matter resulting from different vegetation cover can influence the nitrification potential, as in our study. It is possible that animal excretions, being richer in nitrogen compounds, have influenced the results found in the pas-

ture soil (Garcia and Nahas, 2012) when compared to maize.

With increasing soil depth, MBC content significantly decreased by 32% and 55%, respiratory activity by 55% and 76%, and nitrification potential by 26% and 40% from 0-10 cm to 10-20 and 20-40 cm layers, respectively. However, a significant difference was only observed between the first and the other soil layers (Table 5). Reductions from 7% to 48% in the MBC contents were also showed by Portugal *et al.* (2008) in the 0-10 cm soil layer in relation to the 10-20 cm layer. The decrease in microbial biomass and activity was possibly due to the reduction of TOC and SC of the surface layer compared to the lower layers (Govaerts *et al.*, 2007).

A significant and positive correlation was observed between MBC, nitrification potential and respiratory activity with HA and FA (Table 3). These results demonstrate the influence of biological attributes on the synthesis of HA and FA and can be considered as sensitive parameters of SOM humification in the ecosystems studied. This may suggest that differences in the HA and FA contents of the soils result from TOC and SC, as demonstrated previously, and the efficiency of micro-organisms to metabolize the organic matter available in the different soils.

In conclusion, the results of this study showed the relationship between the formation of humic and fulvic acids and the biological and chemical attributes modeled using three ecosystems. The formation of humic acids was also related to the chemical attributes of TOC and SC of the soil ecosystems studied and to the microbiological attributes of MBC, CO₂ production and nitrification potential.

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