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Comparison of organic carbon from humic and fulvic acids and the degree of humification in five soil orders

Comparación del carbono orgánico proveniente de los ácidos húmicos, ácidos fúlvicos y el grado de humificación en cinco órdenes de suelos

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Abstract

This study compared the organic carbon (OC) content in fractions of humic acids (HA) and fulvic acids (FA) in five soil orders (Aridisol, Entisol, Histosol, Inceptisol and Mollisol) and know their association with the degree of humification. Extraction and fractionation, as well as the degree of humification was carried out by the Nagoya method proposed by Kumada. OC quantification was determined by the Walkley and Black method. The results are: the average OC content of HAs of the order Aridisol differs from that of Histosols, Inceptisols, and Mollisols. The order Entisol presented differences with the Histosols and Mollisols, the soils of the order Inceptisol presented differences with the Aridisols and Histosols and those of the order Histosol differed from the Aridisols, Entisols and Inceptisols. Similarly, those of the Mollisol order differed with the Aridisols, Entisols and Inceptisols. In the fraction of FA the average OC content of the order Aridisol deferred from that found in Histosols, Inceptisols and Mollisols. The Entisol order differed from the Mollisols; likewise, the Inceptisol order differed from the Aridisols and Mollisols and the Histosol order differed from the Aridisols. Finally, the order Mollisol was also different from the Aridisols, Entisols, and Inceptisols. Soil types do not show wetting patterns, because they are not based on pedogenetic processes and these have a wide range of characteristics in surface horizons.

Keywords: *Humus fractions, soils orders, humic substances.*

Resumen

En este estudio se realizaron las comparaciones del contenido de carbono orgánico (CO) en las fracciones de los ácidos húmicos (AH) y los ácidos fúlvicos (AF) en cinco órdenes de suelos (Aridisol, Entisol, Histosol, Inceptisol y Mollisol) y conocer su asociación con el grado de humificación. La extracción y fraccionamiento, así como el grado de humificación se realizó por el método Nagoya propuesta por Kumada. La cuantificación del CO se determinó por el método Walkley y Black. Los resultados son: el contenido promedio de CO de los AH del orden Aridisol difiere del de los Histosoles, Inceptisoles y Mollisoles. El orden Entisol presentó diferencias con los Histosoles y Mollisoles, los suelos del orden Inceptisol presentaron diferencias con los Aridisoles e Histosoles y los del orden Histosol se diferenciaron de los Aridisoles, Entisoles e Inceptisoles. De la misma forma, los del orden Mollisol difirieron con los Aridisoles, Entisoles e Inceptisoles. En la fracción de los AF el contenido promedio de CO del orden Aridisol difirió del encontrado en los Histosoles, Inceptisoles y Mollisoles. El orden Entisol se diferenció de los Mollisoles; así mismo, el orden Inceptisol difirió de los Aridisoles y Mollisoles y el orden Histosol se diferenció de los Aridisoles. Por último, el orden Mollisol se

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diferenció también de los Aridisoles, Entisoles e Inceptisoles. Los tipos de suelo no muestran patrones de humificación, debido a que no se basan en los procesos pedogenéticos y éstos presentan una amplia gama de características en los horizontes superficiales.

Palabras clave: *Fracciones del humus, ordenes de suelos, sustancias húmicas.*

Introduction

The Soil organic matter (SOM) comprises a mixture of residues of plants, microbes, and animals at various stages of decomposition and heterogeneous organic substances closely associated with the mineral fraction (Kononova, 1975; Christensen, 1992; Zaccone et al., 2018; Osorio, 2018; Gallardo, 2016). According to Kononova (1966), this complex process involves two groups: 1. The decomposition of the original components of the tissues and their conversion by microorganisms into simpler chemical compounds identifiable by organic chemistry (proteins and amino acids, simple carbohydrates and compounds, resins, fats, lignins, and others) and partially into products of complete mineralization (CO_2 production by breath, NO_2, NO_3, NH_3, CH_4, H_2O and others). 2. Synthesis of organic compounds with the formation of high molecular weight humic substances. This set of processes are called humification and produces a mixture of substances that have high resistance to subsequent microbial attack and are completely different from any vegetable or animal substance (Duchaufour, 1987). In this way, the soil organic carbon (SOC) content is the component of SOM.

On Earth, the SOC content of the upper horizons of soils (Epipedons) contains between 1200 Pg to 1500 Pg of C (1 Pg = 1015 g = 1012 kg = 109 t) (Gallardo & Merino, 2007). Although the organic carbon of humic substances or humus accounts for 60 % to 80 % of SOC (Piccolo *et al*., 2018; Ismail-Meyer, 2018).

Kumada (1987) was able to obtain information from HA molecules, such as functional groups, elemental composition, and degree of humification. For the degree of humification, he proposes to distinguish four types of humic acids A, B, Rp, and P. This can be known through the formation of humic acids, in which the start of the humification process begins with the type Rp (first humification states of organic material), evolving to type B and finally to type A (each type exhibits a relatively stable form). In strongly acidic soils, the Rp type can be replaced by the P - type.

The brown to black component (humic substances) refers to humic acids (HA), fulvic acids (FA) and humins (HM) which represent the most recalcitrant and stable organic carbon reservoir from the microbial point of view (El-Metwally et al., 2014; Duchaufour, 1987).

Humus or humic substances are the product of the pedogenetic process (Simonson, 1959; Fanning & Fanning, 1989; Zapata, 2001, 2006, 2014; Buol et al., 2011). A slight change in the recalcitrant soil organic carbon reserve will have a major impact on the concentration of atmospheric carbon dioxide (CO_2) (Li et al., 2019).

The objective of this research was to compare the composition of organic carbon content in the fractions of humic acids (HA) and fulvic acids (FA). Also, to know if the degree of humification (A, B, P and Rp) in soils is related to soil orders (Aridisol, Entisol, Histosol, Inceptisol, and Mollisol).

Materials and Methods

Description of the study area

The study was conducted in the district of San Rafael, Ambo Province, Huanuco Department of Peru. This area covers an area of 44,189.73 ha, located between the coordinates: lower-right end is 10’27’41.83” S and 76’1’32.14” O; and the top-left end is 10’12’17.43” S and 76’15’16.33” O, datum WGS-84 (Figure 1). The 42 simple samples were analyzed in the Soil Laboratory of the School of Geosciences of the Faculty of Sciences of the Universidad Nacional de Colombia, Medellin.

The study area is located between 2100 masl and 4890 masl. The average monthly air temperature in the low and medium area (2100 masl to 3500 masl) ranges from 12 °C to 20 °C, in the high areas (3,500 masl to 4,200 masl) it ranges from 8 °C to 16 °C, and in areas above 4,200 masl the temperature ranges from 4 °C to 10 °C (Ministerio de Agricultura y Riego [MINAGRI], 2016).

Soils were classified according to the keys to soil taxonomy (Soil Survey Staff, 2014). In the San Rafael district, the orders Aridisol, Entisol, Histosol, Inceptisol, and Mollisol were identified (Figure 2).

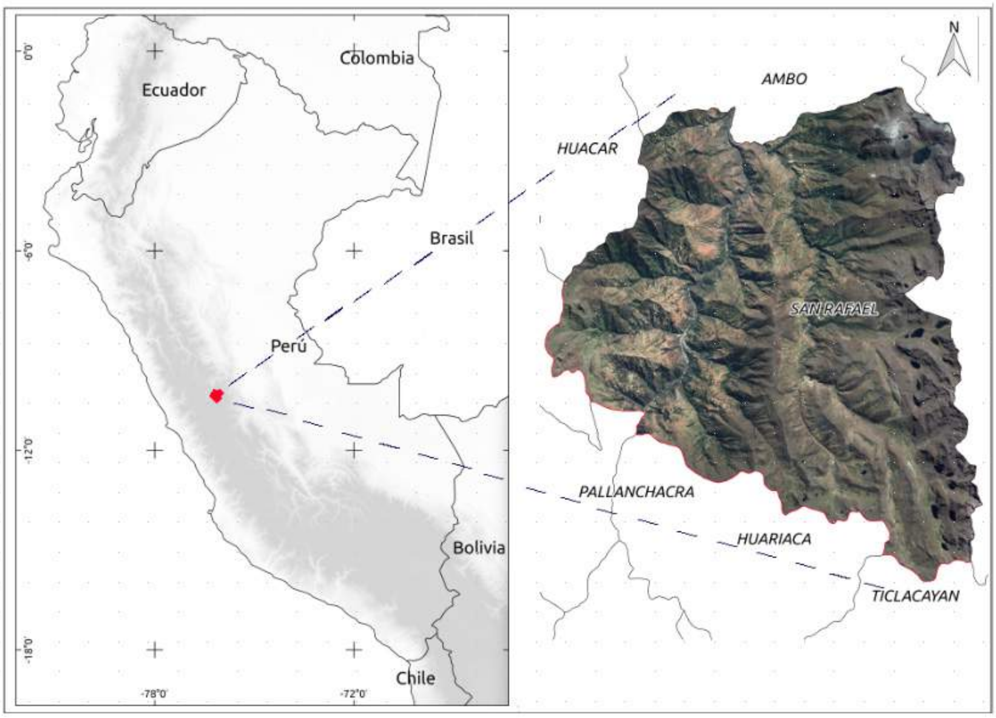
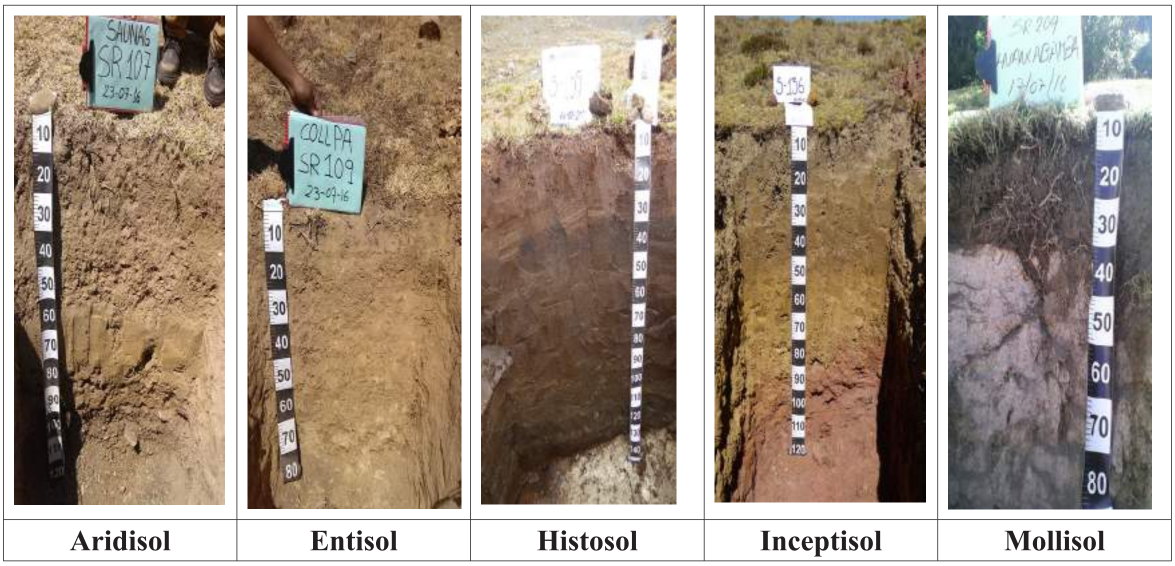


Figure 1: Location of the research area.



Aridisol | **Entisol** | **Histosol** | **Inceptisol** | **Mollisol**

Figure 2: Soil orders in the San Rafael district.

These soils developed from weathering profiles with highly variable evolution, from incipient to evolved, formed from sedimentary rocks (sandstones, lutites, limestones, and clusters), metamorphic rocks (shale), plutonic rocks (diorite), and from deposits of alluvial, colluvial, and glacial materials.

The district presents a mountainous relief of slopes or long and inclined slopes with narrow valleys that present flat accumulation bottoms.

Sampling

Forty-two simple samples were collected in the different soil orders (Table 1). The sampling points were distributed in a random way supported by the previous results of soil organic matter and according to the area that corresponds to it. The samples were taken from the first horizon, their morphological characteristics were described and those of the surrounding relief, also have been georeferenced with a GPS Navigator.

Table 1: Location of the sampled points.

Soil sample	Land order (Soil Survey Staff)	South latitude	West length
m1	Aridisol	10°17’32.7”	76°9’32.6”
m2		10°16’57.7”	76°9’38.7”
m3		10°17’18.1”	76°9’32.9”
m4		10°17’49.6”	76°9’54.1”
m5	Entisol	10°18’5.6”	76°9’42.4”
m6		10°14’51.9”	76°7’34.9”
m7		10°21’44.3”	76°11’58.8”
m8		10°20’10.3”	76°11’9.8”
m9		10°14’8.7”	76°10’3.7”
m10		10°14’4.7”	76°10’15.7”
m11		10°14’12.8”	76°11’30.4”
m12		10°14’20.3”	76°5’50.2”
m13		10°20’50.7”	76°13’21.9”
m14		10°19’23.8”	76°11’56.5”
m15		10°14’47.1”	76°7’52.3”
m16		10°16’3.0”	76°7’40.6”
m17		10°18’11.9”	76°6’49.9”
m18	Histosol	10°14’26.9”	76°5’31.5”
m19		10°14’34.9”	76°5’20.0”
m20		10°18’44.6”	76°14’33.4”
m21		10°19’49.5”	76°3’2.7”
m22		10°19’50.3”	76°2’30.9”
m23		10°20’1.1”	76°4’38.6”
m24	Inceptisol	10°15’14.8”	76°9’19.9”
m25		10°18’26.4”	76°9’52.8”
m26		10°13’33.0”	76°6’37.8”
m27		10°19’1.1”	76°11’21.3”
m28		10°15’25.4”	76°7’39.9”
m29		10°14’19.8”	76°6’18.4”
m30		10°14’36.7”	76°6’46.8”
m31		10°18’10.4”	76°6’53.1”
m32		10°21’32.9”	76°13’7.7”
m33		10°20’24.1”	76°7’22.6”
m34		10°21’36.4”	76°6’56.9”
m35		10°23’0.5”	76°4’50.1”
m36		10°23’15.7”	76°6’48.3”
m37	Mollisol	10°20’49.6”	76°13’57.8”
m38		10°20’59.3”	76°14’43.3”
m39		10°19’47.3”	76°15’11.4”
m40		10°19’21.7”	76°14’48.8”
m41		10°19’31.4”	76°14’42.0”
m42		10°19’45.7”	76°14’40.2”

Extraction and fractionation of organic matter from the soil

The extraction and fractionation of organic matter from the soil were carried out by the Nagoya method, described by Kumada (1987). Soil samples dried outdoors and sifted. MOS fractionation was performed by mixed extraction with two basic solutions at 0.1N of NaOH + 0.1M $Na_2P_2O_7.10H_2O$ (soil to extracting ratio, 1:300, w/v), this ratio depends on the organic carbon content in the soil. It is then boiled (100 ^{o}C) for 30 min by shaking the Erlenmeyer several times. After boiling 1 g of Na_2SO_4 is added as a coagulating agent, after cooling in a water bath plus ice, it was centrifuged at 11,000 rpm by 15 min.

The alkaline extract was then decayed and the soil residue was washed twice with 20 mL of extracting agent containing Na_2SO_4 by centrifugation, as in the previous step.

Once the soluble extract and washes were combined, the extract was acidified with a concentration of H_2SO_4 (1 mL:100 mL) and left to rest for 30 min. Subsequently, acidified extracts were filtered through the filter (Toyo Roshi No. 5C) into a 100 mL volumetric Erlenmeyer, then the precipitate is washed with H_2SO_4 (ratio, acid to water, 1 mL:100 mL) and the filtered volume (FA) to 100 mL is completed. The precipitated HA (on the filter paper) is dissolved with 0.1N NaOH, the solution is collected in an Erlenmeyer of 100 mL or 250 mL volume (depending on the HA content).

Chemical characterization of humic acids

The characterization of humic acids was performed according to Kumada (1987). This system groups HAs into four types: A, B, P, and Rp, according to their position in the orthogonal axis diagram whose coordinates correspond to the RF and Δlog K parameters. These parameters are obtained by the following expression:

$$\Delta log\ K = log\ K_{400} - log\ K_{600}$$

$$RF = K_{600}\ x\ 1{,}000/c$$

Where:

Log K_{400} and log K_{600} is the optical density of an HA solution at 400 nm and 600 nm, both spectrum ranges are obtained with a GENESYS Visible Spectrophotometer™ 20.

c = volume in mL of 0.1N of $KMnO_4$ consumed by 30 mL of HA solution used to determine the absorption spectrum. In this investigation, $KMnO_4$ was replaced by 0.1N of $K_2Cr_2O_7$.

The humification process begins with the Rp type (first humification states of organic matter), evolving to type B, and finally to type A (each type exhibits a relatively stable form). In strongly acidic soils, the Rp type can be replaced by P - type soils.

Quantification of organic carbon

The organic carbon (OC) content in humic and fulvic acids was determined by the wet combustion method by Walkley and Black (1934). The organic forms of soil C oxidize in the presence of excess dichromate in the middle of a strong acid. After the C oxidation step, at the reaction temperature for a certain amount of time, the non-reduced Cr^{+6}, added in excess, was titrated with ferrous sulfate Fe^{+2}. The difference between these two states of Cr oxidation is equal to the organic carbon content of the sample (Allison et al., 1965; Walkley, 1947).

Statistical analysis

The organic carbon results in the HA and FA were determined by mean comparison analysis with the *t* Student test. To know the differences in the predominance of the degree of humification in the soils were calculated by Pearson's chi-square independence test. These tests used a significance level of ($p < 0.05$) according to R software.

Results and Discussion

The results of the quantification of organic carbon from humic acids (HA) and fulvic acids (FA), and the determination of the degree of humification in five soil orders, are summarized in Table 2.

Comparison of the organic carbon content of HA and FA in soils

The dispersion or variability of organic carbon content in HA and FA fractions in the soils studied are presented in boxplot (Figure 3). In the fraction of the HAs (Figure 3a), the order Histosol and Mollisol show greater dispersion of the values, while in the fraction of the FA (Figure 3b), the order Histosol has greater dispersion. The soils of the order Mollisol have a dark horizon (Epipedon) and without diagnostic sub horizon (Endopedon) expressed in an Ap/AC/C type profile located on the slopes or mountain slopes, while the order Histosol presents a histic Epipedon that is expressed with highly decomposed surface horizons and with ustic, udic, and acuic moisture regimes; developed from morrenic deposits, metamorphic rocks, plutonic rocks, and organic materials. Both soils are located in very diverse contexts that include the life zones of very humid paramo and pluvial paramo (MINAGRI, 2016).

According to the Soil Survey Staff (1999) each order is differentiated by the presence or absence of diagnostic horizons or characteristics that reflect the formation processes, thus being very heterogeneous with respect to properties. Therefore, the organic matter content and nature of soils is highly variable (Stevenson, 1994; Lindsay, 1979; Spain et al., 1983).

Table 2: Organic carbon content from humic and fulvic acids, and the degree of humification in different soil orders.

Soil sample	Land order (Soil Survey Staff)	Organic carbon in the HA (g 100 g^{-1})	Organic carbon in FA (g 100 g^{-1})	RF	Δlog K	Type of HA
m1	Aridisol	0.05	0.05	75	0.57	B
m2		0.15	0.10	23	0.67	P
m3		0.15	0.05	57	0.68	B
m4		0.15	0.20	34	0.56	P
m5	Entisol	0.15	0.15	42	0.64	P
m6		0.20	0.35	60	0.67	B
m7		0.10	0.05	45	0.55	P
m8		0.15	0.05	26	0.65	P
m9		0.15	0.10	41	0.66	P
m10		0.05	0.05	62	0.55	P
m11		0.10	0.05	70	0.63	B
m12		1.00	1.50	53	0.45	P
m13		0.15	0.05	28	0.70	P
m14		0.15	0.10	66	0.54	P
m15		0.75	0.10	18	0.55	P
m16		0.15	0.10	61	0.62	B
m17		0.45	0.20	24	0.61	P
m18	Histosol	6.00	1.75	29	0.55	P
m19		3.75	1.25	20	0.54	P
m20		2.10	0.30	26	0.57	P
m21		11.00	3.50	10	0.96	Rp(2)
m22		5.25	1.00	29	0.51	P
m23		5.25	0.75	23	0.50	P
m24	Inceptisol	0.20	0.20	35	0.63	P
m25		0.15	0.30	73	0.69	P
m26		1.20	0.10	15	0.58	P
m27		0.05	0.05	17	0.63	P
m28		0.90	0.25	17	0.57	P
m29		0.25	0.30	80	0.58	B
m30		2.10	0.50	26	0.59	P
m31		0.15	0.20	123	0.62	A
m32		0.15	0.05	77	0.56	B
m33		0.60	0.05	20	0.62	P
m34		0.90	0.40	29	0.53	P
m35		1.00	1.00	76	0.52	P
m36		4.50	1.00	23	0.62	P
m37	Mollisol	0.15	0.35	104	0.64	A
m38		2.50	0.75	27	0.53	P
m39		4.50	0.50	13	0.57	P
m40		1.50	0.50	51	0.50	P
m41		3.00	0.75	20	0.54	P
m42		5.25	1.50	29	0.50	P

The average content of OC in the HA in the soils of the Aridisol order is 0.13 g 100 g^{-1}, in Entisol 0.27 g 100 g^{-1}, in Histosol 5 .56 g 100 g^{-1}, in Inceptisol 0.93 g 100 g^{-1} and Mollisol is 2.82 g 100 g$^{-1.}$ The average content of OC in the FA is slightly lower than that of HAs (Figure 4). The order Aridisol is 0.10 g 100 g^{-1}, in Entisol 0.22 g 100 g^{-1}, in Histosol 1.43 g 100 g^{-1}, in Inceptisol 0.34 g 100 g^{-1} and in Mollisol is 0.73 g 100 g^{-1}.

Previous studies, conducted by several authors, have emphasized that prairie soils (Mollisol) and

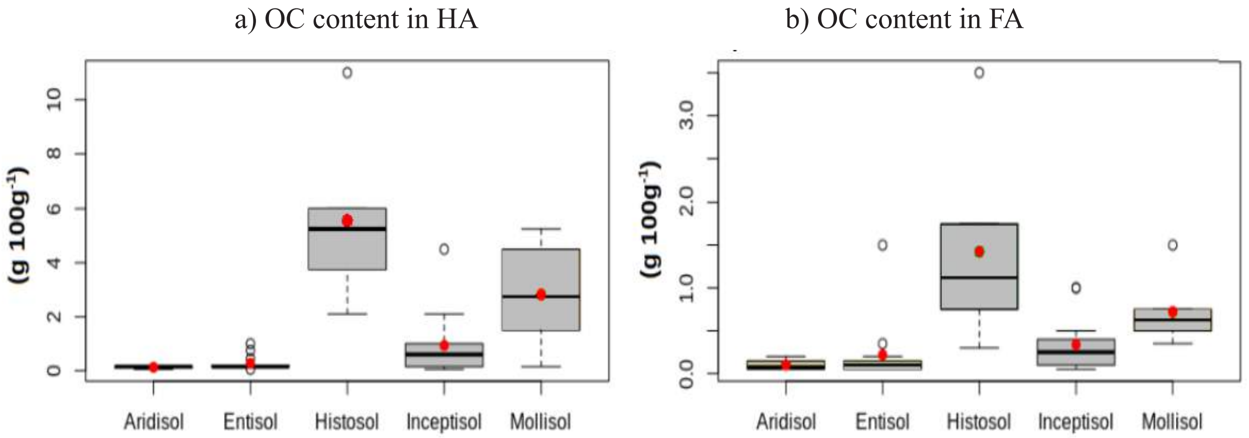


Figure 3: Box plots of carbon content from humic acids (a) and fulvic acids (b) in five soil orders.

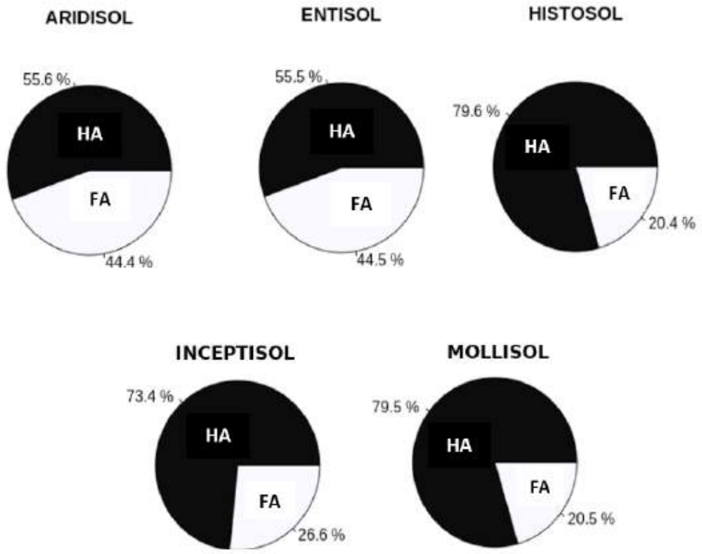


Figure 4: Distribution of fractions of humic acids (HA) and fulvic acids (FA).

peat soils (Histosol) have higher HA content than FA content (Kononova, 1966; Gallardo, 2016).

Also, Stevenson (1994) and Zamboni et al. (2006) found that Mollisol soils have high HA content than other soil types. In Peru, León (2016) reports higher organic carbon content in humic substances in six high Andean wetland soils (Histosol) located in the departments of Ancash, Huancavelica, Arequipa, and Puno. The high content OC in soils does not guarantee the quality of humus or soil organic matter.

When comparing the OC content in the HA and FA fractions (Table 3 and Table 4), the Aridisol and Entisol soils have no significant difference. Aridisol soils do not have water available for most of the time so they are warm enough (Soil Survey Staff, 1999, 2014; Buol et al., 2011; Jaramillo, 2014), these soils have an ochric epipedon and are located in the mountainous steppe life zona – Tropical low montane and evapotranspiration exceeds precipitation for most of the year (MINAGRI, 2016). Generally, in these soils, microbial populations are scarce, due to the limited amounts of water available in the soil profile, the reactions are relatively less intense (Buol et al., 2011).

The soils of the order Entisol can be presented in a wide diversity of climates, on very variable slopes, some of them very pronounced and associated with very diverse natural plant cover. The absence of characteristic features of the different pedogenetic processes is expressed in these soils (Soil Survey Staff, 1999) and presents an ochric Epipedon (MINAGRI, 2016). These conditions of variability can influence the organic carbon content as well, presenting minimum carbon content in the HA and FA fractions. While the order Aridisol presents differences with the orders of Histosol, Inceptisol, and Mollisol.

The contents of the fraction of the HA in the Entisol and Inceptisol orders are similar. This may respond that both orders have a wide range of characteristics such as one or more pedogenetic horizons and develop over a wide range of climates (Soil Survey Staff, 1999).

Comparison of the degree of humification in soils

According to Table 2 and Figure 5, two HA samples are Type P and two HA samples are Type B from the arid sun order, being the degree of immature humification and evolving into the mature form. The mature form in Aridisol occurs in many semi-arid regions, which have lower concentration values of C, MOS is highly transformed and resilient to possible climate change (Gallardo, 2016).

Table 3: Comparison of means of organic carbon in humic acids (HA), in the five soil orders.

	Entisol	Histosol	Inceptisol	Mollisol
Aridisol	p 0.09 ns	p 0.01 *	p 0.03 *	p 0.02 *
Entisol		p 0.01 *	p 0.08 ns	p 0.02 *
Histosol			p 0.01 *	p 0.08 ns
Inceptisol				p 0.02 *

Note: ns: Not significant; * p < 0.05

Table 4: Comparison of means of organic carbon in fulvic acids (FA), in the five soil orders

	Entisol	Histosol	Inceptisol	Mollisol
Aridisol	p 0.32 ns	p 0.03 *	p 0.03 *	p 0.01 *
Entisol		p 0.05 ns	p 0.40 ns	p 0.02 *
Histosol			p 0.06 ns	p 0.20 ns
Inceptisol				p 0.04 *

Note: ns: Not significant; * p < 0.05

In the Inceptisol order, ten HA samples are type P, two HA samples are Type B and one HA sample is type A, their degree of maturation being immature to mature. This immature to mature state may coincide with soil development.

The Mollisol order consists of five Type P HA samples and one Type A HA sample. The degree of humification corresponds to the immature and mature states. The immature state corresponds to anthropically intervened soils, which have superficial horizons of Ap. The type A humification grade is presented in soils with horizon A (soils in grasslands), without anthropic intervention. In these soils of the order Mollisol, humic acids are often preserved for relatively long periods (>3000 years), linked to clay-humus complexes (Tsutsuki et al., 1988; Bockheim, 2014). In these soils, it is possible to detect highly moistened humified humic acids in the outer horizons.

When performing Pearson's chi-square independence test, the soil variables and degree of humification are independent or not associated (p = 0.266, being non-significant).

Conclusion

The work carried out, establishes the comparison of the organic carbon content in the fractions of humic acids (HA), fulvic acids (FA) and the degree of humification for five soil orders in the region of San Rafael (Peru). The most significant results are as follows: 1) In the order Aridisol, the low availability of water for most of the time and where evapotranspiration exceeds precipitation, constitute conditions very unfavorable to the development of pedogenetic processes. Therefore, it is inferred that minimum organic carbon content is presented in HA

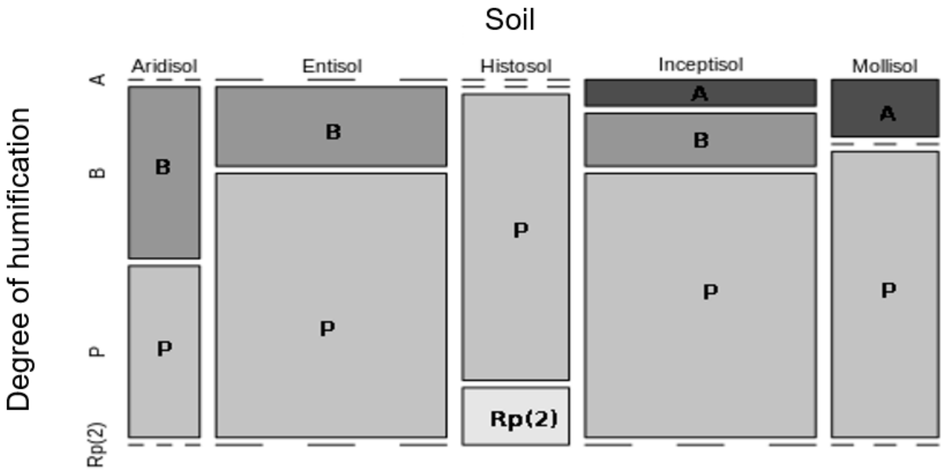


Figure 5: Distribution of the degree of humification in the different soil orders.

and FA fractions and differs from other soil orders. 2) In the soils of the order Entisol and Inceptisol, it has characteristics of a wide diversity of climates, inclined slopes, various types of vegetation that lead to the incipient formation of pedogenetic horizons. Therefore, they have a minimal carbon content in humic fractions. 3) In the order, Mollisol, located under a cover of pastures that annually provide a large amount of organic matter, forming clay-humus complexes, leads to the development of highly moistened humified humic acids and high carbon content in humic fractions. 4) In the order Histosol, due to continuous input, accumulation of organic matter, and cold climates delay the humiliation process. Therefore, they have a high carbon content in the humic fraction and the recent start of the humification process. Finally, soil types are not associated or do not show wetting patterns, which may be because the distinctions made when classifying them are not based on pedogenetic processes and these have a wide range of characteristics in surface horizons; they also develop over a wide range of climates, biota, relief and parental material in an elapsed time.

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