DOI: 10.5433/1679-0359.2017v38n4p1799

Fractions of soil organic matter in the vineyards of altitude regions in Santa Catarina

Compartimentos da matéria orgânica do solo em vinhedos altomontanos em Santa Catarina

Denilson Dortzbach[1]; Shirlei Almeida Assunção[2];
Marcos Gervasio Pereira[3*]; Eduardo Carvalho da Silva Neto[4]

Abstract

The implementation of agricultural systems such as viticulture can quantitatively and qualitatively affect the contents of soil organic matter (SOM). These changes may modify the edaphic features of the soil as well as the soil quality. The objective of this study was to evaluate the chemical and physical fraction of SOMand to analyze changes in the carbon stock and C management index in areas of implanted vineyards in altitude regions of Santa Catarina. Four regions were selected: Region I (Urubici); Region II (San Joaquim); Region III (Campos Novos) and Region IV (Água Doce). In each region, we selected vineyards implanted between 2001 and 2005 as well as surrounding forested areas. Disturbed and undisturbed samples were collected from the 0-5, 5-10, and 10-20 cm layers of the soil. Samples were prepared in the laboratory to obtain air-dried soft soil, which was then used for the analysis of several parameters, namely total organic carbon (TOC), carbon stock,and chemical fractionation of the soil. The chemical fractionation was then used to determine carbon content in the fulvic acid fraction (C-FAF), humic acid fraction (C-HAF), and humin fraction (C-HUM). We also analyzed particle size, quantified the levels of particulate carbon (COp) and carbon associated with clay and silt (COam), and calculated the carbon management index (CMI). We evaluated normality and homogeneity for all data. The results were evaluated with an analysis of variance and subsequent F-test. Mean values were compared using a 5% Student's *t*-test and subsequently submitted to a Tukey's test. The highest TOC levels were observed in Region II in the 0-5 cm layer in both vineyard and forested areas. Vineyard areas exhibited lower values of TOC, Cop, and COam compared to forested areas indicating that the management adopted in these areas contributed to the reduction of these fractions. Forested areas exhibited a higher proportion of Cop compared to vineyard areas. The humin fraction represented the largest portion of the TOC and comprised the highest values in both forested and vineyard areas. The carbon management index indicated a low contribution of vineyard areas or a reduction in carbon storage in their soils.
Key words: Soil management. Organic matter fractionation. Viticulture.

Resumo

A implantação de sistemas agrícolas como a viticultura pode afetar quantitativamente e qualitativamente o conteúdo de matéria orgânica no solo (MOS). Estas transformações podem modificar os

[1] Engº Agrº, Dr. em Agronomia Ciência do Solo, Pesquisador, Epagri, Florianópolis, SC, Brasil. E-mail: denilson@epagri.sc.gov.br

[2] Discente de Doutorado, Curso de Pós-Graduação em Agronomia, Ciência do Solo, CPGA/CS, Universidade Federal Rural do Rio de Janeiro, UFRRJ, Seropédica, RJ, Brasil. E-mail: shirleiaassuncao@gmail.com

[3] Prof. Titular, UFRRJ, Departamento de Solos, Seropédica, RJ, Brasil. E-mail: mgervasiopereira01@gmail.com

[4] Discente de Mestrado, Curso de Pós-Graduação em Agronomia, Ciência do Solo CPGA/CS, UFRRJ, Seropédica, RJ, Brasil. E-mail: netocseduardo@gmail.com

[*] Author for correspondence

Received: July 18, 2016 - Approved: Jan. 09, 2017

atributos edáficos do solo, bem como sua qualidade. Assim, o objetivo desse estudo foi de avaliar a compartimentalização da MOS, com o emprego do fracionamento químico e físico da MOS e verificar as mudanças no estoque de carbono e no índice de manejo de C em áreas de vinhedos implantados nas regiões de altitude de SC. Foram selecionadas quatro regiões de altitude: Região I (Urubici); Região II (São Joaquim); Região III (Campos Novos); Região IV (Água Doce). Em cada região foram selecionadas áreas de vinhedos implantados entre os anos de 2001 e 2005 e também áreas de floresta. Foram coletadas amostras deformadas e indeformadas nas camadas de 0-5; 5-10;10-20 cm. As amostras foram preparadas em laboratório para obtenção da terra fina seca ao ar, material este que foi utilizado para realizar as análises de carbono orgânico total (COT), o estoque de carbono no solo, fracionamento químico, determinando-se os teores de carbono na fração ácido fúlvico (C-FAF), fração ácido húmico (C-FAH) e fração humina (C-HUM) e granulométrico, quantificando-se os teores de carbono particulado (COp) e o associado a argila e silte (COam), além do cálculo do índice de manejo do carbono. Para todos os dados foi realizada a avaliação da normalidade e homogeneidade. Os resultados foram submetidos à análise de variância com aplicação do teste F e os valores médios comparados entre si pelo teste t a 5%, e, posteriormente, submetidos ao teste de Tukey. Os maiores teores de COT foram observados na região II, na camada de 0-5cm, tanto nas áreas dos vinhedos como na área de floresta. As áreas dos vinhedos apresentaram menores valores de COT, COp e COam quando comparadas as de floresta o que indica que o manejo adotado nessas áreas está contribuindo para a redução dessas frações. A área de floresta apresentou maior proporção de COp, quando comparado as áreas dos vinhedos. A fração humina representa a maior porção do COT e apresentou os maiores valores, tanto nas áreas de floresta como nos vinhedos. O índice de manejo do carbono indica baixo aporte ou redução no armazenamento de carbono no solo nas áreas dos vinhedos.

Palavras-chave: Manejo do solo. Fracionamento da matéria orgânica. Viticultura.

Introduction

The implantation of agricultural systems such as viticulture can reduce the annual accumulation of organic carbon in the soil due to the disturbance created when a crop is planted and there is low vegetative input from vines. This change in soil use may cause a decline in the carbon stock, which changes the content of soil organic matter (SOM) in a quantitative and qualitative manner before the SOM achieves an equilibrium as is usually observed in unaltered natural systems (CALONEGO et al., 2012).

The inadequate use and management of soil that results from soil disturbance and the use of crops that do not contribute to SOM content can promote changes in the environment due to SOM degradation.This can have a negative impact on physical and chemical attributes of the soil as well as upon its biodiversity (COSTA et al., 2015). It also contributes to the increase in greenhouse gas emissions (OLSON et al., 2014).

In this context, knowing the different chemical and physical fractionations of SOM can assist in evaluating changes induced by soil use and management. Humic substances (HSs) – subdivided into the fulvic acid fraction (C-FAF), humic acid fraction (C-HAF), and humin fraction (C-HUM) (STEVENSON, 1994) – may indicate the impact of the management system on soil quality. Given that HSs make it possible to differentiate SOM fractions as a function of their stability (MARTINS et al., 2015), variations in the distribution of HSs can be considered an indicator of the process and degree of SOM humification (Stevenson, 1994).

The granulometric fractionation of SOM consists of the separation of two organic fractions: particulate organic carbon (C-COp) and organic carbon associated with minerals (C-COam). The COp is the most labile fraction and is associated with coarser fractions, and C-COam is more stable and slower cycling and is strongly bound to mineral particles (CAMBARDELLA; ELLIOTT, 1992; ROSCOE; MACHADO, 2002).

A possible explanation for a high accumulation of C-COam is that there is greater decomposition of C-COp levels the product of C-COp decomposition is associated with finer textured soil minerals (silt and clay fractions) resulting in higher levels of C-COam (FIGUEIREDO et al., 2010). Granulometric fractionation can be an efficient tool to evaluate soil quality, especially over a short period of time (GAZOLLA et al., 2015).

Different pedo environments exhibit significant differences in SOM content. The altitude regions of the state of Santa Catarina(SC) have high organic matter (OM) content in the surface horizon, low amounts of calcium and magnesium, low pH values, and high levels of exchangeable Al (ALMEIDA, 2009). These features, in addition to the intensity of soil use and management, can influence the dynamics of SOM changes and, consequently, its interaction with the mineral matrix (BENITES et al., 2003).

SOM has been suggested as a key indicator of soil quality as has the amount of total carbon stock or its fractions and the comparison with soil conditions. However, these parameters do not provide values that can be extrapolated for different locations, climates, and soils. The Carbon Management Index (CMI), a relative measure of changes to the soil caused by management, was developed to compare such situations to those considered original or ideal (BLAIR et al., 1995).

Thus, the objective of this study was to evaluate SOM compartmentalization using its chemical and physical fractionation and to investigate changes in the carbon stock and C management index in vineyard areas in altitude regions of SC.

Material and Methods

Our work was conductedin four altitude regions in the state of Santa Catarina-Region I (Urubici), Region II (São Joaquim), Region III (Campos Novos), and Region IV (Água Dulce)-which are the main areas for the production of altitude fine wines.

The study area was located in the Formação Serra Geral where rhyodacite and basalt are predominant. We also observed the presence of some production areas in regions of Paleozoic gonduan sedimentary rocks corresponding to the Paraná Basin. Two geomorphological units occurred in the study regions: the Geomorphological Upland Unit of Campos Gerais, which is distributed in blocks of isolated relief, and the Geomorphological Unit of Dissected Upland Rio Iguaçu/Uruguay River (SANTA CATARINA, 1986). According to the climatic classification of Köeppen, the climate of the region is Cfb (subtropical climate with mild summers), is characterized by evenly distributed rainfall, lacks a dry season, and has an average temperature of below 22ºC in the warmer months.

The vegetation was composed of subtropical forests, namely the Araucaria Forest (Mixed Ombrophylous Forest), which crosses the coastal mountains and extends through the Santa Catarina uplands, and is typically located at altitudes higher than 500m. The subtropical fields occur predominantly in the area of the Santa Catarina uplands (SANTA CATARINA, 1986). The upland fields (altitude fields) appeared as isolated areas intercalated with the Araucária Forest and coincided with altitudes between 900 and 1400 meters. In these regions, short and medium grasses were predominant as well as plants of the Cyperaceae and Verbenaceae, leguminous plants, and plants in the Asteraceae family (SANTA CATARINA, 1986).

Regions I, II, and IV were characterized by the presence of Cambisol soils associated with Nitosol and Leptsol soils, where as Region III was comprised predominantly of Nitosol soils associated with Cambisol and Ferralsol soils. In general, these soils are characterized by a large accumulation of SOM in the superficial horizons, are invariably acidic, and present high levels of aluminum. All soils in the study regions were classified according to Santos et al. (2013). In Region I, soils had, in general, a coarser granulometry compared to the other regions. Soils had a low sum and base saturation and high aluminum content (above 4.0 $cmol_c\ kg^{-1}$), and some presented analytic character (SANTOS et al., 2013) and high clay activity ($T \geq 27.0\ 0\ cmol_c$

kg^{-1} clay). The wet and cold climate also contributed to the high carbon content resulting in horizons that were classified as A humic (P3 and P4) and A prominent (P2) (SANTOS et al., 2013). The altitude in this region varied from 864 to 1,144 m. In Region II, soils originated from rhyodacites and were located at altitudes ranging from 1,109 to 1,325 m. The association of factors such as climate, origin material, and relief material led to the formation of soils with high clay content, low sum (1.2 to 3.6 $cmol_c$ kg^{-1}) and saturation per base (11 to 39%), high aluminum content in most soils, and an allytic character and high clay activity in some soils. The cold and humid climate of this region favored low rates of OM mineralization providing high levels of organic C to the soil. In Region III, soils developed from basalt and were located at lower altitudes compared to other regions: between 832 and 1,128 m. The profiles presented textures that varied from clayey to very clayey depending on the source material. In general, the profiles had low base sum values – lower than 3.0 $cmol_c$ kg^{-1} the exchangeable Al contents were highly variable among the soils with values ranging between 0.4 and 6.5 $cmol_c$ kg soil. For base saturation (V), values ranged from 2% to 81%. In Region IV, soils were locatedbetween 1,183 and 1,260 m. These solids were formed from igneous rocks composed of different origin materials and exhibited a high clay content, varying in the subsurface horizon from 489 to 613 g kg^{-1}. All profiles presented an allytic character, low base saturation, high H + Al content, and high aluminum saturation with values ranging from 66 to 88%. These characteristics indicate a high degree of leaching. Further information on the morphological, physical, chemical, and mineralogical attributes and soil management can be found in Dortzbach (2016).

In each region, we selected vineyards implanted between 2001 and 2005 from three rural properties producing wine grapes; we also collected samples in the forested areas adjacent to the vineyards.

In the vineyard areas, deformed samples of the culture lines were collectedin April and May 2013 from the 0-5, 5-10, and 10-20 cm soil layers. Five composite samples composed of five simple samples from each depth were collected. The same procedure was carried out in the forested areas. After collection, samples were identified, conditioned in plastic bags, transported to the laboratory, air-dried, shredded, and passed through a 2 mm mesh sieve to obtain the air-dried fine soil (ADFS).

Total organic carbon (TOC) was quantified according to Donagema et al. (2011). For the fractionation of humic substances, we used the differential solubility technique established by the International Society of Humic Substances according to the technique adapted and presented by Benites et al. (2003) This technique is based on the solubility of the sample in alkaline and acidic media and the subsequent determination of carbon in each fraction, that is, in humin (C-HUM), fulvic acid (C-FAF), and humic acid (C-FAH). The granulometric fractionation of SOM was performed according to Cambardella and Elliot (1992), and approximately 20 g of soil and 60 mL of a sodium hexametaphosphate solution (5 g L^{-1}) were stirred for 15 hours on a horizontal shaker. The suspension was then passed through a 53 µm sieve. The particulate organic carbon (COp) trapped in the sieve was dried in an oven at 50°C, and quantified in relation to its mass, milled with a porcelain mortar and pestle, and analyzed in terms of C content according to Yeomans and Bremner (1988). The organic carbon associated with minerals (COam) was obtained from the difference between TOC and COp.

Based on the TOC data, the total stock of organic carbon (TSOG; Mg ha^{-1}) was calculated for the 0-5, 5-10, and 10-20 cm layers taking into account equal masses of soil between the treatments and using the forest areas as a reference (correction by equivalent mass) given that, when compacted, samples taken from soil layers of the pasture, for example, were no longer directly comparable with samples from the forest for the same depth of soil. This way, we tried to eliminate the effect of soil use and management systems on the soil density (Ds) following the guidelines of Ellert and Bettany (1995). TSOGs were calculated based on equivalent masses of soil (SISTI et al., 2004).

$$Cs = \sum_{i=1}^{n-1} Cti + \left[Mtn - \left(\sum_{i=1}^{n} Mti - \sum_{i=1}^{n} Msi\right)\right] x\, Ctn$$

In which Cs: total stock of C, corrected for the soil mass of a reference area;

$\sum_{i=1}^{n-1} Cti$: sum of the C stocks of the soil from the first to the penultimate sampled layer in the treatment evaluated (Mg ha^{-1}).

Mtn: soil mass of the last layer sampled in the treatment (Mg ha^{-1});

$\sum_{i=1}^{n} Mti$:sum of the total soil mass sampled in the treatment (Mg ha^{-1});

$\sum_{i=1}^{n} Msi$:sum of the total soil mass sampled in the reference area (Mg ha^{-1});

Ctn: C content of the soilof the last layer sampled (Mg C Mg^{-1} solo).

Before the correction by soil mass, the C stock of each layer in all studied areas was calculated using the mathematical expression proposed by Veldkamp (1994): TSOG = (TOC × Ds × e)/10, in whichTSOG was the organic C stock at a given depth (Mg ha^{-1}),TOC was the total organic C content at the depth sampled (g kg^{-1}), Ds was the soil depth density (Mg m^{-3}), and e was the thickness of the layer considered (m). Soil density analyses were performed according to Veiga (2011).

Based on the TSOG results, the CMI was calculated, following Blair et al. (1995). To obtain the CMI, the C stock index (CSI) was calculated from the relationship between the TSOG of each vineyard and the TSOG of the forested area that was used as reference. The lability (L) of SOM was determined from the relationship between C-COp and C-COam, and the lability index (LI) was determined from the relationship between the lability of each area and the lability of the reference area. The CMI of each area was obtained using the expression CMI = IEC × LI × 100 (BLAIR et al., 1995).

For all data at each depth, homogeneity (Bartlett) and normality (Kolmogorov-Smirnov-Ks) were evaluated. Subsequently, results were submitted to analyses of variance using an F–test, and mean values were compared first using a Student's *t*-test at a significance level of 5% and then with a Tukey's test with the assistance of the ASSISTAT program (SILVA; AZEVEDO, 2002).

Results and Discussion

TOC levels exhibited significant differences among the regions evaluated. For the 0-5 cm layer, the highest values were observed in the forested areas in Regions I and II, which were different from those in Region III (Table 1).

This difference may be associated with the lower altitudes observed in Region III, which consequently were impacted by different climatic conditions. The regions where higher temperatures prevailed exhibited greater SOM decomposition. According to the climatic classification defined by Köeppen and modified by Braga and Guellere (1999), Regions I and II are in temperate climate types 4A and 5, while Region III is in climate type 3A.

Table1. Mean values of TOC, TSOG, C-COam, and C-COp levels in vineyard and forested areas in regions of altitude fine wine production the state of Santa Catarina.

Depth (cm)	Regions							
	Vineyards				**Forest**			
	I	II	III	IV	I	II	III	IV
	TOC (g kg^{-1})							
0 – 05	17.98Ba	21.48Aa	15.41Ca	18.05Ba	31.14Aa	32.13Aa	25.21Ba	29.51ABa
05 – 10	18.33Ba	22.22Aa	14.50Ca	17.71Ba	30.40Aa	30.58Aa	23.10Ba	28.10ABa
10 – 20	16.59Ba	20.32Aa	14.50Ca	17.53Ba	28.95Aa	28.16Aa	22.51Ba	26.88ABa
	TSOG (g kg^{-1})							
0 – 05	7.28Bb	8.70Ab	6.24Cb	7.31Bb	12.61Ac	13.01Ab	10.21Bc	11.95Ac
05 – 10	8.43Bb	10.22Ab	6.67Cb	8.15Bb	26.60Ab	27.08Ab	20.84Bb	24.88ABb
10 – 20	18.08Ba	22.15Aa	15.80Ca	19.11Ba	58.15Aa	57.78Aa	45.37Ba	54.18Aa
	C-COp (g kg^{-1})							
0 – 05	4.64Aa	5.08Aa	4.72Aa	5.5A1a	15.12BCa	18.10Aa	13.58Ca	16.18Ba
05 – 10	3.78Bb	4.74ABa	5.60Aa	4.89ABa	13.15ABab	14.15ABab	12.01Bab	14.95Aab
10 – 20	4.84Aa	4.93Aa	4.99Aa	4.90Aa	11.18ABb	13.10Ab	10.11Bb	12.02ABb
	C-COam (g kg^{-1})							
0 – 05	13.34Bb	0 – 05	13.34Bb	0 – 05	13.34Bb	0 – 05	13.34Bb	0 – 05
05 – 10	14.55Aa	05 – 10	14.55Aa	05 – 10	14.55Aa	05 – 10	14.55Aa	05 – 10
10 – 20	11.75BCb	10 – 20	11.75BCb	10 – 20	11.75BCb	10 – 20	11.75BCb	10 – 20

Mean values followed by the same letter (uppercase for rows; lowercase for columns) are not statistically different from each other as determined by a Tukey's test at 5% probability.

A similar pattern was observed in the vineyard areas, and in Region II, we observed higher values that were different from those of the other regions. The lowest values of TOC were obtained in Region III. In this type of soil use, in addition to climatic factors, the lower values may be due to both management practices and soil disturbance at the time of crop implantation. These factors, in addition to lower altitudes, may have promoted the rapid mineralization of OM compared to the other regions.

When we compared the TOC values of the forested area with those of the vineyard areas, a similar pattern was observed among the regions. We observed lower values in the vineyard areas, which demonstrated a reduction in TOC values from 39% in the superficial layer of Region IV to 27% in the subsurface layers of Region II. However, the greatest reductions were observed in Region I in which values varied from 40 to 43%, probably due to the higher sand contents that were associated with management and may have promoted greater C loss in these areas. This reduction in carbon and nutrient content of the OM is faster in the initial phase of soil use for agricultural system cultivation than its return via waste deposition is, which can be reversed with adequate soil management (BRUN, 2008). Thus, the use of appropriate management techniques (non-disturbance and soil cover) can increase TOC contents in the vineyards considering the low levels of TOC observed in comparison with forested areas.

Among the depths evaluated, we observed that forested areas exhibited a decrease in the TOC levels in all regions (Table 1). In the vineyards, this pattern was not observed, which may be related to soil disturbance that influenced in some cases higher values of TOC at depth when compared to the superficial layer.

The TSOG values (Table 1) were higher in the forested areas compared to the vineyards, similar to what was observed with the TOC values. However, these values increased with depth in most cases. This fact may be related to higher density values that may have promoted the increase of TSOG with depth.

In a similar study performed in the Atlantic Forest of Santa Catarina, Dortzbach et al. (2015) evaluated TSOG in forest and pasture with different times of use. The authors reported higher values of TSOG in the soil surface layer than in the deeper layers, which is in accord with the greater deposition of residues that occurs on the soil surface.

Regarding quantification through granulometric fractionation, C-COp values were higher in the forested areas than in the vineyard areas. The pattern of C-COp differentiated in that in the forested area, values were higher in the superficial layer in all regions analyzed, while this was not observed in the vineyard areas demonstrating that this is a system subject to anthropic alteration.

Results obtained by Nicoloso (2005) and Loss et al. (2009) showed that the variation of C-COp levels between different areas as well as within the 0-5 cm layer were dependent of the addition of plant residues. Thus, soil use systems that promoted the addition of residues to the surface layers influenced the maintenance of C-COp values. This pattern was not observed in the vineyard areas, however, probably due to the low rate of addition of crop residues.

Some authors consider that C-COp can serve as an indicator of SOM quality in relation to management changes to the superficial layers in the short term (CONCEIÇÃO et al., 2005; LOSS et al., 2009).

C-COam values were higher than those of C-COp in both the forested and vineyard areas. According to Nicoloso (2005), more than 80% of TOC stocks are composed of C-COam, a condition not observed in this study given that in the vineyard areas, values ranged from 65 to 79%, and in the forested areas, values were even lower, ranging from 44 to 61% of the TOC.

C-COam levels are modified less by land use and management practices, especially in the short term (BAYER et al., 2004), than C-Cop levels, and they respond slowly to the effects of soil management and cropping. The lower variation of the C-COam contents is due to the fact that this fraction strongly interacts with the mineral fractions of the soil thus becoming more stable, especially in soils with a clayey or very clayey texture. This out come was observed by Figueiredo et al. (2010) in a study that evaluated the effects of different management systems on the labile and stable fractions of the organic matter of dystrophic Red Latosol in the Federal District.

The low CSI values observed, those less than 1 (Table 2), demonstrated the lower capacity of these plantations to accumulate C, and we observed a similarity in this index among the regions evaluated.

Regarding the CMI, the indices for vineyard areas across the four regions were less than those of the forest, which was used as a reference, thus indicating a possible decline in TSOG. All values observed were less than 25 (Table 2), which indicated minimal contribution or a reduction of carbon storage in the soil.

The low CMI values represented a greater than 75% difference in relation to the reference system reaching 90% in the 5-10 cm layer in Region II. Thus, the CMI was sensitive to changes resulting from the substitution of forest in vineyard areas, the use of which had a negative impact on the C levels in the soil.

Table 2. CSI, L, LI, and CMI values in vineyard soil in Santa Catarina production regions.

Depth (cm)	CSI	L	IL	IMC
	REGION I			
0 – 05	0.58	0.35	0.37	21.28
05 – 10	0.32	0.26	0.31	9.76
10 – 20	0.31	0.41	0.57	17.77
	REGION II			
0 – 05	0.67	0.31	0.24	16.05
05 – 10	0.38	0.27	0.26	9.79
10 – 20	0.38	0.32	0.34	12.95
	REGION III			
0 – 05	0.61	0.44	0.38	23.11
05 – 10	0.32	0.63	0.56	17.93
10 – 20	0.35	0.52	0.56	19.34
	REGION IV			
0 – 05	0.61	0.44	0.36	22.10
05 – 10	0.33	0.38	0.33	10.65
10 – 20	0.35	0.39	0.40	14.26

CSI–Carbon stock indexes; L – lability; LI–lability index; CMI – carbon management index.

These results indicate the need to use vegetative cover in vineyard areas to increase these indices given that the system of current use is far from creating a balance compared to the original condition and that the crop is causing a reduction in SOM levels.

As for chemical fractionation, most of the TOC present in the three soil depths evaluated was observed in the HUM fraction (Table 3) both in the forested areas and in the vineyards. The highest values were observed at depths of 0-5 and 5-10 cm in the forested areas of Region III and in the vineyards of Region I. In the forested areas, C-HUM represented about 50% of the HSs. However, in the vineyard areas this value was always higher than 60% of the total HSs reaching a maximum value of 74%.

The highest percentages of C-HUM observed in the vineyard areas may be related to the cultural residues of the grapevine, which are mainly due to pruning andthat produced a greater amount of vegetative residues with higher C/N and lignin/N ratios. These residues require a longer time to decompose and may favor the increase of recalcitrant fractions in the soil. Due to the high stability of the C-HUM fraction, there may have been a carbon increment in this fraction caused by the previous use of the area before the implantation of the vineyards, such as use of the grasses as cover and the presence of previous cover including the native forest it self. In other words, the C-HUM fraction is a product of the various uses and coverages of the soil.

The grasses have a dense root system that is in contact with the mineral particles and that contributes to the stabilization of SOM. This promotes an increase in OM added to the soil, which favors an increase of humidified fractions (PINHEIRO et al., 2003). Root-derived C has a mean residence time that is 2.4 times greater than the C from the aerial part of the plant, and the contribution of the roots to the SOM is about 30% higher than that of the aerial part (ROSSI et al., 2011).

Table 3. Mean carbon content of the fulvic acid fraction (C-FAF), humic acid carbon (C-FAH), humic fraction carbon (C-HUM), and the C-FAH/C-FAF ratio in vineyard and forested areas in altitude regions of fine wine production in Santa Catarina state.

Depth (cm)	Regions							
	Vineyards				Forest			
	I	II	III	IV	I	II	III	IV
	C-HUM (g kg^{-1})							
0 – 5	11.87Aa	0.98Ba	9.27Ba	9.49Bb	12.01Ba	14.87Aa	12.85Ba	12.14Ba
5 – 10	11.12Aa	0.69ABa	6.23Bb	12.13Aa	11.36ABab	13.25Aa	10.05Bb	10.14Bb
10 – 20	9.11Ab	0.27Aa	8.50Aab	9.90Ab	10.75Ab	8.54Bb	9.98ABb	11.20Aab
	C-FAF (g kg^{-1})							
0 – 5	2.51Aa	0.16Aa	2.36Aa	2.28Aa	7.14Aa	7.41Aa	5.84Ba	6.12ABa
5 – 10	1.79Bb	0.83Aa	2.17ABa	2.28Aa	5.98ABb	7.82Aa	5.14Aa	5.12Aa
10 – 20	2.64Aa	0.55Aa	1.77Bb	2.13ABa	6.51ABab	7.46Aa	4.96Ba	5.24Ba
	C-FAH (g kg^{-1})							
0 – 5	2.62Aa	0.22Aa	2.13Aa	2.70Aa	7.23Aa	6.89Aa	4.12Bb	6.25ABa
5 – 10	2.16ABa	0.67Aa	1.66Ba	2.43Aa	6.14ABa	7.12Aa	5.82Ba	5.87Ba
10 – 20	2.48Aa	0.36Aa	2.21Aa	2.70Aa	6.78ABa	7.89Aa	5.25Bab	5.14Ba
	Recovery rate							
0 – 5	94.55Aa	2.20Bb	89.29Aa	80.17ABb	84.71Aa	90.79Aa	90.48Aa	83.06Aa
5 – 10	82.21ABb	3.86Bb	74.52ABb	95.09Aa	77.24Ba	92.18Aa	90.95Aa	75.20Ba
10 – 20	85.77Ab	4.70Aa	86.07Aa	84.03Ab	83.04Aa	84.84Aa	89.69Aa	80.28Aa
	C-FAH/C-FAF							
0 – 5	1.04ABab	0.03ABa	0.90Bb	1.18Aab	1.01Aa	0.93Aa	0.71Bb	1.02Aa
5 – 10	1.21Aa	0.94ABa	0.76Bc	1.07ABb	1.03Aa	0.91Aa	1.13Aa	1.15Aa
10 – 20	0.94Bb	0.93Ba	1.25Aa	1.27Aa	1.04Aa	1.06Aa	1.06Aab	0.98Aa

Mean values followed by the same letter (uppercase for rows; lowercase for columns) are not statistically different from each other as determined by the Tukey's test at 5% probability.

The high percentages of C-HUM in vineyard areas indicate that, in these areas, fractions with high resistance to microbial degradation are predominant due to the strong interaction between C-HUM and the mineral fraction of the soil (STEVENSON, 1994), which is more closely associated with the mineral colloids of the soil (CANELLAS et al., 2000).

The highest absolute values of C-HUM were observed in the superficial layers of the forest areas, which followed the same pattern as observed for TOC. This result can be attributed to the higher contribution of litter and to the absence of anthropic influences in these areas. In natural environments, HS formation is associated with microbial activity and humification over time, which is the result of the microbiological process (MACHADO, GERZABECK, 1993). The accumulation of C-HUM occurs when SOM levels are higher, a response not observed in this study given that the highest values of this fraction were observed in the vineyard areas, which exhibited lower TOC contents when compared to the forested areas.

A higher C-HUM fraction in comparison to the other fractions was also observed in several studies on tropical soils (LOSS et al., 2009, ROSSI et al., 2011, GAZOLLA et al., 2006). In a study that aimed to characterize the HS distribution and edaphic attributes in the profile of soils from different regions of Brazil, Ebelling et al. (2011) reported that the C-HUM fraction is found in most Brazilian soils and in greater quantity than other fractions are. The C-HUM fraction, with high molecular mass (FERREIRA et al., 2004), is the most significant fraction as a reservoir of organic carbon in the soil and is the least soluble fraction as well (CANELLAS; SANTOS, 2005).

The high clay contents present in the soils of the current study may have provided a greater degree of OM humification in the C-HUM fraction demonstrating the effect of texture on SOM maintenance. Some authors include relief as one of the factors influencing C-HUM formation noting that a higher clay content in regions of increased relief stabilizes and protects much of the organic C against mineralization (VOLKOFF et al., 1984; CANELLAS et al., 2000; EBELLING et al., 2011). However, our study found no difference in the C-HUM fraction among the regions evaluated, which have significant differences in relief forms.

Considering the high aluminum and acidity levels of these soils (TESKE, 2010), these characteristics may have strongly influenced the pattern of humic fractions. According to Volkoff et al. (1984), the participation of aluminum in the process of accumulation of humus must be considered.

The C-FAF and C-FAH contents were similar in both forested and vineyard areas (Table 3). However, the values of these fractions were higher in the forested areas.

C-FAF levels represented 12 to 22% of total HSs in vineyard areas and 24 to 31% in forested areas. The latter consisted of soils with textures varying from clayey to very clayey and usually contained a higher carbon content, especially in the form of C-FAF, since these textures have a higher retention capacity for these acids (ASSIS et al., 2006).

In a study that evaluated the effects of soil management practices on SOM and microbial biomass in a Latosol soil, Marchiori Junior and Melo (2000) also observed that the highest TOC values were found in the soiland in the C-HUM fraction in forested areas,.

Fulvic acids are more stable in the soil than humic acids are due to their increased ability to form clayey-humic complexes. In this way, the molecules of soils with a more clayey texture are more important regarding the formation of aggregates (MENDONÇA; ROWELL, 1996). The climate, which is influenced by altitude, may have been reflected in the higher levels of the C-FAF fraction in some cases. The low temperatures reduced the rate of the mineralization process of soil HSs contributing to an increase of the contents of this fraction.

A characteristic of the C-FAF fraction is its greater solubility and mobility through the soil (STEVENSON, 1994). However, in some cases, no increase in this fraction was observed in the deeper layers (Table 3). There were no differences among the soil depths regarding the levels of C-FAF and C-FAH. According to Fontana et al. (2006) because these fractions have lower stability, they can be translocated to deeper layers, polymerized, or mineralized and thus have a reduced residual content in the soil.

C-FAH levels ranged from 14 to 19% in relation to the TOC content in vineyard areas and from 18 to 28% in forested areas. It is possible that the highest values may refer to areas with lower clay content. In a study evaluating the composition of the humid OM of a dystrophic Yellow Argisol in the Coastal Trays under cultivation, Canellas and Façanha (2004) observed that the C-FAH content was always lower in comparison to the C-FAF fraction and that the C-FAH fraction was observed in the superficial layers. They also observed that the highest levels were observed in the C-HUM fraction and that the C-FAH/C-FAF ratio was always less than one and decreased with depth.

The decline in the C-FAH fraction in the vineyard compared to the forested areas may also be related to liming, which interferes with the dynamics of HSs when acidic soils are corrected and after which the C-FAH usually decreases. This result instead may be related to the decrease in the exchangeable aluminum content since liming decreases the forms of exchangeable aluminum and those associated with organic molecules (MENDONÇA; ROWELL, 1996). Cunha and Ribeiro (1998), who worked with soils from calcareous rocks in the state of Bahia in the municipality of Irecê, observed a strong influence of calcium on humic substances, mainly on C-FAF (calcium precipitates C-FAF), followed by polymerization caused by iron, which favor more condensed HS formations. This may explain the higher C-FAH values found in that study.

The C-FAH/C-FAF ratio was used by Kononova (1982) as an indicator of humus quality, since it expresses the degree of evolution in the OM humification process. In tropical soils, lower values of this ratio are typically due to the lower intensity of the humification process, i.e., condensation and synthesis, attributed to the intense mineralization of residues, edaphic restrictions, and the low content of exchangeable bases to the biological activity in the most weathered soils (CANELLAS et al., 2002).

Through analysis of the C-FAH/C-FAF ratio, we assessed the degree of SOM humification and found values close to or greater than 1 in most of the regions and depths evaluated both in vineyard and forested areas (Table 3). The higher the value of the C-FHA/C-FAF ratio, the more intense the polymerization and condensation processes are (GIÁCOMO et al., 2008). Values below 0.80 were observed only in Region III in both the superficial layer of the forested areas (0.71) and the 5-10 cm layer of the vineyard areas (0.76).

Low values of the C-FAH/C-FAF ratio indicate limited evolution of OM added due to management or to pedogenetic processes or even possibly due to recent OM contributions that promote the formation of the C-FAF fraction in relation to the C-FAH fraction. These low values may also indicate less leaching or a translocation of HSs in the soil profile (CANELLAS et al., 2000).

The recovery rate, which is used to evaluate the degree of SOM humification through the C-FAH + C-FAF + HUM/TOC ratio, was between 75 and 92% in the forested areas and 62 and 95% in the vineyard areas. According to Moreno (1996), values between 65 and 92% are considered normal.

Low values such as those observed in the surface layers of Region II (62 and 64%) may indicate organic residues that had been recently added to the soil that had not yet decomposed. On the other hand, the highest values-those observed in the superficial layer of Region I (95%) and in the 5-10 cm layer in Region IV (95%)-may indicate areas where there is less OM contribution or a higher decomposition rate.

Conclusions

Inadequate management of vineyard areas contributed to a reduction in the TOC, TSOG, COp, and COam values.

The forested areas exhibited a higher proportion of vegetative residues in the soil compared to the vineyard areas.

The carbon management index indicated a low or reduced contribution to carbon storage in the vineyard soils.

The humin fraction represented the largest portion of the TOC, and the AF and AH fractions were present in similar amounts.

References

ALMEIDA, J. A. Fatores abióticos. In: BOLDRINI, I.I. (Org). *Biodiversidade dos campos do Planalto das Araucárias.* Brasília: Ministério do Meio Ambiente, 2009. p. 19-38. (Série Biodiversidade, 30).

ASSIS, C. P.; JUCKSCH, I.; SÁ MENDONÇA, E.; NEVES, J. C. L. Carbono e nitrogênio em agregados de Latossolo submetido a diferentes sistemas de uso e manejo. *Pesquisa Agropecuária Brasileira*, Brasília, DF, v. 41, n. 10, p. 1541-1550, 2006.

BAYER, C.; MARTIN-NETO, L.; MIELNICZUK, J.; PAVINATO, A. Armazenamento de carbono em frações lábeis da matéria orgânica de um Latossolo Vermelho sob plantio direto. *Pesquisa Agropecuária Brasileira*, Brasília, DF, v. 39, n. 7, p. 677-683, 2004.

BENITES, V. M.; MADARI, B.; MACHADO, P. L. O. A. *Extração e fracionamento quantitativo de substâncias húmicas do solo:* um procedimento simplificado de baixo custo. Rio de Janeiro: Ministério da Agricultura, Pecuária e Abastecimento, 2003. 7 p.

BLAIR, G. J.; LEFROY, R. D. B.; LISLE, L. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. *Australian Journal of Agricultural Research,* Melbourne, v. 46, n. 7, p. 1459-1466, 1995.

BRAGA, H. J.; GHELLERE, R. Proposta de diferenciação climática para o estado de Santa Catarina. In: CONGRESSO BRASILEIRO DE AGROMETEOROLOGIA E REUNIÃO LATINO AMERICANA DE AGROMETEOROLOGIA, 11., 1999, Florianópolis. *Anais...* Florianópolis: Sociedade Brasileira de Agrometeorologia, 1999. p. 20-31.

BRUN, F. G. K. *Percepção sobre a arborização urbana no Bairro Camobi, Santa Maria – RS:* estudo de um caso. 2008. Especialização (Educação Ambiental) – Universidade Federal de Santa Maria, Santa Maria.

CALONEGO, J. C.; SANTOS, C. H.; TRITAN, C. S.; CUNHA JÚNIOR, J. R. C. Estoques de carbono e propriedades físicas de solos submetidos a diferentes sistemas de manejo. *Revista Caatinga*, Mossoró, v. 25, n. 2, p. 128-135, 2012.

CAMBARDELLA, C. A.; ELIOTT, E. T. Particulate soil organic matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal*, Madison, v. 56, n. 3, p. 7 77-783, 1992.

CANELLAS, L. P.; SANTOS, G. A. (Ed.). *Humosfera:* tratado preliminar sobre a química das substâncias húmicas. Campos dos Goytacazes: Universidade Estadual do Norte Fluminense. 2005. 309 p.

CANELLAS, L. P.; SANTOS, G. A.; RUMJANEK, V. M.; MORAES, A. A.; GURIDI, F. Avaliação de características de ácidos húmicos de resíduos sólidos urbanos I. Métodos espectroscópicos (IV, UV e RMN 13C) e microscopia eletrônica de varredura. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 24, n. 4, p. 741-750, 2000.

CANELLAS, L. P.; OLIVARES, F. L.; OKOROKOVA-FAÇANHA, A. L.; FAÇANHA, A. R. Humic acids isolated membrane H^+-ATPase activity in maize roots. *Plant Physiology*, Lincoln, v. 130, n. 4, p. 1951-1957, 2002.

CANELLAS, L. P.; FAÇANHA, A. R. Chemical nature of soil humifiel fractions and their bioactivity. *Pesquisa Agropecuária Brasileira*, Brasília, v. 39, n. 3, p. 233-240, 2004.

CONCEIÇÃO, P. C.; AMADO, T. J. C.; MIELNICZUK, J.; SPAGNOLLO, E. Qualidade do solo em sistemas de manejo avaliada pela dinâmica da matéria orgânica e atributos relacionados. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 29, n. 5, p. 777-788, 2005.

COSTA, N. R.; ANDREOTTI, M.; LOPES, K. S. M.; YOKOBATAKE, K. L.; FEREIRRA, J. P.; PARIZ, M. C.; BONINI, C. S. B.; LONGHINI, V. Z. Atributos do solo e acúmulo de carbono na integração lavoura-pecuária em sistema plantio direto. *Revista Brasileira Ciência Solo*, Viçosa, MG, v. 39, n. 3, p. 852-863, 2015.

CUNHA, T. J. F.; RIBEIRO, L. P. Qualidade e relações pedogenéticas da matéria orgânica de alguns solos da região de Irecê/BA. *Revista Brasileira Ciência Solo*, Viçosa, v. 22, n. 4, p. 693-704, 1998.

DONAGEMA, G. K.; CAMPOS, D. V. B. de; CALDERANO, S. B.; TEIXEIRA, W. G.; VIANA, J. H. M. (Org.). *Manual de métodos de análise de solos.* 2. ed. rev. Rio de Janeiro: Embrapa Solos, 2011. 230 p. (Embrapa Solos. Documentos, 132).

DORTZBACH, D. *Caracterização dos solos e avaliação da aptidão agrícola das regiões produtoras de vinhos finos de altitude de Santa Catarina*. 2016. Tese (Doutorado em Ciência do Solo) – Universidade Federal Rural do Rio de Janeiro, Seropédica.

DORTZBACH, D.; PEREIRA, M. G.; BLAINSKI, É.; GONZALEZ, A. P. Estoque de C e abundância natural de ^{13}c em razão da conversão de áreas de floresta e pastagem em bioma Mata Atlântica. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 39, n. 6, p. 1643-1660, 2015.

EBELLING, A. G.; ANJOS, L. H. C.; PEREZ, D. V.; PEREIRA, M. G.; GOMES, F. W. F. Atributos químicos, carbono orgânico e substâncias húmicas em Organossolos Háplicos de várias regiões do Brasil. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 35, n. 2, p. 325-336, 2011.

ELLERT, B. H.; BETTANY, J. R. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Canadian Journal Soil Science*, Ottawa, v. 75, n. 4, p. 529-38, 1995.

FERREIRA, J. A.; SIMÕES, M. L.; MILORE, D. M. B. P.; MARTIN-NETO, L.; HAYES, M. H. B. *Caracterização espectroscópica da matéria orgânica do solo*. São Carlos: Embrapa, 2004. (Circular técnica, 24).

FIGUEIREDO, C. C.; RESCK, D. V. S.; CARNEIRO, M. A. C. Labile and stable fractions of soil organic matter under management systems and native Cerrado. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 34, n. 3, p. 907-916, 2010.

FONTANA, A.; PEREIRA, M. G.; LOSS, A.; CUNHA, T. J. F.; SALTON, J. C. Atributos de fertilidade e frações húmicas de um Latossolo Vermelho no Cerrado. *Pesquisa Agropecuária Brasileira*, Brasília, DF, v. 41, n. 5, p. 847-853, 2006.

GAZOLLA, P. R.; GUARESCHI, R. F.; PERIN,A.; PEREIRA, M. G.; ROSSI, C. Q. Frações da matéria orgânica do solo sob pastagem, sistema plantio direto e integração lavoura-pecuária. *Semina: Ciências Agrárias*, Londrina, v. 36, n. 2, p. 693-704, 2015.

GIÁCOMO, R. G.; PEREIRA, M. G.; BALIEIRO, F. C. Estoques de carbono e nitrogênio e distribuição das frações húmicas no solo sob diferentes coberturas florestais. *Revista Brasileira de Ciências Agrárias*, Recife, v. 3, n. 1, p. 42-48, 2008.

KONONOVA, M. M. *Materia orgânica del suelo*: su naturaleza, propiedades y métodos de investigación. Barcelona: Oikos-tau, 1982. 364 p.

LOSS, A.; PEREIRA, M. G.; SCHULTZ, N.; ANJOS, L. H. C. dos; SILVA, E. M. R. Atributos químicos e físicos de um Argissolo Vermelho-Amarelo em sistema integrado de produção agroecológica. *Pesquisa Agropecuária Brasileira*, Brasília, v. 44, n. 1, p. 68-75, 2009.

MACHADO, P. L. O. A.; GERZABEK, M. Tillage and crop rotation interactions on humic substances of a typichaplorthox from southern Brazil. *Soil & Tillage Research*, Amsterdam, v. 26, n. 1, p. 227-236, 1993.

MARCHIORI JUNIOR, M.; MELO, W. J. Alterações na matéria orgânica e na biomassa microbiana em solo de mata natural submetido a diferentes manejos. *Pesquisa. Agropecuária Brasileira*, Brasília, v. 35, n. 3, p. 1177-1182, 2000.

MARTINS, C. M.; COSTA, L. M.; SCHAEFER, C. E. G. R.; SORAES, E. M. B.; SANTOS, S. R. Frações da matéria orgânica em solos sob formações deciduais no norte de Minas Gerais. *Revista Caatinga*, Mossoró, v. 28, n. 4, p. 10-20, 2015.

MENDONÇA, E. S.; ROWELL, D. L. Mineral and organic fractions of two Oxisols and their influence on effective cation-exchange capacity. *Soil Science Society of America Journal*, Madison, v. 60, n. 6, p. 1888-1892, 1996.

MORENO, J. L. *La matéria orgânica em los agrosistemas.* Madrid: Ministerio de Agricultura Pesca y Alimentación, 1996. 174 p.

NICOLOSO, R.S. *Dinâmica da matéria orgânica do solo em* áreas *de integração lavoura-pecuária sob sistema plantio direto*. 2005. Dissertação (Mestrado em Ciência do Solo) – Universidade Federal de Santa Maria, Santa Maria.

OLSON, K. R.; AL-KAISI, M. M.; LAL, R.; LOWERY, B. Experimental consideration, treatments, and methods in determining soil organic carbon sequestration rates. *Soil Science Society of América Jounal*, Madison, v. 78, n. 2, p. 348-360, 2014.

PINHEIRO, E. F. M.; PEREIRA, M. G.; ANJOS, L. H. C.; PALMIERI, F.; SOUZA, R. C. Matéria orgânica em Latossolo Vermelho submetido a diferentes sistemas de manejo e cobertura do solo. *Revista Brasileira de Agrociência*, Pelotas, v. 9, n. 1, p. 53-56, 2003.

ROSCOE, R.; MACHADO, P. L. O. de A. *Fracionamento* físico do solo em estudos da matéria orgânica. Dourados, MS: Embrapa Agropecuária Oeste e Embrapa Solos, 2002. 86 p.

ROSSI, C. Q.; PEREIRA, M. G.; GIÁCOMO, S. G.; BETTA, M.; POLIDORO, J. C. Frações húmicas da matéria orgânica do solo cultivado com soja sobre palhada de braquiária e sorgo. *Bragantia*, Campinas, v. 70, n. 3, p. 622-630, 2011.

SANTA CATARINA – GABINETE DE PLANEJAMENTO E COORDENAÇÃO GERAL, SUBCHEFIA DE ESTATÍSTICA, GEOGRAFIA E INFORMÁTICA. *Atlas de Santa Catarina. Florianópolis*. Rio de Janeiro: IBGE, 1986. 173 p.

SANTOS, H. G.; JACOMINE, P. K. T.; ANJOS, L. H. C.; OLIVEIRA, V. A.; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A.; CUNHA, T. J. F.; OLIVEIRA, J. B. Sistema brasileiro de classificação de solos. 3. ed. Brasília, DF: Embrapa, 2013.

SILVA, F. A. S.; AZEVEDO, C. A. V. Versão do programa computacional Assistat para o sistema operacional Windows. *Revista. Brasileira de Produtos Agroindustriais*, Campina Grande, v. 4, n. 1, p. 71-78, 2002.

SISTI, C. P. J.; SANTOS, H. P.; KOHHANN, R.; ALVES, B. J. R.; URQUIAGA, S.; BODDEY, R. M. Change in carbon and nitrogen stocks in soil under 13 years of conventional or zero tillage in southern Brazil. *Soil & Tillage Research*, Amsterdam, v.76, n. 1, p. 39-58. 2004.

STEVENSON, F. J. *Humus chemistry:* genesis, composition, reactions. 2. ed. John Willey, New York, USA. 1994. 496 p.

TESKE, R. *Relações solo – litologia numa sequência de solos desenvolvidos de rochas efusivas no Planalto Sul de Santa Catarina*. 2010. (Dissertação de Mestrado em Ciência do Solo) – Universidade do Estado de Santa Catarina, Lages.

VEIGA, M. *Metodologia para coleta de amostras e análises físicas do solo.* Florianópolis: Epagri, 2011. 52 p. (Epagri. Boletim técnico, 156).

VELDKAMP, E. Organic carbon turnover in three tropical soils under pasture after deforestation. *Soil Science Society American Journal,* Madison, v. 58, n. 1, p. 175-80, 1994.

VOLKOFF, B.; CERRI, C. C.; MELFI, A. J. Húmus e mineralogia dos horizontes superficiais de três solos de campo de altitude dos estados de Minas Gerais, Paraná e Santa Catarina. *Revista Brasileira de Ciência do Solo*, Viçosa, v. 8, n. 3, p. 277-283, 1984.

YEOMANS, J. C.; BREMNER, J. M. A rapid and precise method for routine determination of organic carbon in soil 1. *Communications in Soil Science & Plant Analysis*, Philadelphia, v. 19, n. 13, p. 1467-1476, 1988.