Fluorescence lifetime evaluation of whole soils from the Amazon rainforest

GUSTAVO NICOLODELLI,[1,*] AMANDA MARIA TADINI,[1,2] MARCELO SAITO NOGUEIRA,[3] SEBASTIÃO PRATAVIEIRA,[3] (ID)
STEPHANE MOUNIER,[4] JOSE LUIS CLABEL HUAMAN,[1] CLÉBER HILÁRIO DOS SANTOS,[1] CÉLIA REGINA MONTES,[5]
AND DÉBORA MARCONDES BASTOS PEREIRA MILORI[1]

[1]Embrapa Instrumentation, São Carlos, SP, Brazil
[2]São Carlos Institute of Chemistry, University of São Paulo, São Carlos, SP, Brazil
[3]São Carlos Institute of Physics, University of São Paulo, P.O. Box 369, 13560-970 São Carlos, SP, Brazil
[4]Laboratoire PROTEE, EA3819, Université de Toulon, 83957 La Garde, France
[5]Centro de Energia Nuclear na Agricultura and NUPEGEL, University of São Paulo, Piracicaba, SP, Brazil
*Corresponding author: gunicolodelli@hotmail.com

Time-resolved fluorescence spectroscopy (TRFS) is a new tool that can be used to investigate processes of interaction between metal ions and organic matter (OM) in soils, providing a specific analysis of the structure and dynamics of macromolecules. To the best of our knowledge, there are no studies in the literature reporting the use of this technique applied to whole/non-fractionated soil samples, making it a potential method for use in future studies. This work describes the use of TRFS to evaluate the fluorescence lifetimes of OM of whole soils from the Amazon region. Analysis was made of pellets of soils from an oxisol–spodosol system, collected in São Gabriel da Cachoeira (Amazonas, Brazil). The fluorescence lifetimes in the oxisol–spodosol system were attributed to two different fluorophores. One was related to complexation of an OM fraction with metals, resulting in a shorter fluorophore lifetime. A short fluorescence lifetime (2–12 ns) could be associated with simpler structures of the OM, while a long lifetime (19–66 ns) was associated with more complex OM structures. This new TRFS technique for analysis of the fluorescence lifetime in whole soil samples complies with the principles of green chemistry. © 2017 Optical Society of America

OCIS codes: (300.2530) Fluorescence, laser-induced; (300.6280) Spectroscopy, fluorescence and luminescence; (300.6500) Spectroscopy, time-resolved.

https://doi.org/10.1364/AO.56.006936

1. INTRODUCTION

Numerous studies of the fluorescence of soil organic matter (SOM) have been made in recent years in different regions around the world [1–7]. The fluorescence techniques that have been successfully employed for this purpose include conventional fluorescence [1,2,5], laser-induced fluorescence spectroscopy (LIFS) [8–10], and three-dimensional emission-excitation matrix (EEM) fluorescence spectroscopy [11,12]. Fluorescence methods are suitable for the investigation of SOM in its natural condition or after extraction of the different fractions [1,12–15].

Knowledge of the composition and global distribution of SOM provides a valuable storehouse of information on vegetation, parent materials, climate, and disturbance [16]. An interesting region for the study of SOM is the Amazon forest, the largest rainforest in the world, with about 60% of its area in Brazil. More than 18% of the Amazon region is covered by

oxisol–spodosol systems [17], characterized by the juxtaposition of spodosols and oxisols in the same landscape units [18,19]. Oxisols are soils of equatorial regions where high rates of erosion and long-term evolution have resulted in leaching of all the main elements, except Al, Fe, and Ti [20]. Spodosols appear and develop where the dissolved organic matter (DOM) can percolate through the soil horizons, reaching the rivers, allowing the leaching of Al and Fe, and favoring the dissolution of clay and iron oxides [21–23]. These soil systems make a significant contribution to the global CO_2 cycle, in which the dynamics and accumulation of organic matter are still poorly understood.

The current methods of humic substance analysis require lengthy and complex sample preparation procedures (such as chemical fractionation of the soil), making them time-consuming and expensive [10]. Spectroscopic techniques such as time-resolved fluorescence spectroscopy (TRFS) offer

promising alternatives for application in soil analyses, since they are fast and require minimal sample preparation.

Time-correlated single photon counting (TCSPC) is a highly sensitive TRFS technique. After excitation of the substrate, low intensity light signals can be detected, enabling determination of the emission dynamics of fluorescent molecules with picosecond resolution and with extremely high precision [24]. The fluorescence lifetime, τ (commonly called the decay time), can be understood as the mean length of time that the fluorophore remains in an excited state, following excitation [25]. The fluorescence lifetime measurement does not provide the number of excited molecules in the sample, but reflects the fluorescence efficiency of these molecules [25]. Measurements of this lifetime can be used as indicators of these parameters and can yield information about the molecular microenvironment of the fluorophore [26].

There are many studies in the literature concerning the use of TRFS to characterize the structures, dynamics, and interactions of biological molecules [27–29]. In the case of soil, the TRFS method is suitable for investigating the dynamic processes of interaction between metal ions and natural organic matter (NOM) [29,30]. The high complexity of the fluorescence decay of NOM can be due to various intra- and intermolecular processes and structural characteristics of the matrix, such as many different fluorophores or a small number of fluorophores in many molecular environments [29]. TRFS is a new tool in the field of spectroscopy, allowing specific analysis of the structure and dynamics of macromolecules [28]. However, this is the first time, to the best of our knowledge, that TRFS has been applied in analysis of the OM of whole soil, making this present paper of high scientific interest. The soils studied were from the Amazon region, which is considered one of the world's largest carbon reservoirs [31]. Thus, the aim of this study was to evaluate the OM fluorescence lifetime in whole soils of the Amazon region, using the TRFS technique.

2. EXPERIMENTAL PART

A. Study Area and Soil Samples

The samples were collected in the municipality of São Gabriel da Cachoeira, Amazonas State, Brazil (0°6′24.5″ S, 66°54′19.3″ W). A total of 18 samples of two soil profiles, a humiluvic spodosol (HS) at the top of a hill and a yellow oxisol (YO) on the hillside, were collected for subsequent analyses. This small number of samples was due to access difficulties in the collection areas. The humiluvic spodosol had a typical vertical succession of horizons: An (organic-mineral surface); E (albic); Bh (spodic); Tr (transition between albic horizons); and K (the underlying kaolin). The yellow oxisol presented the following sequence of horizons: An (organic-mineral surface); BA (transition); Bw (intermediate set of oxic horizons, sandy clay loam to silty clay loam); and Kn (lower set of layers of kaolin with gibbsitic nodules).

The soil samples were dried at 35°C in an oven with renewed air circulation, sieved to remove roots, crushed, and then sieved again to 2.0 mm to obtain similar samples. The soil samples were then ground and passed through a 100-mesh sieve, and pellets were prepared from the homogenized samples using a pressure of $10 \, \text{ton} \, \text{cm}^{-2}$ for 30 s.

B. Time-Resolved Fluorescence and Laser-Induced Fluorescence Measurements

The time-resolved measurements were performed using the TCSPC technique. Two diode lasers were used as excitation sources, one emitting at 378 nm and the other at 445 nm (BDL-375-SMC and BDL-445-SMC, Becker and Hickl, Berlin, Germany), in pulsed mode, with a repetition rate of 20 MHz. The average powers of the lasers were 80 µW for the 378 nm laser and 20 µW for the 445 nm laser. These two wavelengths were chosen to match the absorptions of fluorophores "1" and "2" found in a previous study [11]. The pulsewidth of the lasers was around 80 ps. Delivery and collection of the light employed a bifurcated optical fiber (Y-fiber) with a 600 µm core (Ocean Optics). A long pass filter was used to remove the backreflected excitation light (405 nm long pass for 378 nm excitation and 475 nm long pass for 445 nm excitation). The collected light was transferred to a high-speed hybrid photomultiplier tube detector (HPM-100-50, Becker and Hickl, Berlin, Germany). The experimental setup is shown in Fig. 1. The sample was placed on a flat surface, and the probe was positioned perpendicularly 2 mm above the surface of the sample. The data were processed using SPCM software (Becker and Hickl, Berlin, Germany) and were analyzed with a home-made script in MATLAB v. R2012a (Mathworks, Natick, MA, USA). Five measurements were performed for each sample, and an average characteristic decay time was obtained for each decay curve.

A spectrometer (USB 2000, Ocean Optics, USA) was used to measure the steady state laser-induced fluorescence spectra transmitted by the optical fiber.

The collected fluorescence decay curve was best fitted using a bi-exponential function (Fig. 2):

$$F(t) = a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}, \tag{1}$$

where τ_1 and τ_2 are the fluorescence lifetimes of the short and long lifetime components, respectively, and a_1 and a_2 are the coefficients associated with these components. To compare the decays, the average fluorescence lifetime, $\langle \tau \rangle_f$, was calculated as follows [26]:
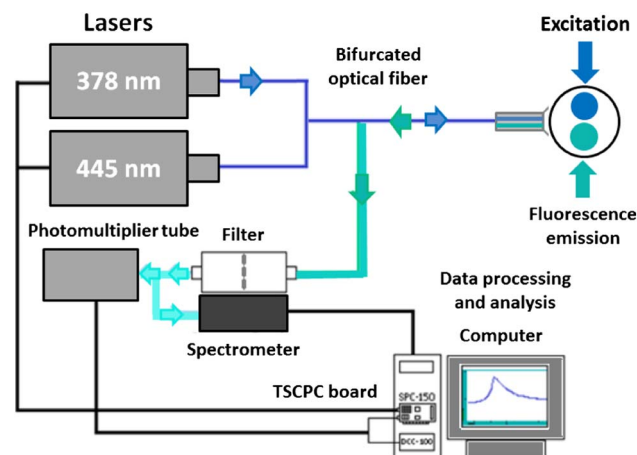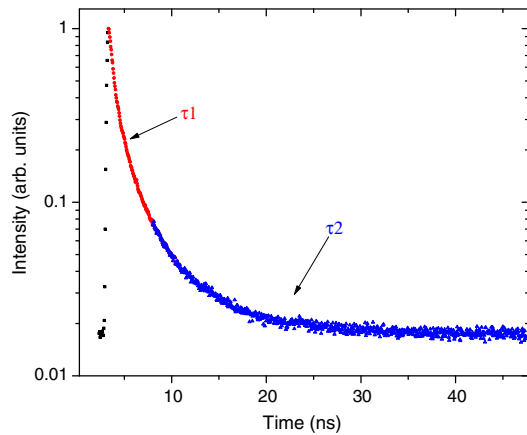

Fig. 1. Experimental setup for fluorescence lifetime determination.

Fig. 2. Illustrative fluorescence decay curves obtained for lifetimes τ_1 and τ_2, highlighted in red and blue, respectively, for the YO excited at 445 nm.

$$\langle\tau\rangle_f = \frac{a_1\tau_1^2 + a_2\tau_2^2}{a_1\tau_1 + a_2\tau_2}. \tag{2}$$

C. Inductively Coupled Plasma Optical Emission Spectrometry

The decomposition of the soil samples was performed as described in EPA Method 3052 (1996), followed by determination of the aluminum (Al) concentrations using ICP-OES.

3. RESULTS AND DISCUSSION

The fluorescence spectra for the HS (horizon Tr), obtained using laser excitation at 378 and 445 nm, showed that the fluorescence profiles differed for the two excitations (Fig. 3). For excitation at 378 nm, the emission spectrum presented two peaks at around 440 and 520 nm, while the excitation at 445 nm resulted in a single peak at around 530 nm.

Santos *et al.* [11] obtained EEM fluorescence spectra of samples of humic substances extracted from these same soils. Application of parallel factor analysis (CP/PARAFAC) revealed that two fluorophores ("1" and "2") were responsible for the
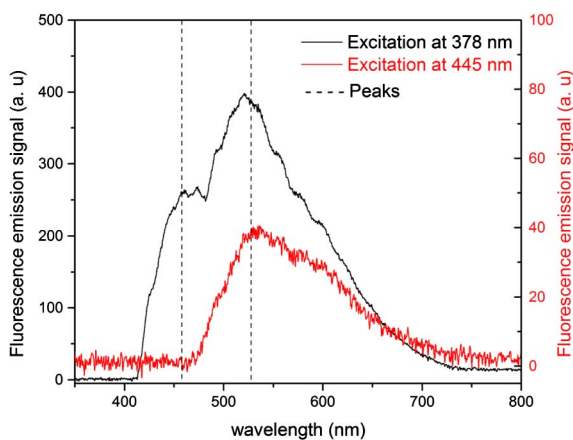

Fig. 3. Fluorescence emission spectra of the HS soil (horizon Tr) using laser excitation at 378 nm (black line) and 445 nm (red line).

fluorescence. Hence, the fluorescence data obtained with excitation at 378 nm (emission at around 420 nm) corresponded to fluorophore "2," equivalent to the peak at $\lambda_{em}/\lambda_{exc} = 420\,\text{nm}/375\,\text{nm}$ found for humic acids [11,12,32,33], which is usually associated with terrestrial humic substances containing simpler fluorophores. The fluorescence peak at $\lambda_{em}/\lambda_{exc} = 530\,\text{nm}/445\,\text{nm}$, corresponding to fluorophore "1" [11], showed a dominant contribution for these samples, reflecting typical terrestrial humic acids derived from lignin, whose structures are more complex [32,33].

Since the importance of these excitation wavelengths for characterization of the SOM was demonstrated previously, the lifetime measurements for the oxisol–spodosol system were performed using these two excitations. The results are shown in Figs. 4 and 5.

Figure 4 shows the average fluorescence lifetimes for the HS and YO soils, with excitation at 445 nm. The humiluvic spodosol presented different fluorescence lifetimes as a function of depth [Fig. 4(a)]. Horizons A, E, and Bh showed the highest average lifetime values, in the range 47–66 ns, and smaller amounts of aluminum were present in these horizons. The shortest average lifetimes and the highest aluminum contents were found for horizons Tr and K of this soil, which could be attributed to complexation of the organic matter with the
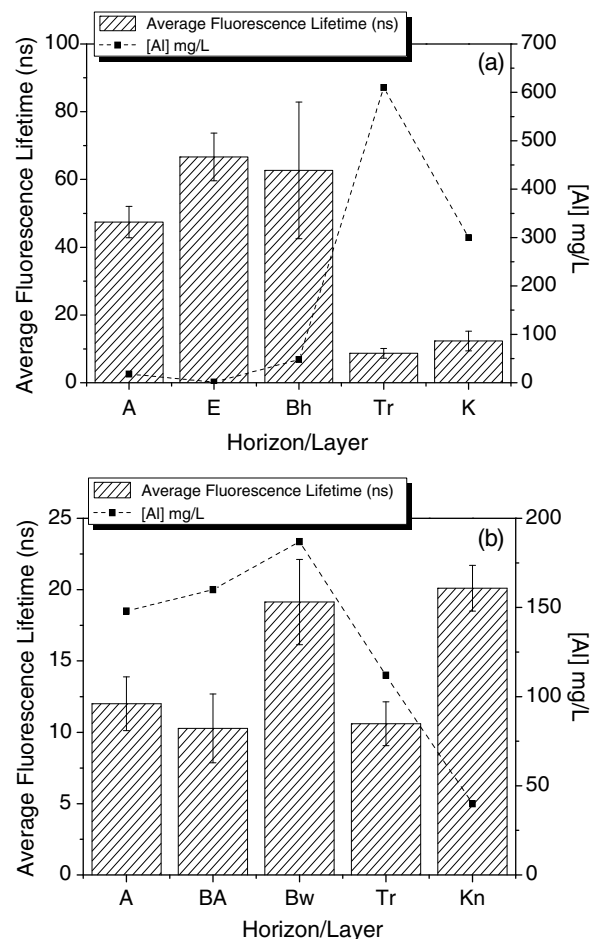
Fig. 4. Average fluorescence lifetimes with excitation at 445 nm, and Al concentrations, for (a) HS and (b) YO.
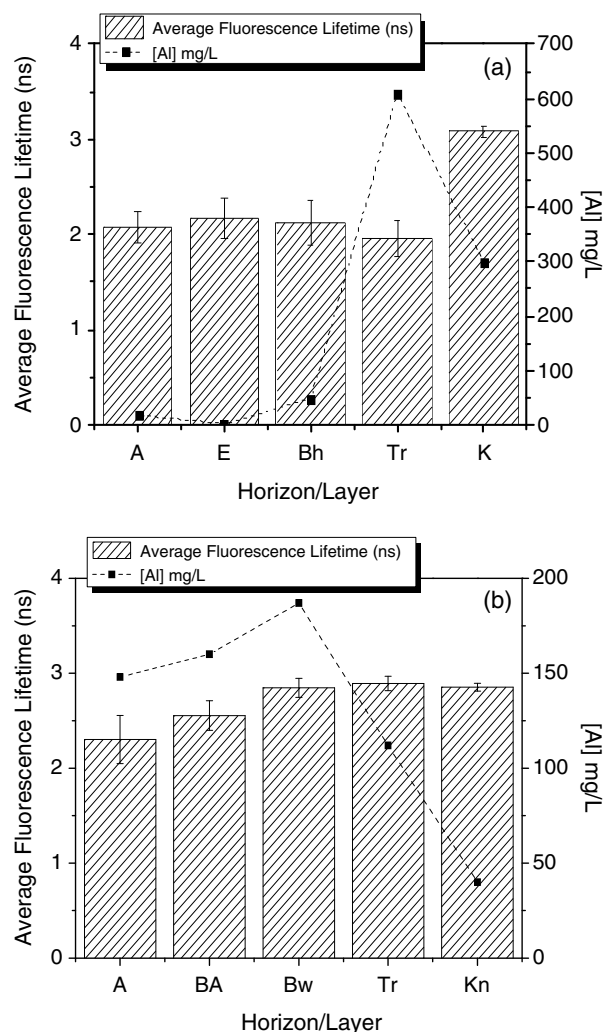
Fig. 5. Average fluorescence lifetimes with excitation at 378 nm, and Al concentrations, for (a) humiluvic spodosol and (b) yellow oxisol.

metal, resulting in a shorter fluorophore lifetime. The Al atoms are strongly bound to –OH groups at the surfaces of the octahedral sheets of kaolinite. According to depth, the octahedral sheets undergo rearrangements and fracturing along the layers, hence increasing the quantity of exposed functional groups, the ion exchange capacity, and the complexation of organic matter [34–36]. Margenot et al. [37] found that the A, E, and Bh horizons presented smaller amounts of kaolinite mineral and higher concentrations of functional groups on the mineral surface. However, in the case of kaolinite-rich horizons, such as Tr and K, penetration of water and cations between the horizons is difficult, so the cation exchange capacities are lower. This can be associated with low concentrations of functional groups [38,39]. The interaction of the fluorophore with the environment can either increase or decrease the efficiency of energy transfer, which can affect the fluorescence decay time of the fluorophore, as shown in Figs. 4 and 5. The dominant minerals in YO are kaolinite and gibbsite, which have low cation exchange capacities, compared to HS horizons A, E, and Bh, with much smaller values for horizons Bw and Kn. The differences

in cation exchange capacity can be mainly ascribed to pH variation, as suggested by Oliveira et al. [40]. As shown in Fig. 4(b), lifetime values of around 11 ns were obtained for horizons A, BA, and Tr. The highest average lifetime values were found for the Bw and Kn horizons, with values of around 20 ns for the YO samples excited at 445 nm. The highest lifetime value obtained for the Kn horizon could be explained by the low amount of metal present, as indicated by the aluminum content of this horizon, since a longer fluorophore lifetime is associated with the presence of the metal ions, which act as fluorescence suppressors. However, different behavior was observed for the Bw horizon, which presented higher values for both fluorophore lifetime and aluminum content.

Figure 5 shows the average fluorescence decay lifetimes for soils HS and YO with excitation at 378 nm. The HS soil presented different lifetimes as a function of depth [Fig. 5(a)]. Horizons A, E, Bh, and Tr displayed similar behaviors, with average lifetimes around 2 ns. There were no significant differences among the average lifetimes for these horizons. However, horizon K showed a small increase in the lifetime, which could be attributed to the degree of humification [11]. For laser excitation at 378 nm, the main fluorophore contribution was related to the humic fraction with simpler structures (such as fulvic acids).

In the case of the YO soil [Fig. 5(b)], there were no significant differences among the average lifetimes from the surface to deeper horizons (A to Kn), with values in the range from 2.30 to 2.89 ns. As for the HS soil [Fig. 5(a)], this suggested that the main fluorophore contribution was associated with the humic fraction containing simpler structures.

The average fluorescence lifetimes (2–12 ns) related to simpler structures of the SOM support the identification of fluorophore "2" proposed by Santos et al. [11], while the average lifetimes of 47–66 ns (HS) and 19–20 ns (YO) could be attributed to greater contributions of fluorophore "1," associated with more complex organic structures. It can be seen from Figs. 4 and 5 that laser excitation at 445 nm revealed greater structural differentiation according to depth, compared to excitation at 378 nm, because structures of greater complexity were excited. Power et al. [41] associated a 2 ns lifetime component to a fulvic acid with phenolcarboxylate constituents, while a longer lifetime was attributed to other unspecified chromophores. Single aromatic rings with oxygen, amine, and methyl substituents have fluorescence decay lifetimes of 2 to 4 ns, with characteristic emission spectra [42].

Fluorescence lifetimes are highly sensitive to the microenvironment of the molecules, and the same fluorophore molecule can exhibit different lifetimes in different microdomains (such as hydrophobic and hydrophilic environments). For example, phenol is a potential model for DOM fluorophores and has fluorescence lifetimes of 2.1 and 7.4 ns in cyclohexane and methanol, respectively [42,43]. Alcala et al. [27] reported that fluorescence decay rates are strongly dependent on the structure and conformation of the sample. For example, in the case of a protein, its conformation is influenced by its environment, and inhibitory mechanisms that affect the protein will also affect the excited state of the molecule, hence altering its fluorescence lifetime. Therefore, in order to apply fluorescence analysis to soil

samples, a fundamental understanding of the fluorophores/soil interaction is essential [44]. As pointed out by Kumke *et al.* [29], the high complexity of the fluorescence decay of NOM can be caused by various intra- and intermolecular processes, together with structural characteristics of the matrix, such as the presence of many different fluorophores, or a small number of fluorophores in many molecular environments. In the present case, the OM from the oxisol–spodosol system showed different lifetimes that could be attributed to two dominant fluorophores ("2" and "1," respectively), when two laser excitations were used.

4. CONCLUSIONS

The fluorescence lifetimes in whole soils are reported here for the first time, to the best of our knowledge. TRFS was shown to be a viable new technique for the analysis of fluorescence lifetimes in whole soil samples in the solid state. No previous articles on this theme were found in the literature. Fluorescence lifetimes in an oxisol–spodosol system from the Amazon region were associated with two different fluorophores, one of which was related to the fraction of OM complexed with Al metal (which caused a decrease in the fluorophore lifetime), while the other was attributed to terrestrial humic substances, consisting of groups with simpler fluorophores. The results presented in this study show the promising potential of the TRFS technique for *in situ* analysis of humic substances using an apparatus capable of portability. Additional advantages of this technique are that the analyses are fast and inexpensive and comply with the principles of green chemistry.

REFERENCES

1. N. Senesi, T. M. Miano, M. R. Provenzano, and G. Brunetti, "Characterization, differentiation and classification of humic substances by fluorescence spectroscopy," Soil Sci. **152**, 259–271 (1991).
2. H. H. Patterson, C. S. Cronan, S. Lakshman, B. J. Plankey, and T. Taylor, "Comparison of soil fulvic-acids using synchronous scan fluorescence spectroscopy, FTIR, titration and metal complexation kinetics," Sci. Total Environ. **113**, 179–196 (1992).
3. A. A. S. C. Machado and J. C. G. Esteves da Silva, "Factor analysis of molecular fluorescence data of marine and soil fulvic acids," Chemom. Intell. Lab. Syst. **19**, 155–167 (1993).
4. A. A. S. C. Machado and J. C. G. Esteves da Silva, "Multivariate curve resolution analysis excitation-emission matrices of fluorescence of humic substances," Anal. Chim. Acta **546**, 52–59 (2005).
5. M. Fuentes, G. González-Gaitano, and J. M. García-Mina, "The usefulness of UV-visible and fluorescence spectroscopies to study the chemical nature of humic substances from soils and composts," Org. Geochem. **37**, 1949–1959 (2006).
6. C. Xiaoli, L. Guixiang, Z. Xin, H. Yongxia, and Z. Youcai, "Fluorescence excitation-emission matrix combined with regional integration analysis to characterize the composition and transformation of humic and fulvic acids from landfill at different stabilization stages," Waste Manage. **32**, 438–447 (2012).
7. F. Tivet, J. C. de Moraes Sá, R. Lal, D. M. B. P. Milori, C. Briedis, P. Letourmy, L. A. Pinheiro, P. R. Borszowskei, and D. da Cruz Hartman, "Assessing humification and organic C compounds by laser-induced fluorescence and FTIR spectroscopies under conventional and no-till management in Brazilian oxisols," Geoderma **207–208**, 71–81 (2013).
8. M. G. Pérez, D. M. B. P. Milori, L. Martin-Neto, L. A. Colnago, O. A. de Camargo, R. Berton, and W. Bettio, "Laser-induced fluorescence of organic matter from a Brazilian oxisol under sewage-sludge applications," Sci. Agricola **63**, 269–275 (2006).
9. D. M. B. P. Milori, H. V. A. Galeti, L. Martin-Neto, J. Dieckow, M. González-Pérez, C. Bayer, and J. Salton, "Organic matter study of whole soil samples using laser-induced fluorescence spectroscopy," Soil Sci. Soc. Am. J. **70**, 57–63 (2006).
10. C. H. Santos, R. A. Romano, G. Nicolodelli, C. M. Carvalho, P. R. Villas-Boas, L. Martin-neto, C. R. Montes, A. J. Melfi, and D. M. B. P. Milori, "Performance evaluation of a portable laser-induced fluorescence spectroscopy system for the assessment of the humification degree of the soil organic matter," J. Braz. Chem. Soc. **26**, 775–783 (2015).
11. C. H. Santos, G. Nicolodelli, R. A. Romano, A. M. Tadini, P. R. Villas-Boas, C. R. Montes, S. Mounier, and D. M. B. P. Milori, "Structure of humic substances from some regions of the Amazon assessed coupling 3D fluorescence spectroscopy and CP/PARAFAC," J. Braz. Chem. Soc. **26**, 1136–1142 (2015).
12. A. M. Tadini, G. Nicolodelli, S. Mounier, C. R. Montes, and D. M. B. P. Milori, "The importance of humin in soil characterisation: a study on Amazonian soils using different fluorescence techniques," Sci. Total Environ. **537**, 152–158 (2015).
13. D. M. B. P. Milori, L. M. Neto, and C. Bayer, "Fluorescência Induzida por Laser para Análise da Matéria Orgânica de Solos Intactos," Boletim de Pesquisa e Desenvolvimento EMBRAPA **3**, 1–18 (2002).
14. M. González Pérez, L. Martin-Neto, S. C. Saab, E. H. Novotny, D. M. B. P. Milori, V. S. Bagnato, L. Colnago, W. J. Melo, and H. Knicker, "Characterization of humic acids from a Brazilian oxisol under different tillage systems by EPR, 13C NMR, FTIR and fluorescence spectroscopy," Geoderma **118**, 181–190 (2004).
15. T. Martins, S. C. Saab, D. M. B. P. Milori, A. M. Brinatti, J. A. Rosa, F. A. M. Cassaro, and L. F. Pires, "Soil organic matter humification under different tillage managements evaluated by laser induced fluorescence (LIF) and C/N ratio," Soil Tillage Res. **111**, 231–235 (2011).
16. E. A. Paul, "The nature and dynamics of soil organic matter: plant inputs, microbial transformations, and organic matter stabilization," Soil Biol. Biochem. **98**, 109–126 (2016).
17. Brasil, Departamento Nacional da Produção Mineral, *Projeto RADAMBRASIL. Levantamento de Recursos Naturais, v. 18* (Folha SA.20 Manaus; geologia, geomorfologia, pedologia, vegetação e uso potencial da terra, 1978).
18. Y. Lucas, A. Chauvel, R. Boulet, G. Ranzani, and F. Scatolini, "Transição latossolos-podzois sobre a formação barreiras na região de Manaus, Amazônia," Revista brasileira de ciencia do solo **8**, 325–335 (1984).
19. Y. Lucas, C. R. Montes, S. Mounier, M. Loustau-Cazalet, D. Ishida, R. Achard, C. Garnier, and A. J. Melfi, "Biogeochemistry of an Amazonian podzol-ferralsol soil system with white kaolin," Biogeosciences **9**, 3705–3720 (2012).
20. Y. Lucas, D. Nahon, S. Cornu, and F. Eyrolle, "Genèse et fonctionnement des sols en milieu equatorial," C. R. Acad. Sci. Paris Ser. IV **322**, 1–16 (1996).
21. Y. Lucas, R. Boulet, and A. Chauvel, "Intervention simultanée desphénomènes d´enfoncement vertical et de transformation latérale dans la mise en place de systems de sols de la zone tropicale humide. Cas des systèmes sols ferrallitiques—podzols de l´Amazonie Brésilienne," C. R. Acad. Sci. **306**, 1395–1400 (1998).
22. U. S. Lundström, N. van Breemen, and D. Bain, "The podzolization process," Geoderma **94**, 91–107 (2000).
23. N. R. Do Nascimento, G. T. Bueno, E. Fritsch, A. J. Herbillon, T. Allard, A. J. Melfi, R. Astolfo, H. Boucher, and Y. Li, "Podzolization as a deferralitization process: a study of an Acrisol-Podzol sequence derived from Palaeozoic sandstones in the northern upper Amazon Basin," Eur. J. Soil Sci. **55**, 523–538 (2004).
24. W. Becker, *The bh TCSPC Handbook*, 5th ed. (Becker & Hickl GmbH, 2012).
25. X. F. Wang and B. Herman, *Fluorescence Imaging Spectroscopy & Microscopy* (Wiley-Interscience, 1996).
26. L. Marcu, P. M. W. French, and D. S. Elson, *Fluorescence Lifetime Spectroscopy and Imaging: Principles and Applications in Biomedical Diagnostics* (CRC Press, 2014).

27. J. R. Alcala, E. Gratton, and F. G. Prendergast, "Fluorescence lifetime distributions in proteins," Biophys. J. **51**, 597–604 (1987).

28. F. G. Prendergast, "Time-resolved fluorescence techniques: methods and applications in biology," Curr. Opin. Struct. Biol. **1**, 1054–1059 (1991).

29. M. U. Kumke, C. Tiseanu, G. Abbt-Braun, and F. H. Frimmel, "Fluorescence decay of natural organic matter (NOM)—influence of fractionation, oxidation, and metal ion complexation," J. Fluoresc. **8**, 309–318 (1998).

30. S. Lukman, T. Saito, N. Aoyagi, T. Kimura, and S. Nagasaki, "Speciation of Eu 3+ bound to humic substances by time-resolved laser fluorescence spectroscopy (TRLFS) and parallel factor analysis (PARAFAC)," Geochim. Cosmochim. Acta **88**, 199–215 (2012).

31. C. C. Cerri, M. Bernoux, D. Arrouays, B. J. Feigl, and M. C. Piccolo, "Carbon stocks in soils of the Brazilian Amazon," in *Global Climate Change and Tropical Ecosystems*, J. M. Kimble and B. A. Stewart, eds., Advances in Soil Science (CRC Press, 2000), pp. 33–50.

32. B. J. H. Matthews, A. C. Jones, N. K. Theodorou, and A. W. Tudhope, "Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs," Mar. Chem. **55**, 317–332 (1996).

33. P. Coble, J. Lead, A. Baker, D. Reynolds, and R. G. M. Spencer, eds., *Aquatic Organic Matter Fluorescence, Environmental Chemistry* (Cambridge University, 2014).

34. C. Chenu, C. Rumpel, and J. Lehmann, "Chapter 13–methods for studying soil organic matter: nature, dynamics, spatial accessibility, and interactions with minerals," in *Soil Microbiology, Ecology and Biochemistry*, E. A. Paul, ed., 4th ed. (Academic, 2015), pp. 383–419.

35. N. R. Osornio-Rubio, J. A. Torres-Ochoa, M. L. Palma-Tirado, H. Jiménez-Islas, R. Rosas-Cedillo, J. C. Fierro-Gonzalez, and G. M. Martínez-González, "Study of the dehydroxylation of kaolinite and alunite from a Mexican clay with DRIFTS-MS," Clay Miner. **51**, 55–68 (2016).

36. F. Franco, L. A. Pérez-Maqueda, and J. L. Pérez-Rodríguez, "The effect of ultrasound on the particle size and structural disorder of a well-ordered kaolinite," J. Colloid Interface Sci. **274**, 107–117 (2004).

37. A. J. Margenot, F. J. Calderon, K. W. Goyne, F. N. D. Mukome, and S. J. Parikh, "IR Spectroscopy, soil analysis applications," in *IR Spectroscopy, Soil Analysis Applications*, 3rd ed. (2017), Vol. **2**, pp. 448–454.

38. C. Ma and R. A. Eggleton, "Cation exchange capacity of kaolinite," Clays Clay Miner. **47**, 174–180 (1999).

39. S. Bravard and D. Righi, "Characteristics of clays in an oxisol-spodosol toposequence in Amazonia (Brazil)," Clay Miner. **23**, 279–289 (1988).

40. F. C. Oliveira, M. E. Mattiazzo, C. R. Marciano, and R. Rossetto, "Organic carbon, electric conductivity, ph and CEC changes in a typic hapludox, after repeated sludge application," Braz. J. Soil Sci. **26**, 505–519 (2002).

41. J. F. Power, R. LeSage, D. K. Sharma, and C. H. Langford, "Fluorescence lifetimes of the well characterized humic substance, armdale fulvic acid," Environ. Technol. Lett. **7**, 425–430 (1986).

42. I. B. Berlman, *Handbook of Fluorescence Spectra of Aromatic Molecules*, 2nd ed. (Academic, 1971).

43. C. D. Clark, J. Jimenez-Morais, G. Jones II, E. Zanardi-Lamardo, C. A. Moore, and R. G. Zika, "A time-resolved fluorescence study of dissolved organic matter in a riverine to marine transition zone," Mar. Chem. **78**, 121–135 (2002).

44. M. U. Kumke, G. Li, L. B. McGown, G. T. Walker, and C. P. Linn, "Hybridization of fluorescein-labeled DNA oligomers detected by fluorescence anisotropy with protein binding enhancement," Anal. Chem. **67**, 3945–3951 (1995).