COMPOSIÇÃO DA MATÉRIA ORGÂNICA EM AGREGADOS DE SOLOS SOB DIFERENTES SISTEMAS AGROFLORESTAIS DE CACAU NO SUL DA BAHIA, BRASIL

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MARÇO – 2020

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"Tese apresentada ao Centro de Ciências e Tecnologias Agropecuárias da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para obtenção do título de Doutora em Produção Vegetal".

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FICHA CATALOGRÁFICA
UENF - Bibliotecas
Elaborada com os dados fornecidos pela autora.

V632 Vicente, Laís de Carvalho.

Composição da matéria orgânica em agregados de solos sob diferentes sistemas agroflorestais de cacau no Sul da Bahia, Brasil / Laís de Carvalho Vicente. - Campos dos Goytacazes, RJ, 2020.

105 f. : il.
Bibliografia: 80 - 93.

Tese (Doutorado em Produção Vegetal) - Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, 2020.
Orientadora: Emanuela Forestieri da Gama Rodrigues.

1. Frações densimétricas. 2. Seringueira. 3. Eritrina. 4. Infravermelho. 5. Ressonância Magnética Nuclear. I. Universidade Estadual do Norte Fluminense Darcy Ribeiro. II. Título.

CDD - 630

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Aprovada em 24 de março de 2020

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À minha mãe, hoje minha estrela guia.

DEDICO

AGRADECIMENTOS

A Deus, pela força para ter seguido até aqui.

Aos meus pais, por todo amor e ensinamentos ao longo de todo o meu crescimento pessoal.

Aos meus irmãos, Murilo e Gabriel, que são um norte para mim, me passando tranquilidade e incentivando ao longo desses anos.

As minhas irmãs, encontradas durante a graduação, Amanda Justino Acha e Francielle de Souza Guimarães, e que seguem comigo nessa jornada ao longo desses 10 anos, me dando força e ombro amigo sempre que precisei.

Aos amigos de laboratório, principalmente ao Seldon Aleixo e Laís Chierici, que além dos assuntos científicos sérios, deixam os dias de trabalho mais leves, com os assuntos aleatórios e engraçados.

A minha orientadora, Emanuela Forestieri da Gama-Rodrigues, por ter acreditado no meu potencial ao longo de todos esses anos. Agradeço a parceria, os ensinamentos e todos os puxões de orelha necessários. Agradeço também por ter sido uma amiga, me orientando e não me deixando desistir, mesmo diante de uma das situações mais difíceis da minha vida.

À Katia Regina do Nascimento Sales, por toda a ajuda nas análises e pelas conversas animadas na hora do café, que ajudaram a aliviar os momentos mais tensos. Assim como ao Ederaldo, Vanilda e André, pelo auxílio nas mais diversas questões e pelos momentos de descontração.

Ao Marcelo, técnico do Laboratório de Química da UENF, por ceder o acessório necessário para a análise no Infravermelho.

Ao Dr. Gustavo Shimamoto, técnico responsável pela utilização da Ressonância Magnética Nuclear do ^{13}C em estado sólido, na Universidade de Campinas, por todo o suporte e ensinamento, além da paciência em ajudar nas análises.

Aos membros da banca, Dr. Bruno José Rodrigues Alves, Dr. Gabriel Nuto Nóbrega e Dr. Antonio Carlos Gama-Rodrigues, por todas as sugestões e apontamentos para melhoria dos trabalhos apresentados, assim como por todas as palavras de incentivo.

À Universidade Estadual do Norte Fluminense Darcy Ribeiro, ao Programa de Pós-Graduação em Produção Vegetal e ao Laboratório de Solos, pela oportunidade de realização do doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa.

E a todos que de alguma forma contribuíram ao longo desses anos, muito obrigada

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RESUMO

VICENTE, Laís de Carvalho, D.Sc., Universidade Estadual do Norte Fluminense Darcy Ribeiro, Março de 2020. Composição da matéria orgânica em agregados de solos sob diferentes sistemas agroflorestais de cacau no sul da Bahia, Brasil. Orientadora: Prof.ª Emanuela Forestieri da Gama-Rodrigues.

Sistemas agroflorestais (SAFs) de cacau apresentam alto potencial de acumulação de carbono orgânico no solo. No entanto, para que o C permaneça sequestrado é necessário que fique protegido do ataque microbiano. Além disso, o efeito de diferentes sistemas no sequestro de C, não está apenas na quantidade, mas também em sua composição química. Sendo assim, o presente estudo foi dividido em dois trabalhos e, em ambos, os sistemas avaliados foram: floresta natural, pastagem (30 anos), cacau+seringueira (20 anos), cacau+Eritrina (35 anos), cacau 'cabruca' (35 anos), cacau+seringueira (4 anos) em substituição a floresta natural, e cacau+seringueira (4 anos) em substituição a pastagem. No primeiro trabalho, os objetivos foram determinar a distribuição e acumulação de C nas frações de macro- (2000-250 μm) e microagregados (250-53 μm) do solo, assim como sua composição química, utilizando o Infravermelho por transformada de Fourier e Refletância Difusa (DRIFTS, sigla em inglês), nas profundidades de 0-10 cm e 80-100 cm. Para a separação das frações foi realizado o fracionamento densimétrico dos macro e microagregados, sendo obtidas frações dos macro- (fração leve livre – FLL, matéria orgânica particulada grosseira e fina – MOPc e MOPf, C associados aos minerais – C_m) e frações dos microagregados (FLL, MOPf e C_m). A FLL não foi analisada. Os resultados mostraram que a fração C_m é o maior pool de C, apresentando compostos mais lábeis em 0-10 cm e bandas de absorção de compostos mais aromáticos (1700-1400 cm^{-1}) em 80-100 cm. Esse resultado na camada superficial sugere que a fração C_m é a principal forma de proteção do C nesses solos. A conversão de pastagem e floresta natural em SAFs de cacau promoveu perdas de C, principalmente formas alifáticas. No entanto, quando esses sistemas já estão estabelecidos, eles contribuem para a acumulação e estabilização do C no solo, sendo similar a floresta natural. No segundo trabalho, para responder algumas perguntas que não foram esclarecidas pelo DRIFTS e compreender, de forma mais detalhada, a dinâmica do C nas frações dos agregados sob os diferentes sistemas analisados, os objetivos foram avaliar a composição química da matéria orgânica oclusa nas frações densimétricas e avaliar a composição da fração $C_{m,}$ sob os diferentes sistemas, utilizando a Ressonância Magnética Nuclear (RMN). As MOPs apresentaram alta concentração de formas aril-C e baixas concentrações de O/N-alquil, em comparação com a fração C_m, devido ao processo de formação dos agregados. Analisando a fração C_m dos macroagregados, a floresta natural apresentou maior proporção de alquil-C, o que indica preservação seletiva do C. Os SAFs de cacau mais antigos e o de 4 anos, em substituição a floresta natural na linha do cacau, apresentaram contribuições relativas próximas. O SAF cacau+seringueira, em substituição a floresta natural na linha da seringueira, apresentou os maiores valores de aril-C, possivelmente devido à maior labilidade do material vegetal da seringueira. A pastagem e o SAF cacau+seringueira, em substituição a pastagem, apresentaram distribuição relativa similar. A fração C_m dos microagregados apresentou maior proporção de O/N-alquil e alquil-C. Sistemas conservacionistas como os SAFs de cacau cessam a perturbação do solo após a implantação, o que contribui para a redução da ciclagem dos macroagregados, promovendo a proteção de compostos mais lábeis.

ABSTRACT

VICENTE, Laís de Carvalho, D.Sc., Universidade Estadual do Norte Fluminense Darcy Ribeiro, March, 2020. Organic matter composition in soil aggregates under different cacao agroforestry systems in Southern of Bahia, Brazil. Advisor: Prof.ª Emanuela Forestieri da Gama-Rodrigues.

Cacao agroforestry systems (AFS) have a high potential for organic carbon accumulation. However, for C remains sequestered, it must be protected from microbial attack. In addition, the effect of different systems on C sequestration is not only in quantity, but also in its chemical composition, since it contributes to stabilization process understanding. Thus, the present research was divided into two studies and, in both, the land use systems evaluated were: natural forest, pasture (30 years), cacao+rubber (20 years), cacao+Erythrina (35 years), cacao 'cabruca' (35 years), cacao+rubber (4 years) replacing natural forest and cacao+rubber (4 years) replacing pasture. In the first study, the aims were to determine the distribution and accumulation of C in the macro- (2000-250 μm) and micro-aggregate (250-53 μm) density fractions, as well as, the chemical composition, using Fourier Transform Infrared and Diffuse Reflectance (DRIFTS), at 0-10 cm and 80-100 cm depths. Density fractionation of macro- and microaggregates was performed, obtaining macro- fractions (light free fraction - LFF, coarse and fine particulate organic matter - POMc and POMf, C associated with minerals - C_m) and microaggregates fractions (FLL, MOPf and Cm). FLL has not analyzed. The results show that C_m fraction was the largest C pool, with more labile compounds in 0-10 cm and absorption bands for more aromatic compounds (1700-1400 cm^{-1}) in 80-100 cm. This result in superficial layer suggests that C_m fraction is the major form of C protection in these soils. The conversion of pasture and natural forest to cacao AFS promoted C losses, mainly more aliphatic forms. However, when these systems are already established, they contribute to C accumulation and stabilization, being similar to natural forest. In second work, to answer some questions that were not clarified by DRIFTS, the aims were to evaluate the chemical composition of occluded organic matter associated to density fractions and to evaluate the composition of C_m fraction under the different systems using Nuclear Magnetic Resonance (NMR). The POMs showed a high concentration of aril-C forms and low concentrations of O/N-alkyl, in comparison with the C_m fraction, due to aggregates formation process. Analyzing the macroaggregates C_m fraction, natural forest showed the highest proportion of alkyl-C, which indicates selective preservation of C. The oldest cacao AFS and 4-year-old replacing the natural forest in the cacao row presented close relative contributions. The cacao+rubber tree (4 years) replacing the natural forest in the rubber tree row showed highest Aril-C values, possibly due to the greater rubber plant material quality. Pasture and cacao+rubber (4 years) replacing pasture showed similar relative distribution. The microaggregates C_m fraction showed a higher proportion of O/N-alkyl and alkyl-C. Conservation systems, such as cacao AFS, cease disturbing soil after implantation, which contributes to reducing the turnover of macroaggregates, promoting the protection of more labile compounds.

1. INTRODUÇÃO

Sistemas agroflorestais (SAFs) apresentam elevado potencial de acumulação de carbono orgânico no solo (COS). De acordo com Nair et al. (2010), o estoque de C em SAFs pode variar de 30 a 300 Mg C ha^{-1}, a depender do sistema e da profundidade analisada. Por isso, é considerada uma opção para lidar com problemas de uso inadequado do solo que promovem a erosão, assim como para mitigação do aquecimento global induzida pelo excesso de CO_2 na atmosfera (Albrecht e Kandji, 2003). Em regiões tropicais, uma cultura que vem sendo utilizada com sucesso em SAFs é o cacau (*Theobroma cacao* L.), nesse sentido, alguns trabalhos na literatura demonstram o potencial de acumulação de C nos solos sob esses SAFs, apresentando números na faixa de 150 a 302 Mg C ha^{-1} (Gama-Rodrigues et al., 2010; Monroe et al., 2016).

De forma geral, o COS pode estar na forma de resíduos frescos, em diversos estágios de decomposição ou compostos humificados, podendo estar associados ou não à fração mineral, como também à porção viva, que é composta pelas raízes e fauna do solo. Com isso, o COS, nas suas mais diversas formas, compreende a matéria orgânica do solo (MOS) (Roscoe e Machado, 2002), sendo a sua quantidade e composição química dependente tanto do material vegetal que é aportado, decomposto e incorporado ao solo, quanto pelas propriedades do ecossistema, tais como, umidade, temperatura e superfícies minerais reativas (Schmidt et al., 2011).

Para que o C permaneça estocado no solo é necessário que esse elemento fique protegido da decomposição pelos microrganismos (Six et al., 2004; Nair et al., 2010). Os agregados do solo, que são formados pela ligação das partículas de areia, silte e argila com substâncias orgânicas e inorgânicas do solo, promovem proteção física (atuando como barreira) e química (pela formação de complexos organominerais), sendo considerados como pools de reserva de carbono orgânico, promovendo a estabilização do C e, consequentemente, o sequestro desse elemento no solo (Bronick e Lal, 2005).

De acordo com Six et al. (2000), sistemas que apresentam alto aporte de material vegetal e não promovem perturbações no solo, contribuem para a formação e manutenção dos agregados, visto que os resíduos frescos aportados ao solo contribuem para a atividade da fauna. Portanto, os seus subprodutos são importantes agentes de ligação dos macroagregados, que apresentam baixa taxa de ciclagem em sistemas que não apresentam revolvimento do solo.

No caso dos SAFs de cacau, a perturbação no solo ocorre apenas na fase de implantação do sistema (duas arações e uma gradagem) (Marques et al., 2012), assim, após o processo de implantação, as perturbações são reduzidas e há estudos que comprovam que esses sistemas atuam como sumidouros de C (Somarriba et al., 2013; Lorenz e Lal, 2014). Todavia, o efeito de diferentes sistemas de uso da terra não está apenas na quantidade de matéria orgânica associada às frações dos agregados do solo, mas também a sua composição química, que contribui para o entendimento do processo de estabilização, visto que a estrutura química está relacionada com a taxa de decomposição da MOS, sendo dependente também dos fatores ambientais e da mineralogia do solo (Schmidt et al., 2011).

Alguns procedimentos, como o fracionamento granulométrico e densimétrico, são utilizados para verificar a influência das frações de diferentes classes de agregados nos processos de estabilização da MOS, devido à quantificação do C acumulado, à influência da parte mineral do solo e as diferenças na composição química da MOS associada as diferentes frações (Six et al., 1998; Helfrich et al., 2006). Para detectar as diferenças na estrutura química da MOS associada a diferentes frações, são utilizadas técnicas espectroscópicas, como o Infravermelho por transformada de Fourier (FTIR, sigla em inglês) e a Ressonância Magnética Nuclear do ^{13}C (^{13}C RMN).

O FTIR é uma técnica rápida, de baixo custo e muito utilizada na ciência do solo, que realiza a determinação de grupos funcionais associados à MOS, como compostos nitrogenados, além dos grupos de compostos aromáticos e alifáticos (Filep et al., 2016; Chen et al., 2018). No entanto, apenas com o FTIR não é possível obter todas as informações necessárias para a identificação e quantificação dos grupos químicos presentes na MOS associada as frações densimétricas. O FTIR não permite determinar com clareza a porcentagem de influência dos vários grupamentos funcionais na MOS e nem a sua origem (Forouzangohar et al., 2013). Com isso, em associação, é possível a utilização da técnica de ^{13}C RMN em estado sólido para contribuir para uma melhor compreensão da dinâmica do C nos solos, sob diferentes sistemas de uso da terra.

A técnica de ^{13}C RMN possibilita uma avaliação muito confiável, o que contribui para determinar a abundância relativa dos grupos funcionais C-alquil, O-alquil, $C_{\text{aromático}}$ e $C_{\text{carboxílico}}$, que são utilizados como indicadores dos diferentes compostos químicos, assim como para estabelecer o nível de aromaticidade e, consequentemente, avaliar os efeitos dos sistemas na estabilização do C no solo (Boeni et al., 2014; Mastrolonardo et al., 2015; Mao et al., 2017). Dessa forma, os resultados obtidos pelo FTIR, juntamente com a RMN, permitem um maior entendimento sobre a reatividade, estrutura e composição da MOS associada as frações dos agregados do solo.

Diante do exposto, a hipótese principal desta tese foi que os sistemas agroflorestais de cacau, em decorrência de um aporte contínuo e significativo de resíduos vegetais, oriundos de diferentes composições arbóreas, apresentam elevado potencial de estabilizar o C no solo, devido à proteção física e química nos agregados ao longo do perfil. Sendo assim, esses sistemas podem ser considerados como ferramentas socioeconômicas e ambientais viáveis, visando o desenvolvimento sustentável. Logo, o objetivo principal consistiu em avaliar a composição química da matéria orgânica associada as frações de macro- e microagregados de solos sob floresta natural, pastagem e SAFs de cacau de diferentes idades e composições, a partir da utilização de técnicas espectroscópicas, como o Infravermelho e a Ressonância Magnética Nuclear.

2. REVISÃO DE LITERATURA

2.1 Sistemas agroflorestais (SAFs)

Os sistemas agroflorestais (SAFs) podem ser definidos como um sistema de uso da terra que envolve uma mistura de espécies florestais com culturas agrícolas ou pastagem (Albrecht e Kandji, 2003). Esses sistemas de uso múltiplo já são reconhecidamente uma estratégia para a acumulação de carbono orgânico no solo (COS). De acordo com Nair et al. (2010) e Albrecht e Kandji (2003), os efeitos positivos de SAFs no sequestro de C dependem do manejo utilizado e da qualidade e quantidade de serapilheira depositada no solo, tanto das espécies florestais como das espécies não florestais.

Os SAFs que vêm sendo amplamente adotados em regiões de clima tropical, como no Sul da Bahia, são os SAFs de cacau (*Theobroma cacao* L.), por apresentarem elevada capacidade de acumulação de carbono no solo. A opção por este sistema ocorre por ele ser comparativamente semelhante na taxa de acumulação de C, com florestas naturais (Gama-Rodrigues et al., 2010; Monroe et al., 2016). Além disso, os SAFs são considerados uma importante ferramenta para a mitigação do aquecimento global e uso sustentável do solo (Albrecht e Kandji, 2003; Nair et al., 2010).

Esse potencial dos SAFs de cacau em acumular C ocorre, principalmente, devido a algumas características que contribuem para o balanço positivo entre as entradas e saídas desse elemento do sistema. Uma dessas características é a habilidade de captura e utilização dos recursos para seu crescimento e desenvolvimento (luz, nutrientes e água), o que promove a alta acumulação de biomassa e, posteriormente, a alta deposição de serapilheira, assim como o baixo nível de perturbação dos solos sob esses sistemas agroflorestais (Nair et al., 2010).

De forma geral, o alto aporte de serapilheira contribui para formação de agregados, pois promove a maior atividade da fauna do solo, que apresenta um importante papel nesse processo, visto que os compostos microbianos produzidos funcionam como agentes ligantes dos macroagregados (2000-250 μm) do solo. Essa afirmação foi observada por Gama-Rodrigues et al. (2010), a partir da avaliação de SAFs de cacau no Sul da Bahia. Os autores verificaram que a maior quantidade (em gramas) e estoque de C (Mg ha^{-1}), ocorreram nos agregados dessa classe de maior tamanho, nas camadas mais superficiais (0-10 e 10-30 cm).

Associado ao aporte de material vegetal, o baixo nível de perturbação do solo contribui para a manutenção e diminuição da ciclagem dos macroagregados, o que promove o maior acúmulo de C no solo, a partir da proteção física desse elemento (Six et al., 2000; Six et al., 2004). Apesar dos SAFs de cacau promoverem perturbações em razão da aração e gradagem no processo de implantação (Marques et al., 2012), esses sistemas, após estabelecidos, cessam o uso de maquinário e favorecem a acumulação de C no solo (Somarriba et al., 2013).

Além do potencial para acumular C, os SAFs de cacau têm se mostrado um importante sistema de exploração que contribui para a diminuição da pressão pelo desmatamento de florestas naturais (Montagnini e Nair, 2004). No estado da Bahia, esses sistemas são considerados uma importante ferramenta para a proteção ambiental e diminuição dos gases do efeito estufa (GEE) na atmosfera, além de apresentarem um cunho social, visto que funcionam como um incentivo econômico para pequenos produtores de cacau (Gama-Rodrigues et al., 2010).

2.2 Agregados do solo: pools de C orgânico

Os agregados do solo podem ser definidos como partículas secundárias, que se formam pela combinação de partículas minerais, substâncias orgânicas e inorgânicas (Bronick e Lal, 2005). E, de forma geral, são considerados como um mecanismo de estabilização de C no solo (Christensen, 2000), visto que promovem a proteção física desse elemento (Six et al., 2004). Assim, os agregados do solo são classificados de acordo com o tamanho que apresentam, podendo ser: macroagregados, se apresentarem tamanho entre 2000-250 µm; microagregados, se apresentarem tamanho variando entre 250-53 µm, e; fração silte-argila, de menor tamanho, sendo <53 µm (Six et al., 2000).

A proteção física do C está relacionada com a estabilidade dos agregados, de acordo com as teorias de formação. Primeiramente, Tisdall e Oades (1982) propuseram a Teoria da Hierarquização, em que afirmavam que há formação dos microagregados (250-53 µm), a partir de agentes ligantes persistentes, como ligações entre óxidos, partículas de argila e a MOS; enquanto os macroagregados (2000-250 µm) são formados a partir da junção de vários microagregados, pela ação de agentes ligantes temporários e transitórios, como raízes finas, hifas de fungos e polissacarídeos.

No entanto, Oades (1984) postulou uma modificação ao modelo proposto por Tisdall e Oades (1982), afirmando que primeiramente ocorre a formação de macroagregados, sendo os microagregados formados no interior dos macroagregados. Isso acontece porque raízes e hifas não persistem e se decompõe em fragmentos, e estes ficam incrustados com os minerais de argila, resultando na criação dos microagregados no interior dos macroagregados.

Essa modificação proposta por Oades (1984) foi confirmada por Six et al. (2000), ao demonstrarem, a partir de um modelo conceitual de formação de agregados (Fig. 1), que os macroagregados são formados em torno de resíduos frescos, formando a matéria orgânica intra-agregado ‘grosseira’ (MOPi ‘grosseira’) (t1). Portanto, a formação dos macroagregados, ao contribuir para a oclusão desses resíduos vegetais, promove a redução da quantidade de oxigênio e de entrada de água, o que diminui a atividade da microbiota no interior dessa classe de agregado e, consequentemente, ocorre redução da taxa de decomposição desse material. Contudo, mesmo com a baixa atividade da microbiota, ainda acontece a decomposição, embora mais lenta, e a fragmentação da MOPi ‘grosseira’, transformando-a em MOPi ‘fina’ (t2).

Sabe-se que quanto maior a quantidade de MOPi ‘fina’ nos macroagregados, mais antigos eles são, o que indica que se houver uma grande quantidade de macroagregados mais antigos, menor é sua ciclagem. Este é um parâmetro importante quando se analisa diferentes sistemas de uso da terra, devido à importância da manutenção dos macroagregados, pois essa classe promove a estabilização do C novo incorporado. Com o tempo, a MOPi 'fina' torna-se incrustada nos minerais de argila e nos compostos microbianos, promovendo a formação dos microagregados no interior dos macroagregados (t3). Como os agentes ligantes dos macroagregados são temporários e transitórios, eles podem ser degradados e decompostos, podendo haver a liberação dos microagregados estáveis (t4), capazes de contribuir como novos blocos no ciclo de formação dos macroagregados (Fig. 1).

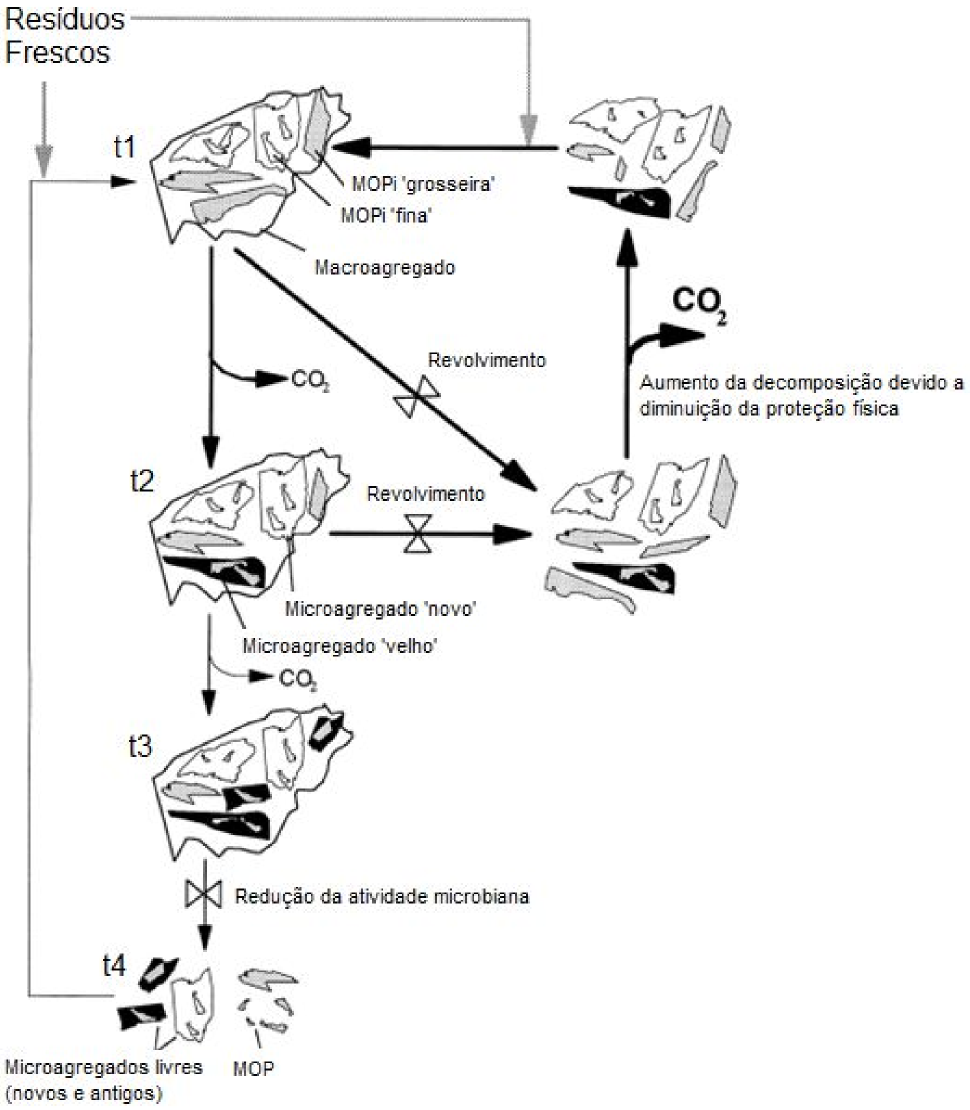

Figura 1: Modelo conceitual proposto por Six et al. (2000) (adaptado). A ciclagem ocorre quando o macroagregado é formado, se torna instável e, eventualmente, é quebrado. MOPi = matéria orgânica particulada intra-agregado; MOP = matéria orgânica particulada; t = tempo.

No entanto, qualquer tipo de perturbação no solo, como por exemplo, aração e gradagem, que são promovidas no processo de implantação de sistemas agroflorestais de cacau (Marques et al., 2012), acarreta quebra dos macroagregados, aumento da ciclagem e perda da matéria orgânica associada a essa classe de agregado (Mastrolonardo et al., 2015) (Fig. 2).

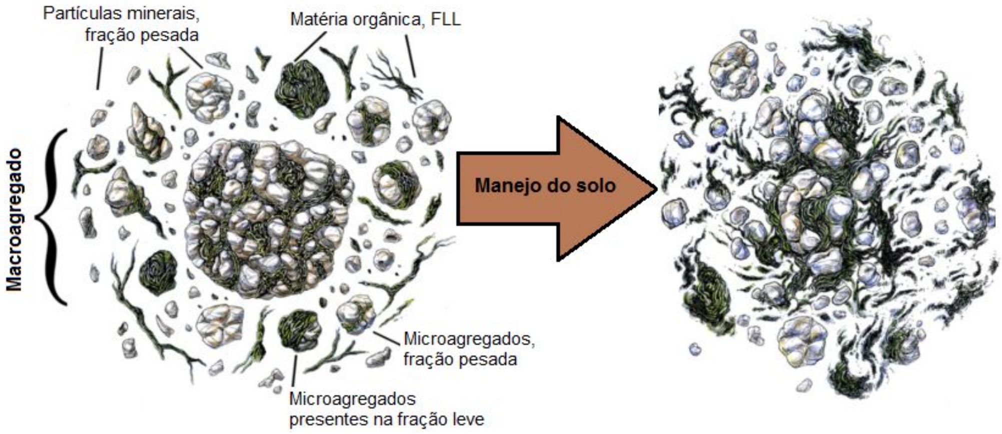

Figura 2: Esquema de rompimento do macroagregado devido ao manejo do solo, levando a liberação e potencial perda da matéria orgânica pelo processo de decomposição. Adaptado de Mastrolonardo et al. (2015).

Em sistemas de uso da terra que promovem uma menor perturbação, como sistemas agroflorestais de cacau já estabelecidos e produtivos, ocorre menor ciclagem dos macroagregados e, consequentemente, há maior proteção física do C e formação dos microagregados, no interior dos macroagregados. Six et al. (2000) ao analisarem o sistema de cultivo convencional e o sistema conservacionista do solo, observaram que no sistema convencional, apenas cerca de 27% do peso dos macroagregados correspondiam a microagregados, enquanto no sistema conservacionista, quase metade do peso dos macroagregados eram microagregados. Desta forma, em sistemas de plantio conservacionistas há maior taxa de acumulação de C, quando comparado com sistemas convencionais do solo, pois os macroagregados formam-se e degradam-se muito mais lentamente, favorecendo a manutenção e estabilização do C no solo (Six et al., 2000; Six et al., 2004).

No entanto, é importante destacar que as teorias de formação abordadas (Tisdal e Oades, 1982; Oades, 1984; Six et al., 2000), tendem a ocorrer em superfície onde os macroagregados são formados a partir de processos biológicos. Em subsuperfície, os macroagregados podem se formar de forma abiótica, a partir das interações físicas e eletrostáticas, mesmo na ausência de entrada de matéria vegetal ou atividade radicular, sendo muito comum em solos cauliníticos. Porém, os macroagregados formados de forma abiótica tendem a ser mais instáveis, quando comparados com aqueles formados de forma biótica (Denef e Six, 2005).

2.3 Composição química da matéria orgânica nos agregados do solo

A composição química da MOS é complexa e varia de acordo com o material vegetal que é depositado, decomposto e incorporado ao solo. Dessa forma, pode haver incorporação de resíduos vegetais com alta relação C/N, em solos mais ricos em lignina ou outras moléculas com estruturas aromáticas ou alifáticas ou de materiais mais ricos em açúcares e proteínas. Esses diferentes compostos, associados às propriedades bióticas e abióticas do solo, assim como as questões climáticas, contribuem para o aumento do tempo de residência do C no solo e, consequentemente, para o sequestro de C a partir da estabilização do C orgânico (Schmidt et al., 2011).

Sendo assim, devido à importância da MOS e dada a sua complexidade química, utilizar apenas as informações quantitativas acerca da acumulação de C é uma avaliação limitada. Por isso, técnicas de análise da composição em escala molecular, como as espectrométricas, são necessárias para uma melhor compreensão dos compostos presentes tanto nos diferentes compartimentos do solo, como na matéria orgânica associada aos macro- e microagregados do solo.

2.3.1 Técnicas espectrométricas

2.3.1.1 Infravermelho por Transformada de Fourier (FTIR)

A técnica espectroscópica de Infravermelho por transformada de Fourier (FTIR) é bastante utilizada na ciência do solo, sendo considerada rápida e de baixo custo para caracterização da composição (Baumann et al., 2016; Filep et al., 2016) e reatividade da MOS. A FTIR possibilita determinar grupos reativos como COOH, OH-fenólico, -NH_2, C=O e anéis aromáticos (Stevenson, 1994), distinguindo, desta forma, os grupos funcionais que indicam a presença de estruturas aromáticas e alifáticas, assim como polissacarídeos, ésteres e compostos nitrogenados (Dick et al., 2008).

De forma geral, as bandas de absorção características para os grupos funcionais, presentes na matéria orgânica do solo, são as da região do infravermelho médio (mid-infrared), situada entre 4000-400 cm^{-1} (Baumann et al., 2016). A representação das bandas de absorção observadas nos espectros de FTIR, em solos sob SAFs de cacau, destacadas por Sales (2009) são apresentadas na tabela 1.

Como o nome da técnica já deixa explicito, a FTIR utiliza a energia eletromagnética na região do infravermelho, de forma que cada molécula, ao receber a radiação, fica excitada e emite um determinado comprimento de onda. Desta forma, a partir do número de onda obtido, é possível determinar quais grupos funcionais estão presentes nas amostras, sendo a sua intensidade expressa em Transmitância (T) ou Absorbância (A) (Stevenson, 1994).

Na literatura é possível encontrar vários trabalhos avaliando composição química com FTIR, em diferentes sistemas de uso da terra (Soriano-Disla et al., 2014; Filep et al., 2016; Rennert et al., 2018). Um estudo avaliando áreas de pastagem e mata nativa (Dick et al., 2008), demonstrou que o aumento da aromaticidade da MOS tem relação com a profundidade do solo, e que o sítio da mata foi o que apresentou os compostos mais aromáticos. Esses mesmos autores observaram que a MOS apresentou as principais bandas de absorção, semelhantes àquelas observadas nos espectros da vegetação. Ao avaliarem a MOS associada aos agregados do solo, Sarkhot et al. (2007) encontraram como resultado, bandas que sugerem a presença de polissacarídeos, amidas, ésteres e compostos aromáticos.

Ao avaliar solos sob SAFs de cacau no Sul da Bahia, Sales (2009) observou que a MOS oclusa nos agregados apresentou intensidade no sinal que representa os grupos funcionais aromáticos (1620 cm^{-1}), na profundidade 0-10 cm, e indicou os principais grupos funcionais sob esses sistemas (Tabela 1). Já Jindaluang et al. (2013), avaliando a composição química em frações densimétricas, demonstraram que houve um aumento na proporção de ácidos carboxílicos e C aromático, e diminuição dos compostos mais alifáticos e polissacarídeos, com o aumento da densidade das frações (<1,85 cm^3, 1,85-2,60 cm^3 e >2,60 cm^3).

Tabela 1: Bandas de absorção observadas nos espectros do Infravermelho por transformada de Fourier (FTIR) em solos sob sistemas agroflorestais de cacau no Sul da Bahia (Adaptado de Sales, 2009)

Banda (cm)	**Atribuições dos Grupos**
3692 e 3652	ν AlO-H, ν FeO-H e ν SiO-H (argilas)
3622	ν N-H de $R\text{-}NH_2$ (argilas)
3463-3413	ν CH- de aromáticos
2940-2850	ν C-H (CH_3 e CH_2 de estruturas alifáticas)
1820-1700	ν C=O (ésteres, cetonas, COOH e amida)
1660-1620	ν C=O de COO; C=C (anel aromático); ν C=N
1590-1517	ν N-H; ν C=N
1489	δ fora do plano C-H de aromático; ν N=O
1158-1113	ν C-O de estruturas, tipo polissacarídeos
900-500	ν Si-O (impurezas inorgânicas)

ν = frequência

No entanto, com a técnica de FTIR não é possível indicar nem quantificar, com total clareza, quais os compostos estão presentes no solo (lignina, cutina, entre outros). Por meio do uso da técnica de FTIR, a partir dos grupos funcionais presentes, que representam compostos químicos característicos, é possível apenas predizer se é um composto orgânico aromático, alifático de cadeia aberta ou fechada.

Sendo assim, é possível, realizar a técnica da FTIR associada a outra técnica espectroscópica, a Ressonância Magnética Nuclear. A RMN possibilita determinar as abundâncias relativas dos compostos químicos a partir da integralização dos espectros, sendo possível, dessa forma, complementar os resultados obtidos no FTIR, permitindo a melhor compreensão da estrutura molecular da matéria orgânica associada aos agregados do solo (Dick et al., 2008; Forouzangohar et al., 2013; Mastrolonardo et al., 2015).

2.3.1.2 Ressonância Magnética Nuclear de ^{13}C (RMN ^{13}C)

A RMN é considerada uma técnica não invasiva e muito sensível para a caracterização de estruturas químicas, a partir da absorção de uma radiação radiofrequente, em MHz, por núcleos magnéticos que são expostos por um determinado tempo, a um campo magnético. Essa técnica é uma das mais relevantes para a caracterização e quantificação dos tipos de C que formam a matéria orgânica do solo (Novotny, 2002; Dick et al., 2005).

O carbono possui dois isótopos estáveis, o ^{12}C e o ^{13}C. No entanto, apenas o isótopo ^{13}C apresenta um momento magnético e, por isso, a técnica de espectroscopia de RMN limita-se a esse isótopo. Por apresentar uma baixa abundância natural (1,108%) e um momento magnético cerca de quatro vezes menor que o do ^{1}H, a detecção do ^{13}C é muito mais difícil pela RMN. Porém, essa técnica tem como vantagem analisar a MOS, sem extraí-la do solo, assumindo-se assim, que o espectro obtido na análise do RMN representa o que ocorre naturalmente no solo, em sua totalidade (Novotny, 2002).

A técnica RMN é realizada, na ciência do solo, a partir da combinação de Polarização Cruzada (CP, em inglês: cross-polarization) e Rotação com Ângulo Mágico (MAS, em inglês: magic-angle spinning), visando melhorar a resolução do espectro obtido nas amostras sólidas e aumentar o sinal do núcleo menos abundante (^{13}C), a partir da alta polarização obtida pelo núcleo, com alta relação giromagnética (^{1}H) (Kogel-Knabner, 1997; Dick et al., 2002). Alguns estudos indicam que a interpretação quantitativa dos grupamentos químicos, a partir da utilização da Polarização Cruzada, deve ser realizada com cautela, em razão da influência do ^{1}H (Baldock et al., 1992; Ussiri e Johnson, 2003; Berns e Conte, 2011).

Sendo assim, os resultados obtidos pela RMN CPMAS, apresentam uma aproximação da abundância relativa dos grupos químicos da MOS, que são utilizados como indicadores de diferentes componentes. Tratam-se os resultados como 'abundância relativa' ou 'distribuição relativa', devido ao fato de a área de cada sinal proporcionar um determinado número de prótons, sendo possível integralizar a área do espectro. No entanto, a integral informa o número relativo de prótons, não o absoluto.

Dessa forma, os espectros obtidos são organizados em quatro regiões, são elas: (1) C-alquil (0-45 ppm), proveniente de lipídeos, ácidos graxos e polímeros alifáticos provenientes de plantas; (2) O-alquil (45-110 ppm), derivado de polissacarídeos, como celulose e hemicelulose, e proteínas; (3) aromáticos ou aril-C (110-165 ppm), tipos de C derivados de lignina, e; (4) carboxila (165-220 ppm), derivado de ésteres alifáticos e grupos carboxílicos. Algumas dessas regiões químicas apresentam sub-regiões características, conforme indicado na figura 3 e na tabela 2 (Song et al., 2008; Knicker, 2011; Panetieri et al., 2013; Mastrolonardo et al., 2015; Mao et al., 2017; Simpson e Simpson, 2017).

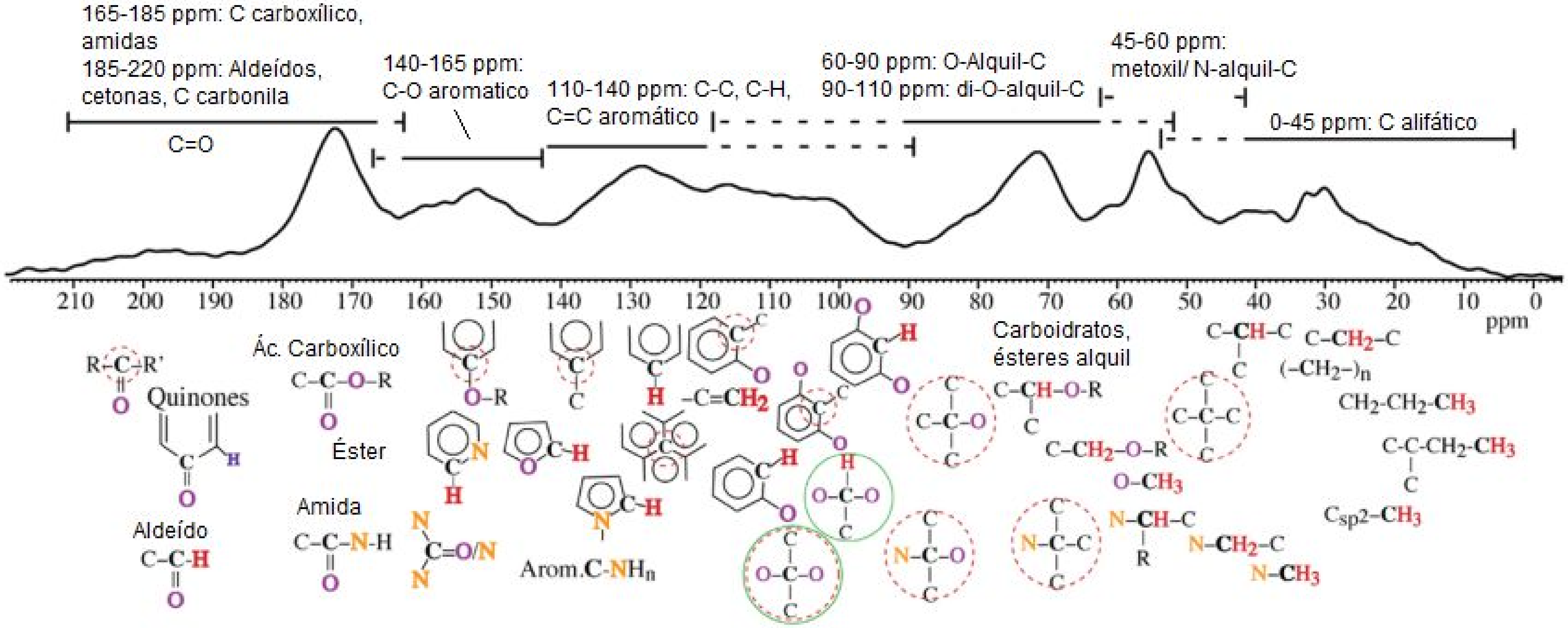

Figura 3: Esquema demonstrativo das regiões (e sub-regiões) químicas identificadas pela técnica da Ressonância Magnética Nuclear do ^{13}C. Adaptado de Mao et al. (2017).

Tabela 2: Regiões e sub-regiões químicas típicas dos constituintes encontrados na matéria orgânica do solo (MOS), com a utilização da Ressonância Magnética Nuclear do ^{13}C.

ppm	Região	Sub-região	Composição química e componentes da MOS
0-45	**Alquil-C**	**-----------**	Grupos com terminal Metil; Anéis e cadeias alifáticas sem O ou N em substituição; Alquil-CH em R-(CH2)-CH2-CH3 de lipídeos; aminoácidos encontrados em proteínas e biopolímeros, como a lignina, cutina, suberina e taninos.
45-60	**N e/ou O-Alquil-C**	**Metoxil/N-Alquil**	Presença de O ou N em cadeias alifáticas; grupos metoxílicos (R-O-CH3); indica presença de aminoácidos, celulose/hemicelulose e lignina (C metoxílico).
60-90		**O-Alquil**	Anéis de C em carboidratos (polissacarídeos); C em éteres; açúcar, celulose e hemicelulose.
90-110		**di-O-Alquil**	Di-O em cadeias alifáticas; C anomérico em carboidratos; C em polissacarídeos e açúcar; celulose e hemicelulose; C-lignina.
110-140	**Aril**	**C-H e C-C aromáticos**	C-H e C-C aromáticos; alguns aminoácidos; lignina; suberina.
140-160		**O-substituído**	C fenólico (OH-anel aromático); Grupos C-O-R ou C-N-R; lignina e suberina.
160-85	**Carboxila**	**Carboxila/Amida/Ester**	Ácido carboxílico; carboxilatos; ester COO-C-; amida CO-N; amidas alifáticas; presença de aminoácidos/proteínas, ácidos graxos (moléculas menores; encontradas em biopolímeros) e éster ligado a lipídeos.
185-220		**Aldeídos e/ou Cetonas**	Grupo Carbonila; Aldeídos, -((CH)=O)-; Cetonas, -(C=O)-; presença de lignina, amino=ácidos/proteína e produtos de degradação.

Compilado de Mastrolonardo et al. (2015), Mao et al. (2017) e Simpson e Simpson (2017).

Boeni et al. (2014), por exemplo, ao avaliarem frações da matéria orgânica no Cerrado, observaram maior quantidade do grupo O-alquil, representando 50% de todo o C orgânico total do solo, em relação a todas as frações em estudo. É válido ressaltar que neste estudo, o grupo C-alquil e o C aromático foram intermediários, e a carboxila foi o grupo menos representativo, raramente passando de 10% do C orgânico total do solo.

Dick *et al.* (2005), em estudo com diferentes solos sob vegetação nativa, encontraram, em todos os espectros avaliados, que o mais pronunciado sinal ocorreu na região de 45 a 110 ppm, indicando o grupo O-alquil. Nos horizontes A, os autores encontraram que: 28-38% da intensidade total do ^{13}C representaram O-alquil, 24-30% representaram C-alquil, seguido pelo C aromático e C carbonila, com 11-14% e 9-12%, respectivamente.

Courtier-Murias et al. (2013), avaliando a MOS intra-agregado e a MOS livre, observaram maior quantidade do grupo C-alquil e carboxila na MOS intra-agregado, principalmente nos microagregados, do que na MOS livre. Isso pode ser explicado devido à maior humificação do material intra-agregado, que é ainda mais perceptível nos microagregados do que nos macroagregados. Em outro estudo, realizado por Helfrich et al. (2006), foram avaliados os compostos de C presentes nas frações de solos, sob diferentes sistemas de uso da terra (milho, pastagem e conífera – *Picea abies* L.), e concluiu-se que a matéria orgânica particulada intra-agregado apresentou maiores proporções de C-alquil e menores proporções de O-alquil, do que a matéria orgânica particulada livre.

No entanto, ainda são escassos os estudos que se dedicam à utilização de RMN em agregados, principalmente em sistemas agroflorestais de cacau em climas tropicais. Diante deste contexto, é importante ressaltar que o uso dessa técnica contribui para elucidar o efeito e a contribuição dos diferentes sistemas agroflorestais de cacau na acumulação de C orgânico, uma vez que, além de ser possível determinar os tipos de C e o nível de aromaticidade associada aos agregados, o uso da RMN também permite quantificar a abundância relativa dos compostos e suas respectivas contribuições na acumulação de C no solo.

3. TRABALHOS

3.1 Chemical composition of organic matter in aggregates fractions under cacao agroforestry systems[1]

ABSTRACT

Agroforestry systems (AFS) have a high potential for carbon (C) accumulation in strongly weathered soils at tropical region, but the chemical characterization of organic C stored remains poorly understood. For this purpose, the chemical composition of soil organic matter was determined in natural forest, pasture and different cacao AFS (*Theobroma cacao* L.) from the Atlantic forest region of Brazil. The physicochemical fractionation was combined with the diffuse reflectance infrared Fourier transformation spectroscopy (DRIFTS) in soil samples at 0-10 (topsoil) and 80-100 cm (subsoil). The studied AFS were: cacao in thinned Atlantic forest (cacao 'cabruca' with 35-year-old), cacao+erythrina (35-year-old) and cacao (20-year) + rubber tree (40-year) AFS, which were compared with natural forest and pasture (30-year-old). The results show that organic C associated to soil

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[1] Artigo submetido na revista Geoderma (ISSN: 0016-7061) em 31 de outubro de 2019

minerals (C_m) fraction was the largest pool of C protected within soil aggregates in the cacao based AFS. Furthermore, this fraction presented labile compounds in 0-10 cm and aromatic band absorbance intensities at 1700-1400 cm^{-1} region in the DRIFTS analysis in 80-100 cm depth. These results in topsoil suggest that the organo-mineral complex is the major form of C protection against microbial decomposition in the soil studied. The conversion of pasture and natural forest to cacao AFS lead to losses in organic C storage, mostly C aliphatic compounds, due to soil management for system implantation. However, the results showed that once established, the cacao AFS systems contributes to C accumulation and stabilization, with data values statistically similar to natural forest, which demonstrates the potential of these systems to mitigate the global warming by sequester of atmospheric CO_2.

Keywords: cacao AFS, rubber tree, *Erytrina*, soil organic carbon, density fractionation, infrared spectroscopy

INTRODUCTION

Tropical agroforestry systems are considered as a strategy for soil organic carbon (SOC) sequestration, with higher SOC values storage when compared to agriculture fields and pasture (Nair et al., 2010). In Bahia State, Brazil, cacao (Theobroma cacao L.) based agroforestry systems (AFS) have presented high potential for C sequestration, with stock values ranging 100-300 Mg ha^{-1}, due to AFS composition and age (Gama-Rodrigues et al., 2010; Barreto et al., 2010; Monroe et al.,2016). This high potential of cacao AFS is based on two main characteristics: (1) deposition of great amounts of plant material – above and belowground – and (2) non-tillage after implantation (Nair et al., 2010; Marques et al., 2012).

However, for SOC stabilization and maintenance in soil, the organic matter must be protected by the interaction with soil mineral particles (clay and/or silt, forming an organomineral complex) and/or physically protected in long-term pools, such as soil aggregates (Sollins et al., 1996; Six et al., 1998; Six et al., 2000). Aggregates are soil morphological units formed by the combination of primary particles (sand, silt and clay) with organic and inorganic substances (Bronick and Lal, 2005; Buol et al., 2011). They are classified according to their size, such as: macroaggregates (2000-250 µm) and microaggregates (250-53 µm) (Elliot, 1986; Six et al., 2000).

A conceptual model displays that macroaggregates are formed around fresh plant residues, which are deposited in soil, by transient (polysaccharides) and transitory (roots and fungus hyphae) binding agents, forming coarse particulate organic matter (POMc). POMc is fragmented and decomposed by microbiota into fine POM (POMf), which becomes encrusted with soil minerals (such as clay particles), forming microaggregates within macroaggregates. Thus, soil aggregates separation by physicochemical fractionation, into pools with different turnover rates, contribute to understand how different cacao-based AFS create a C flow into aggregates density fractions and lead to C stabilization within soil aggregates in topsoil. Nonetheless, some soil characteristics, such as clay content, control the stability in soil depth (Oades, 1984; Six et al., 2000).

In addition, it is necessary to analyze the chemical composition of the organic matter associated to soil aggregates fractions, since the organic C within aggregates is from heterogeneous cacao AFS, composed by different shade trees, such as rubber tree and *Erytrina*. The organic substances vary with functional groups, structure and complexity, which may contribute to C stabilization by chemical and/or physical C protection (Helfrich et al., 2006).

Spectroscopic techniques, such as Infrared Fourier Transformation Spectroscopy, may be useful for organic C chemical characterization (Chen et al., 2018; Rennert et al., 2018), including the diffuse reflectance infrared (Diffuse Reflectance Infrared Fourier Transformation Spectroscopy – DRIFTS). Although DRIFTS presents some limitations, studies have demonstrated its usefulness for rapid and low cost organic C characterization (Nguyen et al., 1991; Gholizadeh et al., 2013; Soriano-Disla et al., 2014; Filep et al., 2016). The knowledge of C chemical composition by DRIFTS may be used to explain more about the persistent forms of C associated to soil aggregates. Thus, DRIFTS may be used to verify the effects of soil management practices, such as cacao AFS implantation, and changes in soil characteristics with depth on C composition at molecular scale.

In this research, we hypothesized that cacao AFS contribute to C sequestration by C stabilization of more aromatic and less reactive forms in soil aggregates. Thus, we seek to answer the questions: How much organic matter is associated with POM (coarse and fine) and C_m fractions and stabilized in soils due to physical protection promoted by aggregates? What are the differences associated with SOM reactivity in different soil aggregates density fractions under different cacao AFS? What is the impact of cacao-based AFS implantation in C stabilization within macro- and microaggregates density fractions? What is the influence of depth for C stabilization in aggregates density fractions (80-100 cm)?

Understand the complex chemical characterization of soil organic matter associated to density fractions is important to comprehend how soil aggregates fractions (POMc, POMf, C_m) and different land-use systems contributes to C sequestration, favoring the soil structure improvement and mitigating global warming. Accordingly, our objectives were: (1) to determine the distribution and the organic C amount; and (2) to evaluate the presence of aliphatic and/or aromatic functional groups and SOM reactivity associated to density fractions in macro- and microaggregates in soils under different cacao-based AFS in topsoil (0-10 cm) and subsoil (80-100 cm), comparing with natural forest and pasture, using DRIFT spectroscopy.

MATERIAL AND METHODS

Study area, soil sampling and analysis

Samples were collected in the cacao growing region of the municipality of Uruçuca (14° 35′ 34″ S, 39° 17′ 2″ W) in southern part of the state of Bahia, Brazil. The region's climate is type *Af* in Köppen's classification. The mean annual temperature is 25 °C and annual rainfall is 1500 mm without a water deficit. The selected soils are classified as Oxisol according to the USDA Soil Taxonomy (Soil Survey Staff, 2009) and equivalent to *Latossolo Amarelo* according to the Brazilian System of Soil Classification (Embrapa, 2013).

Brief description of the land use systems selected:

(1) natural forest are one primary Tropical Ombrophilous Dense Forest
(2) 30-year-old pasture without grazing and replacing a natural forest
(3) cacao (20-year-old) + rubber tree (40-year-old) AFS
(4) 35-year-old cacao+*Erythrina* (*Erythrina glauca* Lour.) AFS
(5) 35-year-old cacao AFS in thinned forest (‘cabruca’)

(6) Rows of cacao planted in 5 lines and (7) double rubber tree hedgerows AFS with 4-year-old replacing an unfertilized pasture without grazing (26 years)

(8) Rows of cacao planted in 4 lines and (9) double rubber tree hedgerows AFS with 4-year-old replacing a natural forest.

The systems were not fertilized until 2002. From 2003, all systems were fertilized every three years. For younger systems (4-year) implantation, the soil was ploughed once and harrowed twice.

Four field replicates (30 x 30 m) were defined per land use system. Soil samples were collected in two depths: 0-10 (topsoil) and 80-100 cm (subsoil), to verify dissimilarities on organic matter composition among land-use systems and with depth. In 4-year-old agroforestry systems (replacing a pasture and forest; sites 6, 7, 8 and 9), the soil samples were collected in both depths and analyzed in cacao and rubber tree rows.

Samples from the two depths were air dried and passed in 2 mm sieve. The soil sample that passed through 2 mm sieve was used to determine soil particle size by pipette method (EMBRAPA, 2017; table 1) and to physical fractionation (topic below). Bulk density (BD) was determined by volumetric ring method (EMBRAPA, 2017; table 1). The procedure for soil sampling, areas description and cited analyses are detailed by Monroe et al. (2016).

Table 1: Particle size-fractions and soil bulk density, in both depths, in soils under cacao agroforestry systems, natural forest and pasture in Southern region of Bahia, Brazil.

Depth (cm)	**Particle size fraction (%) and bulk density (kg m³)‡**	**Land Use Systems**								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0-10	**Sand**	27	38	29	21	39	53	50	60	51
	Silt	13	17	9	13	8	11	14	9	9
	Clay	60	45	62	66	53	35	36	31	40
	BD	1110	980	1140	1050	1110	1090	1100	1090	1100
80-100	**Sand**	17	26	23	23	22	32	35	34	34
	Silt	9	13	12	12	11	8	8	8	8
	Clay	74	61	65	65	67	60	56	57	58
	BD	1040	990	1120	1010	1120	1120	1140	1120	1140

‡ Data obtained from database of Monroe et al. (2016)

1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and *Erytrina;* 5: 35-year-old cacao AFS in thinned forest (‘cabruca’); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: : 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

Physical and size density fractionation

Physical fractionation in aggregates classes was carried out following the method of Elliot (1986), adapted and described by Gama-Rodrigues et al. (2010). 100 g of soil sample was sunk into 500 ml of deionized water for 5 minutes. Soil samples were fractionated by wet-sieving, passing through two sieve sizes (250 µm and 53 µm) and three aggregates classes were obtained: macroaggregates (2000-250 µm), microaggregates (250-53 µm) and silt+clay size fraction (<53 µm). The sieving was done manually. All classes were oven dried at 60 ºC. In this study, silt+clay size fraction was not analyzed, because the aim was to verify the physical and/or chemical protection promoted by macro- and microaggregates density fractions. Then, macroaggregates and microaggregates were used for density fractionation, following an adapted method from Six et al. (1998). Previously, samples were oven dried at 105ºC over-night. A 5.0 g subsample was combined with 35 ml of 1.85 g cm^{-3} sodium iodide and centrifuged (2600 rpm) for 60 minutes. The free light fraction (FLL) was filtered onto nylon filter, rinsed with deionized water and was not analyzed. The heavy fraction was washed five times with deionized water to remove sodium iodide and dispersed using hexametaphosphate (0.5%, v/v) by shaking for 18h. For macroaggregates, the heavy fraction was passed through two sieves: 250 and 53 µm, obtaining three size density fractions: coarse intra-particulate organic matter (POMc; 2000-250 µm), fine intra-particulate organic matter (POMf; 250-53 µm) and mineral-associated organic matter (soil organic carbon associated with the mineral fraction - C_m; <53 µm). For microaggregates, the heavy fraction was passed through only in 53 µm sieve, obtaining POMf (250-53 µm) and C_m (<53 µm). All size density fractions were dried at 60ºC and weighed.

Sand correction

The procedure was performed using 2.0 ml of sodium hydroxide and 20 ml of deionized water for 2.0 g of aggregate sample. The suspension was shaken for 16 hours and the solid passed through sieves (250 µm and 53 µm for macroaggregates and only 53 µm for microaggregates). All fractions were oven dried at 60 ºC. The weights were used to correct aggregate-sized sand, using the equation (Vicente et al., 2019):

$$Sand\ Free\ aaggregate\ (g) = aggregate\ fraction\ (g)\ x\ [1 - sized\ sand\ proportion]$$

The recovery rates were, on average, 96%.

Hydrofluoric acid pre-treatment

Prior to DRIFTS analysis, a pre-treatment using hydrofluoric acid (HF) was carried out in order to remove paramagnetic compounds (i.e. Fe, Mn) and to concentrate organic C in aggregates density fractions (Gonçalves et al., 2003; Dick et al., 2005; Helfrich et al., 2006; Madhavan et al., 2017). Thus, the method for pre-treatment uses a 1:10 sample-to-solution ratio (Rumpel et al., 2006; Sanderman et al., 2017) using 10% (v/v) HF solution with 2 hours of agitation on horizontal shaker (Tecnal TE-240), followed by 10 minutes of centrifugation (2600 rpm). This procedure was repeated eight times (Gonçalves et al., 2003), removing the supernatant in each HF cycle. Then, samples were washed six times with deionized water to remove all HF solution. All samples were over dried at 60ºC and weighted. After pretreatment, the recovered mass, the C and N enrichment and recovered factor (R factor) were calculated following Dick et al. (2005). The results of R factor (ranging from 0.8 to 1.2), C enrichment (1.5-11 times) and low Fe content (removed rate was 97%, on average) confirmed that pretreatment were efficient (Table 2).

Table 2: Parameters calculated after the hydrofluoric acid pretreatment in all density fractions in soils of cacao agroforestry systems, natural forest and pasture in Southern region of Bahia, Brazil.

0-10 cm	**RM‡**	**CE‡**	**NE‡**	**CR‡**	**NR‡**	**Fe†**		**R‡**
						Before$_{HF}$	**After$_{HF}$**	
POMc$_{Ma}$	44.7	3.06	3.47	129.9	159.6	95.87	1.25	1.20
POMf$_{Ma}$	37.1	1.89	3.38	68.5	126.2	56.49	1.46	1.14
C$_{mMa}$	7.1	7.01	5.50	51.7	39.5	65.80	1.63	0.89
POMf$_{Mi}$	39.7	1.55	2.86	58.8	117.2	74.22	1.45	1.19
C$_{mMi}$	5.3	11.09	8.32	54.4	43.6	45.04	1.60	0.95
80-100 cm								
POMc$_{Ma}$	22.0	1.94	2.55	46.5	62.2	69.99	1.08	1.18
POMf$_{Ma}$	19.7	2.88	3.31	50.1	70.2	41.21	0.41	1.11
C$_{mMa}$	1.1	3.71	2.10	3.0	2.6	77.30	1.40	0.99
POMf$_{Mi}$	18.1	2.26	3.09	44.1	50.4	110.51	0.99	1.20
C$_{mMi}$	1.4	4.87	2.84	7.3	4.1	88.97	1.44	0.98

‡Calculated following Dick et al. (2005); †EMBRAPA (2017): values in mg dm^{-3}

Ma: macroaggregates; Mi: microaggregates; POMc: coarse particulate organic matter; POMf: fine particulate organic matter; C$_m$: mineral associated soil organic matter; RM: recovered mass; CE and NE: enrichment in C and N; CR and NR: recovered C and N; R: factor used to verify selective losses in organic matter (should range between 0.8-1.20).

C, N and Fe determination

C and N contents in the different density fractions (before and after HF pretreatment) were determined by dry combustion using an automated analyzer (Perkin-Elmer Series II 2400 CHNS/O). Organic C storage determination in each density fraction, before HF treatment, was calculated as follows:

$$C\ accumulation\ (g/kg) = \frac{\mathrm{C}\ x\ \mathrm{WDf}\ x\ \mathrm{WSF}}{10\ x\ \mathrm{iWf}}$$

Where:

C = C concentration measured in each density fraction (g/100g)

W_{Df} = weight of density fraction (g)

W_{SF} = weight of size fraction obtained in fractionation (g/100g)

iW_f = initial weight of size fraction (5 g for macro and microaggregates)

Total soil organic carbon (SOC) stock data presented in this research was obtained from database of Monroe et al. (2016). Fe was determined using sulfuric acid, following the method of EMBRAPA (2017).

Infrared Spectroscopy

Spectra of the chemical composition of soil organic C were recorded with Shimadzu IRAffinitty-1 spectrometer plus DRS-8000A accessory, and acquired by diffuse reflectance infrared Fourier transformation spectroscopy (DRIFTS) on a spectral range of 4000 to 400 cm^{-1}, using a resolution of 4 cm^{-1} and 40 scans. KBr was used as internal background standard. Spectra were processed with IRsolution FTIR version 1.4 (Shimadzu Inc., Kyoto, JP). The fully automatic baseline correction was applied. The data points were exported to Excel (Microsoft Office Excel 2010) and were used to build the DRIFTS spectra with data analysis software package Origin version 8.0 (OriginLab Corp., Northampton, USA). Band assignments were performed according to literature database. The 2700-2400 cm^{-1} and 900-400 cm^{-1}spectral regions were excluded from the individual spectra because the bands assigned to organic matter compounds can be masked by CO_2 noise or water and to soil minerals, respectively (Haberhauer et al., 2000).

In this study, spectra of all land use systems are similar and followed a similar pattern in each density fraction. Therefore, spectra were reorganized and presented for each fraction for better observation (Fig. 2).

The relative absorbance (% rA) was calculated by dividing the corrected absorbance of main bands (that were assigned in all fractions, with differences in absorbance intensity), which represent the functional groups of aromatic (i.e. 1620, 1510, 1460 and 1260 cm^{-1}) and aliphatic (i.e. 2920, 2850, 1160 and 1050 cm^{-1}), with the sum of absorbance total of all bands and multiplied by 100 (e.g. $[\%rA_{2920} = Abs_{2920}/\Sigma Abs_{2920-1050}]*100$) (Gerzabek et al., 2006; Jindaluang et al., 2013; Dhillon et al., 2017).

Statistical analysis

The density fractions distribution and associated C storage was assessed using Shapiro-Wilk normality test, using STATISTIC 8.0 software. As some variables were not normally distributed, $\sqrt{X}$ transformation was done. For density distribution and C storage, trenches were treated as pseudo-replications, such as performed in other studies on forest and agroforestry systems (Gama-Rodrigues et al., 2010; Fontes et al., 2014; Monroe et al., 2016; Vicente et al., 2016; Vicente et al., 2019). One-way analysis of variance (ANOVA) and Scott-Knott test ($p<0.05$) were used to evaluated, as a completely randomized design with four replicates, the differences of density fractions distribution and associated-C storage. Sigmaplot 12.0 (Systat Sofware inc.) was used to build the graphics. Principal Component Analysis (PCA) was subjected to verify the dissimilarity degree between the different land use systems, only at 0-10 cm layer, considering the

%rA of selected bands from macro- and microaggregates (2920, 2850, 1620, 1510, 1460, 1260, 1160 and 1050 cm^{-1}).

RESULTS

Distribution and C storage in density fractions

Density fractions distribution (POMc, POMf and C_m) and soil organic carbon accumulation (g kg^{-1}) showed differences between older (systems 1 to 5) and younger cacao AFS (systems 6 to 9; both cacao + rubber intercropping) (Fig. 1 and Table 3).

In all macroaggregates density fractions, at 0-10 cm, younger cacao AFS showed the lowest C accumulation values (g kg^{-1}) (Table 3). In subsoil, natural forest presented the highest C storage in all macro-fractions, being statistically equal to ‘cabruca’ only in C_m fraction. Microaggregates density fractions do not present tendency in C accumulation among the land-use systems (Table 3).

In all systems, C_m from macro- and microaggregates was the largest fraction in C accumulation: approximately 12% (old systems) and 2% (young systems) of the total soil organic carbon (database from Monroe ate al., 2016) is found in mineral-association organic matter.

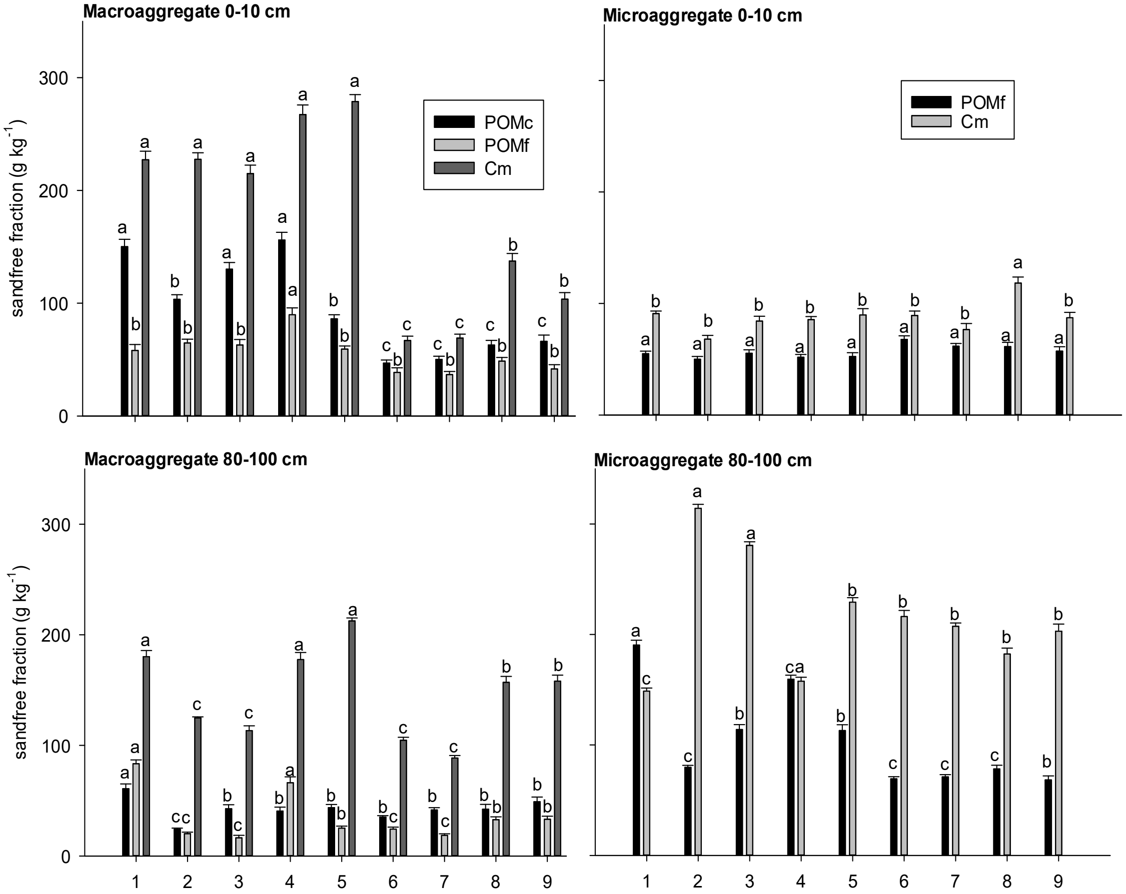

Fig. 1: Distribution of soil macroaggregates and microaggregates density fractions under cacao agroforestry systems, natural forest and pasture in Southern region of Bahia, Brazil. Values followed by the same letter within density fraction and each depth are not different according to Scott-Knott test (*P = 0.05*). POMc: coarse particulate organic matter; POMf: fine particulate organic matter; C_m: mineral associated soil organic matter. 1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and Erytrina (35-year-old); 5: 35-year-old cacao AFS in thinned forest (‘cabruca’); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: : 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

Table 3: Organic carbon accumulation (g kg^{-1}) in macroaggregates and microaggregates density fractions under cacao agroforestry systems, natural forest and pasture in Southern region of Bahia, Brazil.

		Density fractions					
Land Use Systems	**Depth**	**$POMc_{Ma}$**	**$POMf_{Ma}$**	**Cm_{Ma}**	**$POMf_{Mi}$**	**Cm_{Mi}**	**Total**
1	**0-10 cm**	3,69 a	1,21 a	8,28 b	1,20 a	2,60 a	16,98
2		2,39 b	1,34 a	13,01 a	0,92 a	1,89 b	19,55
3		1,91 b	1,06 a	7,32 b	0,44 b	1,61 b	12,35
4		2,41 b	1,47 a	7,64 b	0,65 b	1,92 b	14,10
5		1,25 c	0,64 b	9,02 b	0,54 b	2,29 a	13,73
6		0,33 d	0,43 c	2,21 c	0,83 a	2,00 b	5,81
7		0,14 d	0,19 c	2,34 c	0,30 c	1,52 b	4,49
8		0,21 d	0,25 c	3,15 c	0,63 b	2,26 a	6,50
9		0,58 d	0,67 b	5,18 c	0,26 c	2,46 a	9,16
1	**80-100 cm**	0,40 a	0,63 a	1,85 a	1,05 a	1,28 c	5,21
2		0,05 b	0,05 c	1,14 b	0,14 c	2,61 a	3,99
3		0,16 b	0,05 c	0,76 c	0,19 c	1,45 c	2,61
4		0,06 b	0,34 b	1,46 b	0,41 b	0,96 c	3,24
5		0,13 b	0,08 c	2,08 a	0,51 b	2,02 b	4,82
6		0,07 b	0,11 c	0,71 c	0,14 c	1,82 b	2,85
7		0,10 b	0,05 c	0,72 c	0,14 c	1,53 c	2,54
8		0,12 b	0,08c	1,48 b	0,14 c	1,41 c	3,23
9		0,17 b	0,10 c	1,26 b	0,13 c	1,44 c	3,10

Values followed by the same letter within density fraction and each depth are not different according to Scott-Knott test (*P = 0.05*). Ma: macroaggregates; Mi: microaggregates; POMc: coarse particulate organic matter; POMf: fine particulate organic matter; C_m: mineral associated soil organic matter. Values followed by the same letter within density fraction and each depth are not different according to Scott-Knott test (*P = 0.05*). 1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and Erytrina (35-year-old); 5: 35-year-old cacao AFS in thinned forest ('cabruca'); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: : 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

Chemical C composition: identification of DRIFTS spectral bands

Some spectral peaks assigned to organic compounds were identified in all density fractions (POMc, POMf and C_m) in both depths (0-10 and 80-100 cm) (Fig. 2). Bands at 2920-2840 cm^{-1} are assigned to aliphatic vibrations (Barbosa, 2007; Calderón et al., 2011). Bands at 1680-1500 cm^{-1} represent aromatics (C=C; C=O) and COO^- stretching vibrations (Haberhauer et al., 2000; Calderón et al., 2011), while the band 1160 cm^{-1} represents aliphatic compounds linked to hydroxyl groups (C-OH) or can be assigned to C-O vibration of polysaccharides or other groups, such as alcohols and esters (Janik et al., 2007). Bands at 1070-1050 cm^{-1} are associated to cellulose presence (Calderón et al., 2011).

The signal peaks among the fractions showed different absorbance intensities, more pronounced mainly in 80-100 cm, which allow distinction among fractions and depths. In general, POMs fractions (all POMs from macro- and microaggregates) showed same pattern in all land-use systems, in the two studied depths, differing from C_m (Fig. 2). In C_m fraction from macro- and microaggregates, at 0-10 cm depth, the band at ~1280 cm^{-1} was identified and represents –COOH and C-O stretching vibrations, indicating the presence of carboxylic acids and phenols functional groups (Bomermann et al., 2010). In addition, C_m fraction showed high absorption intensity in 1700-1400 cm^{-1} region in 80-100 cm depth, which suggests more relatively recalcitrant forms of C (Ellerbrock et al., 1999), and showed the intense band (“bell curve”) at 3350-3300 cm^{-1} represents hydroxyl groups (O-H) stretching vibration of alcohol and phenolic compounds (Haberhauer et al., 2000). Moreover, in C_m of macroaggregates at 80-100 cm, and in the two depths for microaggregates, a band at 1460 cm^{-1} was identified, which may indicate the presence of aliphatic compounds preserved in soil (Lorenz et al., 2007).

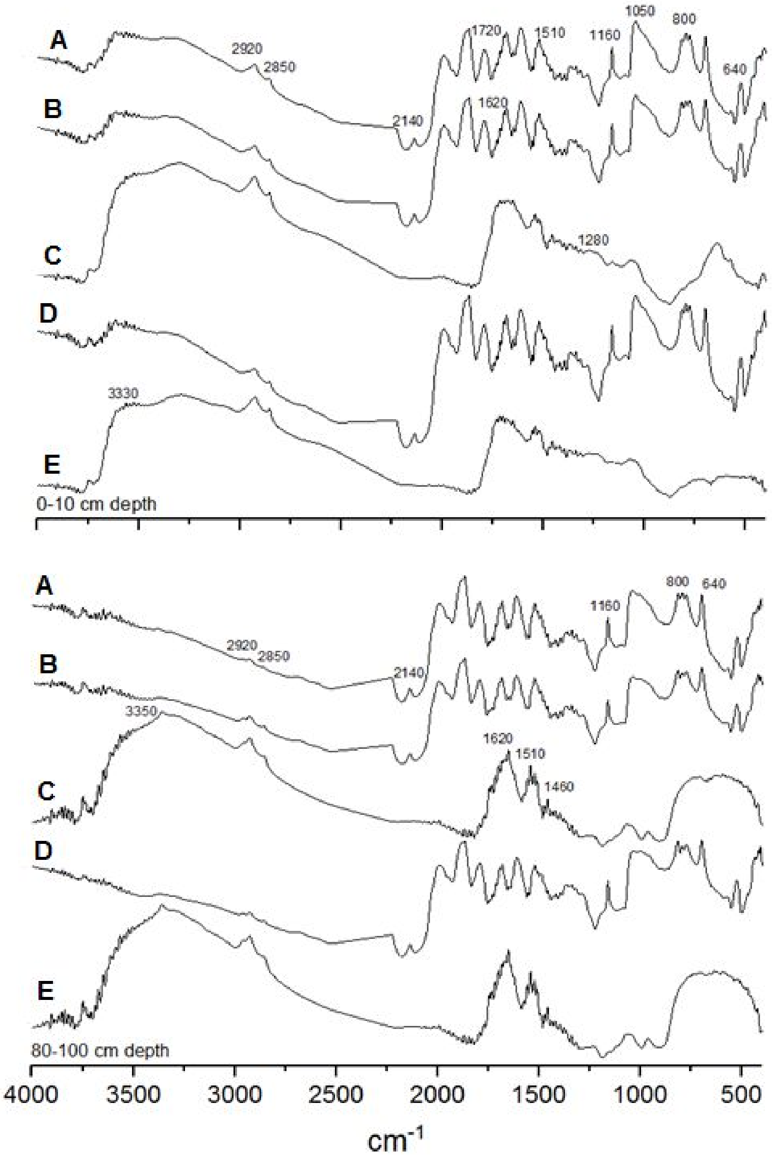

Fig. 2: DRIFTS spectra of density fractions of soils under cacao agroforestry systems, natural forest and pasture in Southern region of Bahia, Brazil. A, B and C, respectively: coarse particulate organic matter, fine particulate organic matter and mineral associated soil organic matter, respectively, from macroaggregates; D and E: fine particulate organic matter and mineral associated soil organic matter from microaggregates.

In all spectra, below 950 cm^{-1}, bands are assigned to a mixture of organic, such as aromatic ring C-H bonds, and inorganic functional groups likely associated to quartz (Calderón et al., 2011), which makes difficult to assign accurately which bands can be attributed to soil organic matter (Fig. 2).

Land-use systems dissimilarity

The fig. 3 display results for the ordination diagram based on Principal Component Analysis (PCA) and represents the land-use systems dissimilarity, considering the relative absorbance (%rA) of the main observed bands (2920, 2850, 1620, 1510, 1460, 1260, 1160 and 1050 cm^{-1}) of each topsoil (0-10 cm) density fraction (POMs and C_m from macro- and microaggregates). The land-use systems were distributed into the four quadrants according to three components (PC1 explained 38.8% of the total variance, PC2 explained 31.3% and PC3 explained 29.9%), which separated the reference areas (lefts quadrants) and old cacao AFS from young cacao AFS (rights quadrants). Sites 1 (natural forest), 2 (pasture) and 3 (40-year-old rubber tree+cacao AFS) comprised one group, whereas sites 4 (*Erythrina*+cacao AFS) and 5 (cacao 'cabruca' AFS) formed another group. On the other hand, sites 6 and 8 (cacao row from AFS replacing pasture and forest, respectively) comprise one group and the rubber tree row of these respective young systems (7 and 9) formed another group. This separation is due to the predominance of C_m and POMf fractions contribution, considering only loadings ≥ 0.70, $p<0.05$ *and* $n=9$ (Fig. 3).

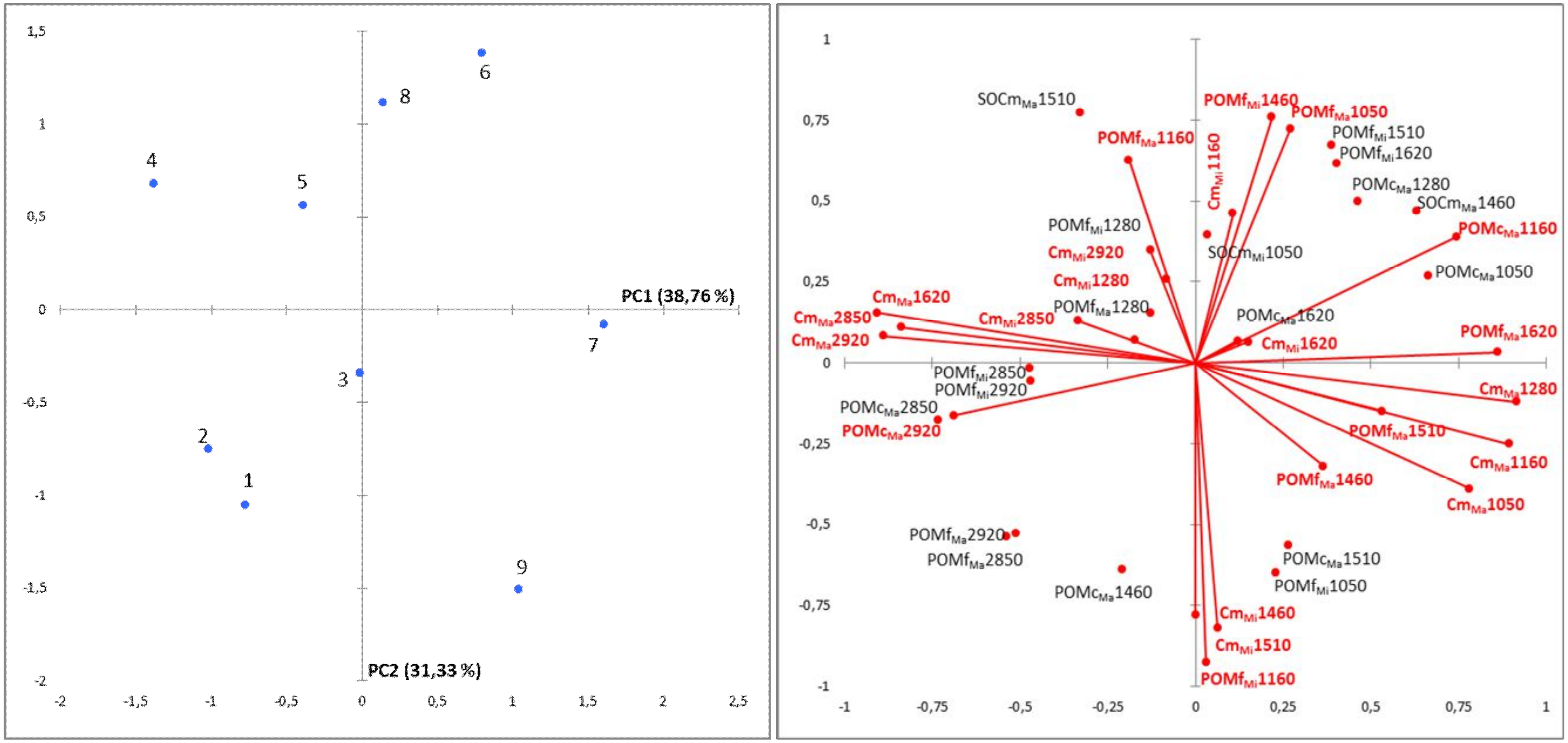

Fig. 3: Principal Component Analysis (PCA) representation obtained considering the relative absorbance (%rA) of the main observed bands (0-10 cm) in DRIFTS spectra of density fractions under cacao agroforestry systems, natural forest and pasture in Southern region of Bahia, Brazil. Variables red marked: only factor loading ≥ 0.70. POMc: coarse particulate organic matter; POMf: fine particulate organic matter; C_m: mineral associated soil organic matter. 1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and Erytrina (35-year-old); 5: 35-year-old cacao AFS in thinned forest (‘cabruca’); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

DISCUSSION

Differences are observed among POMs (coarse and fine – POMc and POMf) and C associated to soil mineral (C_m) and between the two depth (0-10 and 80-100 cm) (Fig. 1 and Table 3). In topsoil (0-10 cm), tillage in younger systems clear reflected in distribution (g kg^{-1}) and, consequently, in C accumulation (Mg ha^{-1}), demonstrating that macroaggregates were broke down and the associated-C in all density fractions was partially lost. The soil disturbance for younger systems (4-year) implantation contributed to increase macroaggregates turnover (Six et al., 2000). Grüneberg et al. (2013) also observed a higher SOC stock under forest systems with no tillage, confirming that the absence of soil disturbances contributes to C stabilization in long-term pools, such as aggregates density fractions. However, tillage influence was not observed in fractions associated with microaggregates because they are more stable than macroaggregates (Six et al., 2000; Six et al., 2002).

In all land use systems, C accumulation followed the order: C_m >POMc>POMf for macroaggregates and C_m >POMf for microaggregates, at both depths (0-10 and 80-100 cm) (Table 3). Consequently, C_m fraction is the major contribution to total SOC, enhancing the relevance of C_m fraction in the soils studied. On average, 12% of total SOC stock under the old AFS, natural forest and pasture is accumulated and stabilized in C_m fraction from macro- and microaggregates, while in young AFS this value decreases to 2% (total SOC database from Monroe et al., 2016). Therefore, old systems promote C protection in the aggregates, with statistical similarity to natural forest. In these systems, the aggregates formation process is occurring from the plant residue deposition time and POMc formation until the final phase, regarding the encrusting of clay minerals (Table 3), which demonstrate the importance of tillage absence to POMs maintenance for aggregates formation process, and, consequently, to C accumulation and stabilization in soil by the physical and chemical protection.

In the chemical characterization of SOM is possibly to note that some bands were attributed to almost all macro- and microaggregates density fractions, with differences in absorbance intensity, such as: 3300, 2920, 2850, 1630, 1510, 1160 and 1050 cm^{-1}, which contributes to differentiation among POMs and C_m fraction (Fig. 2). The 3300 cm^{-1} band, that was more accentuated in C_m fraction, represents the O-H stretching (Calderón et al., 2011) or the presence of N-H in amides vibrations (Fan et al., 2015), which indicates recalcitrant functional groups due to bonds between O-H, NH and closed-chain C-H (Kleber et al., 2007; Lorenz et al., 2007). The bands 2920 and 2850 cm^{-1}, which indicate more aliphatic functional groups (Calderón et al., 2011), were also detected in the more stable fractions, such as C_m of microaggregates until depth 80-100 cm, indicating that part of the organic matter associated with these fractions is labile. Similar results were obtained by Rennert et al. (2018), which explained due to periodic water saturation in deeper soil layers that slows down decomposition and contributes to more labile compounds maintenance. Oxygen limitation with depth has already been reported by Rumpel and Knabner (2011), which observed that C availability for soil microorganisms is affected by specific conditions, such as anaerobiosis, temperature and available nutrients, which contribute to decrease C turnover.

Bands of approximately 1630 and 1510 cm^{-1} was assigned in all fractions studied showed absorption peaks that represent aromatic functional groups. However, C_m fraction presented higher intensity of absorption values than particulate fractions (POMs) only in 80-100 cm (Fig. 2). An absorbance increase in bands 1650-1600 cm^{-1}, along with band 1510 cm^{-1}, possibly indicates aromatic material accumulation (Calderón et al., 2011), demonstrating that POMs possibly have aromatic functional groups associated when compared to C_m. On the other hand, the C_m fraction in 0-10 cm depth has high C storage values, but possibly with low aromaticity degree in topsoil. This is related to a low turnover rate in the C_m fraction due to organomineral complexes formation, which decreases the decomposition process (Boeni et al., 2014).

The absorption band of approximately 1160 cm^{-1} (C-H, O-H, C-OH; Rumpel et al., 2001) and 1050 cm^{-1} band (simple C-H bond or O-H deformation), also presented absorbance intensity in POMs fractions, at both depths, in all the systems studied. It suggests that POMs fractions present functional groups related to polysaccharides derived from plant residues (Calderón et al., 2011), which are considered effective stable aggregates binding agents (transient organic agents) and, in addition, is an important C source to microorganisms whose byproducts also act as binding agents (Tisdall and Oades, 1982; Six et al., 2000).

However, some bands were only attributed to some fractions, such as: 1280-1270, 1420 and 1730 (Fig. 2). Bands at ~1280 cm^{-1} (–COOH, C-O vibrations, O-H deformation, aromatic ring or lignin) (Bonermann et al., 2010; Calderón et al., 2011) are assigned in C_m fraction at topsoil (0-10 cm layer). The presence of ~1420 cm^{-1} band assigned to aliphatic functional groups, phenolic C-O bonds, or the amide groups (Bornemann et al., 2010) were detected only in the C_m fraction at 0-10 cm and in all fractions at 80-100 cm depth (Fig. 2). According to Lorenz et al. (2007), this 1420 cm^{-1} band may represent the presence of stabilized aliphatic polymers preserved in soils. The band at ~1720 cm^{-1}, observed in POMs in the two depths, occurs due to esters with carbonyl presence (Sharkot et al., 2007), representing an organic material more easy to decompose.

The Principal Component Analysis (PCA) revealed dissimilarities between natural forest, pasture and older cacao AFS from younger cacao AFS (4-year-old). C_m fraction was the more relevant for dissimilarity, followed by POMf (considering only factor loadings >0.70) (Fig. 3). These PCA results confirm that the cacao-based AFS implantation, replacing pasture and natural forest, affected SOM storage, chemical composition and reactivity (Table 3 and Fig. 2). The %rA obtained by DRIFTS suggest that the more labile functional groups (such as aliphatic functional groups: 2920 and 2850 cm^{-1} bands) have been metabolized in soil under young cacao AFS, favoring the aromatic C functional groups maintenance (C_{mMi}1620, C_{mMi}1510, C_{mMi}1460 and C_{mMa}1280 cm^{-1}). It suggests that the macroaggregate turnover promoted by tillage increased biological oxidation of organic labile biomolecules (mostly associated to POMc) and, consequently, C losses associated to aggregates. Despite the young systems showed the same behavior previously mentioned there is some dissimilarity between cacao row and rubber tree row, what highlights the role of each specie in the quality and accumulation of large amounts of fresh residues. Cacao residues are more persistent than rubber tree residues, which contribute to maintenance of the organic material in topsoil and, consequently, C input, flow and stabilization in density fractions in soils under cacao. On the other hand, rubber tree residues are easily decomposed and more aliphatic C compounds are rapidly lost (Solomon et al., 2007; Jindaluang et al., 2013; Vicente et al., 2019).

Nevertheless, after implantation, cacao AFS contributes to maintain or even increase SOC associated within aggregates (Gama-Rodrigues et al., 2010) due to the absence of tillage and soil disturbance regimes. The %rA suggest that old cacao AFS contributed to C input and maintenance of labile C functional groups, which suggest that physical protection promoted by soil aggregates is efficient in these soils and the aggregation follow until the formation of C_m fraction. This assertion is based on the clear trends presented in PCA, showing the relevance of $POMf_{Ma}1160$, $C_{mMa}2920$, $C_{mMa}2850$, $C_{mMi}2920$ and $C_{mMi}2850$ cm^{-1} for dissimilarity between older from younger cacao AFS (Fig. 5). A new equilibrium was observed after soil disturbance after cacao AFS systems implantation, with the recovery of C storage associated with aggregates at different organizational levels (represented by the density fractions) in old AFS (20, 35 and 40 years old), closer to natural forest (Table 3 and Fig. 3) (Solomon et al., 2007).

The PCA results also indicate that, in pasture, the main functional groups of organic matter are similar to those under natural forest (Fig. 3), which may be consequence of the well development of grasses root system, allowing high C flow in the aggregates density fractions (Kuzyakov and Domanski, 2000). According to Jobbágy and Jackson (2001), the grassland annual turnover of root system is higher than in trees. In addition, the studied pasture is a no-grazing system, which provides continuous soil coverage.

The combination between physical fractionation and chemical characterization provides important information on the interaction and stabilization processes of soil organic matter associated to aggregates, especially regarding the interaction between organic matter and soil mineral fraction. Our results demonstrate the presence of labile compounds associated with C_m, which demonstrate the importance of C_m pool for protection and stabilization of SOC, mainly in 0-10 cm. Therefore, the results suggest that chemical composition also reflected soil disturbance in cacao AFS implantation. However, it is important to highlight that despite the quantitative loss and differences in %rA, these systems can act as C sinks, being able to C sequestrate in aggregates density fractions when are already established and producing.

CONCLUSION

The organic C associated to soil minerals (C_m) fraction is the largest pool of C protected within soil aggregates in natural forest, pasture and old cacao-based AFS. Moreover, in topsoil, C_m contributes to the maintenance and stabilization of aliphatic functional groups by protection against microorganisms attack, and in subsoil presented more aromatic band absorbance intensities at 1700-1400 cm^{-1} region. Although macro- and microaggregates POMs fractions presented the lowest C storage values, these fractions are relevant for aggregate formation and stability.

The conversion of pasture and natural forest to cacao-based agroforestry system with tillage leads to losses in organic C storage, mostly C aliphatic forms in the top layer. On the other hand, once established, the cacao based AFS contribute to C stabilization within different soil aggregates density fractions, which demonstrate the potential of these systems to mitigate global warming effects through CO_2 sequestration and, consequently, to regulate responses to environmental changes.

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3.2 Chemical assessment of organic matter occluded in soil aggregates under cacao agroforestry systems using solid-state ^{13}C CPMAS NMR[1]

ABSTRACT

Cacao agroforestry systems (AFS) can sequester high amount of soil organic carbon (SOC), but information about these systems influence on chemistry of soil organic matter (SOM) associated to soil aggregates fractions still scarce. The aims were (1) to evaluate the chemical composition of soil organic matter occluded in macro- and microaggregates density fractions (particulate organic matter – POM and C associated to soil minerals – C_m); and (2) to determinate the composition of C-mineral association (C_m) under natural forest, pasture and cacao AFS. Macro- (2000-250 µm) and microaggregates (250-53 µm) density fractions were obtained by chemical fractionation to separate the free light fraction (FLL) from particulate organic matter (POM: coarse and fine) and organic matter bound to mineral (C_m fraction). Chemical characterization was done using solid-state ^{13}C CPMAS NRM. Although relative distribution was observed in all spectra, differences among POMs and Cm fraction were verified. POMs had higher concentration of aryl-C (on average 18.8%) and lower concentrations of O/N-alkyl and alkyl-C (36.7% and

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[1] Artigo a ser submetido na revista Organic Geochemistry (ISSN: 0146-6380)

23.7%, respectively) than Cm (13.5%, 39.6% and 31.5%, respectively). More aryl-C in POM may be occurs due to decomposition process until C becomes encrusted with soil minerals, in aggregates formation process. Fresh residues and/or microbes' debris can, previously, bind with soil minerals, which forms occluded C_m fraction rich in labile compounds. A simple model describing the possible aggregates formation process in soil studied is proposed and uses to explain the variations of O/N-alkyl-C, alkyl-C and aryl-C among density fractions. In macroaggregates Cm fraction, natural forest showed the higher proportion of alkyl-C (43.6% of total intense), which indicates selective preservation of stable C. Cacao 'cabruca' and 4-year-old cacao AFS replacing natural forest in cacao row represented more O/N-aryl and alkyl-C, in detriment of aryl-C. On the other hand, 4-year-old cacao AFS replacing natural forest in rubber tree row showed high proportion of aryl-C (almost 20%), probably due to rubber tree fresh residues that may be rich in labile compounds, easily decomposed and lost. Pasture and 4-year-old cacao AFS replacing pasture showed similar relative distribution in spectra, probably due to Brachiaria sp influence. C_m from microaggregates showed same trend among chemical shifts. No-tillage systems, such as natural forest and pasture with no grazing, and the absence of soil disturbance after cacao AFS implantation contributes to slow macroaggregates turnover, which promote the C labile compounds protection and time to microaggregates formation within macroaggregates.

Keywords: density fractions, POM, C-mineral complex, cacao AFS, NMR spectroscopy.

INTRODUCTION

Soil organic matter (SOM) is composed of heterogeneous organic C compounds and these different C forms display turnover time ranging from days to thousands of years (Kölbl and Kögel-Knabner, 2004). The SOM residence time may be related to several factors, such as hydrophobicity, soil acidity, aggregates formation and organo-mineral interactions (Schmidt et al., 2011). Some researchers have been demonstrated that SOC accumulation by physical protection associated to organo-mineral interactions is the more important mechanism to C sequestration and maintenance in soil (Kogel-Knabner et al., 2008; Six and Paustian, 2014; Angst et al., 2018; Gartzia-Bengoetxea et al., 2020).

Assuming that organo-mineral complex formation is a major stabilization mechanism, particulate organic matter (POM) and C-associated to soil minerals (C_m) may be assigned to more active and more passive pools in soil, respectively (Six et al., 1998; Helfrich et al., 2006; Boeni et al., 2014). Therefore, to investigate the POM and C_m chemical composition, it is necessary to isolate theses fractions from whole soil, which can be approached by physical (separation in aggregates classes: macroaggregates – 2000-250 μm and microaggregates 250-53 μm) and chemical fractionation combined (separation by density: free light fraction, POMs – coarse and fine – and organic C adsorbed in soil minerals) (Six et al., 1998; Gama-Rodrigues et al., 2010). Thus, it is possible to assume the access of physical and chemically protected organic C by aggregates and mineral adsorption.

Some agroecosystems, such as agroforestry systems (AFS), are especially relevant due to higher potential to SOC sequestration (Albrecht and Kandji, 2003;

Nair et al., 2010; Lorenz and Lal, 2014). Moreover, AFS is considered as a viable sustainable soil cover to deal with degradation, in addition to other environmental services, such as increase in soil fertility and biodiversity conservation (Lorenz and Lal, 2014). In Brazil, mostly in Bahia State (Northeast region of country), in cacao (*Theobroma cacao* L.) based AFS studies have been demonstrated that they may accumulate a significant SOC amount up to 1 m (Gama-Rodrigues et al., 2010; Monroe et al., 2016; Salgado et al., 2019), including in soil fractions (Barreto et al., 2010; Rita et al., 2011).

Therefore, this research is part and continuation of a sequence of researches already conducted in soils under cacao AFS in Bahia, Brazil (Gama-Rodrigues et al., 2010; Monroe et al., 2016; Salgado et al., 2019; Oliveira et al., 2019) and was designed to investigate the chemical composition of soil organic matter in macro- and microaggregates density fractions to better understand aggregates formation in tropical soils and how the cacao AFS implantation influence the SOC composition in more stable C pool. Among available techniques to investigate, the solid-state ^{13}C cross polarization with magic angle spinning (CPMAS) NMR is the most useful for chemical characterization of soil organic matter and for chemical shifts semi-quantitative determination (Mao et al., 2017).

In the first article of this thesis, we have yielded chemistry information from the functional groups of SOM under natural forest, pasture and cacao AFS assessed by DRIFTS (Diffuse reflectance infrared Fourier transformation spectroscopy). We showed the relevance of organo-mineral complex for SOC sequestration in these soils, concluding that was the largest C pool, and also provide evidences that differences among all density fractions occur. In addition, we demonstrated that soil disturbance for cacao AFS implantation influenced SOC accumulation in aggregates density fractions, promoting C losses, in more labile functional groups (C-C; C-H; C-O).

Thus, in order to obtain more insights and comprehension about SOC chemical composition in soils studied, we seek out to analytical characterization for individual comparison of SOM fractions within aggregates and to indicate how land-use systems influence the maintenance of C associated with the mineral interaction. Therefore, in this study, we search to answer some questions, such as: What are the chemical differences among all density fractions (POMs and C_m), in macro- and microaggregates, and between them? Are there differences in percentage of chemical shift among the density fractions? What are the differences among land-use systems from chemical composition of C_m fraction, which is an important pool of C sequestration in the soils studied? For this purpose we used the physicochemical fractionation followed by solid-state ^{13}C Nuclear Magnetic Resonance (NMR) analyses, which provides more accurate results at a molecular scale.

Thus, the objectives were: (1) to evaluate the chemical composition of organic compounds and their relative quantities associated to macro- and microaggregates density fractions, seeking out to establish the differences between the fractions and to better understand the aggregates formation process in these soils; and (2) to determinate the composition and relative quantity of organic compounds in organomineral complex (C_m fraction) under natural forest, pasture and different cacao AFS, aiming to facilitate a better understanding of compositional changes promoted in SOM by cacao AFS.

MATERIAL AND METHODS

Sites descriptions, soil collection and analysis

Soil samples were collected, in municipality of Uruçuca, in Bahia state, more precisely at Porto Seguro farm (14° 35′ 34″ S, 39° 17′ 2″ W). Region's climate is classified in Köppen's classification as *Af* (tropical rainforest climate), with annual temperature an average 25ºC and 1500 mm of rainfall. Following USDA Soil Taxonomy (Soil Survey Staff, 2009), the soils were classified as Oxisol, which is equivalent to *Latossolo amarelo* in Brazilian System of Soil Classification (Embrapa, 2013).

The land-use systems selected was: (1) natural forest (Tropical Ombrophilous Dense Forest); (2) pasture (with 30-year-old without grazing, replaced a forest); (3) cacao+rubber tree AFS (cacao with 20-year and rubber tree with 40-year-old); (4) cacao+Erytrina AFS (with 35-year-old; *Erytrina glauca* Lour.); (5) cacao 'cabruca' (cacao in thinned forest); (6 and 7) cacao+rubber tree AFS replacing pasture (4-year-old system; 6 indicate cacao row and 7 rubber tree row); and (8 and 9) cacao+rubber tree AFC replacing natural forest (4-year-old system; 8 indicate cacao row and 9 rubber tree row).

In 4-year-old systems process of implantation, the soil was ploughed and harrowed. The cacao AFS were fertilized since 2003 and the soils were collected in 2011. For soil collection, four plots (30 x 30 m) were delimitated per land-use systems. Trenches were open in each plot, which provides four replications per site. Soils were collected in 0-10 cm depth. All collected samples were air dried and passed in 2000 μm sieve.

Combined physical and density size fractionation

The physical and density fractionation were carried out following Gama-Rodrigues et al. (2010) and Six et al. (1998), respectively. Briefly, 100 g of soil sample (<2000 µm) was fractionated by wet-sieving, passing to through 250 and 53 µm sieves sizes, obtaining macroaggregates (2000-250 µm), microaggregates (250-53 µm) and silt+clay fraction (<53). Afterwards, macro and microaggregates were density fractionated. 5.0 g of an aggregate subsample was centrifuged using 2600 rpm, with 35 ml of sodium iodide (1.85 g cm^{-3}) for 60 minutes, separating the free light fraction (FLL) from heavy fraction (HF). The FLL was filtered, separated and was not analyzed. The heavy fraction was dispersed by shake for 18h on a horizontal shaker (Tecnal TE-240). After shaking, the samples were passed in the sieves (250 and 53 µm-sieves for macroaggregates and 53 µm-sieve for microaggregates). Thus, three fractions were obtained for macroaggregates (coarse intra-particulate organic matter -POMc; 2000-250 µm -, fine intra-particulate organic matter - POMf; 250-53 µm - and mineral-associated organic matter - C_m; <53 µm) and two fractions for microaggregates (POMf; 250-53 µm and C_m; <53 µm). Fractions were dried at 60ºC. The all fractionation procedure is more detailed in the first article of this thesis.

Hydrofluoric acid pretreatment of density fractions

The pretreatment was carried out using a 1:10 sample-solution ratio of 10% (v/v) hydrofluoric acid (HF) (Rumpel et al., 2006; Sanderman et al., 2017; Vicente et al., submitted), aiming to increase the C content and to remove paramagnetic compounds (Baldock et al., 1992; Gonçalves et al., 2003; Dick et al., 2005;

Simpson et al., 2017; Mastrolonardo et al., 2015; Chiti et al., 2019). Samples were combined with HF, agitated in horizontal shaker for 2 hours followed by centrifugation for 10 minutes (2600 rpm). The procedure was repeated eight times and the supernatant was removed in each cycle (Gonçalves et al., 2003). Then, samples were washed to remove hydrofluoric acid solution and were dried at 60 ºC.

C, N and Fe measurements

Carbon and nitrogen contents were analyzed before and after pretreatment, using a Perkin-Elmer Series II 2400 CHNS/O analyzer. C and N enrichment and recovered factor (R factor) were calculated following equations proposed by Dick et al. (2005). Fe was determined following EMBRAPA (2017), using sulfuric acid (Table 1). The results confirmed that pretreatment was efficient.

Table 1: Parameters obtained after the pretreatment in all density fractions of soils under cacao agroforestry systems, natural forest and pasture in Bahia, Brazil.**

0-10 cm	**RM‡**	**CE‡**	**NE‡**	**CR‡**	**NR‡**	**Fe†**		**R‡**
						Before$_{HF}$	**After$_{HF}$**	
POMc$_{Ma}$	44.7	3.06	3.47	129.9	159.6	95.87	1.25	1.20
POMf$_{Ma}$	37.1	1.89	3.38	68.5	126.2	56.49	1.46	1.14
C$_{mMa}$	7.1	7.01	5.50	51.7	39.5	65.80	1.63	0.89
POMf$_{Mi}$	39.7	1.55	2.86	58.8	117.2	74.22	1.45	1.19
C$_{mMi}$	5.3	11.09	8.32	54.4	43.6	45.04	1.60	0.95

** Database in first work in this thesis

‡Calculated following Dick et a. (2005); †EMBRAPA (2017): values in mg dm^{-3}

Ma: macroaggregates; Mi: microaggregates; POMc: coarse particulate organic matter; POMf: fine particulate organic matter; C$_m$: mineral associated soil organic matter; RM: recovered mass; CE and NE: enrichment in C and N; CR and NR: recovered C and N; R: factor used to verify selective losses in organic matter (should range between 0.8-1.20).

NMR spectroscopy

The large number of samples, due to the nine sites, was not viable. Moreover, the low C content and sand influence in POMs, from each land-use systems, made the time required in spectrometer impracticable. The macro- and microaggregates C_m fractions contain most SOC (data showed in the first article of this thesis), thus were used to verify land-use systems dissimilarities. As a result, the NMR analysis was divided into two parts: (1) composite samples of density fractions – POMc, POMf and C_m from macro- and POMf and C_m from microaggregates, obtained by joining the samples from each site; (2) C_m fraction from macro- and microaggregates from soils under natural forest, pasture and cacao AFS.

Thus, ^{13}C CPMAS NMR was performed on the Bruker AVANCE II 400 MHz apparatus, equipped with a 4 mm probe (MAS 4BL CP BB for solid-state analyses) and operating in a resonance frequency of 100 MHz. For all measurements, the optimized contact time and recycle delay were set to 4 ms and 1s, respectively. The parameters were chosen based in preliminary analysis. Approximately 50-100 mg of samples was placed into a rotor with Kel-F caps, using a rotation frequency of 10 KHz. The ^{13}C CPMAS NRM chemical shifts were given relative to tetramethylsilane (reference 0 ppm). The areas of each chemical shift range were divided into four regions: 0-45 ppm (alkyl-C); 45-110 ppm (N and/or O-alkyl-C); 110-160 ppm (aryl-C) and 160-220 ppm (carboxyl C) (Song et al., 2008; Panetieri et al., 2013). These regions are, generally, divided in sub regions, as demonstrated by Mastrolonardo et al. (2015), Simpson and Simpson (2017) and Mao et al. (2017). The assignments of peaks in ^{13}NMR spectra in soil samples are displayed in table 2.

Spectra and statistical analyses

The NMR spectra were processed using MestreNova software 8.1.0. (Mestrelab Research Inc., Santiago de Compostela, Spain). Multipoint baseline correction was done manually. Integration of each chemical shift was calculated and expressed as percentage of total area. Thus, comparisons could be made between density fractions and land-use systems (Baldock et al., 1992; Song et al., 2008; Knicker, 2011; Chiti et al., 2019; Gartzia-Bengoetxea et al., 2020).

Despite ^{13}C CPMAS NMR spectra are not per se quantitative due to ^{1}H influence in signal intensity, the relative distribution of chemical shifts calculated by integration may be used for comparisons between the treatments (Berns and Conte, 2011; Chiti et al., 2019), since the same parameters were applied for all samples (Baldock et al., 1992).

In order to verify land-use systems dissimilarity, the relative distribution of signals (%) from chemical shifts analyzed (presented in Table 4) from macro- and microaggregates C_m fraction was submitted to Cluster Analysis, using Euclidean distance as a dissimilarity measure.

Table 2: Assignments of peaks in solid-state ^{13}C NMR spectra to typical C groups in soil samples (referenced to tetramethylsilane = 0 ppm).

Chemical shift range (ppm)	**Assignment**		
0-45	**Alkyl-C**		Terminal methyl groups; Methylene groups in aliphatic rings and chains; Alkyl-CH in R-(CH2)-CH2-CH3 (lipids); side chains – biopolymers (lignin, cutin, suberin, tannins); amino acids
45-60	**N and/or O-Alkyl C**	**Methoxyl/N-Alkyl**	Methoxyl groups C; C in amino groups (lignin and proteins); sugar-C; cellulose/hemicellulose
60-90		**O-Alkyl**	C5 in xylan; C2 and C6 of carbohydrates (polysaccharides); amino acids; alcohol; sugars; cellulose/hemicellulose
90-110		**di-O-Alkyl**	Anomeric C of carbohydrates; C-polysaccharides; cellulose/hemicellulose
110-140	**Aryl C**	**C-H and C-C aromatic**	Aromatic C-H and C-C carbons; C2 and C6 in lignin; olefinic C; amino acids
140-160		**O-substituted**	Aromatic C-O-R or C-N-R groups; lignin; suberin
160-185	**Carboxyl C**	**Carboxyl/Amide/Ester**	Carboxylic acid; carboxylates; ester COO-C-; amide CO-N; aliphatic amides; fatty acids.
185-220		**Aldehydes and/or Ketones**	Aldehydes, -((CH)=O)-; Ketones, -(C=O)-; lignina; amino acids and protein

References: Baldock et al. (1992); Knicker (1996); Mao et al. (2011); Panetieri et al. (2013); Mastrolonardo et al. (2015); Mao et al. (2017); Simpson and Simpson (2017).

RESULTS

Density fractions chemical composition

The ^{13}C NMR spectra of aggregates density fractions and the relative intensities (%) of each chemical shift regions are displayed in Fig. 1 and Table 3, respectively. In spectra, some differences between POMs and Cm fractions were observed. In general, the patterns of POMs showed signal intensities in aryl-C (110-160 ppm region) more prominent, when compared to Cm fractions (Fig. 1), which indicates high proportion of aromatic C. The aryl-C relative distribution was 21.6% in POMf from microaggregtes, followed by 17.7% and 17% in POMf and POMc from macroaggregates, respectively (Table 3). On the other hand, C_m fractions were dominated by O/N-alkyl (45-110 ppm) and alkyl-C (0-45 ppm) (on average 39.6-31.5% of total intensity in macro- and microaggregates, respectively). This indicates high proportion of aliphatic rings, amino acids and carbohydrates (Fig. 1; Table 2 and 3).

The carboxyl-C forms (160-220 ppm) showed relative proportions in POMs and C_m fractions ranging between 20.8% in POMs to 15.4% in C_m (Table 3), with predominance of carboxyl-C/amide/ester forms (160-185 ppm) that are assigned from lignin, fatty acids, hemicellulose and proteins (Table 2).

Among the POMs, macro- (POMc and POMf) fractions showed close relative distribution in chemical shifts regions. However they presented difference with POMf from microaggregates in alkyl-C region (0-45 ppm): almost 10% more in macro-POMs (almost 27%) than in POMf from microaggregate (17.4%). No trend was observed among C_m from macro- and microaggregates (Table 3).

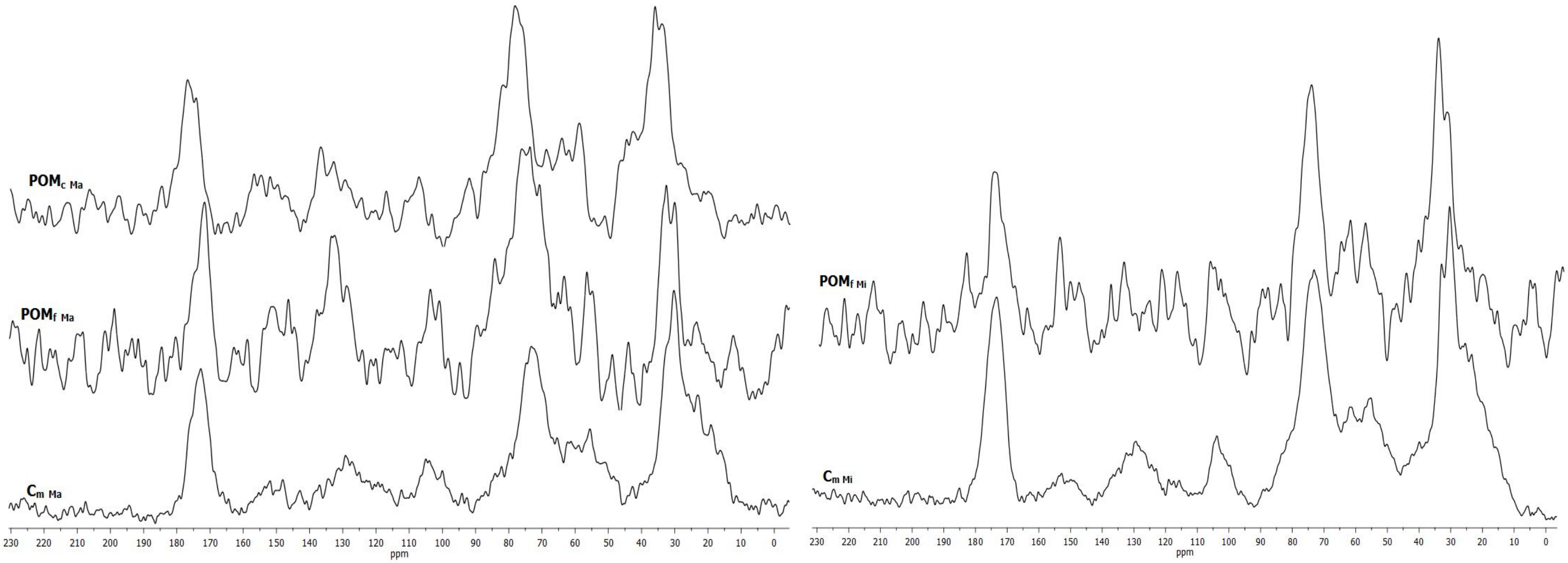

Figure 1: ^{13}C NMR spectra of macro- and microaggregates density fractions (composite samples) of soils under cacao agroforestry systems, natural forest and pasture in Bahia, Brazil. Ma: Macroaggregates; Mi: microaggregates; POM: particulate organic matter (c: coarse; f: fine); C$_m$: mineral associated to soil organic matter.

Table 3: Relative distribution (%) of the signal intensity in selected chemical shifts regions (ppm) of solid-state ^{13}C NMR in aggregates density fractions (composite samples) from soils under cacao agroforestry systems, natural forest and pasture in Bahia, Brazil.

Relative distribution

Density Fractions	Alkyl C (0-45)	N and/or O-Alkyl C			Aril C		Carboxyl C	
		Methoxyl/N-Alkyl (45-60)	O-Alkyl (60-90)	di-O-Alkyl (90-110)	C-H and C-C aromatic (110-140)	O-substituted (140-160)	Carboxyl/ amide/ester (160-185)	Aldehydes and/or ketones (185-220)
POMc$_{Ma}$	26.8	5.9	26.2	4.3	10.3	6.8	12.2	7.6
POMf$_{Ma}$	26.9	7.0	21.2	5.9	10.8	7.0	12.4	8.9
C$_{mMa}$	32.2	9.3	26.8	4.4	8.2	3.0	14.1	2.0
POMf$_{Mi}$	17.4	4.6	28.2	6.7	13.9	7.6	11.8	9.6
C$_{mMi}$	30.8	8.3	24.0	6.5	10.8	4.9	12.0	2.8

Ma: Macroaggregates; Mi: microaggregates; POM: particulate organic matter (c: coarse; f: fine); C$_m$: mineral associated to soil organic matter.

Chemical composition of Cm fraction under natural forest, pasture and cacao AFS

The spectra from land-use systems and the relative abundance of chemical shifts (%) based on ^{13}C NMR spectra from C_m fraction of macro- and microaggregates are display in figures 2-3 and in Table 4. Overall, signal peaks of eight main C forms are observed in spectra from macro- and microaggregates, such as signal in 30 ppm, which indicates lipids and aliphatic biopolymers; signal peak in 70-72 ppm, which are assigned from cellulose and hemicellulose; 95-110 ppm, indicating di-O-alkyl (O-C-O) from sugar rings of carbohydrates (glucose, fructose); and approximately 130 ppm, assigned to lignin presence (Table 2). No trend was clearly noted in carboxyl-C group, but the dominant C-forms were carboxyl-C/amide/ester (160-185 ppm), with a slightly increase in microaggregates (Figs 2 and 3). Although C forms are presented in all spectra, as indicated by relative distribution (%) values, is possible to observe C_m fraction trends in macro- and microaggregates (Table 4), contributing for land-use dissimilarity (Fig. 4).

Our results revealed that the most relevant differences among land-use systems were observed in C_m fraction from macroaggregates (Fig. 2 and Table 4). Natural forest showed high relative proportion of O/N-alkyl (45-110 ppm; 35.6%) and alkyl-C forms (0-45 ppm; 43.6%) than contribution of aryl-C forms (110-160 ppm) (5.3% of total intensity). The same natural forest trend (more O/N-alkyl and alkyl-C; low aryl-C) was observed for cacao ‘cabruca’ (37.7%, 37.2% and 7.3% of the total intensity represents O/N-alkyl, alkyl-C and aryl-C, respectively) and 4-year-old cacao AFS replacing natural forest in cacao row (35%, 40% and 6.5%). On the other hand, 4-year-old cacao AFS replacing natural forest in rubber tree row showed the highest Aryl-C value (almost 20%), while presented 32.5% and 31.9% of the total intensity represented by O/N-alkyl and alkyl-C forms, respectively (Table 4). Older AFS, as cacao (20-year-old) rubber tree (40-year-old) and cacao+Erytrina AFS, showed intermediate values, ranging from on average 31-33.2% representing O/N-alkyl and alkyl-C, while 14.6-15.5% representing Aryl-C, respectively (Table 4). The pasture and 4-year-old cacao AFS replacing pasture in rubber tree and cacao rows presented 43.4-29.5%, 42,4-31.4% and 39.7-27.3% of the total intensity by O/N-alkyl and alkyl-C forms, and 12.5%, 11.3% and 17.5% by aryl-C forms (110-160 ppm), respectively.

Microaggregates C_m fraction showed similar trend to that observed in macroaggregates among land-use systems (predominance of O/N-alkyl and alkyl-C, and low aryl-C forms). On average, O/N-alkyl, alkyl-C and aryl-C represents 39.2%, 31.8% and 11.1% of the total intensity, respectively, less in 4-year-old AFS replacing the natural forest in rubber tree row, which presented 19.2% of the total intensity by aryl-C (Fig. 3 and Table 4).

The dendrogram from Cluster analysis allowed a comparison between land-use systems (Fig. 4). 4-year-old AFS replacing the natural forest in rubber tree row is clearly different from all the other systems, as well as natural forest. The pasture (2) and 4-year-old AFS replacing pasture (both cacao and rubber tree row) showed dissimilarities from other systems, but similarities to each other, forming a group. Cacao (20-year-old) rubber tree (40-year-old) AFS, cacao+Erytrina, cacao ‘cabruca’ and 4-year-old AFS replacing the natural forest in cacao row were also similar, forming a group, being dissimilar from the other systems.

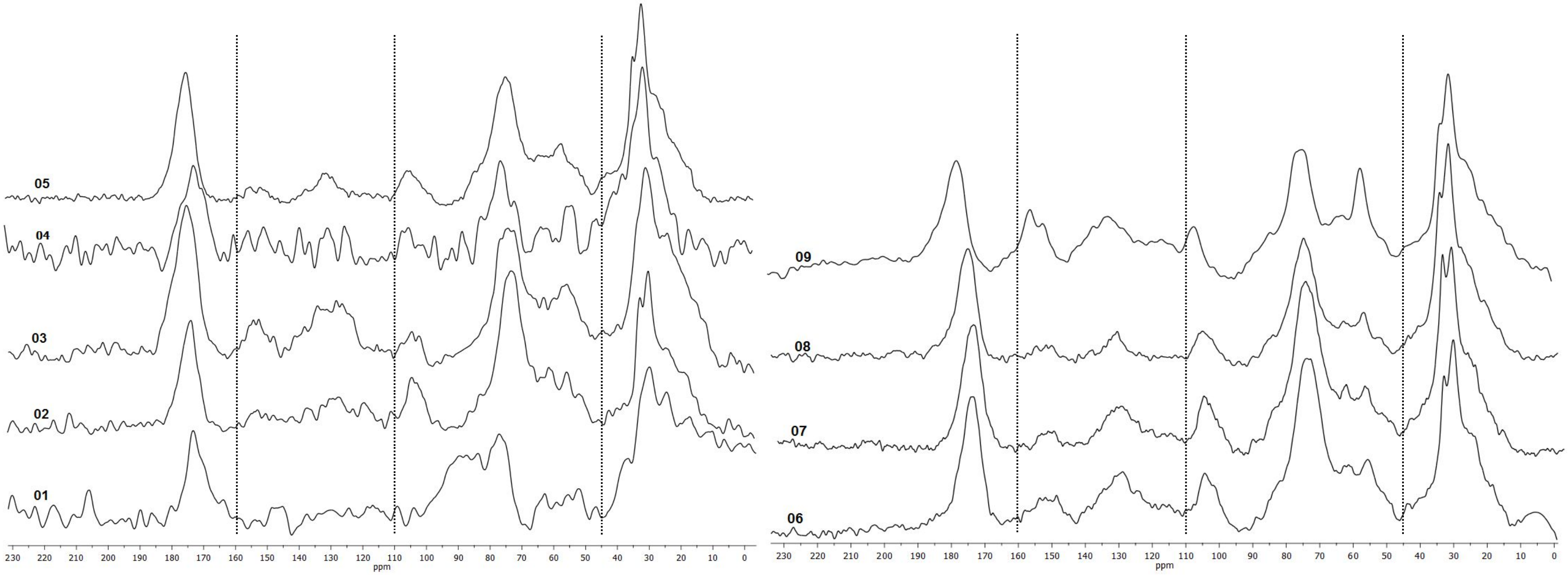

Figure 2: ^{13}C NMR spectra of Cm fractions from macroaggregate in soils under cacao agroforestry systems, natural forest and pasture in Bahia, Brazil. 1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and Erytrina; 5: 35-year-old cacao AFS in thinned forest ('cabruca'); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

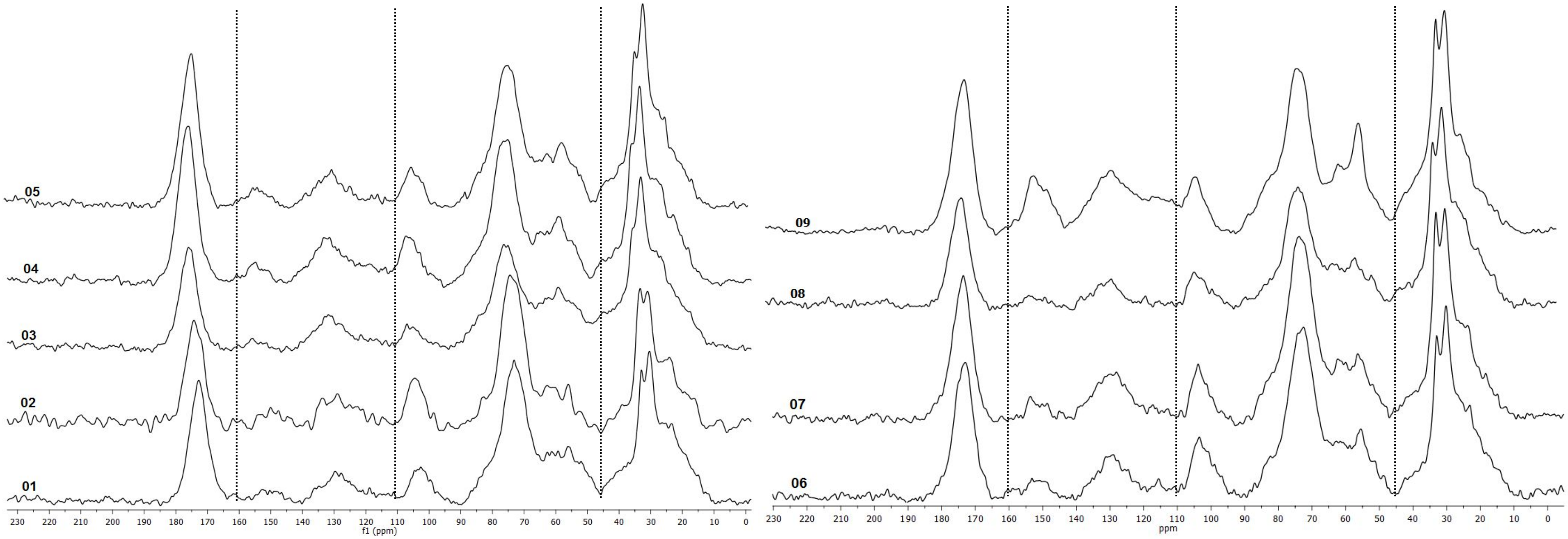

Figure 3: ^{13}C NMR spectra of Cm fractions from microaggregates in soils under cacao agroforestry systems, natural forest and pasture in Bahia, Brazil. 1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and Erytrina; 5: 35-year-old cacao AFS in thinned forest ('cabruca'); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

Table 4: Relative distribution (%) of the signal intensity in selected chemical shifts regions (ppm) in macro- and microaggregate C_m fraction from soils under cacao agroforestry systems, natural forest and pasture in Bahia, Brazil.

		Relative distribution (%)							
			N and/or O-Alkyl C			Aril C		Carboxyl C	
	Land Use Systems	Alkyl C (0-45)	Methoxyl/N-Alkyl (45-60)	O-Alkyl (60-90)	di-O-Alkyl (90-110)	C-H and C-C aromatic (110-140)	O-substituted (140-160)	Carboxyl/ amide/ester (160-185)	Aldehydes and/or ketones (185-220)
Macroaggregates	1	43.6	4.8	20.7	10.1	4.3	1.0	12.2	3.2
	2	29.5	7.3	30.1	5.9	8.9	3.5	12.3	2.4
	3	34.9	8.9	21.9	0.9	10.5	4.1	17.4	1.3
	4	31.6	7.3	16.9	6.1	8.7	6.8	12.9	9.6
	5	37.2	8.6	25.3	3.8	5.4	1.9	15.6	2.2
	6	27.4	7.5	26.5	5.7	12.1	5.4	13.3	2.1
	7	31.4	7.8	29.7	4.9	9.0	2.3	13.2	1.7
	8	40.0	7.2	25.0	2.8	4.4	2.2	15.3	3.1
	9	32.0	9.3	20.7	2.4	12.9	7.1	12.3	3.2
Microaggregates	1	31.0	8.7	27.9	5.0	7.7	2.8	14.6	2.4
	2	27.1	5.2	27.1	6.9	9.2	4.4	14.0	6.1
	3	37.1	10.5	23.2	3.9	8.2	2.0	12.3	2.7
	4	31.7	8.0	24.4	5.5	9.8	2.5	16.0	2.1
	5	32.4	8.3	25.8	4.6	7.3	2.9	16.2	2.5
	6	29.8	7.5	27.9	7.0	8.5	3.1	14.0	2.2
	7	31.5	7.2	29.2	4.6	9.2	2.6	14.2	1.5
	8	37.5	7.3	24.4	5.2	6.4	2.6	13.1	3.5
	9	28.4	8.4	24.6	4.9	13.2	6.1	13.1	1.5

1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and *Erytrina;* 5: 35-year-old cacao AFS in thinned forest ('cabruca'); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

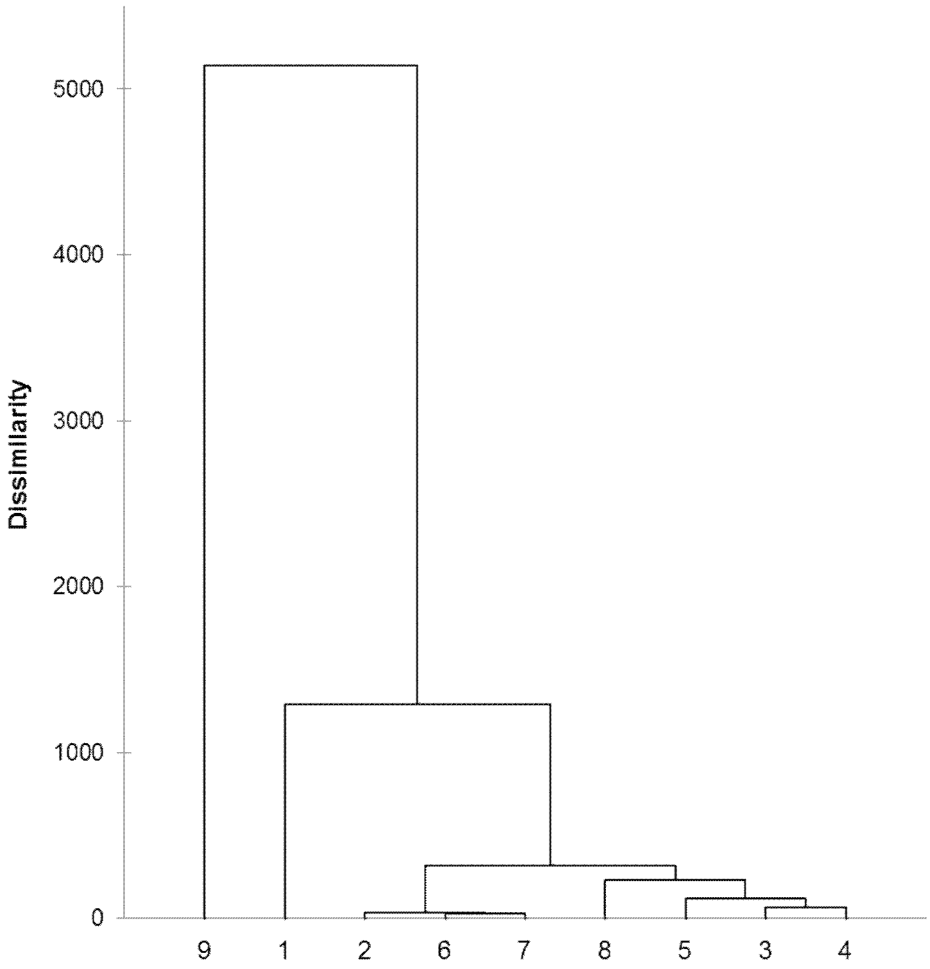

Figure 4: Dendrogram of land-use systems dissimilarity obtained by relative distribution (%) of chemical shifts from macro-and microaggregates C_m fraction, using Euclidean distance as dissimilarity measure (Y axis). 1: natural forest; 2: pasture; 3: cacao (20-year-old) and rubber tree (40-year-old) AFS; 4: cacao and Erytrina; 5: 35-year-old cacao AFS in thinned forest ('cabruca'); 6: 4-year-old cacao and rubber tree AFS replacing pasture (cacao row); 7: 4-year-old cacao and rubber tree AFS replacing pasture (rubber tree row); 8: 4-year-old cacao and rubber tree AFS replacing the natural forest (cacao row); 9: 4-year-old cacao and rubber tree AFS replacing the natural forest (rubber tree row).

DISCUSSION

Chemical composition of SOM associated to aggregates density fractions

The solid-state ^{13}C CPMAS NMR spectra have shown that are differences in soil organic matter chemistry composition in density fractions. Data demonstrated that C_m fractions from macro-and microaggregates preserved more alkyl-C and O/N-alkyl-C forms than POM fraction (Fig.1 and Table 3). The addition of fresh residues below- (roots turnover and exudates) and aboveground (litterfall) promotes soil microorganisms activity, which decompose the more labile C forms, such as aliphatic compounds, polysaccharides and amino acids, which leads to aromatic-C enrichment in POMs (Caricasole et al., 2011; Kurganova et al., 2019). The decomposition process produces mucilage by microbiota activity, which becomes encrusted with soil minerals, forming the organo-mineral complex (C_m: C-mineral) and, therefore, this mucilage act as binding agent, forming microaggregates within macroaggregates. Thus, the smaller relative contributions by aryl-C (110-160 ppm) obtained in C_m fraction from macro- and microaggregates occur because the microbial decomposition rate is stabilized by organo-mineral complex formation, which contributes to reduce the process of C transformation (Cotrufo et al., 2013; Pronk et al., 2013), maintaining C forms from more aliphatic plant components (N/O-alkyl). Moreover, earthworms and termites helps to fragment and transfer plant litter in topsoil, which contributes to incorporation of plant fresh residues in soil minerals (Six et al., 2004).

These results suggest that the macroaggregate formation do not follow a straight line of formation, as according to Six et al. (2000), but may be occur the formation of C_m fraction with the more labile C compounds. The applied physicochemical fractionation enabled us insights into the aggregates formation process at molecular scale in these tropical soils and climate characteristics studied. Thus, we propose a model describing the influence of macroaggregates formation on chemical composition of SOM associated to macro- and microaggregates density fractions, using the data assessed by ^{13}C CPMAS NMR (adapted from Six et al., 2000) (Fig. 5).

In the proposed model we demonstrate that the decomposition process of POMs in macroaggregates promote labile C compounds losses (O/N-alkyl) and, consequently, increment of more aromatic C (aryl-C) in these fractions. On the other hand, C_m fraction may complex with more labile C forms from plant fresh residues source (cellulose, hemicellulose, amino acids from litterfall and root system) or complex with labile microbiota subproduts (extracellular polysaccharides, proteins and/or organic acids). Although the O/N-alkyl and alkyl-C forms are easier to decompose then aryl-C, the complex formation with soil minerals maintains the organic C protected (Kögel-Knabner et al., 2008; Angst et al., 2018).

Soil biota contributes to mineral-organic complex formation due to deposition of polysaccharides and proteins that act as adhesives and some bacteria produce peripheral slime polymers, such also act as glue, linking soil particles and organic matter (Tisdall and Oades, 1982; Rashid et al., 2016). Furthermore, the microbial subproducts produced in decomposition process of SOM contribute to forming the C_m fraction rich in O/N-alkyl and alkyl-C forms (Baldock et al., 1997; Bimüller et al., 2014). Some studies have been already demonstrated the same relevance from clay fractions for C more labile accumulation in soils by organo-mineral complex protection (Dick et al., 2005; Kögel-Knabner et al., 2008; Marschner et al., 2008; Bimüller et al., 2014; Angst et al., 2018; Gartzia-Bengoetxea et al., 2020).

Although POMs (coarse and fine) have been showed the highest aryl-C values, the physical protection promoted by soil aggregates are important to labile and/or aromatic-C maintenance in these fractions, until C becomes encrusted with soil minerals, forming the C_m fraction from macroaggregate or the microaggregate within macroaggregates.

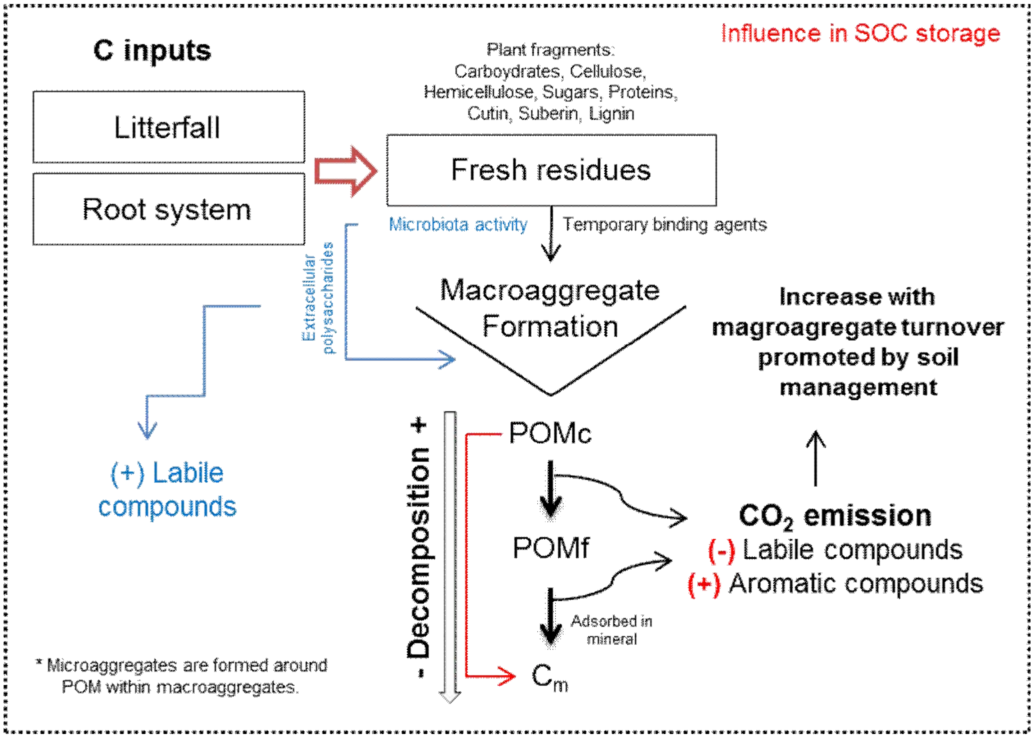

Figure 5: A simple model, adapted from Six et al. (2000), describing the influence of macroaggregate formation on chemical composition of SOM associated to macro- and microaggregates density fractions, using the data assessed by ^{13}C CPMAS NMR. This behavior is result of the interrelations between the quality of fresh residues from land-use systems, soil microbiota and climate factors (temperature and moisture). POMc and POMf: Particulate organic matter coarse and fine, respectively. C$_m$: organic C associated to soil mineral.

Land-use systems dissimilarities: influence of Cm fraction

The ^{13}C RMN spectra of natural forest, pasture and cacao AFS demonstrated some similarities, since signal peaks of eight C main types are displayed in spectra (Figs 2 and 3). However, the signal peaks at each chemical shift display differences in relative distribution (%), least in carboxyl-C group, which allow some distinctions among the land-use systems in this research (Table 4), which are demonstrated by the Cluster analyze by Euclidean distance (Fig. 4).

Overall, the mainly differences in land-use systems were observed in C_m fraction from macroaggregates when compared to C_m from microaggregates (Fig. 2 and Table 4). Regarding macroaggregates differences, the natural forest showed high O/N-alkyl (35.6%) and alkyl-C (43.6%) and, consequently, the smaller values (5.3%) in aryl-C (110-160 ppm). An increase of alkyl-C proportions seems to be consequence of carbohydrates decomposition (decrease of O/N-alkyl) and selective preservation of stable organic materials (Baldock et al., 1992). Angst et al. (2018) showed the importance of alkyl-C forms as a source of stable organic materials that contributes to humus formation, more than aryl-C forms. Added to this, there is a slow and natural macroaggregates turnover, which maintaining the protected-C (Six et al., 2000).

Kögel-Knaber et al. (2008), in an article review about organo-minerals associations in temperate soils, highlighted the major C forms associated to soil minerals are alkyl-C and O/N-alkyl-C. These authors, investigating soils using NMR, observed the intense signal in the aromatic region (110-160 ppm) was low in clay fraction, which is in line with findings by Bimüller et al. (2014). Moreover, Gartzia-Bengoetxea et al. (2020), studying organic matter mineral control in temperate forest soils, observed the major C compounds in soils studied was dominated by O/N-alkyl-C forms (37.6-40.3% of total intensity). Although, the tropical climate contributes to high decomposition rate, the organo-mineral complex formation seems to proceeds and sequestration O/N-alkyl-C and alkyl-C forms. According to Schmidt et al. (2011), organic matter persists in soils by the physicochemistry protection, which contributes to reduce the decomposition rate. Thus, these authors affirmed that labile compounds can persist in soils by mechanisms that reduce microbiota access, such as soil aggregates and organic C adsorbed in minerals.

Cacao ‘cabruca’ and 4-year-old cacao AFS replacing natural forest in cacao row showed a similar pattern in C_m fraction from macroaggregates in natural forest, with more proportion of O/N-alkyl-C and alkyl-C than aryl-C. These cacao AFS are similar to cacao+Erytrina and old cacao+rubber tree AFS (more than 20-year-old) (Fig. 4), possible due to cacao plant residues influence. We did not evaluate litter quality or root extension/exudation in these systems. However, it is well known that cacao AFS deposits large amounts of plant litter and have well-development root systems that may contributes to more activity of microbial communities, due to input of C source, which may promotes the macroaggregates formation and C sequestration (Dawoe et al., 2010; Gama-Rodrigues et al., 2010). Moreover, cacao AFS have a high abundance of soil fauna and microbial communities (Moço et al., 2009; Zaia et al., 2012; Buyer et al., 2017) and the interaction between them improve soil aggregation (Rashid et al., 2016).

The pasture and 4-year-old cacao AFS replacing pasture displayed the same trend in C_m fractions from macro- and microaggregates (Fig. 2-3 and Table 4) and are considered similar (Fig.4), which suggests the influence of C forms from previous pasture that exist before cacao and rubber tree implantation. Thus, these systems showed the highest O/N-alkyl proportion (on average 41.8%), which may be attributed to the maintenance of polysaccharides, amino acids and sugar-C. According to Angst et al. (2018), high proportion of polysaccharides is probably derived from root exudates. On the other hand, these land-use systems also showed great aryl-C proportion (on average 13.7%). The *Brachiaria* sp residues probably contribute to high input of C from aromatic forms, which are linked to soil minerals (Six et al., 2002; Marques et al., 2012; Cyle et al., 2016).

4-year-old cacao AFS replacing natural forest in rubber tree row presented 20% and 19.2% of total intensity represented by aryl-C forms in macro- and microaggregates, respectively (Table 4), being dissimilar from all land-use systems (Fig. 4). These results suggests that rubber tree residues are more rapidly and easily decomposed (Vicente at al., 2019), which promote the more labile compounds losses, such as carbohydrates, and an increase of more aromatic, phenolic and carboxyl-c compounds (Caricasole et al., 2011). Furthermore, Maggioto et al. (2014), evaluating the potential of C sequestration in soils under rubber tree plantation in Brazil, showed that the plantation age influence in biomass accumulation rate. These authors experimentally demonstrated that biomass accumulation ratio in 15-year-old plantation was higher when compared to 4-year-old plantation, reflecting in C accumulation. Soil management for system implementation also may have contributed to more aromatic forms, due to the macroaggregate turnover. The more easy compounds are decomposed, remaining the C-aromatics (Six et al.., 2002b).

The combination of physicochemical fractionation with ^{13}C CPMAS RMN spectroscopy technique provided a good start for the comprehension of soil aggregates chemical composition under different cacao AFS in tropical high weathered soils. Thus, C_m fraction, mostly in macroaggregates, can be considered as an important pool of C accumulation and a complex mixture of different biomolecules, as more labile and recalcitrant C compounds. Our results demonstrated the presence of more O/N-alkyl and alkyl-C compounds in C_m fractions due to SOM-mineral strongly association, which contributes to a non-decomposition of organic C. In addition, natural forest, pasture and cacao AFS also presented predominance of more labile compounds (O/N-alkyl) associated to C_m from macro- and microaggregates, as well as more selective preservation of stable C forms (alkyl-C). These results confirm that conservation systems increase C sequestration and stabilization, reducing the SOM availability for microorganisms by aggregates and organo-mineral complex protections. Therefore, SOM stabilized by mineral associations and occluded in soil aggregates has over long-term turnover time and, thus, can be considered as stable C (Dick et al., 2005; Kogël-Knabner et al., 2008; Marschner et al., 2008; Pronk et al., 2013; Cooper et al., 2016).

CONCLUSION

This study demonstrated a non-linear aggregates formation, from NMR data of density fractions, due to higher O/N-alkyl and alkyl-C forms associated with organo-minerals complex (C_m fraction) from macro- and microaggregates. As well as, indicated the possible influence of the microbial compounds from decomposition process on the amount of labile C forms associated to C_m fraction. Thus, our results could demonstrated a new way to explain the aggregates formation in weathered tropical soils, confirming the importance of C_m fraction for C sequestration in these soils under natural forest, pasture and cacao AFS. Also displayed that pasture-cacao AFS or natural forest-cacao AFS conversion leads to losses in SOM, contributing to increase of aryl-C forms. However, in these conservation systems, the absence of soil tillage after implantation contributes to slow macroaggregates turnover, which promote the C labile compounds maintenance and protections promoted by aggregates and organo-mineral complex formation.

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4. RESUMO E CONCLUSÕES

No primeiro trabalho foi realizada a quantificação do C associado às frações densimétricas e determinação da caracterização química do C, pela utilização da técnica espectroscópica do Infravermelho por transformada de Fourier e Refletância Difusa (em inglês: *DRIFTS*). Os resultados mostraram que a fração C_m foi o maior *pool* de sequestro de C, principalmente nos SAFs mais antigos de cacau (.>20 anos), floresta natural e pastagem. Na camada superficial, considerando os solos e condições climáticas em estudo, a fração C_m demonstrou que o complexo organomineral contribui para a manutenção dos compostos mais lábeis contra o ataque microbiano. A fração C_m também demonstrou maior aromaticidade na camada subsuperficial (80-100 cm).

As frações de matéria orgânica particulada (POMs), tanto dos macro- quanto dos microagregados, apresentaram os menores valores de estoque de C nas frações densimétricas. No entanto, é importante destacar a relevância dessas frações para a formação e estabilidade dos agregados, que são imprescindíveis para a proteção física do C orgânico. Além disso, observou-se diferenças entre os SAFs de cacau mais antigos e mais novos (4 anos), devido ao processo de revolvimento do solo para a implantação dos sistemas em substituição a pastagem e a floresta natural, o que promoveu a quebra dos macroagregados e, consequentemente, a liberação do C associado. Sendo assim, os compostos mais lábeis foram facilmente decompostos, permanecendo os compostos mais aromáticos.

No segundo trabalho, visando responder algumas questões que não ficaram claras com a utilização do DRIFTS, foram realizadas análises utilizando a Ressonância Magnética Nuclear do ^{13}C (RMN), que é uma técnica útil para a caracterização química e determinação quantitativa dos compostos químicos, a partir de suas quantidades relativas. As MOPs apresentaram alta aromaticidade, indicada pela proporção de formas aril-C (em média 18,8%), quando comparado com a fração C_m (13,5%). Além disso, a fração C_m apresentou maior proporção de O/N-alquil (em média 39,6%), o que indica predomínio de celulose, hemicelulose, polissacarídeos, açúcares e aminoácidos.

Na fração C_m dos macroagregados, a floresta natural apresentou a maior proporção de alquil-C, indicando a preservação seletiva de compostos estáveis de C. O Cacau ‘cabruca’ e o SAF cacau+seringueira, em substituição a floresta natural na linha do cacau, apresentaram maior proporção de O/N-alquil e alquil-C. O SAF de cacau+seringueira mais antigo (>20 anos) e o SAF cacau+Eritrina, apresentaram valores intermediários de O/N-alquil e alquil-C. Já o SAF cacau+seringueira, em substituição a floresta natural na linha da seringueira, apresentou os maiores valores de aril-C, possivelmente devido à qualidade do material vegetal da seringueira. A pastagem e o SAF, em substituição a pastagem, apresentaram distribuição relativa similar. Esse resultado indica que ainda há influência da cobertura anterior na fração C_m do SAF de cacau+seringueira recém-implantado. Na fração C_m dos microagregados também foram observadas maiores proporções de O/N-alquil e alquil-C. Também foi observada uma ligeira tendência do aumento na proporção de carboxil-C (160-185 ppm), quando comparado aos macroagregados, o que indica aumento da hidrofobicidade da MOS.

Com os resultados obtidos, foi possível concluir que a fração C_m é o maior *pool* de C nos solos estudados, visto que apresentou o maior estoque e predomínio de compostos mais lábeis. Apesar de serem formas facilmente decompostas, a ligação com os minerais do solo contribui para a manutenção e estabilização do C contra o ataque microbiano.

Além disso, mesmo havendo perdas de C pelo processo de perturbação do solo para a implantação dos sistemas agroflorestais de cacau, foi possível verificar que, após o estabelecimento dos sistemas e cessado o manejo do solo, eles contribuíram para a diminuição da ciclagem dos macroagregados, promovendo a proteção e acumulação do C, resultado similar ao observado no solo sob floresta natural. Sendo assim, observou-se que os SAFs contribuem para a melhoria da qualidade do solo e podem atuar como sumidouros de C, mitigando o aquecimento global por meio do sequestro de CO_2 e, consequentemente, regulando as respostas às mudanças climáticas.

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