Decomposition of Carbon Adsorbed on Iron (III)-Treated Clays and Their Effect on the Stability of Soil Organic Carbon and External Carbon Inputs

Mengyang You

Chinese Academy of Sciences

Xia Zhu-Barker (✉ wyjzhu@ucdavis.edu)

University of California Davis College of Agricultural and Environmental Sciences

https://orcid.org/0000-0001-9050-3696

Timothy A. Doane

University of California Davis

William R. Horwath

University of California Davis

Abstract

The interaction of organic carbon (OC) with clay and metals stabilizes soil carbon (C), but the influence of specific clay-metal-OC assemblages (flocs) needs further evaluation. Clay-metal-OC assemblages representing OC-mineral soil fractions were synthesized using dissolved organic C (DOC) combined with kaolinite (1:1 layer structure) or montmorillonite (2:1 layer structure) clays in the absence or presence of two levels of Fe (III). Flocs were mixed with soil and incubated for 30 days. Total C mineralization from flocs was approximately 70% lower than non-flocced DOC. The flocs made with montmorillonite had 16–43% lower C mineralization rate than those made with kaolinite with no Fe or low Fe. However, when flocs were made with high Fe, clay mineralogy did not significantly affect total C mineralization. A positive priming effect (PE) of flocs on native soil OC was observed in all treatments, with a stronger PE found in lower Fe treatments. The high-Fe clay flocs inhibited ryegrass decomposition, while the flocs made without clay had no impact on it. Interestingly, flocs significantly decreased the PE of ryegrass on native soil OC decomposition. These results indicate that the adsorption of DOC onto clay minerals in the presence of Fe (III) stabilizes it against decomposition processes and its stability increases as Fe in flocs increases. Flocs also protect soil OC from the PE of external degradable plant C input. This study showed that Fe level and clay mineralogy play an important role in controlling soil C stability.

1. Introduction

The adsorption of organic carbon (OC) onto mineral surfaces is an important mechanism of stabilizing soil organic carbon (SOC) (Han et al. 2016; Kahle et al. 2002; Mikutta et al. 2007; von Luetzow et al. 2006). Phyllosilicate clays and iron minerals have long been recognized as reactive surfaces that adsorb OC (Balcke et al. 2002; Kaiser and Guggenberger 2007). The OC stabilization effect in the absence of metals depends on particle size and surface charge (Eusterhues et al. 2005; Feng et al. 2005; Zhang et al. 2012). Kaolinitic clays with a 1:1 layer structure occur as larger particles but carry less charge compared to 2:1 clays (Greenland 1971). Six et al. (2002) found that soil dominated by 1:1 clays exhibited a lower C sequestration potential compared to soil dominated by 2:1 clay minerals. The stability of OC on clay often occurs with hydrous Fe oxides, a common bridging metal. The stability of clay-Fe-OC assemblages (referred to as flocs) differs with clay type, and undoubtedly affects the priming effect (PE) outcomes in the presence of degradable C inputs.

Many studies have investigated the role of phyllosilicate clay minerals in stabilizing DOC through adsorption interactions (Kahle et al. 2004; Saidy et al. 2013; Saidy et al. 2012; Sodano et al. 2016). Dissolved organic carbon (DOC) is a mobile and reactive OC pool in soil that can adsorb and desorb from metal-clay assemblages (Wang et al. 2003). By measuring C mineralization following adsorption, these studies found that different clay minerals imparted various degrees of stability to OC. Moreover, Saidy et al. (2015) showed that plant C adsorbed to pure phyllosilicate clays had a significant lower C mineralization rate compared to the absence of clay. Characteristics of clay minerals such as specific surface area (SSA) and cation exchange capacity (CEC) determine the degree to which DOC is adsorbed (Kahle et al. 2004). For example, coating of kaolinite with hydrous Fe oxide enhanced DOC sorption, but

this phenomenon was not observed with montmorillonite due to its relatively limited available surface after coating of Fe (Saidy et al. 2012).

Co-precipitation of DOC and Fe commonly occurs in soils, sediments, and surface and ground water. It is believed that these floc formations are stable and have limited mobility following formation (Larsen 2009). The stability of flocs is in part promoted by the adsorbed OC's ability to protect Fe oxides from reduction to Fe (II) (Henneberry et al. 2012; Pisani et al. 2015). Eusterhues et al. (2008) mentioned the important role of floc in immobilizing DOC and protecting it from microbial degradation. Studies in paddy soil and aquatic environments (Pisani et al. 2015; Sodano et al. 2016) have shown that the rate of floc C decomposition was faster in wet than in dry seasons. The stability of OC in hydromorphic soils, such as rice paddies and wetlands, can be strongly controlled by its adsorption onto Fe (hydr)oxide (Henneberry et al. 2012; Sodano et al. 2016). These studies shed light on C associated with clays and Fe but do not provide insight into the PE that they potentially exert on other soil C pools, such as litter or other labile sources of C.

The objectives of this study were to understand the decomposition of flocced OC formed through the interaction among DOC, Fe and clay and their effect on the stability of other SOC pools and the decomposition of degradable C in plant residues. We hypothesized that 1) the decomposition rate of C in clay-Fe-OC flocs decreased as Fe content in the flocs increased, 2) OC in pH dependent charge kaolinite flocs decomposed faster than permanent charge montmorillonite flocs, and 3) the flocs made with low Fe and kaolinite stimulated SOC and plant residue decomposition. To test these hypotheses, we conducted a laboratory incubation experiment using flocs made with kaolinite or montmorillonite clays precipitated with DOC in the absence or presence of different levels of Fe. Our results provide information on the role that metals play in stabilizing OC on clay surfaces as a function of clay mineralogy and how SOC and the degradable plant C substrate be affected by flocs containing different Fe contents.

2. Materials And Methods

2.1 Sample preparation

Extraction of dissolved organic carbon: The DOC used to prepare floc products was extracted from mature corn residue (Zea mays L.). Before extraction, the corn residue (approximately 2 cm pieces) was inoculated with 2% soil solution (soil:water = 1:50) and pre-incubated at 40 °C for 30 days. The purpose of this pre-incubation was to allow soil microorganisms to consume labile C in the residue to better reflect characteristics of soil DOC. After the pre-incubation, the residue (2 kg dry mass equivalent) was suspended in deionized water (8 L). The suspension was shaken at 170 r min^{-1}, left to settle for 48 h, and filtered through a 0.45μm glass fiber filter. The DOC concentration in the filtrate was 1.6 g C L^{-1}, determined by UV-persulfate digestion (Teledyne-Tekmar Phoenix 8000) and had a δ^{13}C value of -15.8‰, determined at the UC Davis Stable Isotope Facility.

Preparation of iron-OC floc

A flocculant solution was prepared consisting of 1.75 M $Fe_2(SO_4)_3$ and 9M H_2SO_4. The optimal dose of $Fe_2(SO_4)_3$ required to remove maximum DOC from the corn residue filtrate was determined in a parallel experiment by adding different amounts of $Fe_2(SO_4)_3$ into the filtrate solution until the concentration of the remaining DOC in the solution did not change anymore. Flocs were subsequently prepared using the optimal dose of coagulant and recovered by filtering the suspension through a 0.45μm glass fiber filter. The floc retained on the filter was oven-dried at 50 °C.

Preparation of clay-iron-OC and clay-OC flocs: Two clays were obtained from the Clay Minerals Society: kaolinite (low-defect kaolin, Warren Count, Georgia, USA; SSA: 10.0 $m^2 \, g^{-1}$) and Ca-rich montmorillonite (Gonzales County, Texas, USA; SSA: 83.8 $m^2 \, g^{-1}$). The Fe (hydr)oxide-coated clays were prepared by suspending clay (1 g) in coagulant solution (pH = 2.5, NaOH adjusted) with different concentrations of Fe as $Fe_2(SO_4)_3$. The "low Fe" products (Fe_{low}) contain 5.4 mg Fe g^{-1} clay and 11.4 mg Fe g^{-1} clay for kaolinite and montmorillonite, respectively. The "high Fe" products (Fe_{high}) contain 199.0 mg Fe g^{-1} clay and 219.0 mg Fe g^{-1} for kaolinite and montmorillonite, respectively. The clay-Fe mixtures were shaken for 48 h at 22 °C, and then centrifuged. This process was repeated numerous times until no Fe was detected in the supernatant using phenanthroline indicator (Saidy et al. 2012). The Fe-coated clays were then oven-dried at 50 °C and gently ground by mortar to pass a 200 μm sieve.

Clays with or without Fe coating were used to adsorb DOC from the filtrate solution (described above) by shaking 20 ml DOC solution with 1 g clay (without Fe coating) or Fe-coated clay for 12 hours at 22 °C. The pH of the mixtures was adjusted to 6.0 with NaOH after shaking. The suspensions were then centrifuged and the pellets were washed with deionized water and centrifuged again; this step was repeated until no DOC was detected in the supernatant. The precipitates were oven-dried at 50 °C and then ground to pass 200 μm. The C concentration and $\delta^{13}C$ value analyses of all the floc products were performed at the UC Davis Stable Isotope Facility. All the floc products had a similar $\delta^{13}C$ value of -15.8‰.

Soil incubation preparation: The soil used for incubations was collected from the Russell Ranch Sustainable Agricultural Facility, University of California-Davis, California. The soil is a silty clay loam classified as a Mollic Haploxeralf, with 19.39 g kg^{-1} OC, 2.58 g kg^{-1} total nitrogen, and a $\delta^{13}C$ value of −26.7‰. The sand was washed with hydrochloric acid numerous times to remove nutrients and other ions. No C or N was detected in the washed sand. The washed sand was then mixed with soil at a ratio of 19: 1 (by mass) and air dried. Aliquots of the dried sand/soil (10 g dry mass) mix (hereafter referred to as soil medium) were placed into 60 ml glass serum vials, wetted to 40% water holding capacity with deionized water, and pre-incubated at 22 °C in the dark for 7 days.

2.2 Incubation experiment

After the pre-incubation, floc material or unflocced DOC was added to the soil medium at a rate of 0.2 mg C g^{-1} and mixed thoroughly with a glass rod. The soil medium alone (no floc or DOC additions) was treated as control. A separate set of treatments received enriched ^{13}C-labled mature ryegrass residue

(*Lolium perenne L.*) at a rate of 1 mg C per treatment (equals to 0.4 mg C g^{-1}) at the same time as floc addition. The ryegrass, with a $\delta^{13}C$ value of 538.0‰, was labeled by frequent $^{13}CO_2$ pulse labeling events according to Bird et al. (2003). The use of ^{13}C-enriched ryegrass residue allowed us to sufficiently differentiate C mineralization from ryegrass vs. soil/floc mixtures to estimate the PE of ryegrass on soil/floc C and the effect of floc on ryegrass decomposition during incubation. For each treatment, a nutrient solution containing $(NH_4)_2SO_4$ and K_2HPO_4 was added to reach a final C: N and C: P of the soil/floc mixture of 10: 1 and 25: 1, respectively, to ensure no nutrient limitation. The moisture content of all the treatments was adjusted to 60 % of water holding capacity with deionized water. Sample vials were closed with butyl rubber septa and crimp-sealed following the addition of nutrient solution and moisture adjustment. The headspace of sample vials was then immediately flushed with CO_2-free air to ensure no background CO_2 remained in the headspace. All the vials were incubated at 22 ^oC in the dark for 30 days. The experiment was set up as a completely randomized design with 18 treatments (summarized in Table 1), each with 4 replicates.

Table 1
Summary of the treatments

Treatment	Description
Control	No floc or DOC solution was added into soil medium
DOC	Only DOC solution was added into soil medium
Fe-OC	The floc made with Fe and DOC was added into soil medium
M-OC	The floc made with montmorillonite and DOC was added into soil medium
Felow-M-OC	The floc made with low Fe-coated montmorillonite and DOC was added into soil medium
Fehigh-M-OC	The floc made with high Fe-treated montmorillonite and DOC was added into soil medium
K-OC	The floc made with kaolinite with adsorbed C was added into soil medium
Felow-K-OC	The floc made with low Fe-coated kaolinite and DOC was added into soil medium
Fehigh-K-OC	The floc made with high Fe-treated kaolinite and DOC was added into soil medium

2.3 Gas sampling and analysis

Gas samples were removed from the headspace of the vials on days 1, 3, 5, 7, 9, 11, 14, 17, 20, 23, 26, and 30 and transferred to evacuated exetainers (Labco, Ceredigion, United Kingdom). At each sampling event, all vials were flushed immediately after gas sampling with CO_2-free air to ensure no CO_2 remained in the headspace. The CO_2 concentration in the gas samples was analyzed on a gas chromatograph with a thermal conductivity detector (Model 2014, Shimadzu Scientific Instruments). Stable C isotope analysis of CO_2 in gas samples were analyzed on days 1, 5, 17, and 30 by the UC Davis Stable Isotope Facility, on

a Thermo GC combustion isotope ratio mass spectrometer (GC/C-IRMS) consisting of a Trace GC Ultra gas chromatograph (Thermo Electron Corp., Milan, Italy) coupled to a Delta V Advantage IRAM through a GC/C-III interface (Thermo Electron Corp., Bremen, Germany).

2.4 Soil analysis

At the end of the incubation, all treatments were extracted with 0.5 M K_2SO_4 (2:1 extractant volume-to-soil mass ratio). The extracts were filtered, and the supernatant analyzed for DOC concentration using UV-persulfate oxidation (Phoenix 8000, Teledyne-Tekmar) and ^{13}C-DOC content using a TOC analyzer (1010 OI Analytical, College Station, TX, USA) coupled to a PDZ Europa 20−20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility. Working DOC standards prepared with glucose of known mass and ^{13}C enrichment were used for quality control.

2.5 Calculation

Treatments without ryegrass addition

The difference in $\delta^{13}C$ natural abundance of soil medium (-26.7‰) and flocs (-15.8‰) was used to calculate the contribution of these C pools to CO_2 production. The fraction of CO_2 (f_{floc}) and CO_2 production ($CO_{2,floc}$) derived from flocs was calculated as follows

$$f_{floc} = (\delta^{13}C\text{-}CO_{2,treatment} - \delta^{13}C\text{-}CO_{2\,control}) / (\delta^{13}C_{floc} - \delta^{13}C_{control}) \quad (1)$$

$$CO_{2,floc} = CO_{2,total} \times f_{floc} \quad (2)$$

where $\delta^{13}C\text{-}CO_{2,treatment}$ and $\delta^{13}C\text{-}CO_{2,control}$ are the $\delta^{13}C$ value of mixed CO_2 produced from the treatments of soil medium amended with flocs and control (soil without floc), respectively. $\delta^{13}C_{floc}$ and $\delta^{13}C_{control}$ refer to the $\delta^{13}C$ signature of the flocs and soil medium in the control, respectively. $CO_{2,total}$ represents the total CO_2 production in the floc treatments.

The CO_2 production ($\mu g\ g^{-1}$) derived from soil medium only was derived from native soil organic C ($CO_{2,SOC}$) and was determined as follows:

$$CO_{2,SOC} = CO_{2,total} \times (1 - f_{floc}) \quad (3)$$

The priming effect ($\mu g\ g^{-1}$) of flocs on native SOC mineralization was defined as follows:

$$PE = CO_{2,SOC} - CO_{2,control} \quad (4)$$

where $CO_{2,control}$ was the CO_2 production from the treatment of soil medium alone (control, no floc and DOC addition).

In the DOC only treatment, the fraction of CO_2 (f_{DOC}) and CO_2 production ($CO_{2, DOC}$) derived from DOC was calculated using the same approach as described above by replacing floc pool with DOC pool in the calculation.

Treatments with and without ryegrass straw addition

The fraction of CO_2 derived from ryegrass straw was calculated as follows

$$f_{straw} = (\delta^{13}C\text{-}CO_{2, \text{ with straw}} - \delta^{13}C\text{-}CO_{2, \text{ without straw}}) / (\delta^{13}C_{, \text{ straw}} - \delta^{13}C_{, \text{ without straw}}) \quad (5)$$

where $\delta^{13}C\text{-}CO_{2, \text{ with straw}}$ and $\delta^{13}C\text{-}CO_{2, \text{ without straw}}$ are the $\delta^{13}C$ value of mixed CO_2 produced from the treatments with and without straw for each floc, respectively. $\delta^{13}C_{, \text{ straw}}$ and $\delta^{13}C_{, \text{ without straw}}$ refer to the ^{13}C signature of the ryegrass straw and the soil/floc mixture, respectively. The soil and flocs ^{13}C (– 26.7 to – 15.8‰) were considered as background in the presence of highly enriched ryegrass straw (538‰). We carried out the calculation (Eq. 5) using the ^{13}C signature of soil or flocs and the results did not vary (CV < 1%). Thus, we treated the soil and floc mixture as one C pool, named "soil-floc" pool (with the measured $\delta^{13}C$ value of -16.1‰). By using this approach, we also acknowledge that CO_2 derived from the soil or the flocs in the ryegrass straw addition treatments could not be differentiated. The priming effect (PE) of the ryegrass addition on C mineralization of soil-floc pool was estimated to be the difference between the C-CO_2 derived from soil-floc pool in the ryegrass treatments and the total C-CO_2 production in the treatments without ryegrass. The large difference in the ^{13}C enrichment between the soil-floc and ryegrass was sufficient to estimate reliably the change in the contribution of ryegrass to total CO_2 production.

The fraction of extracted DOC derived from flocs or soil/floc mixture in the treatments without ryegrass or with ryegrass was calculated using the same approach for CO_2 fraction calculation (see above).

2.6 Statistical Analyses

A two-way Analysis of variance (ANOVA) was performed using PROC Mixed procedures in SAS (SAS 9.3, SAS Institute Inc., Cary, N.C., USA). Differences were considered significant at $P < 0.05$, with a separation of mean values by a least significant difference (LSD) test. In the treatments without or with ryegrass residue, clay type and Fe level were treated as fixed factors, and replicate as a random factor, to test their effect on cumulative CO_2 production, priming effect, and DOC content at the end of the incubation.

3. Results

3.1 Properties of flocs

Regardless of Fe levels (none, low, or high), the maximum DOC sorption capacity of montmorillonite flocs is larger than that of kaolinite flocs (Table 2). Coating clays with Fe enhanced DOC sorption capacity of both montmorillonite and kaolinite clays, with the high Fe-treated clays adsorbing more DOC than the low

Fe-treated clays. The CEC decreased in the flocs containing higher Fe content. The floc made without clay adsorbed higher DOC compared to the flocs made with clays.

Table 2
Characteristics of flocs

Treatments		Montmorillonite			Kaolinite		
	Fe-OC[a]	M-OC	Fe_{low}-M-OC	Fe_{high}-M-OC	K-OC	Fe_{low}-K-OC	Fe_{high}-K-OC
CEC[b] (cmol kg^{-1})	-	84.4	49.0	1.9	2.0	1.3	-
Fe (mg g^{-1})	9.0	-	11.4	219.0	-	5.4	199.0
Sorbed C[c] (mg C g^{-1})	340.0	4.0	9.1	26.0	2.4	6.3	22.0
[a] Fe coagulated with dissolved organic carbon							
[b] Cation exchange capacity							
[c] Carbon content of flocs							

3.2 Cumulative CO_2 production and source partitioning

In all treatments, cumulative CO_2 production increased gradually throughout the entire incubation (Fig. S1). In treatments without ryegrass, total CO_2 production in DOC treatments were significantly higher than other floc treatments (Table 3). More CO_2 was produced in treatments containing flocs made with kaolinite alone or combined with low level of Fe than comparable treatments with montmorillonite. In treatments with ryegrass, the total CO_2 production in DOC only treatment was similar to the treatment of flocs made with kaolinite alone but significantly higher than other floc treatments. In floc treatments with no or low Fe, higher CO_2 production was found in kaolinite flocs than montmorillonite flocs (Table 3).

Table 3

Cumulative CO_2 production (ug C g^{-1}) during incubation period and extracted DOC (ug C g^{-1}) at the end of incubation from different treatments without and with ryegrass (n = 4). The letters represent the differences in CO_2 production or DOC among different floc treatments without or with ryegrass addition.

Treatment	Without ryegrass		With ryegrass	
	CO_2 production	DOC content	CO_2 production	DOC content
Control	19.6 (1.2) f	7.35 (0.5) c	302.7 (10.0) e	15.03 (0.6) f
DOC	148.2 (0.6) a	280.13 (35.3) a	463.2 (13.5) a	111.61 (2.5) a
Fe-OC	50.7 (2.4) e	46.13 (0.9) b	344.7 (14.4) d	57.13 (2.7) b
M-OC	87.9 (1.4) d	17.41 (0.7) c	407.2 (11.3) c	31.37 (0.5) c
Fe_{low}-M-OC	82.6 (1.5) d	27.10 (0.9) c	350.9 (7.0) d	39.94 (1.0) c
Fe_{high}-M-OC	25.2 (2.5) f	13.92 (0.7) c	256.1 (17.1) f	17.07 (0.7) f
K-DOC	129.7 (1.8) b	15.38 (1.6) c	457.3 (7.9) ab	14.97 (1.2) f
Fe_{low}-K-OC	106.6 (3.8) c	24.38 (0.7) c	433.2 (8.2) bc	25.92 (1.2) e
Fe_{high}-K-OC	28.5 (2.6) f	17.32 (1.0) c	268.9 (10.7) f	23.31 (1.2) e

The fractional contributions of floc and native SOC to total CO_2 production were determined for all the treatments without ryegrass (Fig. S2 A). In all the treatments of flocs made with no Fe, floc was the dominant source of CO_2 produced while native SOC contributed significantly to the total CO_2 production in floc treatments made with Fe regardless of its level. The total CO_2 production derived from floc in all treatments was significantly lower than the CO_2 produced from DOC decomposition in DOC only treatment. At the end of the incubation, 45.4% of DOC was mineralized in DOC only treatment while 4.0-30.4% of floc-C was mineralized in floc treatments. The floc-derived CO_2 production was 1.2−2.3 times higher in the treatments of flocs made with kaolinite than that in the treatments with montmorillonite (Fig. 1A). Within the same clay treatment, CO_2 produced from floc decomposition decreased as the Fe level increased. Compared with the control (soil only), the addition of flocs or DOC significantly promoted CO_2 produced from native SOC, with higher SOC-derived CO_2 production found in the flocs made with lower level of Fe. The type of clay minerals in the flocs made with Fe did not significantly impact the CO_2 produced from native SOC. In the treatments of flocs made without Fe, higher SOC-derived CO_2 production was found in kaolinite compared to montmorillonite treatments (Fig. 1A).

The fractional contributions of soil-floc mixture and ryegrass to total CO_2 production were determined for all the treatments with ryegrass (Fig. S2B). As expected, ryegrass-derived CO_2 production dominated total

CO_2 production in all treatments, ranging from 59 to 76% (Fig. 1B). In all treatments, 45–78% of ryegrass was mineralized by the end of the incubation, with the highest rate found in DOC only treatment. The total CO_2 produced from ryegrass treatments was significantly increased by flocs made without Fe. However, regardless of clay mineralogy, the flocs made with high Fe significantly inhibited ryegrass C decomposition by 14–19% while no impact of Fe-OC floc on ryegrass decomposition was found. The total CO_2 produced from the soil-floc mixture C pool varied significantly among floc treatments, with higher soil-floc mixture-derived CO_2 production was found in kaolinite than montmorillonite floc treatments.

3.3 Dissolved organic carbon and source partitioning

At the end of the incubation, the fractional contributions of the extracted DOC sources were determined for all treatments (Fig. 2). In the treatments without ryegrass, the floc-derived DOC changed significantly among treatments. For example, the floc-derived DOC was 85–420% higher in Fe-OC floc treatment than others. No significant difference in soil-derived DOC was found among all treatments except DOC only treatment, which had higher soil-derived DOC than control and other floc treatments. Unexpectedly, the addition of ryegrass increased the total extracted DOC was only found in treatments of flocs made with montmorillonite or floc made without clay (Table 3). In ryegrass treatments, 44–95% of the extracted DOC was derived from soil-floc mixture instead of ryegrass (Fig. 2). However, the addition of ryegrass promoted DOC released from soil-floc mixture compared with non-ryegrass treatments.

3.4 Priming effect

The clay mineralogy and Fe level in flocs had a significant impact on the PE of flocs on native SOC in treatments without ryegrass (Table 4). Regardless of Fe level, the flocs made with kaolinite primed 2.9–31.1 ug C g^{-1} more CO_2 from SOC than the montmorillonite flocs (Table 5). Regardless of clay mineralogy, the flocs made with low Fe significantly primed 46.8–49.2 ug C g^{-1} more CO_2 from SOC than the high Fe flocs. The primed SOC-derived CO_2 production in floc treatments made with Fe only or clay only were 11.3–19.7 ug C g^{-1} less than in DOC only treatment. At the end of the incubation, a negative PE on SOC-derived DOC was found in all floc treatments while the DOC only significantly primed DOC released from SOC (Table 5).

Table 4
Results of analysis of variance on the effect of clay mineralogy and Fe level on cumulative CO_2 production, extracted DOC and priming effect in the treatments without and with ryegrass over 30 days incubation

		Variances	df	Mean square	F	P
Without ryegrass	CO_2 production	Clay	2	1640.1	88.9	< 0.001
		Fe	2	18226.2	987.7	< 0.001
		Clay×Fe	3	7912.8	428.8	< 0.001
	PE	Clay	2	5290.5	153.8	< 0.001
		Fe	2	10202.3	296.6	< 0.001
		Clay×Fe	3	1484.1	43.1	< 0.001
With ryegrass	CO_2 production	Clay	2	1904.2	7.0	0.003
		Fe	2	15327.1	56.6	< 0.001
		Clay×Fe	3	7893.2	29.2	< 0.001
	PE	Clay	2	2631.2	22.4	< 0.001
		Fe	2	1005.2	8.6	< 0.001
		Clay×Fe	3	2159.6	18.4	< 0.001

Table 5

Priming effect (PE) of flocs and ryegrass residue on the C mineralization (or extracted DOC) of native soil or soil/floc mixture. PE was calculated as the difference in CO_2 production derived from soil or soil-floc C pool between the treatments and control. Different letters show significant differences ($P < 0.05$) among different floc treatments without or with ryegrass addition.

Treatment	Without ryegrass		With ryegrass	
	PE of SOC		PE of soil-floc mixture	
	CO_2 ug C g^{-1}	DOC ug C g^{-1}	CO_2 ug C g^{-1}	DOC ug C g^{-1}
Control	-	-	48.1(7.5) ab	3.6 (0.6) a
DOC	37.67 (1.5) b	33.4 (11.0) a	-11.5 (3.5) e	-106.2 (21.3) b
Fe-OC	23.0 (2.5) c	-0.4 (0.8) b	46.1 (1.8) ab	7.0 (2.8) a
M-OC	17.9 (1.6) d	-1.6 (0.9) b	9.4 (5.5) d	-3.7 (2.3) a
Fe_{low}-M-OC	46.2 (0.9) a	-3.4 (1.6) b	34.1 (4.3) bc	5.9 (2.5) a
Fe_{high}-M-OC	-2.8 (0.8) f	-4.7 (1.1) b	28.1 (3.8) c	0.5 (1.0) a
K-DOC	49.0 (2.3) a	-1.0 (0.8) b	56.2 (2.1) a	-3.1 (1.6) a
Fe_{low}-K-OC	49.1 (1.8) a	-2.5 (0.8) b	48.7 (8.5) ab	-1.5 (1.2) a
Fe_{high}-K-OC	2.3 (2.1) f	-4.7 (0.6) b	48.6 (7.3) ab	4.1 (0.6) a

The addition of ryegrass stimulated 3–34% more CO_2 production from soil-floc mixtures compared to treatments without ryegrass, while in DOC only treatment, less soil-floc-derived CO_2 production was observed in ryegrass treatments compared with non-ryegrass treatments (Fig. 1A and B, Table 5). In treatment without flocs (control: soil only), the addition of ryegrass residue primed SOC-derived CO_2 production by 48 ug C g^{-1}. Interestingly, the ryegrass primed soil-floc-derived CO_2 in floc treatments was significantly lower than or not different from the ryegrass primed SOC-derived CO_2 (Table 5).

4. Discussion

The interaction of clay and DOC plays a key role in the stabilization of SOC, which influences its potential for C sequestration (Mikutta and Mikutta 2006; Saidy et al. 2015). The properties of clays significantly affect the amount of DOC that can be adsorbed. Pedogenic Fe oxides actively interact with DOC and play a pivotal role in the capacity of soil to adsorb DOC (Kahle et al. 2004). In this study, flocs made by adsorbing DOC to montmorillonite contained relatively more C than those with kaolinite (Table 2). Previous studies attribute the higher cation exchange capacity (CEC) and specific surface area (SSA) of montmorillonite as factors affecting DOC adsorption (Saidy et al. 2013). The role of cation bridging, where Fe is bound to both clay and DOC was summarized by Stevenson (1994). In our treatments, high

Fe-treated clays were found to adsorb more DOC than low Fe-treated clays. A positive correlation was found between Fe content and the amount of C in clay-Fe-C flocs. The floc, made with Fe and DOC without clay, contained the highest amount of C among all the floc treatments (Table 2). The high adsorptive capacity of different flocs has been shown to remove DOC from wetlands and water streams *in situ* (Ye et al. 2015; Zou et al. 2018).

Adsorbing DOC to clay and/or Fe decreased its mineralization rate by up to 85% compared to the DOC alone (Table 3); the extent to which mineralization rate was reduced depends on clay mineralogy and Fe levels (Kaiser and Guggenberger 2007). These results are in line with previous studies, which showed that mineralization of OC adsorbed to clay minerals was reduced by 27–43% (Chenu and Plante 2006; Saidy et al. 2015). Kalbitz et al. (2005) reported that after 365 days of incubation, 13 to 27% of OC adsorbed to clay minerals was mineralized compared to 28–91% of the OC in solution. Generally, simple compounds such as glucose and citrate can be easily mineralized even after they adsorb to clay minerals (Jones and Edwards 1998). The reduction in mineralization rate of adsorbed C over extended periods is most likely due to the reduced mineralization rate of complex compounds that have been adsorbed. In this study, significantly more C was mineralized from flocs made with kaolinite than montmorillonite when no Fe or low Fe occurred in the flocs; however, in flocs made with high Fe, the difference in C mineralization rate between the two types of clay minerals disappeared (Fig. 1). These results indicate that clay mineralogy and Fe levels play a positive interactive role in controlling C stability of flocs. A similar result was also reported by Saidy et al. (2015), who found that microbial degradation differences between OC adsorbed to kaolinite and smectite depended on the presence of Fe in flocs. The mineralization rate of the adsorbed OC decreased as the Fe content in flocs increased (Fig. 1).

In flocs made with clays, Fe (III) adheres to the clay surface or incorporates into the clay lattice without influencing floc adsorptive properties (Bhattacharyya and Sen Gupta 2008; Carroll 1958). In instances where Fe is present as a (hydr)oxide coating, the surface (and adsorption) properties of the floc change. In this study, when the highest amount of Fe was present in flocs, no significant difference in C mineralization was observed between kaolinite and montmorillonite flocs, indicating that mineralization of C in these flocs was controlled predominantly by Fe rather than clay mineralogy. Therefore, while clay mineralogy and Fe both affect C mineralization, the relative amount of each must be considered in defining C stabilization potential.

In general, compared with 1:1 clays (i.e. kaolinite), 2:1 clays (i.e. montmorillonite) have larger CEC, which facilitates greater adsorption of positively charged metals such as ferric ions (Greenland 1971). The net charge on the montmorillonite surface remains negative if a limited amount of Fe is adsorbed. The sorption of DOC to Fe-treated montmorillonite is expected to occur via non-coulombic mechanisms or cation bridging. For kaolinite, a net positive charge occurs when Fe-adsorbs to the surface and allows for absorbing negatively-charged DOC via coulombic mechanisms such as ligand exchange. Consequently, the stability of C adsorbed onto these two types of clays likely differs. This is reflected in our results showing that the PE of flocs made with the two different clays on SOC decomposition varied (Table 5). In this study, the floc made with kaolinite promoted SOC decomposition compared to montmorillonite.

However, when Fe was present in flocs, the floc-primed SOC decomposition was decreased as the Fe content increased in flocs, regardless of clay mineralogy (Table 5). These results indicate that the interaction between Fe and clay can enhance the resistance of OC in flocs and in SOC to microbial decomposition.

As expected, the addition of ryegrass increased total CO_2 production from all treatments, with higher CO_2 production found in kaolinite treatments than montmorillonite treatments (Table 3). Interestingly, the decomposition of ryegrass residue was inhibited by flocs made with high Fe combined with clay. This is probably due to the inhibitory effect of large amounts of Fe on microbial activity. However, no impact of Fe-OC floc on the ryegrass was found while significant higher ryegrass decomposition rate occurred in DOC only treatment (Fig. 1B). These results indicate that the presence of Fe in flocs can protect OC decomposition and therefore slow down microbial C turnover process.

Over the course of the incubation with ryegrass residue, the majority of total CO_2 was derived from ryegrass but the DOC extracted at the end of the incubation was mainly derived from soil-floc mixture (Figs. 2 and 3). This result was in line with Hagedorn et al. (2004), who reported that an external C input was preferentially respired as CO_2 but the majority of DOC originated from native SOC. Toosi et al. (2012) also reported that only 8–14.5% of the total extractable DOC was derived from added ryegrass residue. In their study, the addition of ryegrass residue promoted two times more CO_2 derived from SOC compared with the control (soil only), an example of positive PE. In our study, a similar positive PE was also observed for all floc treatments except DOC only treatment (Table 5), where a negative PE was found. Unexpectedly, the PE of ryegrass on soil-floc-derived CO_2 or DOC in floc treatments was significantly lower than or not different from the ryegrass primed SOC-derived CO_2 or DOC (Table 5), indicating that flocs can decrease the PE of ryegrass on native SOC decomposition and therefore increase the potential of soil C sequestration.

5. Conclusion

In this study, flocs made with montmorillonite absorbed and stabilized more DOC than kaolinite regardless of Fe content in the flocs. The stability of the adsorbed DOC in flocs was also controlled by the nature of the interaction between clay mineralogy and Fe mineral. For flocs made with high Fe content, the stability of adsorbed DOC was not affected by clay mineralogy, showing that higher amounts of Fe overcome the mineralogical characteristics of clay type in adsorbing DOC. The inclusion of synthetic flocs promoted SOC decomposition with kaolinite derived flocs having a higher PE than montmorillonite flocs. Higher primed SOC-derived CO_2 was also found in the floc treatments with low or no Fe compared to those with high Fe content. The addition of ryegrass residue accelerated C mineralization from the soil-floc mixture. On the other hand, flocs significantly decreased the PE of ryegrass on SOC decomposition. The application of high-Fe clay flocs also inhibited the decomposition of ryegrass residue. Considering cumulative C mineralization and extractable DOC remaining in the soil mixture, the flocs made with montmorillonite and/or high Fe content offered better protection of adsorbed C, SOC and recent plant C

inputs against microbial decomposition compared to flocs made with kaolinite and/or low Fe. Overall, clay mineralogy and Fe levels in flocs had a significant effect on C mineralization of clay-Fe-C products and soil C stability and provides insights into the role of Fe in affecting PE.

Declarations

Acknowledgments

Data availability

The datasets are available from the corresponding author on reasonable request.

References

Balcke GU, Kulikova NA, Hesse S, Kopinke FD, Perminova IV, Frimmel FH (2002) Adsorption of humic substances onto kaolin clay related to their structural features. Soil Sci Soc Am J 66:1805–1812. doi:10.2136/sssaj2002.1805

Bhattacharyya KG, Sen Gupta S (2008) Kaolinite and montmorillonite as adsorbents for Fe(III), Co(II) and Ni(II) in aqueous medium. Appl Clay Sci 41:1–9. doi:10.1016/j.clay.2007.09.005

Bird JA, van Kessel C, Horwath WR (2003) Stabilization of C-13-carbon and immobilization of N-15-nitrogen from rice straw in humic fractions. Soil Sci Soc Am J 67:806–816. doi:10.2136/sssaj2003.0806

Carroll D (1958) Role of clay minerals in the transportation of iron Geochimica. Et Cosmochimica Acta 14:1–1&. doi:10.1016/0016-7037(58)90090-5

Chenu C, Plante AF (2006) Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the 'primary organo-mineral complex' European. J Soil Sci 57:596–607. doi:10.1111/j.1365-2389.2006.00834.x

Eusterhues K, Rumpel C, Kogel-Knabner I (2005) Organo-mineral associations in sandy acid forest soils: importance of specific surface area, iron oxides and micropores European. J Soil Sci 56:753–763. doi:10.1111/j.1365-2389.2005.00710.x

Eusterhues K et al (2008) Characterization of ferrihydrite-soil organic matter coprecipitates by X-ray diffraction and mossbauer spectroscopy. Environmental Science Technology 42:7891–7897. doi:10.1021/es800881w

Feng XJ, Simpson AJ, Simpson MJ (2005) Chemical and mineralogical controls on humic acid sorption to clay mineral surfaces. Org Geochem 36:1553–1566. doi:10.1016/j.orggeochem.2005.06.008

Greenland DJ (1971) Interactions between humic and fulvic acids and clays. Soil Sci 111:34–34+. doi:10.1097/00010694-197101000-00004

Hagedorn F, Saurer M, Blaser P (2004) A C-13 tracer study to identify the origin of dissolved organic carbon in forested mineral soils European. J Soil Sci 55:91–100. doi:10.1046/j.1365-2389.2003.00578.x

Han L, Sun K, Jin J, Xing B (2016) Some concepts of soil organic carbon characteristics and mineral interaction from a review of literature. Soil Biol Biochem 94:107–121. doi:10.1016/j.soilbio.2015.11.023

Henneberry YK, Kraus TEC, Nico PS, Horwath WR (2012) Structural stability of coprecipitated natural organic matter and ferric iron under reducing conditions. Org Geochem 48:81–89. doi:10.1016/j.orggeochem.2012.04.005

Jones DL, Edwards AC (1998) Influence of sorption on the biological utilization of two simple carbon substrates. Soil Biol Biochem 30:1895–1902. doi:10.1016/s0038-0717(98)00060-1

Kahle M, Kleber M, Jahn R (2002) Predicting carbon content in illitic clay fractions from surface area, cation exchange capacity and dithionite-extractable iron European. J Soil Sci 53:639–644. doi:10.1046/j.1365-2389.2002.00487.x

Kahle M, Kleber M, Jahn R (2004) Retention of dissolved organic matter by phyllosilicate and soil clay fractions in relation to mineral properties. Org Geochem 35:269–276. doi:10.1016/j.orggeochem.2003.11.008

Kaiser K, Guggenberger G (2007) Sorptive stabilization of organic matter by microporous goethite: sorption into small pores vs. surface complexation European Journal of Soil Science 58:45–59. doi:10.1111/j.1365-2389.2006.00799.x

Kalbitz K, Schwesig D, Rethemeyer J, Matzner E (2005) Stabilization of dissolved organic matter by sorption to the mineral soil Soil. Biology Biochemistry 37:1319–1331. doi:10.1016/j.soilbio.2004.11.028

Larsen KR (2009) Corrosion-resistant coatings incorporate metal oxide nanoparticles. Mater Performance 48:23–23

Mikutta R, Mikutta C (2006) Stabilization of organic matter at micropores (< 2 nm) in acid forest subsoils. Soil Sci Soc Am J 70:2049–2056. doi:10.2136/sssaj2005.0366N

Mikutta R, Mikutta C, Kalbitz K, Scheel T, Kaiser K, Jahn R (2007) Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. Geochimica Et Cosmochimica Acta 71:2569–2590. doi:10.1016/j.gca.2007.03.002

Pisani O, Scinto LJ, Munyon JW, Jaffe R (2015) The respiration of flocculent detrital organic matter (floc) is driven by phosphorus limitation and substrate quality in a. subtropical wetland Geoderma 241:272–278. doi:10.1016/j.geoderma.2014.11.023

Saidy AR, Smernik RJ, Baldock JA, Kaiser K, Sanderman J (2013) The sorption of organic carbon onto differing clay minerals in the presence and absence of hydrous. iron oxide Geoderma 209:15–21. doi:10.1016/j.geoderma.2013.05.026

Saidy AR, Smernik RJ, Baldock JA, Kaiser K, Sanderman J (2015) Microbial degradation of organic carbon sorbed to phyllosilicate clays with and without hydrous iron oxide coating European. J Soil Sci 66:83–94. doi:10.1111/ejss.12180

Saidy AR, Smernik RJ, Baldock JA, Kaiser K, Sanderman J, Macdonald LM (2012) Effects of clay mineralogy and hydrous iron oxides on labile organic. carbon stabilisation Geoderma 173:104–110. doi:10.1016/j.geoderma.2011.12.030

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155–176. doi:10.1023/a:1016125726789

Sodano M, Said-Pullicino D, Fiori AF, Catoni M, Martin M, Celi L (2016) Sorption of paddy soil-derived dissolved organic matter on hydrous iron oxide-vermiculite. mineral phases Geoderma 261:169–177. doi:10.1016/j.geoderma.2015.07.014

Stevenson FJ (1994) Humus Chemistry: Genesis, Composition, and Reactions, 2nd edn. Wiley, New York

Toosi ER, Doane TA, Horwath WR (2012) Abiotic solubilization of soil organic matter, a less-seen aspect of dissolved organic matter production Soil. Biology Biochemistry 50:12–21. doi:10.1016/j.soilbio.2012.02.033

von Luetzow M, Koegel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review European. J Soil Sci 57:426–445. doi:10.1111/j.1365-2389.2006.00809.x

Wang WJ, Dalal RC, Moody PW, Smith CJ (2003) Relationships of soil respiration to microbial biomass. substrate availability clay content Soil Biology Biochemistry 35:273–284. doi:10.1016/s0038-0717(02)00274-2

Ye R, Doane TA, Morris J, Horwath WR (2015) The effect of rice straw on the priming of soil organic matter and methane production in peat soils. Soil Biol Biochem 81:98–107. doi:10.1016/j.soilbio.2014.11.007

Zhang L, Luo L, Zhang S (2012) Integrated investigations on the adsorption mechanisms of fulvic and humic acids on three clay minerals Colloids and Surfaces a-Physicochemical. and Engineering Aspects 406:84–90. doi:10.1016/j.colsurfa.2012.05.003

Zou Y, Zhang S, Huo L, Sun G, Lu X, Jiang M, Yu X (2018) Wetland saturation with introduced Fe(III) reduces total carbon emissions and promotes the sequestration of. DOC Geoderma 325:141–151. doi:10.1016/j.geoderma.2018.03.031

Figures

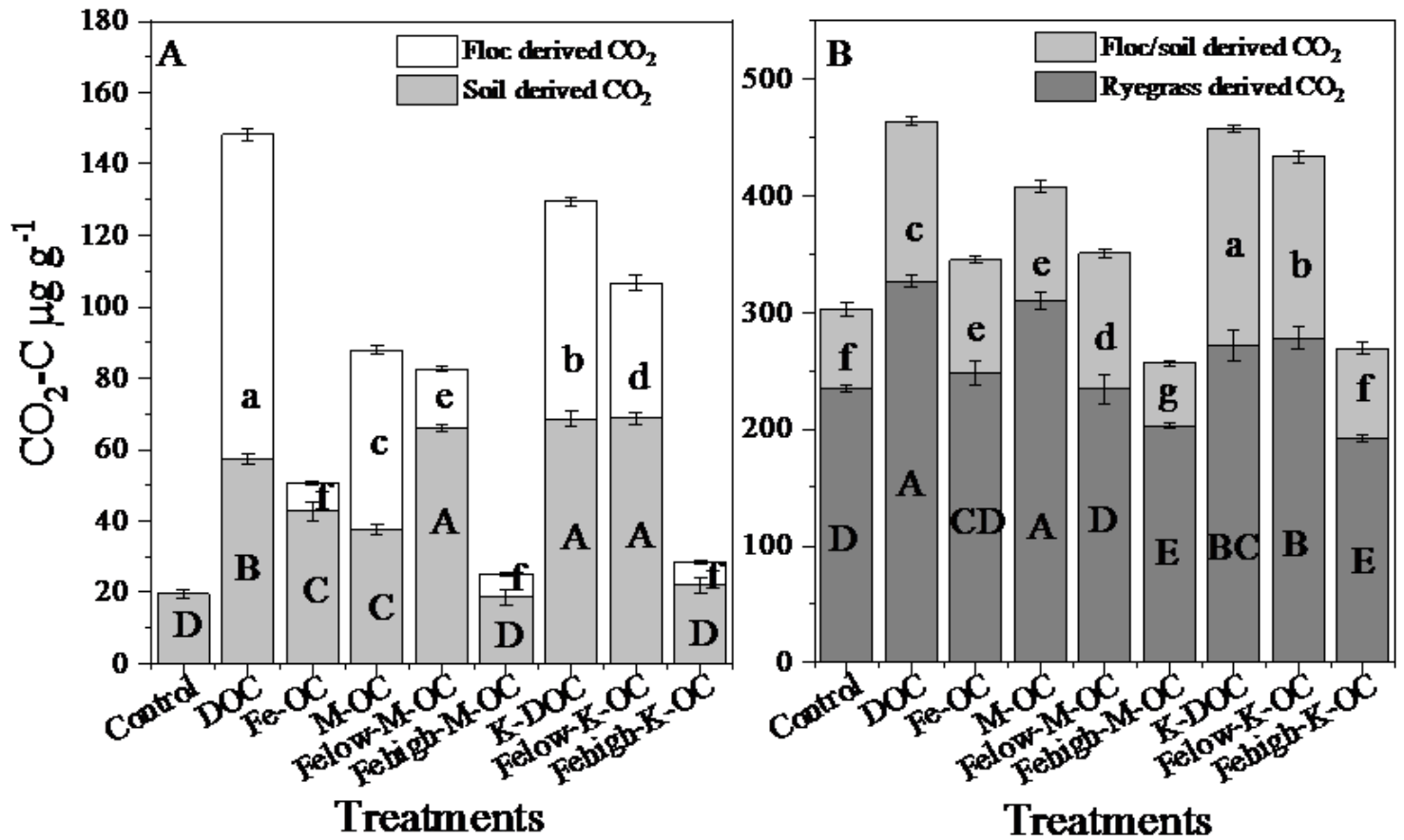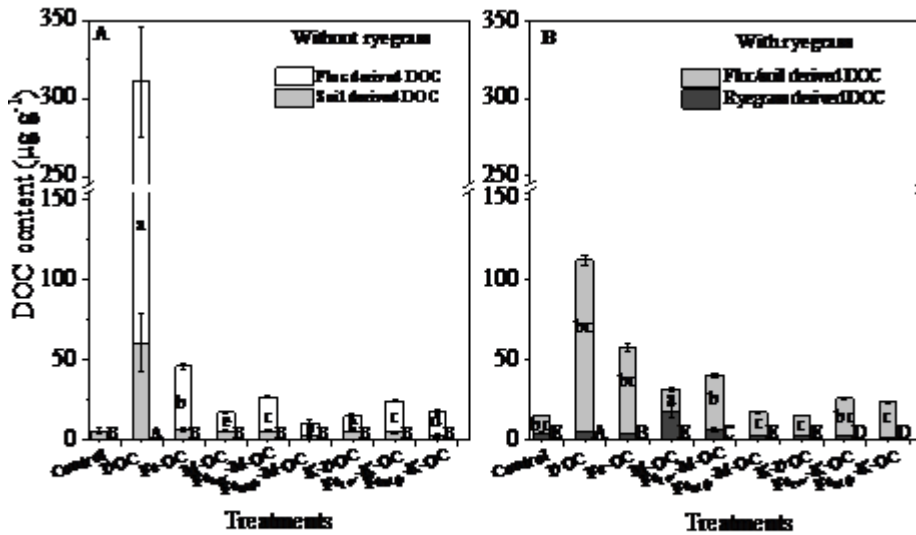

Figure 1

Quantification of the cumulative CO2 production from floc and native soil C pools in the treatments without ryegrass (A) and from ryegrass and soil/floc C pools in the treatments with ryegrass (B). The uppercase letters show significant differences in CO2 derived from soil (A) or ryegrass (B) at P < 0.05 among different floc treatments. The lowercase letters show significant differences in CO2 derived from floc (A) or soil/floc mixture (B) at P < 0.05 among different floc treatments. Error bars represent standard error of the means (n=4).

Figure 2

Extracted DOC at the end of the incubation without (A) or with ryegrass (B). The uppercase letters show significant difference in DOC derived from soil or ryegrass at P < 0.05 among different floc treatments without or with ryegrass. The lowercase letters show significant difference in DOC derived from floc (A) or soil/floc mixture (B) at P < 0.05 among different floc treatments. Error bars represent standard error of the means (n=4).

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