Upland soils in the midwestern United States are often ponded during the spring and remain flooded for days or even weeks. Aquolls and Aqualfs are the suborders most impacted by prolonged saturation and are very common in the Midwest (Franzmeier et al., 1983). Short-term ponding conditions may affect the aggregate state of upland soils through changes in chemical conditions, such as the redox state of the soil. Changes in the redox state of the soil occur under low O₂ levels and are important in waterlogged soils. When soils are flooded, aerobic microbial respiration quickly consumes the stock of O₂ in the saturated soil and a shift from aerobic to anaerobic respiration occurs (Rowell, 1981). A sequential thermodynamic order of reduction (gain of electrons) and oxidation (loss of electrons) reactions, called redox, takes place as a result of O₂ depletion from the soil (Kirk, 2004). The sequential thermodynamic order predicts that after O₂ depletion, NO₃ is reduced first and the reduction sequence usually then proceeds in the following order: Mn, Fe, S, and organic substrates (Ponnamperuma, 1972; Patrick and Jugsujinda, 1992). Reducing conditions may lead to changes in the cation exchange capacity (CEC), pH, organic matter sorption–desorption mechanisms, distribution of cations and anions in the soil and soil solution, and mineralogy and structure of the soils (Ponnamperuma, 1972; Shen et al., 1992; Fiedler and Kalbitz, 2003; Kirk et al., 2003; Favre et al., 2004; Thompson et al., 2006). The redox and associated chemical and physicochemical changes may influence soil structure and aggregation.

Several studies have reported that the CEC of long-term flooded soils was increased under strongly reducing conditions (Favre et al., 2002, 2004; Kirk et al., 2003) and that may lead to dispersion, hence decreasing soil aggregation. Reducing conditions usually bring the pH to near neutrality in flooded soils (Ponnamperuma, 1972). An increase in pH is expected in acid soils after flooding and that may lead to changes in the physicochemical state of the soil. For instance, clay dispersion, and thus soil disaggregation, is generally enhanced with increasing pH (Suarez et al., 1984). Organic substrates can be desorbed from Fe oxides by reduction–dissolution reactions when the soil undergoes more reduction (Fiedler and Kalbitz, 2003) and that may contribute to the soil disaggregation. Alkaline metals concentrations, such as Ca and Mg, tend to increase in the soil.
solution under reducing conditions (Ponnamperuma, 1972; Phillips and Greenway, 1998). A loss of Ca and Mg from the exchange sites of clays may decrease clay flocculation, therefore increasing the instability of aggregates (Heil and Sposito, 1993). Conversely, increasing concentrations of K on exchange sites of clays of wet soils have been attributed to clay dispersion and thus soil disaggregation (Auerswald et al., 1996). Kirk et al. (2003) reported that the tortuosity of pore interspaces, and thus porosity, decreases with increased CEC under reducing conditions. Decreasing soil macroporosity due to tillage, solution concentration, soil composition, or sodicity leads to soil disaggregation (Levy and Mamedov, 2002; Norton et al., 2006).

Thus, transformations in the soil and soil solution caused by reducing conditions may modify the soil aggregation state through dissolution of inorganic stabilizing minerals, changes in pH, changes in the strength of bonds between organic compounds and other soil particles, variations in the concentrations of cations on the soil exchange sites, redistribution of soil particles and charges, and changes in soil porosity. Nevertheless, we have not found a study that specifically addresses the effects of reducing conditions on soil aggregation during short-term ponding conditions.

The objective of this study was to determine how changes in the redox status of the soil under a set of controlled temperature and light conditions can impact soil aggregation during short-term flooding conditions. The hypothesis tested is that the aggregation of upland soils decreases under short-term reducing conditions. To carry out this research, batch experiments were performed in an anaerobic biogeochemical reactor using six upland soils from two management systems with different organic matter contents. The high-energy moisture characteristic (HEMC) procedure (Pierson and Mulla, 1989) was used to determine changes in soil aggregation after short periods of anaerobic incubation.

MATERIALS AND METHODS

Soil Collection and Characterization

Surface horizons (0–10-cm depth) of six upland soils subjected to annually intermittent ponding conditions were collected from the Purdue University Research Farm in early spring shortly before the experiments were conducted (Table 1). Three cultivated soils (soil series Peotone, Soil A; Drummer, Soil B; and Miami, Soil C) and three uncultivated soils (soil series Drummer, Soil D; Whitaker, Soil E; and Miami, Soil F) with different organic matter contents were sampled. The soils had a mixed clay mineralogy, predominantly illitic or smectitic. The cultivated soils have been under a corn (Zea mays L.)–soybean [Glycine max (L.) Merr.] rotation and the uncultivated soils under forest for at least 20 yr. The moist soils were thoroughly mixed and immediately stored at 4°C until further processing. After no more than 2 mo, the soils were air dried and sieved. The average portion of soil aggregates of 1- to 2-mm diameter in the whole soil was ~60% after sieving. These aggregate sizes were chosen as representative of the whole soil and used in this study. Furthermore, this aggregate size range was selected because the HEMC procedure described below is properly performed using aggregate sizes between 0.5 and 1.0 mm, which can be obtained after resieving the treated aggregates of 1- to 2-mm diameter.

Soils properties of pH in water (1:1) and CEC were determined according to Thomas (1996) and Sumner and Miller (1996) (Table 1).
Incubation Procedure

The anaerobic incubation was conducted in a biogeochemical reactor (De-Campos, 2006) designed to hold Eh and pH meters (Fig. 1). Duplicate samples of air-dried aggregates (1–2 mm) from each soil (total of 60 samples) with 80 g of soil plus 120 mL of degassed water were incubated in 250-mL centrifuge bottles for 1 h (0 d, control) and 1, 3, 7, and 14 d under anoxic conditions. The replicates were randomized to allow statistical comparisons. The experiment was performed in the dark at 25°C. The centrifuge bottles were purged continuously with Ar gas at a flow rate of 10 cm$^3$ min$^{-1}$ during the anoxic incubation. The Eh and pH measurements were taken daily until the end of the incubation treatment. The Eh and pH measurements were performed directly in the soil solution using a digital millivolt meter (Accumet Model 15, Fisher Scientific, Pittsburgh, PA). A Pt redox combination oxidation-reduction potential electrode (YSI-PH100ORP) and a pH combined glass electrode with an inner AgCl reference (YSI-PH1001M), were used for the measurements. The Eh was recorded after stabilization of the millivolt meter and was converted to standard H electrode potential (Eh) by adding 199 mV to each value (Patrick et al., 1996). The Eh values were corrected to pH 7 according to equations modified from Rowell (1981). After each incubation treatment, the centrifuge bottles were moved to a glove bag and opened under Ar gas atmosphere for electrical conductivity (EC) measurements.

Aggregate Stability Experiments

The supernatants were poured off from the centrifuge bottle in the glove bag after the EC measurements, and the centrifuge bottles were kept open. This simulated a situation in the field where the soil solution is washed out by a rainfall event, surface runoff, or subsurface leaching. The centrifuge bottles with moist soils were oven dried at 60°C. The soil moisture content after oven drying was considered similar to air-dry conditions. The oven-drying procedure was necessary because in previous trials it was verified that a long period of time (>20 d) was needed to dry the samples at 25°C. That long period of drying could cause changes in the soil and solution chemistry related to prolonged saturation and the changes were not controlled during the incubation. After oven drying, the incubated samples were resieved to select 0.5- to 1.0-mm aggregate sizes for HEMC aggregate stability determination. The HEMC procedure is properly performed using specific aggregate diameters, which allows comparisons between treatments. After previous trials, the aggregate class of 0.5- to 1.0-mm diameters was chosen as suitable to perform the HEMC procedure. Similar aggregate sizes have been selected and used by other researchers for a range of soils with high variability in their stability (e.g., Collis-George and Figueroa, 1984; Pierson and Mulla, 1989; Levy and Miller, 1997; Norton et al., 2006). Duplicate samples of the original soils without previous incubation treatment were also tested for aggregate stability.

High-Energy Moisture Characteristic Theory

The HEMC procedure is used for determining soil aggregate stability through changes in the volume of drainable pores under different suctionss. It was first proposed by Childs (1940), later modified by Collis-George and Figueroa (1984), further evaluated by Pierson and Mulla (1989), and finally modified by Levy and Mamedov (2002). The HEMC procedure has been reported as a sensitive procedure to detect changes in aggregate stability for a range of soils from arid and humid zones (Pierson and Mulla, 1989; Levy and Miller, 1997; Crescimanno and Provenzano, 1999; Levy and Mamedov, 2002; Levy et al., 2003; Norton et al., 2006) and thus was appropriate for this study. The procedure used in this study was from the latest version (Levy and Mamedov, 2002).

The procedure is based on the following main steps. Soil aggregates are wetted either slowly (2 mm h$^{-1}$) or rapidly (100 mm h$^{-1}$) in a controlled manner, and a moisture content curve at high energies (i.e., energies up to 500 mm H$_2$O tension) is constructed (Fig. 2a). An index of aggregate stability is obtained by quantifying differences in the moisture characteristic curves for fast and slow wetting. For a given wetting rate, a structural index is defined as the ratio of the volume of drainable pores to modal suction (Collis-George and Figueroa, 1984). Modal suction corresponds to the matric potential (ψ, J kg$^{-1}$).
at the peak of the specific water capacity curve \( (d\psi/d\theta) \), where \( \theta \) is the water content (kg kg\(^{-1}\)) (Fig. 2b). The volume of drainable pores is defined as the area beneath the specific water capacity curve and above the dotted baseline (Fig. 2b). The dotted baseline represents the volume of water lost because of aggregate shrinkage rather than pore emptiness (Collins-George and Figueroa, 1984). The fast/slow ratio of the structural indexes, termed the stability ratio (SR), is used as an index to estimate the stability of aggregates on a relative scale from zero to one. Thus as the SR increases, the aggregate stability increases.

**High-Energy Moisture Characteristic Procedure**

Fifteen grams of the 0.5- to 1-mm oven-dried soil aggregates were placed in a 60-mm i.d. funnel with a fritted disk to form a 5-mm-thick bed. The fritted disk had a maximum pore size of 20 to 40 \( \mu \)m. Saturation of the fritted disk was ensured prior to placing the aggregates in the funnel. The funnel was connected from its bottom via tubing to a peristaltic pump, which was then used to wet (full volume of water lost because of aggregate shrinkage rather than pore emptiness) the aggregates in the funnel either faster (0-, 1-, 3-, 7-, and 14-d incubation treatments) or slowly (0-d incubation). Distilled water was used for wetting the aggregates in the funnel.

A moisture content curve at a matric potential in the range between 0 and −5.0 J kg\(^{-1}\) was obtained using a hanging water column coupled with a graduated glass pipette for either wetting rate. The height of the water meniscus in the pipette was decreased in increments of 0.1 to 0.2 J kg\(^{-1}\) to increase the suction with time. At each matric potential, and after a 2-min equilibration period, the volume of water drained from the aggregates was recorded. The corresponding water content of the aggregates was calculated for each suction increment. Previous trials showed that under these experimental conditions, no additional water drainage was observed after the 2-min equilibration time.

**Aggregate Stability Data Analysis**

To accurately calculate the volume of drainable pores and modal suction, modeling of the moisture characteristic curves was performed with the following seven-parameter modified van Genuchten model (Pierson and Mulla, 1989):

\[
\theta = \theta_s + \left( \theta_s - \theta_f \right) \left[ 1 + (\alpha \psi)^n \right]^{-1/n} + A\psi^2 + B\psi + C \quad [1]
\]

where \( \theta_s \), and \( \theta_f \) are pseudo saturated and residual gravimetric water contents, respectively; \( \alpha \) and \( n \) control the location and steepness, respectively, of the S-shape inflection of the moisture characteristic curve; and \( A \), \( B \), and \( C \) are the quadratic terms added by Pierson and Mulla, (1989) to improve fitting of the model to the moisture characteristic curve. A specific water capacity curve \( (d\theta/d\psi) \), needed for obtaining the value of modal suction, was computed by differentiating Eq. [1] with respect to matric potential, with the explicit form

\[
(d\theta/d\psi)(\theta, \psi) = (\theta_s - \theta_f) \left[ 1 + (\alpha \psi)^n \right]^{-1/n} \times \left[ \frac{1}{n} (\psi)^{n-1} \left( 1 + (\alpha \psi)^n \right)^{-1/n} \right] \psi^2 + 2A\psi + B \quad [2]
\]

The volume of drainable pores, i.e., the area under the specific water capacity curve and above the soil shrinkage line (Fig. 2b), was calculated by subtracting the terms for pore shrinkage \( (2A\psi + B) \) from Eq. [2] and analytically integrating the remainder of that equation. For each soil, the slow wetting values of the HEMC obtained from the 0-d incubation treatment were used for all treatments to allow comparisons between treatments.

**Chemical Analysis**

In a parallel experiment, using the same incubation procedure, soil solution samples of the supernatant were collected in the glove bag after the anaerobic incubation and analyzed for chemical parameters. The sampling and chemical analytical procedures were as follows. An unfiltered subsample (1 mL) of the solution above the soil was collected for Fe\(^{2+}\) measurement, with 1 mL of solution added to 3 mL of ferrozine plus 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, according to Kirk et al. (2003). The supernatants were poured off in O\(_2\)-free 30-mL syringes and filtered through 0.45-\( \mu \)m filters purged with Ar gas. A filtered acidified solution subsample was collected from each bottle for metal analysis. The acidification was performed by adding two drops of 50% (v/v) HNO\(_3\)-H\(_2\)O. Another filtered, unacidified solution subsample was collected for DOC and dissolved organic molecules molecular weight analysis. Soluble total Mn, Ca, Mg, and K were analyzed in the acidified subsamples by ICP–OES (PerkinElmer Model 2000 DV, Norwalk, CT). Dissolved organic C was analyzed using a total organic C analyzer (Shimadzu TOC-Vws, Shimadzu Corp., Kyoto, Japan). The molecular weight of dissolved organic compounds was calculated from spectrophotometric data (spectrophotometric absorbance measured at 280 nm using a Thermo Model GENESYS 10 UV spectrophotometer, Thermo Scientific, Waltham, MA) according to Chin et al. (1994).

**Scanning Electron Microscope**

Microscopic morphological analysis was performed for an uncultivated soil for the control and 14-d incubation treatments to show changes in aggregate morphology. Images were taken using a JEOL JSM-840 scanning electron microscope (JEOL Ltd., Tokyo).

**Statistical Analysis**

Analysis of variance was used to determine significant effects of soil (six soils), management system (cultivated vs. uncultivated), and time of incubation (0, 1, 3, 7, and 14 d) on the aggregate stability (SAS Institute, 1999). Significant effects were identified at the 0.05 probability level and the mean comparisons were based on Fisher’s protected LSD.

**RESULTS**

**Changes in Redox Potential, pH, and Electrical Conductivity**

The Eh decreased more rapidly in the uncultivated soils than in the cultivated soils (Fig. 3a). On average, the Eh in the uncultivated soils decreased sharply after 1 d of anaerobic incubation, from 397 to −74 mV, then it decreased slowly to −119 mV during the rest of the incubation period. A slower change was observed for the cultivated soils, where the average Eh gradually dropped from 507 to 76 mV during the 14-d incubation period (Fig. 3a).

A small increase in pH was observed for both soil groups during the initial 6 d of incubation (Fig. 3b). After 6 d, the average pH tended to remain near neutrality. Except for Soil B, the pH of the uncultivated soils was slightly higher than that of the cultivated soils for more prolonged incubation periods (Fig. 3b).

Overall, the EC increased for both cultivated and uncultivated soils (Fig. 4). The greatest increase was observed for the
uncultivated soils. The average EC in the uncultivated soils reached 17.07 μS m⁻¹ after 14 d of incubation, whereas the average was 9.11 μS m⁻¹ for the cultivated soils for the same time period.

Changes in the Solution Concentration of Chemical Species

The concentrations of Fe²⁺ and total soluble Mn increased through time and the greatest changes were observed for the uncultivated soils after 3 d of incubation (Fig. 5a and 5b). The concentrations of Mn in the uncultivated soils were always greater than Fe²⁺ for the same incubation period (Fig. 5a and 5b). The solution concentrations of Ca and Mg increased through time (Fig. 6a). This trend was also confirmed by the increase in the EC through the incubation period (Fig. 4). Calcium and Mg consistently increased in solution for all soils except Soil A. The K concentration increased up to 7 d of incubation, then decreased afterward for Soils A, B, D, E, and F (Fig. 6b). The DOC concentrations were always higher for the uncultivated soils and showed a significant increasing trend with incubation time, whereas the DOC concentrations of the cultivated soils remained low and relatively unchanged (Fig. 7). On average, the molecular weight of dissolved organic molecules was higher for the cultivated soils (Fig. 8).

Stability Ratio and Aggregate Stability

Data on the SR for the original soils and control (0 d) and the 1-, 3-, 7-, and 14-d incubation treatments are shown in Fig. 9 and Table 2. In the original cultivated soils, the SR decreased with decreasing organic C content of the soils, i.e., Soil A showed the highest SR and had the highest organic C content and it was followed in order by Soils B and C (Fig. 9). In the original uncultivated soils, however, this trend was not observed. The original Soil D with the highest SR also had the highest organic C content. Soil F had a lower organic C but a higher SR than that of Soil E (Fig. 9).

Overall, for the treated soils the SR decreased with time for all soils in comparison with the controls (Table 2). The cultivated Soil A showed a significant change in the SR only between the control and 1-d incubation treatments. Conversely, the cultivated Soils B and C showed the greatest changes in the
SR with time among all soils. The relative SR, which was calculated by assuming a maximum value of 1 for the control and dividing the SR of the other incubation treatments by the SR of the control, showed 18 and 21% decreases in aggregate stability for Soils B and C after 14 d of incubation (Table 2).

Soils D and F showed the greatest changes in the SR among the uncultivated soils (Table 2). The SR for Soil D decreased during the first 7 d of incubation and it remained stable until the end of the incubation period, while the SR for Soil F had the greatest drop after 7 d of incubation. A slight increase in the SR for Soil F was observed between Days 3 and 7 of incubation. The relative decreases in aggregate stability (Table 2) for Soils D and F after the 14-d incubation treatment were 12 and 13%, respectively. Changes in the SR for Soil E were less pronounced and showed only 6% reduction after the 14-d incubation treatment.

DISCUSSION

The most remarkable finding of this study is that soil aggregate stability decreased with time under short-term flooding and reducing conditions.

In the treated soils, the first significant decrease in aggregate stability was observed between the control and 1-d saturation treatments (Table 2). Overall, the observed decrease in the SR between these two treatments was more pronounced in the cultivated soils and in the uncultivated Soil D and was related to changes in the physical properties of the soil after flooding. It is probable that the fast water saturation of the soil with mixed clay mineralogy at the beginning of the incubation period allowed slaking and swelling mechanisms to occur and that caused a breakdown of aggregates. Previous studies reported that slaking and swelling are important mechanisms of aggregate breakdown when the soil is rapidly wetted (Reichert and Norton, 1994; LeBissonnais, 1996; Levy and Mamedov, 2002). The mechanism of slaking tends to occur in the first stage of saturation when the soil is wetted and the
Table 2. Stability ratio (SR) and relative stability ratio (RSR) as a function of time of anaerobic incubation for the six soils: cultivated (A) Peotone, (B) Drummer, and (C) Miami; and uncultivated (D) Drummer, (E) Whitaker, and (F) Miami (±1 SE in parentheses).

<table>
<thead>
<tr>
<th>Incubation</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>SR</td>
<td>RSR</td>
<td>SR</td>
<td>RSR</td>
<td>SR</td>
<td>RSR</td>
</tr>
<tr>
<td>d</td>
<td>SR</td>
<td>RSR</td>
<td>SR</td>
<td>RSR</td>
<td>SR</td>
<td>RSR</td>
</tr>
<tr>
<td>0</td>
<td>0.771 (0.007)</td>
<td>1.00</td>
<td>0.713 (0.007)</td>
<td>1.00</td>
<td>0.675 (0.007)</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>0.715 (0.003)</td>
<td>0.93</td>
<td>0.657 (0.006)</td>
<td>0.92</td>
<td>0.610 (0.005)</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>0.716 (0.007)</td>
<td>0.93</td>
<td>0.622 (0.006)</td>
<td>0.87</td>
<td>0.574 (0.003)</td>
<td>0.85</td>
</tr>
<tr>
<td>7</td>
<td>0.712 (0.009)</td>
<td>0.92</td>
<td>0.590 (0.009)</td>
<td>0.83</td>
<td>0.557 (0.003)</td>
<td>0.82</td>
</tr>
<tr>
<td>14</td>
<td>0.703 (0.008)</td>
<td>0.91</td>
<td>0.582 (0.009)</td>
<td>0.82</td>
<td>0.534 (0.003)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Table 3. Pearson’s correlation coefficients between the stability ratio and redox potential (Eh) and pH within soil type. The soils are cultivated (A) Peotone, (B) Drummer, and (C) Miami; and uncultivated (D) Drummer, (E) Whitaker, and (F) Miami (P values in parentheses).

<table>
<thead>
<tr>
<th>Soil management</th>
<th>Soil</th>
<th>Eh</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated</td>
<td>A</td>
<td>0.85 (0.0021)</td>
<td>−0.56 (0.0745)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.92 (0.0001)</td>
<td>−0.72 (0.089)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.85 (0.0019)</td>
<td>−0.98 (&lt;0.0001)</td>
</tr>
<tr>
<td>Uncultivated</td>
<td>D</td>
<td>0.60 (0.0639)</td>
<td>−0.33 (0.2998)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.72 (0.0186)</td>
<td>−0.54 (0.0697)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.64 (0.0480)</td>
<td>−0.51 (0.0878)</td>
</tr>
</tbody>
</table>

Soil Aggregation and pH in a Reduced Environment

Overall, the changes in pH had less influence on the observed decrease in aggregate stability than Eh (Fig. 3, Table 3). The observed changes in pH had a negative effect on the stability of aggregates in Soils B and C (Table 3) and that can be attributed to increased dispersion as pH increased. Suarez et al. (1984) reported that at pH values above the point of zero charge (PZC) of soil minerals, the bonding between Fe and Al (hydr)oxides to clay particles decreases and soil dispersion is probable. It is possible that the changes in pH reached the PZC of minerals for Soils B and C and that led to soil dispersion.

Soil Aggregation as Affected by Iron and Manganese Reduction

The reduction of Fe and Mn (hydr)oxides strongly influenced the observed decrease in soil aggregation for some soils (Table 5). The increase in Fe²⁺ concentration in solution was well correlated with the decrease in the stability of the aggregates (SR) for Soils D and F (Table 5). The SR decreased in Soil D between 1 and 14 d of incubation and that was the period when Fe reduction increased in this soil (Fig. 5). In Soil F, the influence of Fe reduction was even greater than in Soil D. A sharp increase in the concentration of Fe²⁺ and a sharp decrease in the stability of aggregates were observed between 7 and 14 d of incubation in Soil F (Fig. 5, Table 2); this indicates a strong effect of Fe (hydr)oxide reduction on the aggregate stability of this soil. Iron (hydr)oxides have been reported to be important aggregators (Suarez et al., 1984; Barberis et al., 1991; Rhoton et al., 2003). Among Fe minerals, poorly ordered Fe oxides and ferrhydrite have been described as strong aggregators.
soil aggregators (Bartoli et al., 1988); their chemical removal leads to increases in the smaller aggregate size fractions (Arduino et al., 1989; Barberis et al., 1991; Duiker et al., 2003) and to changes in the morphology of soil particles (Favre et al., 2004). The Fe mineral dissolution may also lead to colloid dispersion under anoxic conditions (Tadanier et al., 2005; Thompson et al., 2006). We believe that the chemical changes related to Fe reduction contributed to the observed decrease in the aggregate stability of Soils D and F.

The increase in total soluble Mn showed a strong correlation with the decrease in the aggregate stability (SR) for all soils except Soil A (Table 5). The aggregate stability in Soils B, C, D, E, and F significantly decreased between 1 and 14 d when the reduction of Mn occurred (Table 2, Fig. 5). It is probable that Mn reduction had a negative effect on the aggregate stability of these soils (directly or indirectly) and that may be even more important than Fe. This is still an open question, however, and further studies are needed to fully understand the role of Fe and Mn reduction on soil aggregate stability for upland soils subjected to short-term ponding conditions.

**Effect of Cation Exchange on Soil Aggregation in a Reduced Environment**

Exchanges of aggregate-stabilizing cations, such as Ca, Mg, and K, between the soil and the soil solution under reducing conditions may also have contributed to the observed decrease in aggregate stability. Strong linear correlations were found between Ca and Mg solution concentrations and aggregate stability (SR) for Soils B, C, E, and F (Table 5). The SR decreased in these soils as the Ca + Mg concentration increased in solution (Table 2, Fig. 6). It has been postulated that an increase in the concentration of divalent alkaline metals in solution under reducing conditions is favored by solubilization of organic matter and Fe and Mn (hydr)oxides that bind these metals (Phillips and Greenway, 1998). It is probable that cation solubilization occurred during the incubation period and led to increased Ca and Mg concentrations in solution. An increasing Ca concentration in solution as a result of the loss of Ca from the soil may increase soil dispersion (Heil and Sposito, 1993; Amezcketa, 1999), thus leading the soils to disaggregate. Changes in K concentration were linearly correlated with aggregate stability (SR) only for Soil C (Table 5). Except for Soil C, soluble K did not have any significant impact on soil aggregation. The effect of K on soil aggregation, however, has to be investigated for K on the exchange sites of soils instead of its concentration in solution. This is because the presence of K on the exchange sites of clays can increase soil dispersion in wet soils (Auerswald et al., 1996). The tendency for the soluble K concentration to decrease after 7 d of incubation, i.e., under strong reducing conditions, indicated that this cation returned to the exchange sites of these soils (Fig. 6). It is possible that an increase in the concentration of K on the exchange sites of Soils A, B, D, E, and F contributed to increased soil disaggregation.

**Organic Carbon Solubilization and Soil Aggregation under Reducing Conditions**

The observed changes in DOC concentrations influenced soil aggregate stability. The DOC concentrations substantially increased in the uncultivated soils and were strongly corre-

<table>
<thead>
<tr>
<th>Soil management</th>
<th>Soil</th>
<th>Fe²⁺</th>
<th>Mn</th>
<th>Ca+Mg</th>
<th>K</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated</td>
<td>A</td>
<td>0.55</td>
<td>0.00</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.39</td>
<td>0.74</td>
<td>0.83</td>
<td>0.64</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.48</td>
<td>0.81</td>
<td>0.91</td>
<td>0.92</td>
<td>0.45</td>
</tr>
<tr>
<td>Uncultivated</td>
<td>D</td>
<td>0.75</td>
<td>0.70</td>
<td>0.65</td>
<td>0.01</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.09</td>
<td>0.98</td>
<td>0.96</td>
<td>0.13</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.80</td>
<td>0.68</td>
<td>0.85</td>
<td>0.03</td>
<td>0.67</td>
</tr>
</tbody>
</table>

The spectrophotometric data showed that the DOC molecules in the cultivated soils had higher molecular weights than in the uncultivated soils (Fig. 8). Thus, it is probable that the DOC compounds in the cultivated soils consisted of larger and heavier organic compounds, like humic acids, whereas in the uncultivated soils lighter organic molecules predominated in the DOC fraction, such as fulvic acids. High-molecular-weight molecules are preferentially adsorbed to mineral surfaces compared with low-molecular-weight molecules because of their higher hydrophobicity, lower solubility, and the presence of aromatic rings and carboxylic acids (Jardine et al., 1989; Gu et al., 1994; Kalbitz et al., 2000). Thus, more high-molecular-weight organic molecules generated by organic matter mineralization in the cultivated soils were adsorbed by the mineral phases of those soils. The opposite happened in the uncultivated soils, i.e., lower adsorption and a greater concentration of low-molecular-weight molecules in solution.

With the exception of Soil A, the other two cultivated soils (B and C) showed the greatest decrease in aggregate stability (SR) among all soils (Table 2). It is quite possible that the aggregate stability of Soils B and C were negatively affected by the retention of high-molecular-weight DOC molecules on clay surfaces. The findings of Heil and Sposito (1993) further support this hypothesis. These researchers postulated that the steric mechanism, i.e., steric repulsion resulting from the overlap of adsorbed organic substrates, is an important mechanism controlling colloidal and aggregate stability. The idea is that organic substrate coating of soil particles acts as a hard surface and limits the approach of two coated particles by reducing the effectiveness of attractive interparticle van der Waals forces (Heil and Sposito, 1993). Thus, the retention of soluble organic substrates on clay surfaces may have contributed to increased soil dispersion and aggregate breakdown in Soils B and C.

Conversely, the higher loss of low-molecular-weight, particle-binding organic C substrate as DOC molecules from the uncultivated soils may have contributed to the decrease in aggregate stability of these soils. It is known that organic matter is an important soil aggregator and its degradation may lead to increased soil disaggregation (Tisdall and Oades, 1982).
Therefore, the observed decrease in aggregate stability of the uncultivated soils can be partially explained by the degradation of soil organic C, which was shown through the increase in the DOC concentrations in solution (Fig. 7).

Images from the scanning electron microscope for Soil D (Fig. 10) showed that changes in soil aggregation occurred during anoxic incubation due to the mobilization of organic substrates. The microscope images reveal that the pronounced organic coating disappeared after the 14-d incubation treatment (Fig. 10). These findings indicate that changes in soil aggregators under reducing conditions, such as mobilization of organic substrates, occurred and led to an increase in the smaller aggregate size fractions, thus facilitating soil disaggregation; however, the effects of the nature and fate of DOC molecules on aggregate stability under reducing conditions is a complex question that needs further investigation.

CONCLUSIONS

This study demonstrated that the aggregate stability of upland soils subjected to short-term ponding effects decreases under reducing conditions. The decrease in aggregate stability under reducing states reached up to 21% during an incubation period of 14 d, which is quite significant in terms of soil disaggregation. It is important to stress that this study was conducted using natural soils with no chemicals added. Thus the findings reported here have strong connections with field situations when aerobic upland soils remain saturated for several days and the O2 level decreases to a minimum to permit reducing conditions. A companion study (De-Campos, 2006) simulating field conditions showed a decrease in soil aggregate stability after short-term saturation and under reducing conditions (data not shown), which further supports the previous statement. Once reducing reactions take place in the field, we believe the disaggregation processes should be irreversible during the time of flooding because the drainage will wash away the released chemicals and the soil will not return to the original state. Chemical release and soil disaggregation may have a detrimental effect on water quality under reducing conditions through (i) increasing chemical concentrations in surface and groundwater and (ii) reducing soil porosity by different aggregate breakdown mechanisms that can move dispersed and colloidal materials that then clog the pores (Kjaergaard et al., 2004; Norton et al., 2006). Reducing conditions only occur for short periods of time during the year in the upland soils studied here, however, whereas aggregation processes operate for several months, causing reaggregation of soil particles. Thus, the degree of irreversibility in soil disaggregation caused by reducing processes is still an open question that needs further investigation considering the time frame of flooding conditions and reaggregation processes that occur during the year.

Overall, the aggregate stability of the cultivated soils was more affected by reducing conditions than that of the uncultivated soils and this indicates that management plays an important role in the stability of aggregates. When cultivated soils are rich in organic matter, clay, and Fe, however, they tend to be more stable, as was the case of Soil A. Well-aggregated cultivated soils, such as Soil A, have a strong Eh poising capacity, thus more prolonged reducing conditions would be necessary to produce significant changes in the aggregate stability of this type of soil.

The initial concentration, nature, and mobilization of soil organic matter are important factors controlling the overall aggregate stability of the upland soils studied here. This is the case of the uncultivated Soil D, which had initially the highest organic matter concentration and the highest aggregate stability and showed a significant decrease in aggregate stability under reducing conditions. The chemical transformations produced under reducing conditions, however, may have had a great impact on the stability of aggregates during prolonged water saturation and that could be even more influential than the organic matter content in some soils. Further investigation using controlled experiments is needed to fully understand the role of organic matter and redox reactions on soil aggregation under short-term saturation and wetting–drying conditions.

REFERENCES
