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Soil Biology & Biochemistry 37 (2005) 339–347

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Carbon and nitrogen mineralization as affected by drying and wetting cycles

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Received 8 March 2004; received in revised form 19 July 2004; accepted 14 August 2004

Abstract

Drying and rewetting of soil is an important process in soil aggregation, soil organic matter (SOM) decomposition, and nutrient cycling. We investigated the source of the C and N flush that occurs upon rewetting of dry soil, and whether it is from microbial death and/or aggregate destruction. A moderately well drained Kennebec silt loam (Fine-silty, mixed, superactive, mesic Cumulic Hapludoll) was sampled to a 10 cm depth. Soil under constant water content (CWC) was compared with soil subjected to a series of four dry–wet (DW) cycles during the experimental period (96 d) and incubated at 25 °C. Mineralized C and N were measured during the drying and rewetting periods. Aggregate size distributions were studied by separating the soil into four aggregate size classes (>2000, 250–2000, 53–250, and 20–53 μm) by wet sieving. Repeated DW cycles significantly reduced cumulative N mineralization compared with CWC. The reduction in cumulative mineralized C resulting from DW compared with CWC increased as the DW treatments were subjected to additional cycles. The flush of mineralized C significantly decreased with repeated DW cycles. There was no significant effect on aggregate size distributions resulting from to the DW cycles compared with CWC treatment. Therefore, the flush of mineralized C and N seemed to be mostly microbial in origin in as much as aggregate distribution was unaffected by DW cycles.
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Keywords: Dry–Wet cycles; Carbon and nitrogen flush; Aggregate destruction and size distribution

1. Introduction

Dry–wet cycles can stimulate microbial activity and increase mineralization of soil organic matter (SOM) (West et al., 1992; Denef et al., 2001a,b). The increase in mineralization of SOM can be partly attributed to microbial death upon rewetting of dry soil (van Gestel et al., 1991, 1993; Cabrera, 1993; Magid et al., 1999), and partly to the increased exposure of organic residues (van Gestel et al., 1993; Appel, 1998; Denef et al., 2001a,b). Both microbial biomass and organic residue can be simultaneously involved in enhanced mineralization (van Gestel et al., 1993; Pulleman and Tietema, 1999).

Soil microorganisms are subjected to water stress in dry soil (Griffin, 1981; Harris, 1981). Passive equilibration of internal water potential can occur by cellular plasmolysis and by decreases in internal water potential. During dry conditions, microbial cells may die because of this passive equilibration. Accumulation of intercellular solutes (organic or inorganic) is an active cellular response to decreasing external matric potential. Different matric potentials between the soil environment and a microbial cell can result in cell turgor pressure (Harris, 1981). The most rapid changes in soil matric potential occur when dry soil is wetted. The desiccation process usually proceeds slowly, allowing time for microbial accumulation of intracellular solutes. The rewetting of dry soil, by precipitation or irrigation, occurs rapidly as a wetting front penetrates dry soil microsites (Kieft et al., 1987). Harris (1981) hypothesized that cell wall thickness was the major determinant of microbial ability to withstand these rapid changes in matric potential.

Microbial cells that do not survive desiccation are considered to be part of the SOM (Marumoto et al., 1977),

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0038-0717/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.soilbio.2004.08.003

whereas cells that have passively equilibrated to the dry conditions will rehydrate during rewetting (Kieft et al., 1987). Microbial cells that adjust to dry conditions must readjust their internal matric potential to the new environment upon rewetting. Increasing soil matric potential may cause one or more of the following: (i) an influx of water through cell membranes, resulting in increasing turgor and cell lysis due to excessive turgor pressure; (ii) disposal of intercellular solutes by rapid catabolism to CO_2; and (iii) transport of intercellular solutes out of cells. The immediate result is a release of the cell solutes, such as amino acids, ammonium compounds, and glycerol (Kieft et al., 1987). These readily degradable organic compounds could be utilized by surviving soil microorganisms, thus contributing to the pulse of soil respiration after rewetting (Bottner, 1985; van Gestel et al., 1991, 1993).

Dry–wet cycles also affect soil physical properties such as aggregation. The mechanism by which rewetting of dry soil can affect soil aggregates is the result of rapid intake of free water during which air becomes entrapped and compressed in pores, causing swelling or inflation of soil aggregates (Kemper et al., 1985; Gäth and Frede, 1995). Rewetting was found to cause macroaggregate disruption, accompanied by SOM decomposition (Denef et al., 2001b). This disruption could lead to enhanced macroaggregate turnover and loss of macroaggregate-associated organic matter (Denef et al., 2001a,b). Denef et al. (2001b) also reported that, upon disruption of macroaggregates by dry–wet cycles, easily decomposable residue associated with macroaggregates was released for decomposition. The effects of drying and rewetting on soil aggregates are not clear, because water-stable aggregates decrease and increase during wet-dry cycles (Degens and Sparling, 1995; Denef et al., 2001a,b). Denef et al. (2001a,b) observed that most aggregates become slacking resistant after two dry–wet cycles, when SOM remained occluded in macroaggregates and was physically protected against microbial utilization. Although the rewetting effect on C and N mineralization has been studied for decades (Lebedjantzev, 1924; Birch, 1958; van Gestel et al., 1991; Cabrera, 1993; Magid et al., 1999), researchers recently have examined and focused on intraaggregate particulate organic matter (iPOM) and soil aggregate stability (Denef et al., 2001a,b) associated with dry–wet cycles. Adu and Oades (1978) reported that mechanical disruption of aggregates by drying and rewetting might be as important as chemical and biological factors in causing the flush of microbial activity. In most studies, the drying process was rapid (within 1–3 d), which can have an effect on the survival of soil microorganisms. Slow soil drying allows for microbial metabolic adjustment that could reduce mortality (Chao and Alexander, 1984; Hartel and Alexander, 1986; Roberson and Firestone, 1992). Minimum information about C and N dynamic during the drying period and within a few hours of rewetting is available in literature due to the drying technique used.

This study focused on: (i) multiple cycles of drying and rewetting, by a slow-drying and fast rewetting and (ii) collecting detailed data on C and N flushes and aggregate size distribution.

2. Materials and methods

2.1. Soil collection and preparation

In March of 2000, soil was sampled from the 0 to 10-cm depth of a long-term no-tillage study established in 1990 at the North Agronomy Farm located at Kansas State University, Manhattan, KS. The soil was a moderately well-drained Kennebec silt loam (Fine-silty, mixed, superactive, mesic Cumulic Hapludoll) with a texture of 9% sand, 69% silt, and 22% clay and total C content of 16.2 g C kg^{-1}. A sterile (207.9 l) polyethylene bag (United State Plastic Corp., Lima, OH) was filled with soil collected randomly from the field by using a 2-cm diameter Oakfield soil probe (Forestry Supplies, Inc., Jackson, MS). The sampled soil was stored field-moist at 4 °C. Within 24 h, the soil was sieved through a 6-mm sieve and stored at 4 °C until starting the experiment. Before starting the wet-dry experiment, an initial experiment was performed to determine the time required to dry the soil from a matric potential of −0.033 to −1.5 MPa with silica gel. Gravimetric soil water content (SWC) was determined by weight loss at 105 °C for 24 h.

2.2. Initial drying experiment

This experiment was conducted to determine the length of drying period required to dry soil slowly from −0.033 to −1.5 MPa. A proper amount of deionized water was sprayed carefully on the soil and mixed to raise the SWC from 0.24 to 0.26 g H_2O g^{-1} soil (−0.033 MPa). The soil was stored at 4 °C for 24 h to equilibrate the water throughout the entire soil volume. After 24 h, 100 g (oven-dry basis) of soil was added to a glass vessel (Mason jar 930 ml) and covered with a lid with a septum (Fig. 1). A specimen cup with the lid (Fisher Scientific, Pittsburgh, PA) was held above the soil. Four holes were drilled (1.59 cm diameter) in the specimen cup, which contained 37 g of silica gel desiccant (Fisher Scientific, Pittsburgh, PA). The specimen cup's holes allowed diffusion of soil water vapor and absorption of the vapor by the silica gel. The silica gel changed color from purple blue (dry condition) to pink (wet condition) as it absorbed water. A spinal needle (Popper and Sons, Inc., New Hyde Park, NY) with a septum (Shimadzu Scientific Instruments, Inc., Columbia, MD) attached to its upper end was inserted through the septum of the glass vessel's lid, passing through the specimen cup (Fig. 1). The spinal needle was used to hold the specimen cup and the silica gel above the soil and for CO_2 sampling of the headspace. The apparatus was

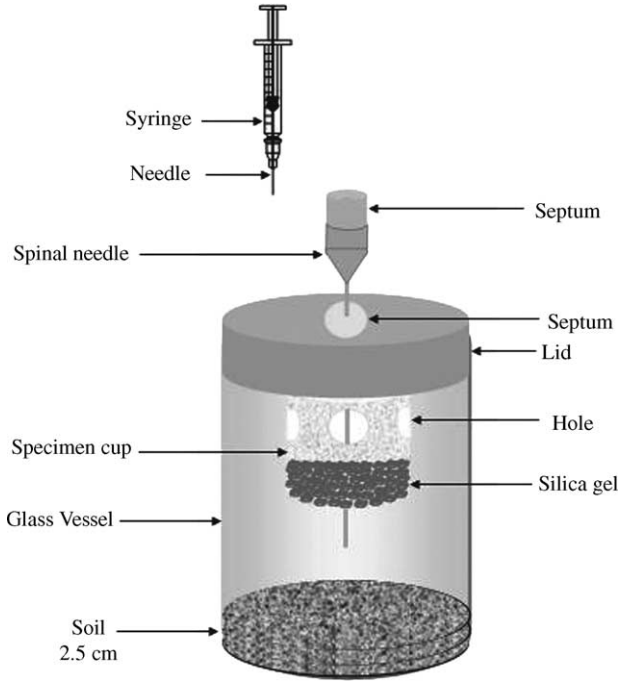

Fig. 1. Drying apparatus used to slowly dry soil from a soil water content 26 g $H_2O\ g^{-1}$ soil corresponding to −0.033 MPa; to 11 g $H_2O\ g^{-1}$ soil corresponding to −1.5 MPa.

closed tightly and incubated at 25 °C for the drying period. Every day, six soil samples were sampled destructively and the SWC was determined as described previously. The silica gel was changed periodically for the rest of the samples until the end of drying period. The total drying period (from −0.033 to −1.5 MPa metric potential) was determined to be 10 d and silica gel was changed three times; the first change was after 4 d and every 3 d thereafter.

2.3. Dry–Wet experiment

Four dry–wet (DW) cycles were implemented during the experimental period (96 d). Each cycle contained two periods, 10 d of drying, followed by 14 d of incubation at 25 °C after rewetting. To determine the effect of DW cycles on C and N flush, eight replicates were prepared for each cycle (four replicates for DW treatment and four replicates for CWC treatment). At the same time, four replicates for each treatment were prepared and maintained undisturbed for aggregate separation after the end of each cycle.

A proper amount of deionized water was sprayed carefully onto the soil and mixed to raise the SWC to 0.26 g $H_2O\ g^{-1}$ soil (−0.033 MPa). The soil was stored at 4 °C for 24 h for equilibration. After 24 h, 100 g soil (oven-dry basis) was used for each replicate in the DW and CWC treatments. The drying apparatus was used for the DW treatment, whereas the CWC treatment was kept in a glass vessel (Mason jar 930 ml) and covered with a lid with a septum.

To determine the effect of silica gel on CO_2 concentration, a check (CH) treatment for drying period (CH_{dry}) was included. Four replicates of the drying container (with silica gel) containing only 26 ml of H_2O (same amount of water content in soil samples corresponding to −0.033 MPa) were prepared. Another four replicates of check treatment with no silica gel added (CH_{wet}) were prepared by using 26 ml of H_2O added to the containers that were covered with a lid to determine the CO_2 (CH_{wet}) of laboratory air.

2.4. Drying period

During the drying period and before changing the silica gel, the CO_2 concentration was determined by taking a 0.5 ml sample with a 1 ml syringe. The headspace was mixed 10 to 15 times before sampling by use of a 10 ml syringe. The concentration of the CO_2–C was measured on a Shimadzu Gas Chromatograph-8A (Shimadzu, Inc., Kyoto, Japan). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and a 2 m Porapak column. The column temperature was 70 °C and the carrier gas was Helium at a flow rate of 14 ml min^{-1}. After the headspace gas was sampled, the jars were opened (under the hood) for approximately 15 min to equilibrate with the atmosphere and to change the silica gel for the DW treatment. This procedure was repeated twice during the 10 d drying period. To calculate mineralized C, the following equation was used

$$\text{Mineralizable C} = [(\text{Soil } CO_2 - \text{Check } CO_2) \times V_{vessel}]/\text{ODW} \quad (1)$$

where mineralizable C measured in (μg g^{-1}), soil CO_2 (μg ml^{-1}) is CO_2 evolved from soil treatment, Check CO_2 (μg ml^{-1}) is CO_2 evolved from check treatment, V_{vessel} (ml) is the volume of the vessel used, and ODW(g) is the soil oven dry weight.

Because silica gel, as a desiccant, will decrease the water partial pressure, the CO_2 partial pressure will increase to maintain the equilibrium inside the closed vessel. Therefore, the CO_2 concentration will be greater in drying soils compared with CWC soils. Therefore, to calculate mineralized soil C throughout the drying period, the difference in CO_2 concentration was adjusted to account for the drying process. Eq. (1) was used for both treatments (DW and CWC), but the CO_2 concentration in CH_{dry} was subtracted from the DW treatment, whereas CH_{wet} was subtracted from the CWC treatment.

2.5. Rewetting period

At the end of drying period, fast rewetting was performed by applying 15 ml of deionized water (=the amount of water required to attain SWC of 0.26 g $H_2O\ g^{-1}$ soil) with a syringe. Subsamples for inorganic N determinations and CO_2 concentration were taken about every 8 h for the first 2 d, then daily through day 8, and every 2 d thereafter.

Soil was mixed thoroughly before sampling. Because the soil was disturbed and the soil weight was decreased by periodic sampling, the CO_2 concentration was determined from the containers that were not disturbed.

Five days after the rewetting period, microbial biomass, aggregate size distribution, and aggregate associated C and N were determined. A treatment set was prepared and maintained undisturbed to determine aggregate size classes. Soil inorganic N was determined by extracting 5 g of soil with 20 ml of 1 M KCl and shaking for 1 h at 300 rev min^{-1} on an orbital shaker. The supernatant was filtered through Whatman filter paper No. 2 (Fisher Scientific, Fair Lawn, NJ) and stored at 4 °C until analyzed for NH_4–N and NO_3–N on an Alpkem Autoanalyzer (Alpkem Corp., Bulletins A303-S021 and A303-S170, Clackamas, OR). The net N mineralized was calculated by subtracting initial soil inorganic N from the amount present at each sampling.

Microbial biomass C (MBC) and N (MBN) were determined after 5 d of the rewetting period by using the fumigation-incubation method (Jenkinson and Powlson, 1976). The distribution of sand-free water-stable aggregate (WSA) were determined after 5 d of each rewetting by using a modified Yoder (Yoder, 1936) wet-sieving apparatus (Fig. 2). Four aggregate size classes were separated; >2000, 250–2000, 53–250, and 20–53 μm diameter. Macroaggregates were defined as >2000 and 250–2000 μm fractions; microaggregates were defined as 53–250 and 20–53 μm fractions. Sieves with mesh openings ≥250 μm in diameter were contained in the oscillation cylinders. Soils (100 g from each treatment) were air dried for 24 h and evenly distributed over the nested sieve surfaces (>2000 and 250–2000 μm mesh). The nest was set at the highest point and the oscillation cylinders were filled with distilled water to the point, where the bottom sieve (250 μm mesh) was completely covered with water, without reaching the top screen (2000 μm mesh). To slake the air-dried soil, 1 l of distilled water was rapidly added to each cylinder until the soil sample and top screen were covered with water. The soils were submerged in water for 10 min before the start of the wet-sieving action. The apparatus specifications of oscillation time (10 min), stroke length (4 cm), and frequency 30-cycle min^{-1} were held constant.

After wet sieving, soil plus water remaining in the oscillation cylinder was poured onto the finer sieves (53 and 20 μm mesh). Each sieve was shaken horizontally for 1 min to allow water and particle fractions smaller than the sieve size to pass through. Material remaining on each sieve was backwashed into a round aluminum pan and dried at 50 °C for 24 h. Aggregates <20 μm diameter were discarded, and soil recovery was calculated. Subsamples (0.2–2.0 g) of WSA from each size class were dried at 105 °C for 24 h to allow correction for dry weight.

Sand-free WSA was measured by using a subsample of intact aggregates (2–5 g) combining it with a fivefold volume (10–25 ml) of 5 g l^{-1} sodium hexametaphosphate, leaving overnight, and shaking on an orbital shaker at 350

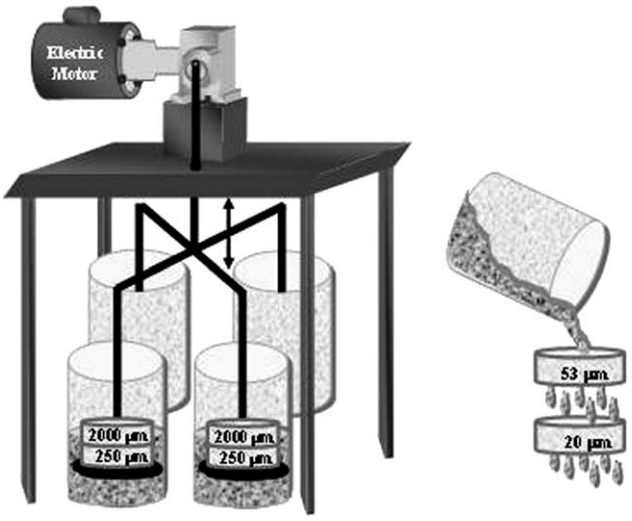

Fig. 2. Wet-Sieving apparatus.

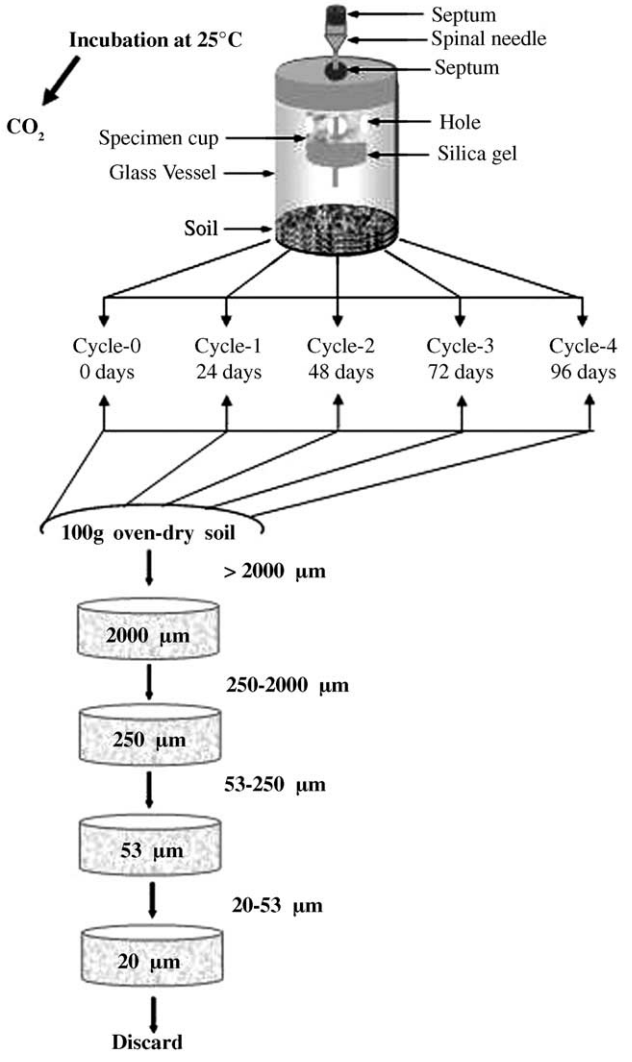

Fig. 3. The schematic diagram for soil separation to four aggregate size classes throughout the experiment period.

rpm for 4 h. The dispersed organic matter and sand was collected on a 53 μm diameter mesh sieve, washed with deionized water, and dried at 105 °C for 24 h, and the sand weights were recorded for estimating the sand-free correction. The schematic diagram for soil separation to four aggregate size classes throughout the experiment period is presented in Fig. 3.

2.6. Statistical analyses

The design of this experiment can be considered as a split–split-plot in which DW and CWC (treatment) were the whole-plot factor, the four cycles were the sub-plot factor, and the days within the cycle were the sub-sub-plot factor. The whole-plot error was rep (treatment) and the sub-plot error term was cycle×rep (treatment) interaction. A first-order autoregressive model was used to model the correlation structure among days within a cycle. The computations were carried out according to the Proc Mixed of the SAS® system (SAS Institute, Inc., 1999).

3. Results

Cumulative C mineralization was greater with CWC, compared with DW cycles (Fig. 4A). For the first 4 d of incubation, cumulative C mineralization was 68 and 53 μg C g^{-1} soil for CWC and DW treatments, respectively, which represents a 22% reduction due to drying. Upon rewetting, a flush of mineralized C was observed, but it did not compensate for the reduction of mineralized C that occurred during the 10-d drying period. At the end of the first cycle, mineralized C was reduced by 85 μg C g^{-1} soil in the DW treatment compared with the CWC treatment

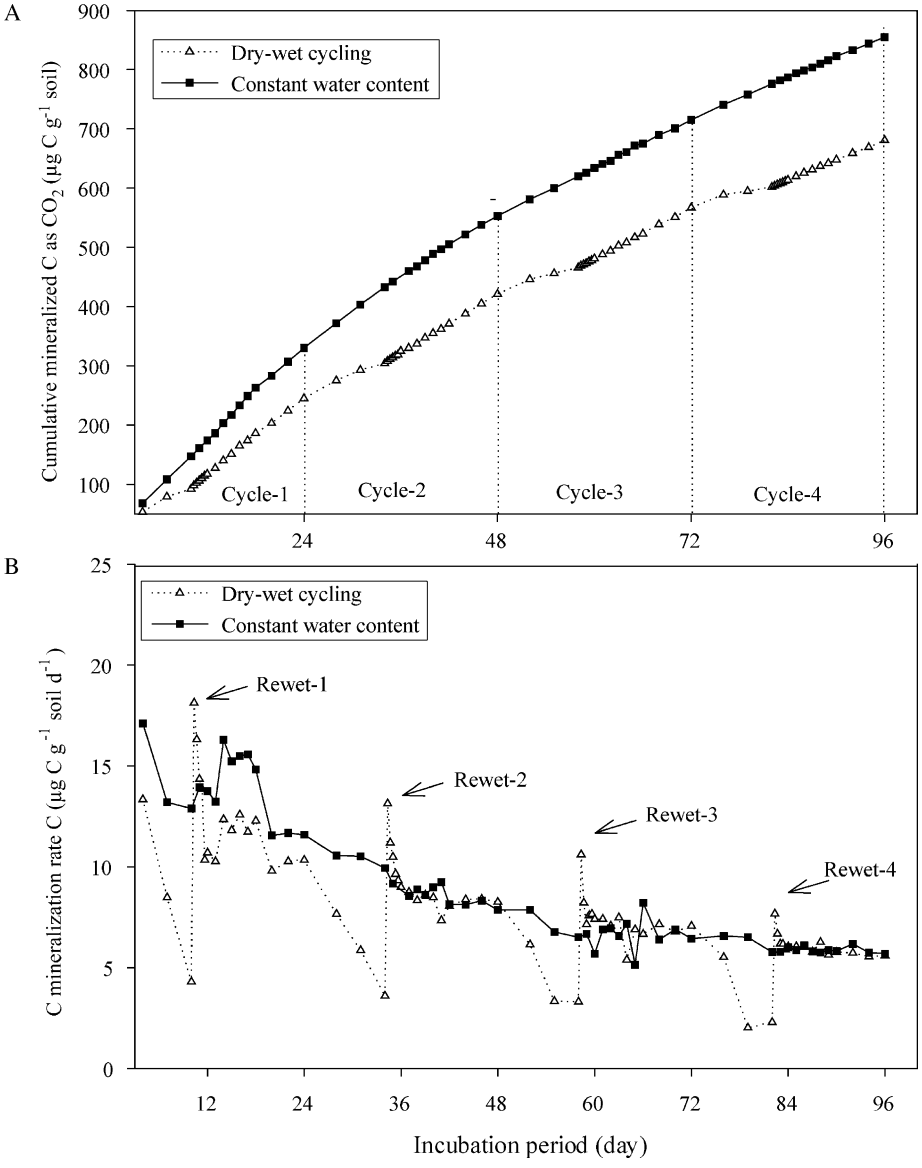

Fig. 4. Mineralized C as affected by moisture treatments. (A) Cumulative C mineralized; (B) C mineralization rate.

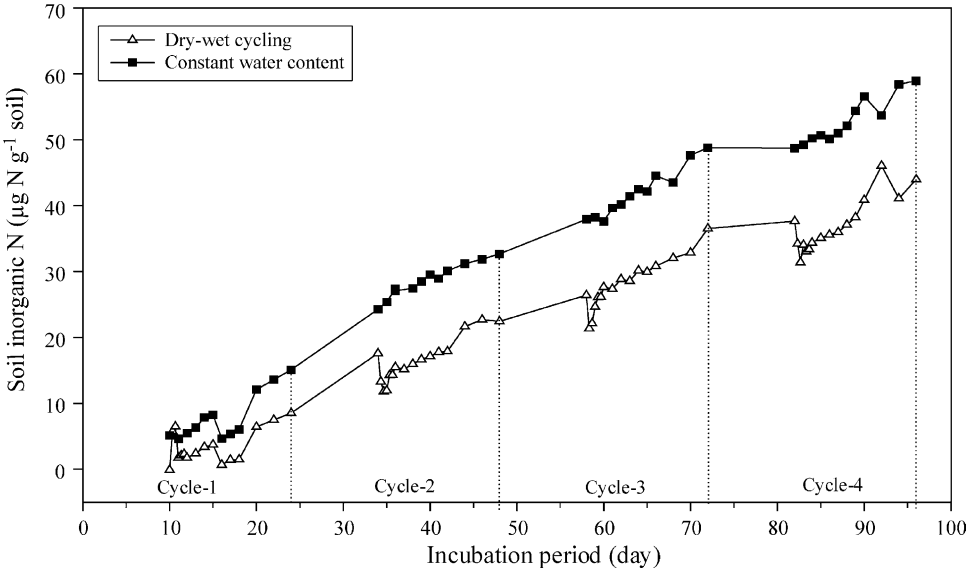

Fig. 5. Soil inorganic N as affected by dry–wet cycles and constant water content treatments.

(Fig. 4A). The differences in cumulative C mineralization between the CWC and DW treatments, increased with additional cycles. Cumulative C mineralization was greater in CWC, compared with DW treatment by 132, 148, and 174 µg C g^{-1} soil at the end of cycles 2, 3, and 4, respectively (Fig. 4A).

Throughout the experimental period, the C mineralization rate significantly ($P<0.05$) decreased with both treatments (Fig. 4B). During the drying period, the C mineralization rate of DW soil significantly decreased, compared with CWC. The reduction in the C mineralization rate was observed in all four cycles (Fig. 4B). As the soil was exposed to more DW cycles, the C mineralization rate at the end of each drying cycle was reduced by 16, 23, and 47% at 34, 58, and 82 d, respectively, compared with the first drying cycle at 10 d (Fig. 4B). The reduction in C mineralization rate was significant at 58 and 82 d, compared with the first 10 d. The continuous reduction in C mineralization rate indicates that the repeated DW cycles affect the available substrate and the microbial activity. Carbon mineralization rate did not decrease after 4 d of drying period (at 55 and 79 d) as the drying period continued for 3 more days (58 and 82 d). A flush of C mineralization was observed during the first 24 h after rewetting. The flush of C mineralization after 8 h of rewetting significantly decreased with each cycle. During the first rewetting cycle, the flush of mineralized C was observed only after 16 h of rewetting. After 24 h of rewetting, there was no significant difference in the C mineralization rate between the DW and CWC treatments. After 48 h of the first rewetting and until the second drying cycle, the C mineralization rate was significantly reduced in DW, compared with CWC treatment (Fig. 4B). With the remaining cycles (cycles 2, 3 and 4), however, and after 48 h of rewetting (at the end of C flush) and before the drying period (36–48, 60–72, and 84–96 d), the C mineralization rate was not significantly different between the treatments.

Net N mineralization was significantly less for the DW, compared with the CWC treatment (Fig. 5). A reduction in mineralized N was observed during the first 24 h after each rewetting period. This reduction in mineralized N could be caused by to increased microbial activity after rewetting the dry soil, resulting in N immobilization (Fig. 5). In general, soil inorganic N significantly decrease in DW treatments compared with CWC treatment by 7, 10, 12, and 15 µg N g^{-1} soil at the end of cycles 1, 2, 3, and 4, respectively. The differences in net N mineralization between treatments DW and CWC increased as the soil was exposed to more cycles (Fig. 5).

Microbial biomass C and N were significantly less at initial (0 d) time, compared with other times during the incubation period (Table 1). Microbial biomass C was not

Table 1
Microbial biomass C (MBM-C) and MBM-N as affected by dry–wet (DW) cycles and constant water content (CWC) treatments

Treatments	MBM-C (µg g^{-1} soil)			MBM-N (µg g^{-1} soil)		
	Initial	Cycle 3	Cycle 4	Initial	Cycle 3	Cycle 4
	303 b[a]			84 C[b]		
DW		1324 a	1409 a		319 A	323 A
CWC		1303 a	1412 a		241 B	261 B

[a] Represents significant difference at ($P<0.05$) between treatments and initial values for MBM-C.
[b] Represents significant difference at ($P<0.05$) between treatments and initial values for MBM-N.

Table 2
Soil aggregate size distribution (g 100 g^{-1} soil normalized to a sand-free basis) as affected by dry–wet cycles (DW) and constant soil water content (CWC) treatments

	Treatments	Aggregate size classes (g 100 g^{-1} soil normalized to a sand-free basis)			
		20–53 μm	53–250 μm	250–2000 μm	>2000 μm
Initial		25 b[a]	46 a	9	0.89
Cycle 4	DW	30 a	37 b	11	0.63
	CWC	30 a	39 b	9	0.97

[a] Lowercase letter represents significant difference at ($P<0.05$) between treatments in cycle 4 and initial values within each aggregate size class.

significantly affected by the treatments (DW vs CWC). But, MBM-N was significantly greater with the DW cycles compared with the CWC at cycles 3 and 4 (Table 1). The distribution of aggregate size classes was not significantly affected by the treatments (DW vs CWC). The DW cycles had a minimal effect on aggregate size distribution until cycle 4. At the end of cycle 4, however, the mass of microaggregate size classes (53–250 μm) was significantly ($P<0.05$) less than the initial mass, whereas, the mass of microaggregate size classes (20–53 μm) was significantly ($P<0.05$) greater than the initial mass (Table 2). The gain in microaggregates (20–53 μm) was probably caused by the loss in aggregates >53 μm; although the loss was not significant with macroaggregates, it was significant with microaggregate 53–250 μm. Aggregate associated C and N were not significantly affected by the treatments (DW vs CWC).

4. Discussion

Repeated drying of the soil to −1.5 MPa and rewetting to −0.033 MPa reduced microbial activity, resulting in reduced cumulative mineralized C and N. Our results agreed with Franzluebbers et al. (1994) in which they observed a reduction in cumulative mineralized C and N from repeated drying and rewetting of cowpea residue-amended soil. Many studies have reported that the reduction in mineralized C and N with drying could be caused by reduced microbial activity (West et al., 1992; Franzluebbers et al., 1994; Pulleman and Tietema, 1999), decreased microbial mobility (Griffin, 1981), and restricted substrate and nutrient availability (Sommers et al., 1981).

Rewetting of the dry soil often causes a flush of C, indicating the microorganisms regained activity upon rewetting (Franzluebbers et al., 1994). Rapid increases in C mineralization rates could be related to the release of readily degraded organic compounds (resulting from microbial death), which, in turn, would be used by the surviving soil microorganisms upon rewetting (Bottner, 1985; van Gestel et al., 1991, 1993). Our results indicated that the increased microbial activity, which occurred after 8 h, was not sufficient to compensate for the reduction in C mineralization during the drying period. Thus, as the number of DW cycles increased, the differences in cumulative C mineralization increased between the DW and CWC treatments. This data agrees with Fierer and Schimel (2002) were they observed a reduction in CO_2 with repeated DW cycles. They also observed that the respiration rates of soil exposed to DW cycles were substantially lower that continuous wet treatment even after 6 weeks of the last DW cycle. According to Magid et al. (1999), microorganisms lose some of their ability to degrade complex substrates during desiccation. They partly regained that activity upon rewetting, but not to the extent maintained by microorganisms in CWC conditions.

A reduction in C flush with repeated DW cycles could be due to the changes in the physiological state of the decomposer community, making them less susceptible to desiccation. Other studies have reported that microbial communities can adjust to the DW cycles by withstanding changes in osmotic potentials (Harris, 1981; van Gestel et al., 1993; Lundquist et al., 1999). Harris (1981), also reported that microorganisms' ability to withstand desiccation is influenced by their cell walls and their growth type. Slow growing soil organisms are less susceptible to the drying condition than fast growing soil organisms (Robinson et al., 1965). This study showed that repeated DW cycles did not significantly reduce the size of the microbial biomass. Therefore, the size of microbial biomass was not the limiting factor for C and N mineralization. Franzluebbers et al. (1994) also observed no change in microbial biomass in response to repeated DW cycles, but, a change in the species composition could result with repeated DW cycles.

The flush of mineralized C after each wetting period was accompanied by a reduction in cumulative N mineralization. The reduction in inorganic N present in soil could indicate an increase in microbial activity and/or rapid growth in microbial biomass (van Gestel et al., 1991, 1993). Thus microbes were assimilating mineral nutrient (inorganic N) to meet microbial demand (multiplication, growth, and maintenance of the living and active biomass). Rapid N immobilization was also observed by Appel (1998), which he related to the easily accessible C source that occurred after rewetting a dry soil. In this study, a significant reduction in soil inorganic N in DW treatment compared with CWC treatment was observed. Thus, as the number of DW cycles increased, the differences in net N mineralization further increased between the DW and CWC treatments (Fig. 5). Changes in soil water potential could

cause the death of some portion of the microbial population (Kieft et al., 1987), and that may cause a shift in the active microbial population and their N needs. In general, microorganisms use nitrogenous organic maternal, proteins, as a source of energy for their metabolic activity. To meet microbial energy demand, the organic material present in soil should have a specific *E* (energy)/N ratio. Therefore, N mineralization should meet the N requirement for microorganisms responding to excess energy, or inorganic N will be drawn from the soil N pool, thus reducing N pool size. In this study, significant increase in microbial biomass N (MBM-N) was observed with DW cycles, compared with CWC at cycles 3 and 4 (Table 1), which indicate that more inorganic N was assimilated by soil microorganisms. Increased N assimilation by microorganisms after cycle 2 caused a reduction of accumulative inorganic N in DW treatments, which caused increases in the differences between the treatments (DW vs CWC) after the second cycle. According to Franzluebbers et al. (1994), repeated DW cycles could cause a reduction in net N mineralization, either because of chemical reactions during the drying period, which reduce the amount of available N or reduce the active microbial biomass, or because of a change in species composition, in which instance more N could be retained in the microbial cells. Further, accumulation of N in a less-available portion of dead microbial biomass after each rewetting event could further reduce net N mineralization (Franzluebbers et al., 1994).

The reduction in cumulative N mineralization observed in this study does not agree with other studies in which there was an increase in mineralization after rewetting of dry soil (Bottner, 1985; Kieft et al., 1987; Cabrera, 1993; Scheu and Parkinson, 1994). The disagreement in C and N mineralization upon rewetting of dry soil between this study and the previous studies could be related to the contribution of the organic residue to the C and N flush after rewetting the dry soil (van Gestel et al., 1993; Appel, 1998; Magid et al., 1999; Denef et al., 2001a,b). In most of these other studies, soil physical disruption and/or changes in temperature accompanied soil drying. Soil physical disruption (van Gestel et al., 1993; Magid et al., 1999) could cause aggregate destruction and a release of protected soil organic matter, which contributes to the nutrient flush upon rewetting. The technique used in this study allowed the soil to remain structurally intact throughout the experiment period and during the DW cycles.

In the current experiment, the lack of significant effect of DW cycles on the distribution of aggregate size classes and aggregate-associated C and N could be caused by many factors that affect soil-aggregate stability, such as DW method, soil texture, and total C content. Drying cycles were performed, where the soil was kept structurally intact. Although fast rewetting was applied in this study, macroaggregates were not significantly affected by rewetting. Other studies have shown a decrease in macroaggregates with rewetting dry soil (Degens and Sparling, 1995; Denef et al., 2001a,b). The lack of change in macroaggregates size distribution in this study may also because of the clay and organic C content of our soil. Both clay and organic C promote stabilization of soil aggregates (Rochette and Gregorich, 1998; Aoyama et al., 1999). The soil in this study had greater organic C and less sand content, compared with soils used by Denef et al. (2001a,b) and Degens and Sparling (1995).

During the course of this study, and after the second cycle, a crust was observed on the soil surface. The crust formation could contribute to the lack of significant effect on aggregate size distribution. The energy of the added water during the rewetting could be absorbed by the crust layer, through which the water penetrated slowly, reducing its effect on soil aggregates. The formation of the crust layer, because of the energy of added water, that we observed in a small scale under the laboratory conditions, could happen on a larger scale under field conditions. Under field conditions, especially in tilled fields, the crust layer may reduce the impact of rainfall on soil aggregates. Residue cover could have a positive affect on absorbing rainfall energy and reduce the effect on soil aggregate destruction. The other possibility could be the rewetting technique used in this experiment, in which the water drops does not have the same energy of the natural rainfall. In general, the technique used in this experiment allowed us to simulate, on a small scale, the slow drying and fast rewetting events that could occur under field conditions.

In general, our results suggested that DW cycles caused a significant C flush, especially within the first 8 h after rewetting; but, the extent of the C flush was significantly reduced with repeated wet-dry cycles. Contrary to other studies and due to different experimental techniques, aggregate destruction and nutrient release associated with residue biomass did not significantly contribute to the C and N flush. The flush of C was mainly related to microbial activity and microbial turnover (microbial origin). Overall, repeated DW cycles reduced microbial activity; upon rewetting they regained activity, but was not sufficient to match the CWC treatment. Further studies using soils of different C and texture are needed to further determine the effect of DW cycles on soil aggregation and nutrient release.

Acknowledgements

This research was partly supported by the Cooperative State research, Education, and Extension Service, US Department of Agriculture, under Agreement No. 2001-38700-11092. Contribution No. 03-211-J of Kansas Agric. Exp. Stn.

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