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Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics

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Abstract

Aggregate dynamics and their relationship to the microbial community have been suggested as key factors controlling SOM dynamics. Dry–wet (DW) cycles are thought to enhance aggregate turnover and decomposition of soil organic matter (SOM), particularly in tilled soils. The objective of this study was to evaluate the effects of DW cycles on aggregate stability, SOM dynamics, and fungal and bacterial populations in a Weld silt loam soil (Aridic Paleustoll). Samples, taken from 250 μm sieved air-dried soil (i.e. free of macroaggregates > 250 μm), were incubated with ^{13}C-labeled wheat residue. In one set of soil samples, fungal growth was suppressed using a fungicide (Captan) in order to discern the effect of dry–wet cycles on fungal and bacterial populations. Aggregate formation was followed during the first 14 d of incubation. After this period, one set of soil samples was subjected to four DW cycles, whereas another set, as a control, was kept at field capacity (FC). Over 74 d, total and wheat-derived respiration, size distribution of water stable aggregates and fungal and bacterial biomass were measured. We determined native and labeled C dynamics of three particulate organic matter (POM) fractions related to soil structure: the free light fraction (LF), and the coarse (250–2000 μm) and fine (53–250 μm) intra-aggregate POM fraction (iPOM). In the fungicide treated soil samples, fungal growth was significantly reduced and no large macroaggregates (> 2 mm) were formed, whereas without addition of fungicide, fungi represented the largest part of the microbial biomass (66%) and 30% of the soil dry weight was composed of large macroaggregates. During macroaggregate formation, labeled free LF-C significantly decreased whereas labeled coarse iPOM-C increased, indicating that macroggregates are formed around fresh wheat residue (free LF), which is consequently incorporated and becomes coarse iPOM. The first drying and wetting event reduced the amount of large macroaggregates from 30 to 21% of the total soil weight. However, macroaggregates became slake-resistant after two dry-wet cycles. Fine iPOM-C was significantly lower in soil after two dry–wet cycles compared to soil kept at FC. We conclude that more coarse iPOM is decomposed into fine iPOM in macroaggregates not exposed to DW cycles due to a slower macroaggregate turnover. In addition, when macroaggregates, subjected to dry–wet cycles, became slake-resistant (d 44) and consequently macroaggregate turnover decreased, fine iPOM accumulated. In conclusion, differences in fine iPOM accumulation in DW vs. control macroaggregates are attributed to differences in macroaggregate turnover.

Keywords: Soil aggregates; Particulate organic matter; Microflora; Dry–wet cycles

1. Introduction

Soil undergoes a complex of physical, chemical, and biological changes under the impact of drying and wetting, including changes to soil structure (aggregation), soil organic matter (SOM) and microflora (Soulides and Allison, 1961; Sorensen, 1974; Utomo and Dexter, 1982). The effects of drying on soil structure are still unclear, since both increases and decreases in water stable aggregation have been observed following drying. Aggregate stability

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Abbreviations: − F, without fungicide; + F, with fungicide; FC, field capacity; DW, dry–wetted; iPOM, intra-aggregate particulate organic matter; LF, light fraction

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measurements are affected by air-drying because: (1) additional intermolecular associations are formed between organic molecules and mineral surfaces (Kemper and Rosenau, 1984); (2) the process of slaking (aggregate disruption under the action of water) is induced by air-drying soil prior to fast rewetting; and (3) since drying is usually not uniform, unequal strains arise throughout the soil mass, which causes incipient failure zones and cracks to develop (Haynes and Swift, 1990). The balance between the counteracting processes induced by drying and wetting determines aggregate stability. For example, Haynes and Swift (1990) observed a reduced aggregate stability in air-dried arable soil samples, but an increased aggregate stability in air-dried pasture soil samples, compared to their field-moist counterparts. Pasture soils have a high SOM content, which results in a reduced wettability because of the hydrophobic characteristics of SOM (Caron et al., 1996) and the formation of many additional intermolecular associations upon drying. These processes seem to offset the slaking-effect in pasture soils. In contrast, arable soils have a lower SOM content and rewet much faster. Therefore, in arable soils, the disruptive effect of slaking outweighs the stabilizing effect of drying, which results in a reduced aggregate stability.

Soil aggregates are more subjected to dry–wet cycles in conventionally tilled soil (CT) compared to no-tilled soil (NT) because tillage, by mixing the plow-layer, continually exposes new soil to dry-wet cycles at the soil surface and incorporates crop residues which serve as a protective barrier in NT (Beare et al., 1994; Paustian et al., 1997). Based on aggregation data from several long term field experiments, Paustian et al. (1999) found a general decrease in aggregation in CT soil compared to NT soil, concomitant with a reduced carbon content in CT soil. These observations indicate a possible interrelation among tillage intensity (and consequently drying and wetting intensity), soil structure and SOM dynamics.

Many studies have focused on the influence of drying and rewetting on microbial activity and decomposition of SOM (Soulides and Allison, 1961; Bloem et al., 1992; Magid et al., 1999). Dry–wet cycles generally cause an increase in decomposition of SOM, because more decomposable organic substrates become available for microbial attack upon drying and rewetting (Soulides and Allison, 1961; Sorensen, 1974). These substrates are derived partially from the death of a portion of soil biota upon drying (Lund and Goksoyr, 1980; Bottner, 1985) and partially from the release of previously inaccessible organic compounds occluded in aggregates (Sorensen, 1974; Van Gestel et al., 1991). The increased availability of organic substrates results in a flush of microbial activity and an increase in length of fungal hyphae and bacterial biomass (Jager and Bruins, 1974).

A physical fractionation method in which free light fraction (LF) (POM outside of aggregates) and intra-aggregate particulate organic matter (iPOM) (POM inside of aggregates) were differentiated, has been used in order to directly relate soil aggregate and associated organic matter fraction dynamics (Jastrow, 1996; Six et al., 1998, 1999a; Gale et al., 2000b). Based on this method, Six et al. (1998, 1999a) proposed a conceptual model in which it is hypothesized that increased aggregate turnover in tilled soil is an important mechanism causing a loss of SOM.

This study was designed to evaluate the effects of dry–wet cycles over time on: aggregate turnover; POM dynamics; and fungal and bacterial populations in a silt loam soil, and to clarify interrelationships between aggregate, POM and microbial population dynamics. The main hypotheses in this study were that: (1) drying and wetting enhances aggregate turnover; and (2) there is more accumulation of fine iPOM in the absence of dry–wet cycles.

2. Materials and methods

2.1. Site description and sampling

Surface soil (0–20 cm) was collected in July 1998 from an experimental site 6.4 km east of Akron, Colorado. The site is located at the Central Great Plains Research Station (40 9′ N, 103 9′ W). The soil is a Weld silt loam soil (Aridic Paleustoll) with a texture of 41% sand, 36% silt and 23% clay. The field has been cultivated (conventional tillage) for more than 90 years (Halvorson et al., 1997). After collection, the soil was air-dried and passed through a 250 μm sieve to reduce macroaggregates to < 250 μm sized particles. The 250–1000 μm sized sand and particulate organic matter (POM) fractions were kept and remixed with the soil after sieving. Material larger than 1000 μm were discarded. Field capacity was measured on three replicates of 50 g air-dried soil (18%). The air dried 250 μm sieved soil was thoroughly mixed and 110.8 g subsamples were taken and packed into cores (volume = 93.5 cm^3) which resulted in a bulk density of approximately 1.2 g cm^{-3}. The cores consisted of two aluminum cylinders (radius = 2.4 cm, height = 2.5 cm) connected with tape. Each end of the core was covered with a hardware cloth ring (0.5 mm mesh size), attached to an empty aluminum cylinder, to hold the soil sample in place.

2.2. Experimental treatments

Soil samples were incubated with the following four treatments: with or without fungicide, and two dry–wet treatments differing in soil disruptive effect: (1) a disruptive treatment where soil was kept in the core, air-dried at 25°C with a fan for 2 d (moisture content after air-drying was 1–2%), and fast wetted by pouring 19.5 ml of water (= the amount of water required to attain field capacity) directly on the soil surface (DW treatment); and (2) a non-disruptive treatment where soil was kept at field capacity at 4°C, to minimize microbial activity, during the period in which DW soil samples were dried and fast wetted (control treatment).

Soil samples were mixed with 0.49 g of ^{13}C-labeled wheat straw, which is twice the amount that would be incorporated under field conditions (Halvorson et al., 1997). Wheat straw was ground to the size of 500–850 μm. Only stems and leaves were used (C/N: 61.4; δ ^{13}C: 1803.0 ± 2.5‰; 1.79 mg wheat-C g^{-1} soil). For the fungicide treatment, soil samples were mixed with 0.33 g of fungicide (Captan). The samples were incubated for 14 d before dry–wet cycles were started to allow sufficient aggregate formation (Bossuyt et al., 2001). Four dry–wet cycles were applied, each consisting of the drying and wetting treatment described above followed by 12 d incubation. Before and after the first (d 14 and d 17) and the third (d 44 and d 47) drying and wetting and at the beginning (d 0) and the end (d 74) of the experiment, three replicates of each treatment were sampled and analyzed for aggregate size distribution. Free light fraction (LF) and intra-aggregate POM (iPOM) separation was done on the same days, except after the third drying and wetting (d 47). Fungal and bacterial biomass was measured at d 0, 14, 44 and 74.

In total, 60 soil samples with added wheat residue were incubated at 25°C in glass jars sealed with aluminum lids having luer lock fittings to allow collection of headspace gas samples. Over the entire incubation period (74 d), respiration was measured on 12 soil samples. These samples were the samples used to measure aggregation, microbial biomass, free LF and iPOM at the end of the experiment (d 74). An extra set of 12 soil samples without added wheat residue was incubated in a similar way and subjected to the four treatments described above. Only respiration was measured on these samples in order to have a control for the measurement of carbon isotope ratios ($^{13}C/^{12}C$) of the respired CO_2. Respiration was measured at d 2, 4, 6, 8 and 12 of each dry–wet cycle, using an infrared gas analyzer (LICOR model LI6252). Gas subsamples (35 ml) were stored in sealed glass vials prior to measurement of carbon isotope ratios ($^{13}C/^{12}C$) of the respired CO_2. After subsampling the 24 samples, all jars were flushed with bottled air of known CO_2 concentration (350 ppm) and δ ^{13}C-signature (−7.5‰).

2.3. Aggregate size distribution analyses

Aggregate size separation was done by a wet sieving method adapted from Elliott (1986). All wet sievings were done on soil samples at field capacity. For the DW treatment, soil samples were rewetted, equilibrated (18 h at 4°C) and brougth back to room temperature (21°C) prior to wet sieving. Soil samples of approximately 50 g were taken from the upper part of the core, to ensure field capacity. A series of three sieves was used to obtain four aggregate size fractions: (1) > 2000 μm (large macroaggregates); (2) 250–2000 μm (small macroaggregates); (3) 53–250 μm (microaggregates); (4) < 53 μm (silt and clay fraction). The 50 g samples were submerged in room temperature water on top of the 2000 μm sieve for 5 min prior to sieving. During sieving, aggregates were separated by manually moving the sieve up and down 3 cm with 50 repetitions during a period of 2 min. Water plus soil that went through the sieve was poured onto the next smaller size sieve and the same sieving procedure was repeated. The aggregate fractions retained on each sieve were oven dried (50°C) for 24 h, weighed and stored in glass jars at room temperature (21°C).

2.4. Size density fractionation

The method for separation of free LF and iPOM is described in detail by Six et al. (1998). We did this separation on microaggregates (53–250 μm). Because no aggregates were present in the 250–2000 μm size class, all the POM in this fraction, which consisted of sand and POM, was considered free LF. Since the added wheat residue was ground to a size between 500 and 850 μm and all native POM was of a size <1000 μm, there was no free LF in the > 2000 μm size class. Therefore, this fraction was only analyzed for iPOM. The free LF was isolated from the microaggregate fraction by flotation in 1.85 g cm^{-3} sodium polytungstate (SPT). After floating off the free LF, the heavy fraction was dispersed in 0.5% Na-hexametaphosphate and passed through 2000 μm and/or 250 μm and/or 53 μm sieves, depending on the aggregate size being analyzed, to isolate the iPOM. Sand and iPOM were oven-dried (50°C) and weighed. The free LF and iPOM fractions were ground and analyzed for C content and carbon isotope ratio.

Sodium polytungstate was cleaned and recycled according to Six et al. (1999b) to remove C, Ca^{2+}, and other soil derived ions. Contamination of SPT with C would significantly bias the $^{13}C/^{12}C$ analysis of the obtained fractions.

2.5. Carbon analyses

Carbon content of aggregate size fractions and iPOM was measured using a LECO CHN-1000 analyzer (Leco Corp., St. Joseph, MI). Carbon content of free LF was measured on a Carlo Erba NA 1500 CN analyzer because of the smaller size of the free LF samples (Six et al., 1999a). According to Elliott et al. (1991), comparisons of C content across aggregate size classes can only be made with sand corrected carbon data because of the difference in amount of sand (with which there is no organic matter associated) between size fractions. Sand correction is also necessary when comparing C contents within one size fraction, over time and between dry–wet treatments, because sand contents of DW and control aggregates are different and change over time. Sandfree C concentration is calculated with the following formula:

$$\text{sand free } C_{\text{fraction}} = \frac{C_{\text{fraction}}}{1 - [\text{sand proportion}]_{\text{fraction}}}$$

2.6. Isotope analyses

Variations in ^{13}C of the CO_2 evolved during incubation of 24 soil samples were determined using a micromass VG optima mass spectrometer (Micromass UK Ltd., Manchester, UK). The CO_2 gas samples were transferred to the IRGC vacuum tube with a syringe. Results were expressed as:

$$^{13}C‰ = \left[\frac{(^{13}R_{sample})}{^{13}R_{standard}} - 1 \right] \times 1000,$$

where $^{13}R = {}^{13}C/{}^{12}C$ and the standard is the international Pee Dee Belemnite (PDB).

The amount of CO_2-C derived from the wheat residue (Q_w) was calculated using the following mass balance:

$$Q_t\delta_t = Q_w\delta_w + Q_s\delta_s + Q_b\delta_b$$

where Q_t = the total amount of CO_2-C; δ_t = its isotopic composition; Q_w = the amount of CO_2-C derived from the wheat; δ_w = its isotopic composition (1803.0 ± 2.4‰); Q_s = the amount of CO_2-C derived from the soil; δ_s = its isotopic composition; Q_b = blank CO_2-C amount; δ_b = its isotopic composition (−7.5‰). The control samples (no wheat added) were used to measure Q_s and δ_s. The changes in δ_s were found to be minor in comparison to the large δ value of the wheat and δ_s was therefore not measured in cycles 4 and 5. For these two cycles, the average δ_s values of the third cycle were used.

Delta ^{13}C in the free LF, iPOM and aggregate size fractions were determined using a Carlo Erba NA 1500 CN analyzer coupled to a Micromass VG isochrom-EA mass spectrometer (Micromass UK Ltd., Manchester, UK) (continuous flow measurement). The proportion of wheat derived C (f) was calculated using the equation:

$$f = (\delta_t - \delta_s)/(\delta_w - \delta_s)$$

where $\delta_t = {}^{13}C$ of the analyzed fraction at time t; $\delta_s = {}^{13}C$ of the original soil (−19.5 ± 0.5‰); $\delta_w = {}^{13}C$ of the wheat (1803.0 ± 2.4‰). When the total C content (C) is known, the quantity of C derived from the wheat (C_w) can be determined: $C_w = C \times f$.

2.7. Bacterial and fungal abundance and biomass

Fungal and bacterial abundance and biomass were determined following the method of Frey et al. (1999). Briefly, a 5 g subsample was taken to prepare two soil smears: one for fungal staining and one for bacterial staining. Fungi and bacteria were stained with calcifluor M_2R fluorescent brightener and DTAF [5-(4,6-dichlorotriazin-2-yl) aminofluorescin], respectively (Bloem et al., 1995). The slides were observed under a Zeiss Axiophot epifluorescent microscope, using a filter set for UV illumination for fungi and a filter set for blue light for bacteria. From each smear, 30 images were randomly collected with an Optronics cooled CCD camera (model DEI-470) and Adobe Photoshop image capturing software. Lengths and widths of fungal hyphae and bacterial cells were measured from the images using IP Lab Spectrum image analysis software (Signal Analytics Corporation, Vienna, Virginia). Fungal and bacterial biovolume was calculated from lengths and average widths (Frey et al., 1999):

$$\text{biovolume} = \pi/4 \times (\text{width})^2 \times (\text{length} - \text{width}/3).$$

2.8. Statistical analyses

The data were analyzed using the SAS statistical package for analysis of variance (ANOVA-GLM, SAS Institute, 1990). Within each experiment, dry–wet treatment was the main factor in the model, with time and replicate as secondary factors. Separation of means was tested using Tukey's honestly significant difference with a significance level of $P < 0.05$.

3. Results

3.1. Aggregation

Aggregate distributions after 14 d of incubation of 250 μm sieved soil maintained at field capacity (controls) were significantly different between fungicide (+F) and no-fungicide (−F) treatments. In +F treated soil, no large macroaggregates (> 2000 μm) were formed (Table 1), whereas −F soil samples had an average mean weight diameter (MWD) of approximately 1.7 mm at d 14 (Fig. 1). During the entire incubation, aggregate distribution did not change in the +F treatment. However, untreated (−F) soil showed significant differences in aggregation between dry–wet treatments: after the first drying and fast wetting (d 17), aggregation was significantly reduced, whereas aggregation in control soil was still increasing (Fig. 1). The differences in MWD between DW and control soil at d 17 were mainly attributed to differences in the large macroaggregate size class (>2000 μm). The amount of microaggregates (53–250 μm) increased after the first drying and wetting and the amount of primary particles (<53 μm) remained the same (Table 1). From d 17 to d 44, aggregation steadily increased in both DW and control soil. After the third drying and wetting (d 47), no further macroaggregate breakdown occurred and DW macroaggregates remained stable after d 47. In contrast, aggregation in control soil decreased between d 44 and d 74. Both DW and control treatments showed similar aggregate distributions at the end of the incubation (d 74) (Table 1).

3.2. Fungal and bacterial populations

Fungicide application suppressed fungal biomass in the first 44 d of incubation. However, fungal biomass significantly increased after d 44 (Fig. 2(a)). No non-target effects of the fungicide on bacterial biomass occurred in this study

Table 1
Effect of fungicide and dry–wet cycles on aggregate size distribution (% aggregate dry weight) in 250 μm sieved soil with incorporated wheat residue

Treatment	Aggregate size (μm)	% aggregate dry weight					
		Day 0	Day 14	Day 17	Day 44	Day 47	Day 74
− F Control	> 2000	0.0 ± 0.0[a]	30.0 ± 1.5	40.0 ± 1.6	50.2 ± 2.8	48.9 ± 1.9	42.1 ± 2.6
	250–2000	20.0 ± 0.3	14.2 ± 0.5	7.6 ± 0.2	6.3 ± 0.2	5.7 ± 0.2	6.5 ± 0.4
	53–250	36.8 ± 0.5	26.8 ± 0.8	26.3 ± 0.9	20.9 ± 1.6	20.8 ± 1.1	23.1 ± 1.2
	< 53	43.2 ± 0.4	29.1 ± 0.7	26.1 ± 0.5	22.5 ± 1.1	24.6 ± 0.6	28.2 ± 1.3
− F DW	> 2000	ND	ND	20.9 ± 1.5	34.9 ± 1.9	40.2 ± 1.4	44.3 ± 0.6
	250–2000	ND	ND	16.2 ± 0.3	12.6 ± 0.4	6.5 ± 0.3	6.2 ± 0.1
	53–250	ND	ND	32.3 ± 0.6	23.6 ± 1.4	23.8 ± 1.0	21.5 ± 0.4
	< 53	ND	ND	30.6 ± 0.6	28.9 ± 0.2	29.5 ± 0.2	28.1 ± 0.1
+ F Control	> 2000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	250–2000	19.2 ± 0.3	20.1 ± 0.1	19.9 ± 0.2	19.5 ± 0.1	20.1 ± 0.1	19.9 ± 0.3
	53–250	38.2 ± 0.2	39.6 ± 0.4	40.2 ± 0.5	39.2 ± 0.5	39.7 ± 0.3	39.0 ± 1.0
	< 53	42.7 ± 0.3	40.4 ± 0.6	39.9 ± 0.4	41.2 ± 0.4	40.2 ± 0.3	41.2 ± 0.9
+ F DW	> 2000	ND	ND	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.3
	250–2000	ND	ND	19.9 ± 0.3	19.8 ± 0.4	20.0 ± 0.3	20.1 ± 0.4
	53–250	ND	ND	41.4 ± 1.0	41.3 ± 0.4	39.7 ± 1.0	40.4 ± 0.2
	< 53	ND	ND	38.8 ± 0.9	38.7 ± 0.5	40.4 ± 1.0	38.9 ± 0.5

[a] Average ± standard error; +F = with fungicide; −F = without fungicide; Control = field capacity; DW = fast wetted; ND = not determined. The data for the 250–2000 μm size class indicate the amount of sand and wheat residue in this fraction. No macroaggregates were found in this size class over the entire incubation period.

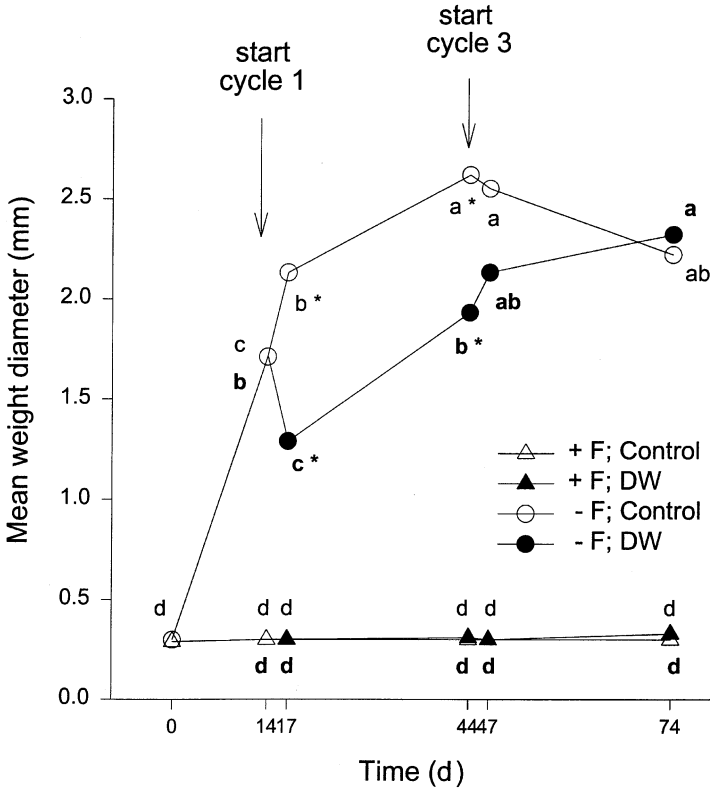

Fig. 1. Effect of fungicide and dry–wet cycles on aggregation (mean weight diameter (mm)). +F = with fungicide; −F = without fungicide; Control = field capacity; DW = dry–wetted. Values followed by * within the same sampling day/fungicide treatment and between dry–wet treatments, are significantly different. Values followed by different lowercase letter within the same fungicide treatment/dry–wet treatment and among sampling days, are significantly different. Statistical significance determined at $P < 0.05$ according to Tukey's HSD mean separation test.

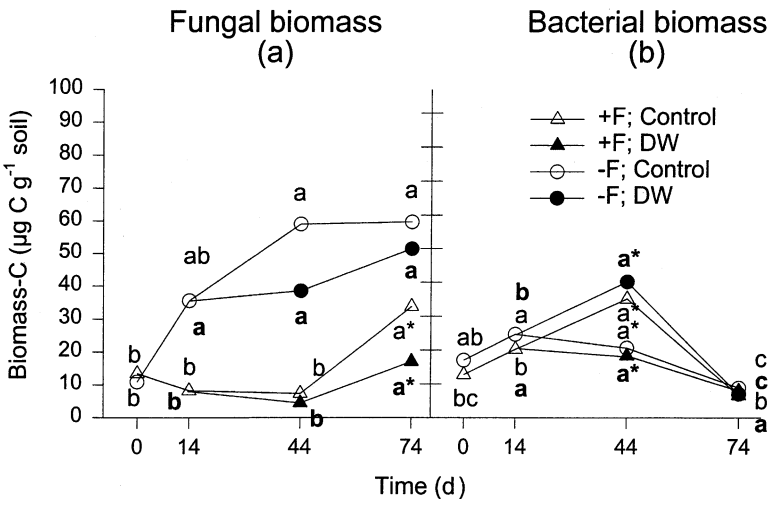

Fig. 2. Effect of fungicide and dry–wet cycles on: (a) fungal biomass (µg biomass-C g^{-1} soil); and (b) bacterial biomass (µg biomass-C g^{-1} soil). +F = with fungicide; −F = without fungicide; Control = field capacity; DW = dry–wetted. Values followed by * within the same sampling day/fungicide treatment and between dry–wet treatments, are significantly different. Values followed by a different lowercase letter, within the same fungicide treatment/dry–wet treatment and among sampling days, are significantly different. Statistical significance determined at $P < 0.05$ according to Tukey's HSD mean separation test.

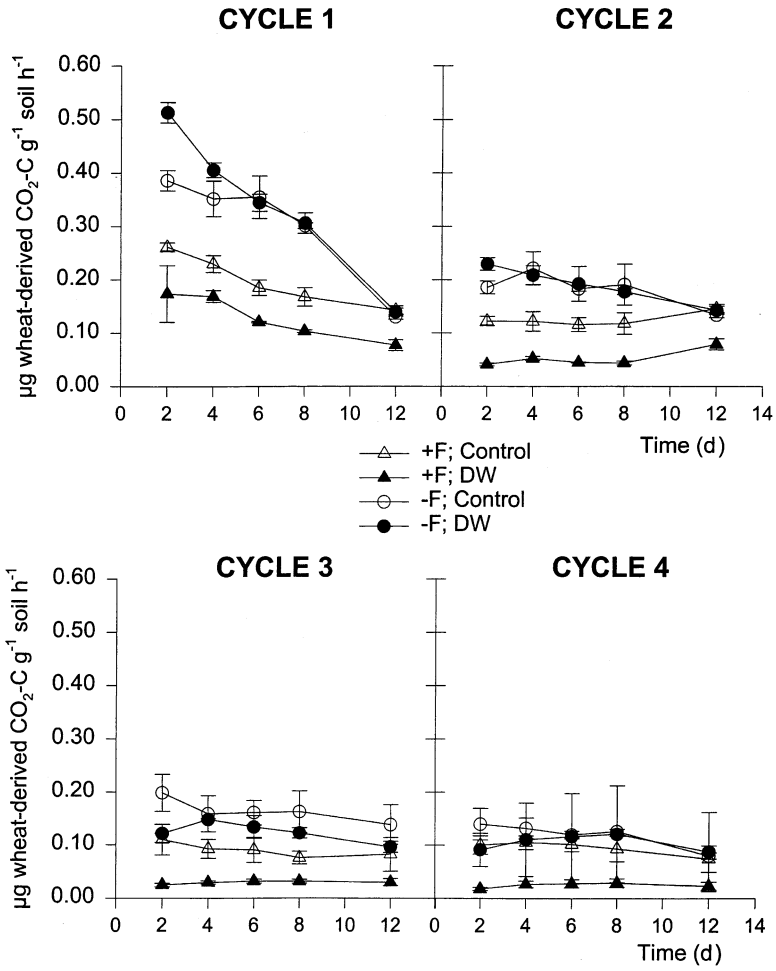

Fig. 3. Wheat-derived respiration rates (µg CO_2-C g^{-1} soil h^{-1}) in different dry–wet cycles, measured during 12 d after each dry–wet treatment. +F = with fungicide; −F = without fungicide; Control = field capacity; DW = dry–wetted. Bars indicate standard deviations.

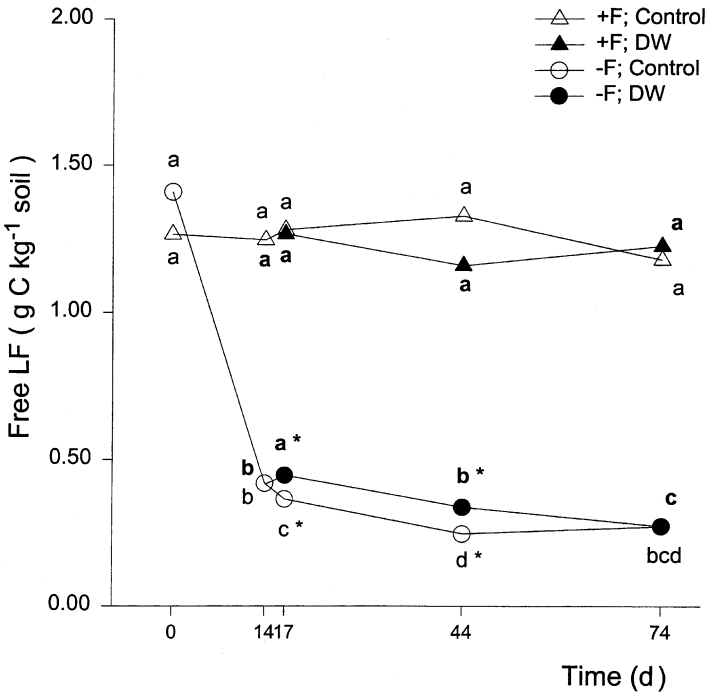

Fig. 4. Effect of fungicide and dry–wet cycles on wheat-derived free LF-C (g C kg^{-1} soil). +F = with fungicide; −F = without fungicide; Control = field capacity; DW = dry–wetted. Values followed by * within the same sampling day/fungicide treatment and between dry–wet treatments, are significantly different. Values followed by a different lowercase letter within the same dry–wet treatment and among sampling days, are significantly different. Statistical significance determined at $P < 0.05$ according to Tukey's HSD mean separation test.

(Fig. 2(b)). In the −F control treatment, fungal biomass-C increased until d 44 (Fig. 2(a)). After day 44, the fungal biomass stabilized. Drying and wetting did not significantly change fungal biomass-C over time in the −F treatment. At d 44, there was a tendency towards lower fungal biomass-C in the DW soil compared to the control soil (38.6 vs. 59.1 μg C g^{-1} soil). In contrast, bacterial biomass-C was significantly higher in DW than in control −F soil (40.1 vs. 20.7 μg C g^{-1} soil) (Fig. 2(b)). In the +F treatment, bacterial biomass-C was significantly lower in DW soil compared to control soil at d 44 (18.4 vs. 35.1 μg C g^{-1} soil).

3.3. Wheat-derived respiration

At d 2 of the first DW cycle, wheat-derived respiration rate was significantly higher in the untreated (−F) DW soil, compared to control soil (Fig. 3). Similar but smaller differences in wheat-derived respiration rate were noticed in the second DW cycle. However, in the third DW cycle, a maximum in wheat-derived respiration rate was no longer observed 2 d after drying and wetting. At day 2 in this third DW cycle, control soil samples had higher wheat-derived respiration rates compared to DW soil samples (Fig. 3). Over the entire incubation period, wheat-derived respiration rates were significantly lower in the +F treatment compared to the −F treatment. Within the +F treatment, significantly lower wheat-derived respiration rates were observed for DW compared to control soil samples during the first three DW cycles.

3.4. Free light fraction (LF)

In the untreated (−F) soil samples, wheat-derived free light fraction (LF) was strongly reduced after the first 14 days of incubation (Fig. 4). After the first dry–wet treatment (d 17), significantly higher free LF-C concentrations were found compared to the control treatment. From d 17 to d 74, the amount of wheat-derived LF-C slightly decreased in both −F control and DW treatments. In the +F treatment, free LF did not significantly change over time.

3.5. Intra-aggregate particulate organic matter (iPOM)

Since no macroaggregates were found in the +F treatment, iPOM did not exist and all POM associated with this fraction was considered free LF. In the −F treatment, a strong increase in wheat-derived coarse iPOM occurred during the first 14 d of incubation (Fig. 5(a)). Between d 14 and 74, wheat-derived coarse iPOM in the control treatment decreased (Fig. 5(a)) with a concomitant increase of wheat-derived fine iPOM (Fig. 5(b)). After the first drying and wetting (d 17), coarse iPOM-C was significantly higher in DW macroaggregates compared to control, but this difference disappeared by d 44. For the fine iPOM-C, significant differences between DW and control macroaggregates only occurred at d 44. However, higher concentrations were found in the control than in the DW treatment.

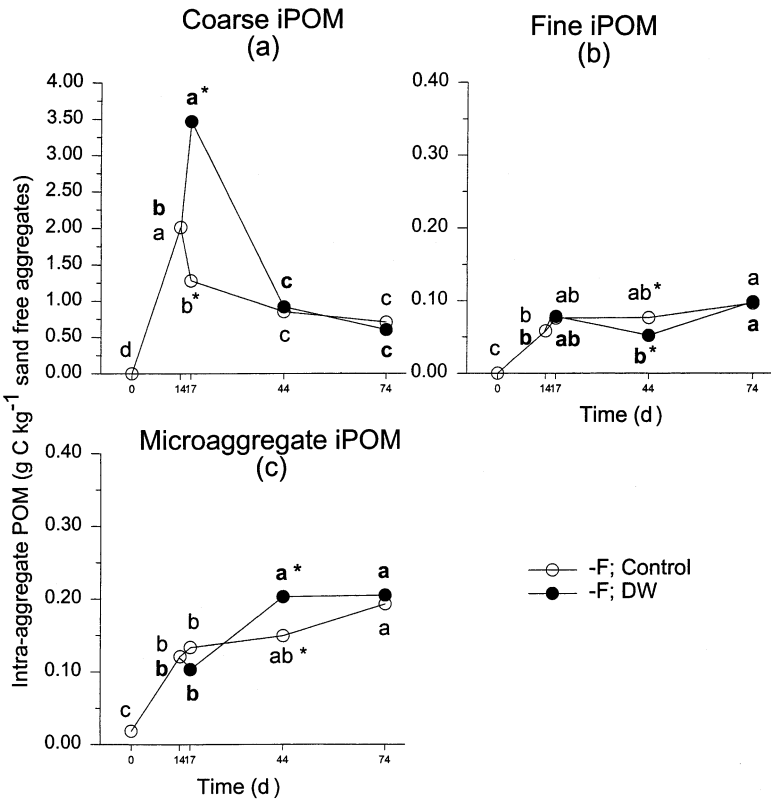

Fig. 5. Effect of dry–wet cycles in −F soil on: (a) wheat-derived coarse iPOM (250–2000 μm) (g iPOM-C kg^{-1} sandfree aggregate) in large macroaggregates (> 2000 μm); (b) wheat derived fine iPOM (53–250 μm) (g iPOM-C kg^{-1} sand free aggregate) in large macroaggregates (> 2000 μm); and (c) wheat-derived microaggregate iPOM (53–250 μm) (g iPOM-C kg^{-1} sandfree aggregate). Control = field capacity; DW = dry–wetted. Values followed by a * within the same sampling day and between dry–wet treatments, are significantly different. Values followed by a different lowercase letter within the same dry–wet treatment and among sampling days, are significantly different. Statistical significance determined at $P < 0.05$ according to Tukey's HSD mean separation test.

Between day 44 and day 74, fine iPOM significantly increased in DW macroaggregates (Fig. 5(b)). Wheat-derived microaggregate iPOM-C significantly increased over time in the control treatment (Fig. 5(c)). In the DW treatment, a strong increase in wheat-derived micro-aggregate iPOM-C was observed during the first two DW cycles, resulting in significantly higher iPOM-C concentrations in DW microaggregates compared to control micro-aggregates at d 44 (Fig. 5(c)). Between day 44 and d 74, changes in wheat-derived microaggregate iPOM-C did no longer occur in DW soil. At d 74, similar concentrations for wheat-derived coarse, fine and microaggregate iPOM-C were found in both control and DW treatments (Fig. 5(a)–(c)).

4. Discussion

4.1. Aggregation and microbial activity

Several studies have reported strong correlations between fungal growth and initial rise in aggregate stability (Molope et al., 1987; Bossuyt et al., 2001). According to Beare et al. (1993), fungal growth is enhanced in the presence of fresh residue and in the absence of soil disruptive forces. Increased fungal hyphal length and the concomitant increased deposition of extracellular polysaccharides contribute to macroaggregate formation (Lynch and Bragg, 1985). We observed no macroaggregate formation (Table 1) and a significant suppression of fungal biomass and activity (Fig. 2(a)) in the fungicide treated soil samples over the entire incubation period. In the −F treatment however, simultaneous increases in fungal biomass and macroaggregation were found (Figs. 1, 2(a), and Table 1). A linear correlation was calculated (data not shown) between fungal biomass and the percentage large macroaggregates ($r^2 = 0.93$). These observations suggest that fungi have a significant, positive effect on macroaggregate formation.

Non-target effects of the fungicide on bacterial biomass were absent in this study (Fig. 2(b)). Since higher amounts of bacterial biomass-C in +F control soil compared to +F DW soil did not lead to higher amounts of macroaggregates (e.g. at d 44) (Figs. 1 and 2(b)), we suggest that bacteria did not significantly affect the formation of macroaggregates in this study.

A first drying and fast wetting event significantly reduced aggregation (Table 1). Other investigators also noted higher rates of aggregate breakdown with faster wetting rates

(Utomo and Dexter, 1982; Bossuyt et al., 2001, in press). In our experiment, the amount of large macroaggregates (>2000 μm) was mostly reduced by drying and wetting (Table 1). Larger aggregates are expected to be more susceptible to drying and wetting due to a larger number of planes of weakness (Kay, 1990). According to the porosity exclusion principle (Dexter, 1988), large aggregates have larger pores and are less dense than small aggregates because large aggregates also contain larger pores between the small aggregates that comprise them. This results in a faster infiltration of water upon wetting and consequently a higher and faster pressure build up by entrapped air in large aggregates compared to small aggregates. Furthermore, stabilization of large aggregates requires larger organomineral associations or stronger attractive forces to bridge the pores between small aggregates composing large aggregates (Dexter, 1988). As a result, macroaggregates break up more easily upon drying and wetting compared to microaggregates.

Since, in our study, the amount of microaggregates (53–250 μm) increased after the first drying and wetting, but the amount of primary particles (<53 μm) stayed the same (Table 1), we suggest that upon drying and wetting, large macroaggregates break down into microaggregates rather than primary particles, and that stabilized microaggregates are not disrupted by rapid wetting. The same observations were made by Elliott (1986), Cambardella and Elliott (1993), and Six et al. (2000). These observations support the Tisdall and Oades (1982) model of aggregate hierarchy, in which macroaggregates are composed of an assemblage of microaggregates that are resistant against the disruptive forces of slaking.

In this study, the direct effect of drying and wetting on fungal and bacterial populations could not be discerned because microbial biomass was not determined immediately after drying and wetting. However, some interesting observations could be made regarding the dynamics of microbial biomass and activity upon drying and wetting. Responses of soil biomass to drying can be attributed to its location in the soil (Van Gestel et al., 1991). Fungi are often located in larger pores and therefore suggested to be more sensitive to drying compared to bacteria, located in smaller pores, because water is retained longer in smaller pores than in larger pores (Tisdall and Oades, 1982; Hattori, 1988). This could be a possible reason why fungal biomass-C was lower in the DW compared to the control treatment after two DW cycles (d 44) (Fig. 2(a)). The sensitivity of fungi to drying and wetting can also explain why fungi are less abundant in tilled soil compared to no-tilled soil (Beare et al., 1993), since tillage continually exposes new soil to dry–wet cycles (Beare et al., 1994; Paustian et al., 1997). In the fungicide treatment, no macroaggregates were formed (Fig. 1) and bacterial biomass-C was significantly lower at d 44 for DW soil compared to control soil (Fig. 2(b)). In the presence of macroaggregates (−F treatment) on the other hand, bacterial biomass-C was higher in DW soil compared to control soil (Fig. 2(b)). Furthermore, in the +F soil, significantly higher respiration rates were observed for the control treatment compared to the DW treatment (Fig. 3), indicating that bacterial activity is reduced by dry–wet cycles in the absence of macroaggregates. Therefore, we hypothesize that macroaggregates physically protect bacterial populations against drying and wetting forces. According to Hattori (1988), bacterial populations are more stable in the inner sites of aggregates than on external surfaces, where they are more exposed to fluctuations in the soil environment (e.g. drying and wetting). Since fungi are located on the outer surfaces of aggregates (Hattori, 1988), they should be more sensitive to dry–wet cycles than bacteria. Interestingly, bacterial biomass-C increased between d 14 and d 44 in the −F DW treatment (Fig. 2(b)). According to Lund and Goksoyr (1980), restoration after air-drying and rewetting can also differ for bacteria and fungi. It has been observed that bacteria are able to use C resources made available by air-drying almost immediately after rewetting for growth (Scheu and Parkinson, 1994).

In this study, it was difficult to evaluate the exact mechanisms for the observed microbial population dynamics because drying and wetting can also have several indirect effects. First, competition for available substrate could exist between fungal and bacterial populations and can be affected by drying and wetting: drying can affect certain microbial groups, and therefore providing an extra pool of substrate (dead biomass) to other microbial groups. When using a fungicide, one group of microorganisms (fungi) was already suppressed and the effect of drying and wetting on substrate availability could be less. Second, soil structure (aggregation) can be altered by physical forces such as drying and wetting which then can affect microbial populations and their sensitivity to drying and wetting.

The observed increase in aggregation in both DW and control soil between d 17 and d 44 indicates that easily decomposable residue was still available for microbial activity and consequently aggregate binding agents were still being produced. In the DW treatment, previously destroyed aggregates were reformed using the once inaccessible organic matter, released by drying and wetting forces (Sorensen, 1974; Van Gestel et al., 1991). This is confirmed by the higher wheat-derived respiration rates observed in the DW treatment compared to the control treatment 2 d after the first drying and wetting (Fig. 3). Several authors have proposed that observed increases in C-mineralization upon drying and rewetting could be attributed to an increase in more water-soluble, easily decomposed material (Birch, 1960; Van Gestel et al., 1993) or to microbial death due to dessication or rapid rewetting (Kieft et al., 1987). However, the effect of drying and rewetting per se on organic matter decomposition may be less than usually assumed. Magid et al. (1999) reported no increase in the respiration from SOM upon drying and rewetting per se. However, the respiration from SOM consistently increased by heating, which accompanies the process of drying and

wetting in most studies (Birch, 1960). According to Adu and Oades (1978), mechanical disruption of aggregates by drying and wetting and the subsequent release of organic matter may be as important as chemical and biological factors in causing a flush of microbial activity upon drying and wetting. This could indicate that temperature change and physical disruption that may accompany drying and wetting experiments may be more important for C-mineralization than drying and wetting per se. In our study, control and DW treated soil samples showed similar amounts of 'total' respired carbon at d 2 of the first dry–wet cycle (data not shown), even though control soil was not subjected to drying and wetting cycles, but was stored for three d at 4°C prior to the start of the incubation. Microbial activity in the control treatment was probably enhanced due to the reactivation of microbial populations after bringing the samples back to room temperature. However, the 'wheat-derived' respiration rate was significantly lower in the control treatment compared to the DW treatment (Fig. 3). This suggests that the difference in wheat-derived respiration rate between DW and control treatments was mainly attributed to a release of previously protected wheat residue upon drying and fast wetting.

Aggregate disruption was increased by drying and wetting, which resulted in a faster aggregate turnover induced by dry–wet cycles. However, no further aggregate breakdown was observed after the third drying and wetting (Fig. 1). Consequently aggregate turnover was no longer affected by drying and wetting forces. We hypothesized that the aggregates at this point no longer broke up upon fast wetting because they became slake-resistant with time. This hypothesis was corroborated by a separate test of slake-resistance of the aggregates obtained by wet sieving. Since the upper half of the core was used to measure the aggregate size distribution of the soil sample at field capacity, we used the bottom part to determine the percentage of aggregates that were slake-resistant. This was done by air-drying the soil sample prior to wet sieving (i.e. slaking). We noticed that slake-resistant aggregates were present at d 44, but were absent at d 0 and 14 (data not shown). Since microbial activity was lower during the second DW cycle than during the first (Fig. 3), we suggest that most of the new aggregates formed after d 17 were formed during the first DW cycle (between d 17 and d 29). Therefore at d 44, DW macroaggregates formed between d 17 and d 44 have already a considerable age and consequently a high stability (Utomo and Dexter, 1981; Kemper and Rosenau, 1984). In contrast, according to Bossuyt et al. (2001), during the first 14 d of incubation, DW macroaggregates were formed after approximately 8 d but were not slaking resistant. Therefore, DW macroaggregates were much younger and consequently less stable against fast wetting at d 14 compared to DW macroaggregates at d 44 (Fig. 1). Greater stability of aged compared to newly formed aggregates has also been observed by Utomo and Dexter (1981) and Kemper and Rosenau (1984). Moreover, the increasing stability of DW macroaggregates over time can be attributed to frequent exposure to drying and wetting. Disintegration of macroaggregates into microaggregates and primary particles due to fast wetting, allows the particles to settle into more packed configurations which results in a greater cohesion upon the next drying event (Kemper and Rosenau, 1984). Interestingly, a maximum in wheat-derived respiration rate was no longer observed two d after the third DW event (d 49 of the incubation) (Fig. 3). This could be partly attributed to the depletion of the fresh and easily decomposable organic matter pool. Furthermore, after two dry–wet cycles, aggregates became resistant to slaking, and therefore physically protected organic matter was no longer released upon drying and wetting and remained inaccessible for microbial attack.

After the third drying and wetting, control soil samples had higher wheat-derived respiration rates compared to DW soil (Fig. 3). This is in accordance with our observations for aggregate turnover. In the DW treatment, most aggregates became slake-resistant after two dry–wet cycles (data not shown). Therefore more organic matter remained occluded in macroaggregates and physically protected against microbial attack. In contrast, after 44 d of incubation of soil at FC, control aggregates became unstable and started disaggregating (Fig. 1). We suggest that after 44 d, free and easily decomposable organic reserves were depleted. The resulting decrease in microbial activity and production of binding agents caused a halt in the formation of new macroaggregates. Perhaps control macroaggregates even became unstable because of the transient nature of binding agents (Tisdall and Oades, 1982). Consequently, macroaggregate disruption outweighed new macroaggregate formation. Upon aggregate disruption, there is a release of microaggregates, mineral particles (Table 1) and POM, which is then readily available for microbial attack.

4.2. Free light fraction (LF) and intra-aggregate particulate organic matter (iPOM)

In this study, one of the objectives was to investigate the effect of drying and wetting on the dynamics of aggregate associated organic matter. More specifically, our hypothesis was that fine iPOM (53–250 μm) is sequestered under control conditions and that drying and wetting cycles cause a loss of fine iPOM due to enhanced macroaggregate turnover. The rationale behind this hypothesis is as follows: when macroaggregates are formed, free LF (= fresh residue) is incorporated as coarse iPOM (250–2000 μm). Under control conditions, this coarse iPOM is further decomposed into fine iPOM, whereas the formation of fine iPOM is inhibited by enhanced macroaggregate turnover (Six et al., 1998, 1999a) imposed by dry–wet cycles in the DW treatment.

In the untreated (−F) soil samples, wheat-derived free light fraction (LF) was strongly reduced after the first 14 d of incubation (Fig. 4). In this period, macroaggregates

were formed (Fig. 1) and wheat-derived coarse intra-aggregate POM (iPOM) increased (Fig. 5(a)). In the +F treatment, there was no formation of macroaggregates during the whole experiment and free LF did not change over time.

These observations confirm the concept of free LF serving as a nucleation site for macroaggregation (Jastrow, 1996; Six et al., 1998). Light fraction material can function as a nucleation site for microbial activity, resulting in the binding of soil particles with concomitant incorporation of the free LF into macroaggregates as coarse iPOM. According to Six et al. (1999a), this coarse iPOM is further fragmented and decomposed into fine iPOM in macroaggregates that turn over more slowly. In the control treatment, we observed a strong decrease of wheat-derived coarse iPOM (Fig. 5(a)) with concomitant significant but small increase of wheat-derived fine iPOM (Fig. 5(b)) between d 14 and d 74. Since iPOM-C concentrations were expressed on a sandfree aggregate basis, the coarse iPOM decrease is partly attributable to a decrease in sand content in large macroaggregates (from 43.2 to 35.8%; data not shown). However, the amount of fine iPOM-C per kg sandfree macroaggregates increased concomitant with decreasing sand content in large macroaggregates, indicating that this is really attributed to an increase in fine iPOM-C.

After the first drying and wetting (d 17), a significantly higher amount of coarse iPOM-C was noticed compared to the control treatment (Fig. 5(a)). According to Six et al. (1999a), the concentration of coarse iPOM in the aggregate is related to the stability of the aggregate. In our experiment, the DW macroaggregates obtained at day 17 all survived the first drying and wetting and have consequently a high stability. In the control treatment, there are probably also macroaggregates that are not stable against DW, and contain consequently less coarse iPOM. Therefore, on an average aggregate basis, there is more coarse iPOM in the DW than in the control treatment. Moreover, we found more coarse sand per aggregate in DW than in control soil at day 17. This is probably due to a dilution of sand in the control treatment, because more macroaggregates were found in the control than in the DW soil at d 17. Since we express iPOM concentrations on a sandfree aggregate base, this causes higher values for the coarse iPOM per sandfree aggregate in the DW treatment.

At d 44, wheat-derived fine iPOM was significantly higher in control macroaggregates compared to DW macroaggregates (Fig. 5(b)). The accumulation of fine iPOM is enhanced in the presence of macroaggregates that turnover more slowly because decomposition and fragmentation of coarse iPOM into fine iPOM occurs at a slow rate within the aggregate (Six et al., 1998, 1999a). The slow rate of fine iPOM formation is partly due to the physical protection within the aggregate and partly due to the more chemically recalcitrant nature of the partially decomposed iPOM. Consequently, fine iPOM could be formed out of coarse iPOM within control macroaggregates because they had a slower turnover compared to DW macroaggregates. The enhanced macroaggregate turnover under DW resulted in a release of coarse iPOM, which became part of the free LF. This is confirmed by the significantly higher free LF-C concentrations that were found after fast wetting compared to the control treatment (Fig. 4). Free LF is more available for microorganisms and therefore more easily decomposable than POM protected within aggregates. After the first drying and wetting, wheat-derived respiration was also higher in DW compared to control soil samples (Fig. 3). However, this fast decomposition of released coarse iPOM can result in a pool of fine POM, which subsequently can become reincorporated in new macroaggregates and accumulate as fine iPOM in DW macroaggregates. Therefore, we hypothesize that the enhanced accumulation of fine iPOM in control versus DW macroaggregates is not only attributed to the degradation of coarse iPOM into fine iPOM, but also to an enhanced formation of microaggregates within macroaggregates, which leads to an increased physical protection of the fine iPOM (Jastrow, 1996; Six et al., 1999a).

From d 17 to d 74, the amount of wheat-derived LF-C slightly decreased in both control and DW treatments (Fig. 4), which can be attributed to further decomposition of fresh wheat residue (LF-C) and further incorporation of free LF into new macroaggregates. However, this decrease in free LF was not as fast as during aggregate formation, where also much higher respiration rates were noted compared to respiration rates in the following period (Fig. 3). These observations suggest that easily decomposable free organic reserves were strongly diminished after d 17.

Wheat-derived microaggregate iPOM-C significantly increased over time in the control treatment (Fig. 5(c)). This suggests microaggregate formation occurs through the binding of primary particles and wheat-derived POM. In the DW treatment, a strong increase in wheat-derived microaggregate iPOM-C was observed during the first two dry–wet cycles, resulting in significantly higher iPOM-C concentrations in DW microaggregates compared to control microaggregates at d 44 (Fig. 5(c)). We hypothesize that the breakdown of DW macroaggregates due to drying and wetting resulted in a release of sand, wheat-derived coarse iPOM, and microaggregates which were previously formed around wheat-derived fine iPOM within the macroaggregates. Consequently, after two DW cycles, more wheat-derived fine iPOM became part of the DW microaggregate fraction, whereas in control soil, fine iPOM accumulated inside macroaggregates. As previously mentioned, DW macroaggregates became slake-resistant after two dry–wet cycles (d 44). Between d 44 and d 74, an increase in wheat-derived fine iPOM occurred in the DW treatment, confirming the hypothesis that coarse iPOM decomposes into fine iPOM when aggregates are not disturbed (Six et al., 1999a). Consequently, no additional increase in DW microaggregate iPOM was observed (Fig. 5(c)).

5. Conclusions

We found that fungi are responsible for the formation of water-stable macroaggregates, as long as fresh wheat residue is readily available in the soil, and that these water-stable macroaggregates could be responsible for the protection of bacterial populations against drying and wetting forces. Bacterial contributions to aggregate formation appeared to be insignificant in our study.

When comparing aggregate dynamics in soil samples kept at field capacity (control) and soil samples subjected to dry–wet cycles, we found that drying and wetting strongly enhanced aggregate turnover in the first two dry–wet cycles. However, after two dry–wet cycles, aggregates became stable and slake-resistant. We suggest that dry–wet cycles do not affect aggregate turnover at this stage in the aggregate 'life' cycle.

During the first 14 d, macroaggregates were formed concomitant with a reduction of free LF and an increase in wheat-derived coarse iPOM. This indicates that during aggregate formation, free LF, derived from fresh applied wheat residue, was incorporated into aggregates as coarse iPOM. Drying and wetting strongly affected the amounts of POM incorporated into aggregates. We found a lower accumulation of wheat-derived fine iPOM in DW soil during the first two dry–wet cycles, compared to soil kept at field capacity (control). This could be attributed to a slower aggregate turnover in control soil and consequently in more time for the formation and accumulation of wheat-derived fine iPOM. In soil samples subjected to dry–wet cycles, on the other hand, macroaggregates turnover too fast for the accumulation of wheat-derived fine iPOM. Only when aggregates became slake-resistant (d 44), did wheat-derived fine iPOM accumulate in these aggregates.

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