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Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil

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Abstract

Aggregate stability often exhibits a large inter-annual and seasonal variability which occurs regardless of residue treatments and is often larger than the differences between soils or cropping systems. Variations in soil moisture and seasonal stimulation of microbial activity are frequently cited as the major causes. The goal of this paper was to evaluate the effects of drying–rewetting cycles on aggregate stability and on its main microbially mediated agents from a mechanistic point of view. The 3–5 mm aggregates of a silty soil were incubated at 20 °C for 63 days with the following treatments and their combinations: (i) with or without straw input and (ii) with or without exposure to four dry–wet cycles. Microbial activity was followed by measuring the soil respiration. We estimated the microbial agents of aggregate stability measuring hot-water extractable carbohydrate-C, microbial biomass carbon and ergosterol content. We measured the water drop penetration time to estimate the hydrophobicity and aggregate stability according to Le Bissonnais [1996. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 47, 425–437] to distinguish three breakdown mechanisms: slaking, mechanical breakdown and microcracking. The addition of straw stimulated microbial activity and increased the resistance to the three tests of aggregate stability, enhancing the internal cohesion and hydrophobicity of aggregates. All the estimated microbial agents of aggregate stability responded positively to the addition of organic matter and were highly correlated with aggregate stability. Fungal biomass correlated better with aggregate stability than total microbial biomass did, showing the prominent role of fungi by its triple contribution: physical entanglement, production of extracellular polysaccharides and of hydrophobic substances. Dry–wet cycles had less impact on aggregate stability than the addition of straw, but their effects were more pronounced when microbial activity was stimulated demonstrating a positive interaction.

Keywords: Aggregate stability; Dry–wet cycles; Le Bissonnais method; Water drop penetration time; Ergosterol; Hot-water extractable carbohydrate-C

1. Introduction

Aggregate stability often exhibits large inter-annual and seasonal variability. Aggregate stability is usually lowest during winter and increases in spring. Such variations occur regardless of residue treatments and are often larger than the differences between soils or cropping systems (Perfect et al., 1990; Angers et al., 1999). Two factors are mainly controlling these fluctuations: climate and organic matter incorporations. Climate can directly influence aggregate stability through its action on soil moisture (Perfect et al., 1990) and indirectly through seasonal stimulation of microbial activity.

Soil moisture affects aggregate stability in several ways. First, the soil water content at the moment of the test impacts slaking. The extent of slaking decreases as the initial moisture content increases until saturation is reached (Panabokke and Quirk, 1957). This effect is particularly evident in soils with low contents of organic matter (Haynes, 2000). Given the impact of sample physical

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conditions, such as aggregate size and soil moisture content before applying the test (Amézketa, 1999), it is obvious that the lack of a satisfactory standard methodology is a problem in this field (Le Bissonnais, 1996). The methods are, as well, often poorly described (Yang and Wander, 1998). The soil water content at the time of sampling thus impacts aggregate stability when it is measured on field moisture samples (Perfect et al., 1990). Furthermore, even though aggregate stability is measured on air-dried samples, the antecedent water content has been shown to affect aggregate stability (Caron et al., 1992). The effect of moisture on aggregate characteristics cannot be generalized and, alone, have no consistent effect on soil aggregate size and stability (Yang and Wander, 1998). Instead, soil moisture interacts with other events to influence aggregation.

It is not easy to link the influence of dry–wet cycles on macroaggregation as they can affect it directly through physical or chemical process (Utomo and Dexter, 1982) and/or indirectly through their action on microbial activity (Denef et al., 2001). Effects of drying on soil structure are still unclear, since both increases and decreases in water stable aggregation have been observed following drying (Denef et al., 2001). The contradictory results found in the literature can be explained by different initial physical conditions of the aggregates, organic matter contents, intensities and durations of drying and rewetting phases and aggregate stability methods. The term 'initial physical conditions' refers particularly to soil water content and aggregate size. Finally, the cohesion of macroaggregates can be modified by aging or thixotropy. As suggested by Suwardji and Eberbach (1998), inter-annual and seasonal variability in aggregate stability may result from seasonal wetting and drying interacting with the accumulation of plant and microbial debris associated with the growing crop.

The incorporation of organic matter to soil may also largely contribute to intra-annual variations in aggregate stability. It is well established that the addition of organic matter to soil increases aggregate stability within a few weeks due to the stimulation of microbial decomposers. Microorganisms increase the stability of aggregates in several ways. Fungi act mainly by mechanical enmeshment of soil particles (Degens et al., 1996), bacteria and fungi may exude extracellular polysaccharides which bond the particles and increase interparticle cohesion (Chenu and Guérif, 1991). Microorganisms have also been observed to increase the repellency of soil aggregates, presumably by exuding hydrophobic substances (Capriel et al., 1990; Hallett and Young, 1999). This may stabilize the aggregates by decreasing their rate of wetting.

Notwithstanding the complexity of factors that govern the short-term variations in aggregate stability in relation to soil moisture and organic matter additions, not too much effort has been given to understand these variations from an aggregate breakdown mechanistic point of view. Predictions of long-term changes in aggregate stability require a good record of the variability encountered within a single year, involving both a statistical description (Caron et al., 1992) and a mechanistic understanding of the factors governing aggregate stability. Such mechanistic approach is facilitated by the use of a method, based on three tests combining two liquids (ethanol and deionized water), that achieves the distinction of three basics breakdown mechanisms: slaking, mechanical breakdown and microcracking (Le Bissonnais, 1996).

The general objective of our study was to better understand short-term variations in aggregate stability for a temperate silty loam cultivated soil in order to improve its prediction. The goal of this paper is thus to evaluate the net effects of dry–wet cycles on aggregate stability and on its main microbially mediated agents. We hypothesized that there was an interaction between dry–wet cycles and microbial agents of aggregation. To evaluate this position, we carried out an experiment over 63 days under controlled conditions in the laboratory where we managed dry–wet cycles and microbial activity separately. We investigated the changes in aggregate stability in terms of slaking, mechanical breakdown and microcracking.

2. Materials and methods

2.1. Soil and sampling

We collected a surface soil (0–20 cm) from the "La Cage" plots of the experimental site of the Institut National de la Recherche Agronomique—INRA—(48°48′29″N, 2°04′58″E), in Versailles city, France. The climate is temperate with an annual rainfall of 639 mm yr^{-1} (1928–2003) and 10.5 °C annual mean temperature.

The soil was a silt loam Luvisol with a texture of 167 g kg^{-1} clay, 562 g kg^{-1} silt and 271 g kg^{-1} sand, with a total carbon content of 9.2 g kg^{-1}, C_t/N_t: 10.5 and pH(H_2O) of 7.0. The plot had been cultivated for more than 50 yr with conventional tillage (mouldboard plow at 0–30 cm) with a rotation based on wheat (*Triticum aestivum* L.), colza (*Brassica napus* L.) and pea (*Pisum sativum* L.).

In September 2003, 1 week after the seeding of colza, we carefully sampled the soil at a water content of 0.18 g H_2O g^{-1} soil. This work was carried out manually, using a shovel, to avoid disturbing the natural structure of the soil as much as possible. The larger clods were gently crumbled by hand at field moisture along their natural fissures and sieved to obtain an adequate amount of aggregates between 3.15 and 5 mm (hereinafter referred to as 3–5 mm aggregates). Great care was taken to avoid damaging the natural aggregates. After sieving, coarse organic matter (free roots and plant debris) was removed with tweezers and the soil was then stored in the dark at 4 °C for 2 months (~0.15 g H_2O g^{-1} soil).

The aggregates were stocked in a plastic box at 20 °C for 10 days before incubation to minimize the variations in

microbial activity due to changes in temperature conditions (Kiem, and Kandeler, 1997).

2.2. Incubation and experimental treatments

Sixty cores with 3–5 mm aggregates were incubated at 20 °C ± 0.5 °C for 63 days with the following treatments and their combinations: (i) without (−ST) or with (+ST) straw input (4 g C kg^{-1} soil) and (ii) without (W) or with (DW) exposure to four dry–wet cycles, characterized by a fast air drying and a slow rewetting to field capacity. Treatment −ST W was the control treatment; it was kept at field capacity (0.195 g g^{-1}, Ψ—10 kPa) and with no straw input. At field capacity the porosity was 42.8% filled by water. The straw was from maize (*Zea mays* L.) stems and leaves ground between 200 and 500 μm (414.5 g C kg^{-1}; C/N: 54.7).

We placed the equivalent of 80 g dry sol (105 °C, 24 h) in plastic cylinders (diameter = 7.9 cm, height = 2.4 cm) closed at the bottom by a nylon cloth (20 μm mesh) suspended in sealed 1 l glass jars with 40 ml of deionized water at the base to minimize desiccation. The samples were carefully packed to obtain a bulk density of 1.2 Mg m^{-3}. A beaker containing NaOH was also placed in the jars to trap and measure the CO_2 produced during incubation.

The samples with added maize straw were cautiously mixed and immediately sprayed with a solution of NO_3NH_4 to adjust the sample C/N ratio to 10 and its water content to field capacity. The added N suppresses the limiting effect of N during the decomposition of crop residues (Recous et al., 1995). The samples with no straw addition were treated in the same way, but with deionized water.

Four dry–wet cycles were started from day 7 of incubation and then every ~15 days (i.e. days 7, 21, 36 and 49). In each cycle (72 h) the samples were removed from the jars and put under a fan at room temperature (20 °C) for ~10 h until reaching the air-dry moisture content (0.012 g g^{-1}). Afterwards, the samples were slowly capillary-wetted to −3.1 kPa over a period of 48 h and to −10 kPa over a period of 14 h. The rewetting process was carried out on tension tables to minimize slaking. All samples were taken right before the start of each dry–wet cycle.

2.3. Measurements

To estimate soil respiration the evolved C-CO_2 trapped in NaOH was measured at days 2, 4, 7, 14, 21, 36, 49 and 63 in the treatments kept at field capacity. In those subjected to dry–wet cycles, the respiration was not measured during the cycles themselves (days 7–9, 21–23, 36–38 and 49–51).

At days 0, 7, 21, 36, 49 and 63 three replications per treatment were used to determine immediately in a moist subsample the microbial biomass carbon (MBC) by fumigation extraction according to Vance et al. (1987). We froze another subsample to measure within the week the ergosterol content (ERG) modified from Djajakirana et al. (1996) and Gong et al. (2001) ERG being a biomarker of fungi. The rest of the soil was dried at 40 °C during 48 h and an aliquot was taken to estimate the hot-water extractable carbohydrate-C (HWEC) (Puget et al., 1999). Then we carefully re-separated the soil into aggregates from 3.15 mm (here referred to as 3 mm) to 5 mm. From these aggregates, we assessed aggregate wettability by measuring the water drop penetration time (WDPT) as performed by Chenu et al. (2000) and aggregate stability (Le Bissonnais, 1996). Subsamples were also taken to measure and express all results at 105 °C 24 h standard moisture content.

To estimate MBC, the difference between soil carbon (C) extracted with 0.03 M K_2SO_4 from chloroform-fumigated and unfumigated soil samples (20 g) were measured with a TOC-5050A Shimadzu elemental analyzer ($K_c = 0.45$). The ERG content was estimated with a Waters 2695 HPLC using 3.5 g of moist soil extracted with 120 ml ethanol agitated with glass beads for 30 min. HWEC were obtained from the extract of 1 g of soil suspended in 20 ml of hot water (80 °C) for 24 h, the carbohydrate content of the extract was analyzed by the H_2SO_4/phenol method (Dubois et al., 1956).

2.4. Aggregate stability

We measured the soil aggregate stability according to Le Bissonnais (1996) to distinguish three breakdown mechanisms: slaking, mechanical breakdown and microcracking. Briefly, the test is performed on 3–5 mm aggregates, dried at 40 °C for 48 h. It involves three pre-treatments with different subsamples before sieving in alcohol at 50 μm and dry sieving of the resulting fraction (>50 μm): (i) Slow-wetting, where the aggregates are capillary rewetted with water on a tension table at a potential of −0.3 kPa for >60 min; (ii) fast-wetting, where 5 g of aggregates are immersed in deionized water for 10 min; and (iii) stirring after prewetting, where the aggregates are saturated in ethanol for 30 min, then agitated in deionized water in an Erlenmeyer end over end for 20 times. Dry sieving was performed by hand with a nest of six sieves (2000, 1000, 500, 200, 100 and 50 μm) and the mean weight diameter (MWD) was calculated as the sum of the mass fraction remaining on each sieve after sieving, multiplied by the mean aperture of the adjacent sieves.

2.5. Statistical analyses

We used analysis of variance (ANOVA, $4 \times 6 \times 3$ factorial design) at 6 sampling dates with three replications to determine the effects of straw and dry–wet cycles on MBC, ERG, WDPT, HWEC and structural stability. When effects were significant at a level of 0.05, means were tested with the Tukey test. For WDPT, we show the

medians of 20 measures per repetition analyzed by a non-parametric test (Kruskal–Wallis one-way ANOVA). Data are presented as mean or median values with standard errors. Pearson's correlations were made among variables.

3. Results

3.1. Biological variables

The respiration rates in +ST treatments were significantly ($P<0.05$) higher than the −ST ones throughout the entire incubation except for the last period (49–63 days) (Fig. 1). In the −ST samples the total microbial activity varied slightly during incubation whereas the +ST treatments showed a peak of the respiration rate at day 3 and its stabilization from day 28 at $<20\,\mu g\,C\text{-}CO_2\,g^{-1}$ soil per day. Only the first dry–wet cycle significantly increased the respiration rates, and it was more important in the +ST treatment.

The addition of straw strongly increased MBC at all sampling dates (Fig. 2), with a more persistent effect than on respiration rates. Incubations without straw affected MBC only slightly, showing a cyclical pattern over the incubation period. DW cycles affected MBC negatively up to the end of the experiment while maintaining a similar difference between +ST W and +ST DW treatments averaging $45\,\mu g\,C\,g^{-1}$ soil in the last three DW cycles. The effect of straw addition was even stronger on the ERG content, with a peak at day 7, and stabilization from day 49 (Fig. 3). It was the biological variable that showed the largest difference between the +ST and the −ST treatments, about ten times at day 7 and nearly six times at the end of the experiment. However, almost no effects of dry–wet cycles were observed on ERG content over the 63 days of the trial.

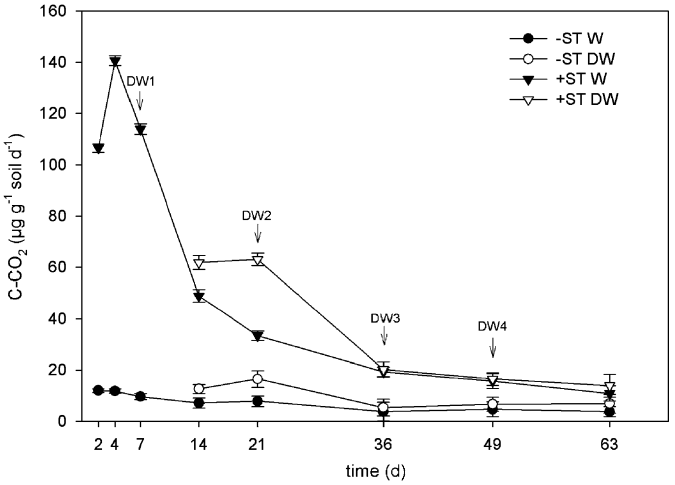

Fig. 1. Soil respiration during incubation. No added straw (−ST), added straw (+ST), dry–wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means. DW events are indicated with arrows.

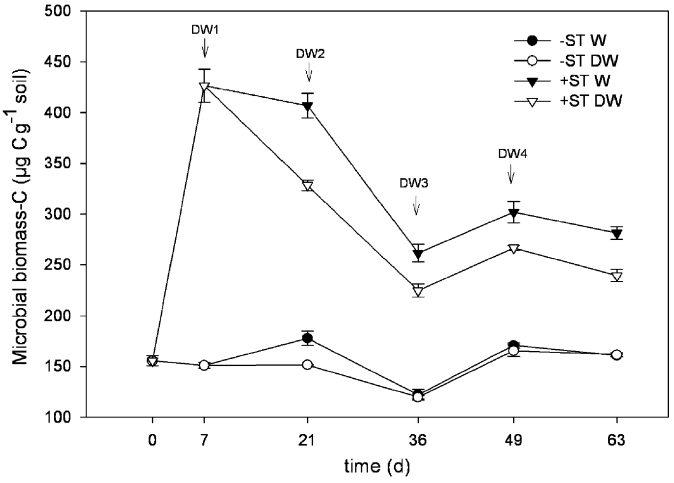

Fig. 2. Microbial biomass carbon in control (−ST), added straw (+ST), dry–wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means ($n=3$). DW events are indicated with arrows.

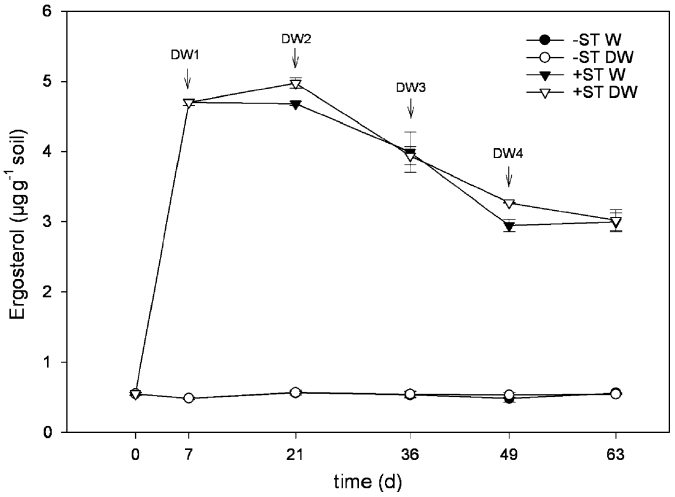

Fig. 3. ERG content in control (−ST), added straw (+ST), dry–wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means ($n=3$). DW events are indicated with arrows.

The amount of HWEC remained almost constant in the −ST treatments, averaging ca. $180\,mg\,C\,kg^{-1}$ (Fig. 4). Adding straw increased polysaccharide contents by 57% at day 7 to 47% at the end of incubation, with respect to −ST treatments. In the +ST treatments, HWEC decreased significantly after the second dry–wet cycle and remained low until after the fourth cycle, at which time the amounts of HWEC of both treatments became statistically the same.

3.2. Aggregate stability and WDPT

The silty soil used in this experiment exhibited very small MWDs after the tests at the day 0, showing its low aggregate stability: 0.8 mm after the slow wetting test, 0.3 mm after the fast wetting test and 0.9 mm after stirring

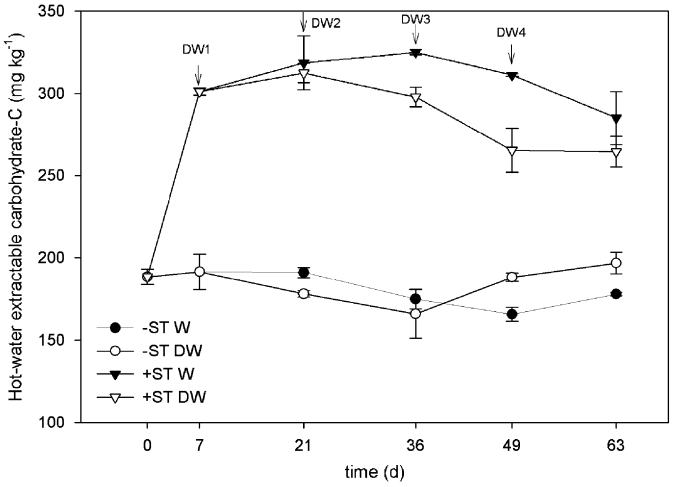

Fig. 4. HWEC in control (−ST), added straw (+ST), dry–wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the means ($n = 3$). DW events are indicated with arrows.

following the prewetting test (Fig. 5). The MWD of the −ST samples varied little during incubation.

Both slow and fast wetting tests showed a similar response over the incubation time after the addition of straw but at different scales of MWD, showing the different intensities and kinds of stresses applied (Fig. 5a and b). After the slow wetting test the MWD ranged between 0.6 and 2 mm, whereas after the fast wetting it was between 0.2 and 0.6 mm. In both cases, the addition of straw significantly increased the MWD by >110% and 60%, respectively, as an average from day 7 to day 63. The addition of straw also increased the resistance of aggregates to mechanical breakdown by about 28% (Fig. 5c).

The size distribution of aggregates after the tests, from which MWDs were calculated, is plotted in Figs. 6–8. When the slow wetting test was applied, the initial 3–5 mm aggregates were disrupted mostly to small macroaggregates 0.2–2 mm (69.6% mass), large macroaggregates >2 mm (9.7% mass), microaggregates (0.05–0.2 mm, 14.3% mass) and to <50 μm fraction (6.4% mass). The fast wetting test was much more disruptive and released mostly microaggregates (41.6% mass) and small macroaggregates (42.3% mass) (Fig. 6). The progressive breakdown of aggregates when subjected to increasing stresses (from slow rewetting and stirring after prewetting to fast rewetting) shows the hierarchical character of soil structure.

After the addition of straw, more macroaggregates (>2 mm) resisted the slow wetting test than in the control −ST W treatment (Fig. 7a and b). These were mainly formed at the expense of small macroaggregates. These large macroaggregates did not resist the fast wetting test (Fig. 8). However, small macroaggregates were stabilized at the expense of microaggregates.

In the −ST samples, the dry–wet cycles had no significant ($P < 0.05$) effect on aggregate stability (Fig. 5). However, in the +ST samples, they decreased the MWD from the first cycle in the slow wetting test. No variations

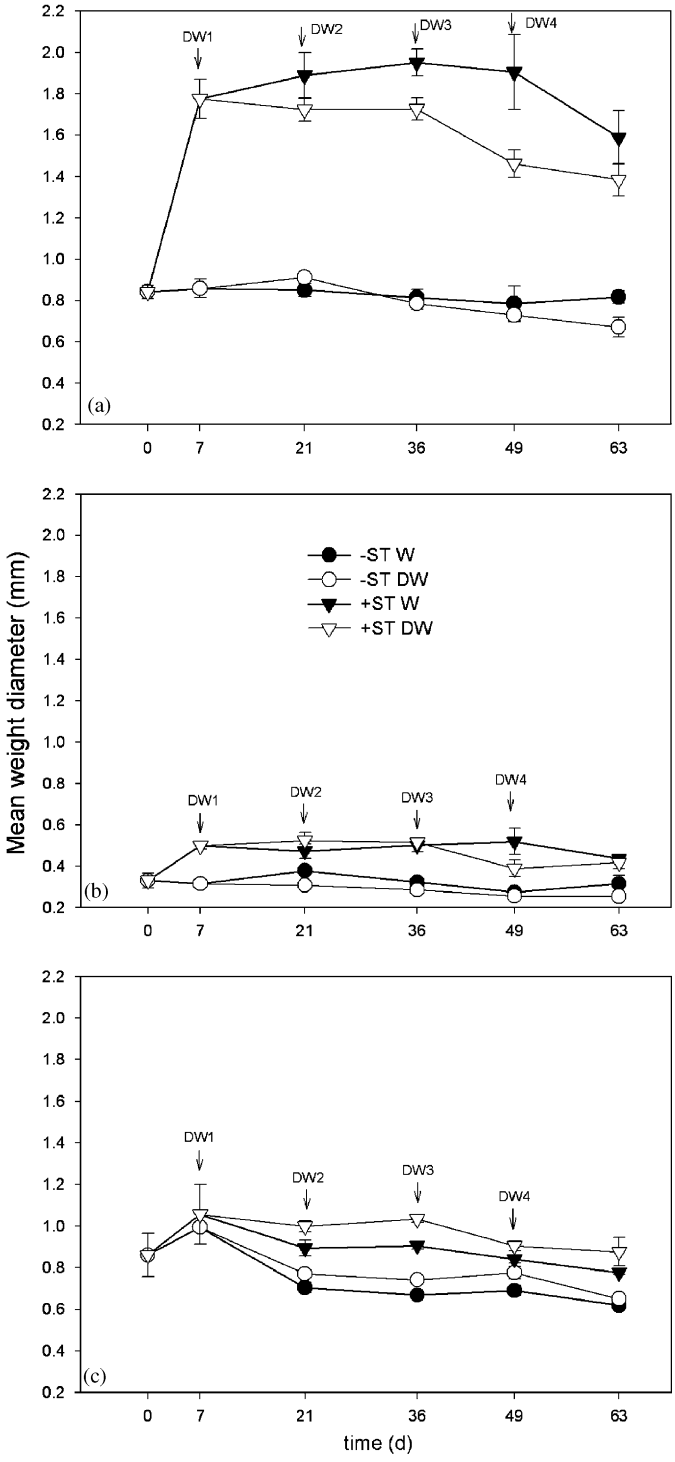

Fig. 5. MWD in control (−ST), added straw (+ST), dry–wet cycles (DW) and continuously wet (W) treatments for (a) slow wetting test, (b) fast wetting test and (c) stirring after prewetting test. Error bars represent the standard error of the means ($n = 3$). DW events are indicated with arrows.

could be measured in the fast wetting test but the dry–wet cycles increased the MWD in the stirring after prewetting test because of the increase in the proportion of macroaggregates (>0.2 mm). MWD decreased with incubation time for all treatments. Dry–wet cycles only slightly affected the size distribution (Fig. 7b and c).

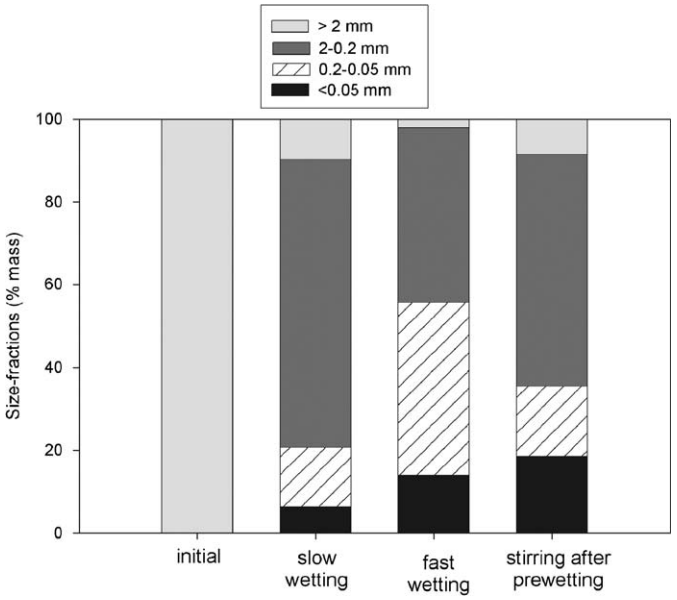

Fig. 6. Aggregate size distribution of the control sample at time 0, initially and after the three aggregate stability tests.

The aggregates of the silty soil were highly wettable, as shown by small WDPT (Fig. 9). The addition of straw increased WDPT up to 10 s after 1 week of incubation and it then remained stable from day 7 to day 63 (Fig. 9). The error bars of + ST treatments were higher than the control ones, showing a physical heterogeneity of the organic components that determine this variable. The WDPT was not affected by the DW cycles.

4. Discussion

4.1. Effect of organic matter addition on aggregate stability

Aggregate dynamics occur in three phases: formation from non-aggregated material, stabilization and breakdown (Tisdall and Oades, 1982). Here, by adding organic matter to 3–5 mm pre-existing aggregates, we focused on the stabilization of these pre-existing aggregates. These had very low stability, which is typical of silty cultivated soils from northern Europe.

Many studies showed a positive, short-term effect of adding organic matter on soil aggregate formation and stabilization (e.g. Kiem and Kandeler, 1997; De Gryze et al., 2005), which is due to the activity of microbial decomposers. Our results also showed a rapid increase of aggregate stability, which peaked 7 days after the beginning of the incubation. Straw had no effect at time 0. Therefore, all observed effects on aggregate stability were due to microbial activity. Furthermore, the method used here enabled us to analyze the results in mechanistic terms. Le Bissonnais (1996) reviewed the theories about the mechanisms of aggregate breakdown, highlighting three main elementary mechanisms for non-dispersed soils: (i) slaking, breakdown caused by compression of entrapped air during

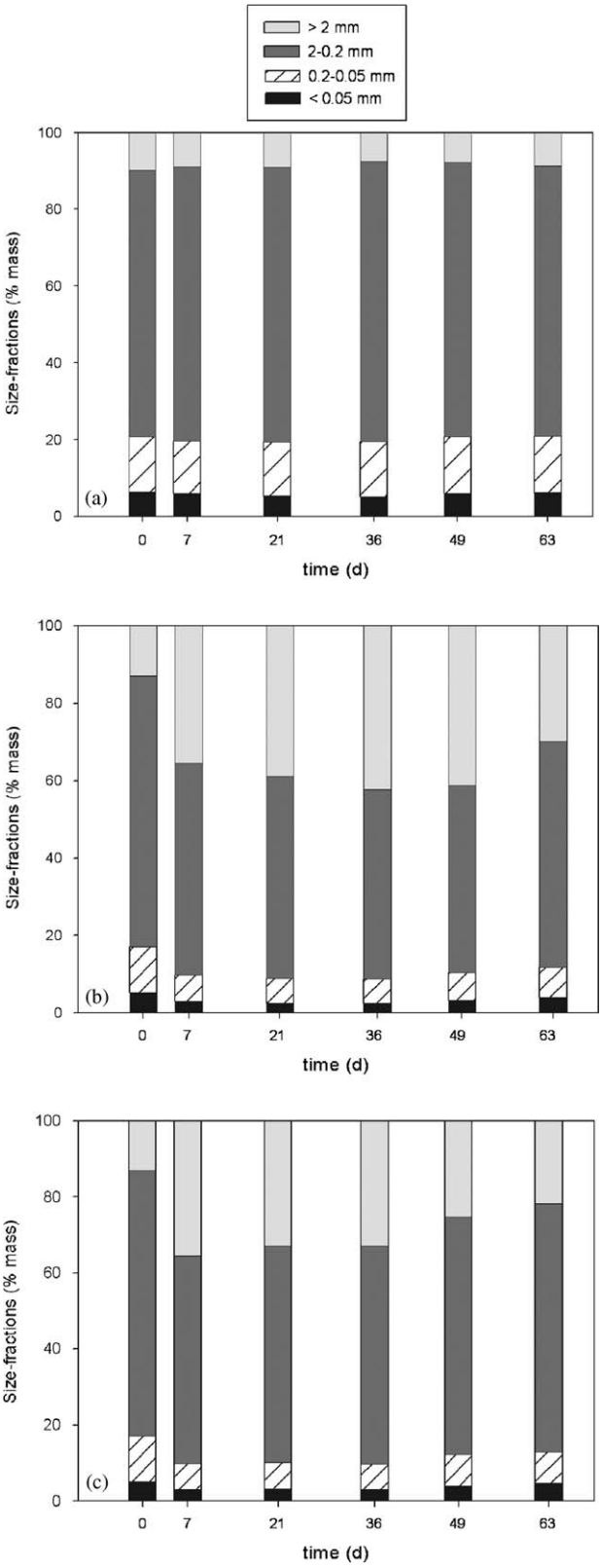

Fig. 7. Aggregate size distribution after the slow wetting test. (a) control continuously wet; (b) added straw, continuously wet and (c) added straw with dry–wet cycles.

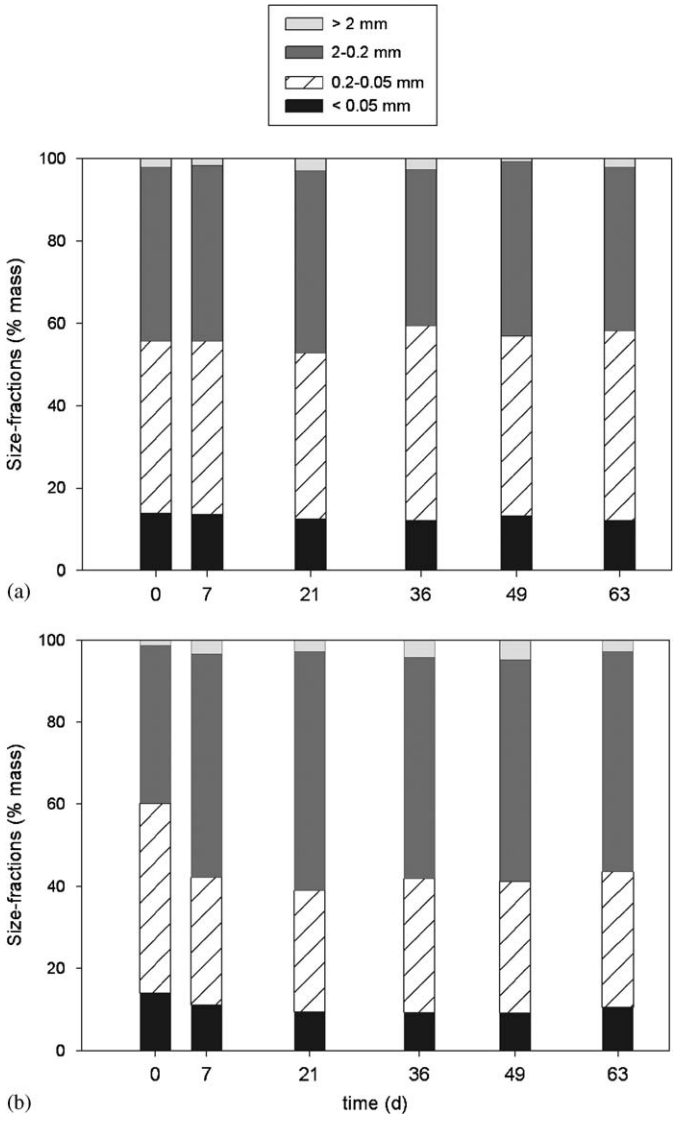

Fig. 8. Aggregate size distribution after the fast wetting test in continuously wet treatments; (a) control and (b) added straw.

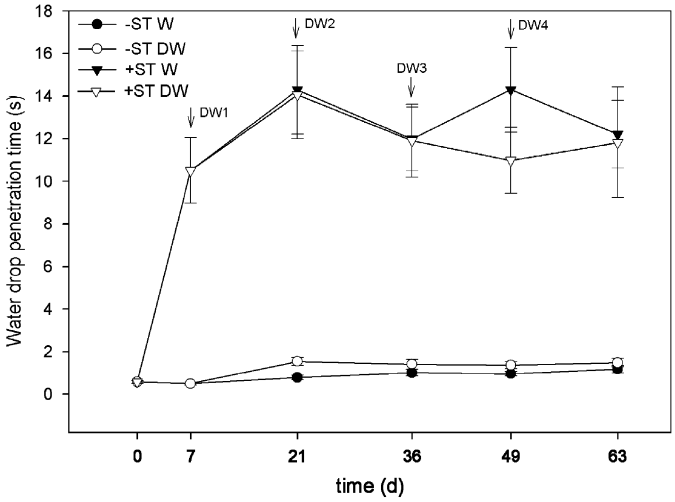

Fig. 9. WDPT in control, added straw (+ST), dry–wet cycles (DW) and continuously wet (W) treatments. Error bars represent the standard error of the medians ($n = 20$). DW events are indicated with arrows.

Table 1
Correlation coefficients (r) between measured variables

	Microbial biomass C	Respiration rate	Ergosterol	HWEC	WDPT
Fast wetting	0.785*	0.575	0.889*	0.844*	0.887*
Stirring after prewetting	0.560	0.618	0.613	0.645*	0.467
Slow wetting	0.868*	0.603	0.950*	0.957*	0.956*
WDPT	0.836*	0.521	0.942*	0.911*	–

HWEC: hot-water extractable carbohydrate-C; WDPT: water drop penetration time.
*Significant values at $P < 0.001$ ($n = 24$).

wetting; (ii) mechanical breakdown by raindrop impact and (iii) microcracking, or breakdown by differential swelling. The method we used attempts to separate these mechanisms (Le Bissonnais, 1996). The fast wetting test emphasizes the slaking, the stirring after prewetting test isolates the wet mechanical cohesion independently of slaking and the slow wetting test expresses mainly the breakdown of the aggregates by microcracking, although some slaking also takes place. Organic matter may stabilize aggregates in two ways: by increasing the interparticle cohesion and by increasing their hydrophobicity, thus decreasing their rate of wetting (Robert and Chenu, 1992). In addition, organic matter may modify the porosity of aggregates and thus the extent of slaking.

The net, although small effect of straw addition on MWD after the stirring after prewetting test (Fig. 5c), showed that microbial activity increased the internal cohesion of aggregates. WDPT increased after straw addition, which showed that microbial activity increased the hydrophobicity of aggregates, in line with results from Hallett and Young (1999). Increased stability of aggregates to fast and slow wetting tests, i.e. increased resistance to slaking and microcracking, involved increased hydrophobicity of aggregates. Accordingly, in our experiments, the WDPT correlated well with the MWD after fast wetting test and with the MWD after slow wetting test, but it was not with the MWD after the stirring after prewetting test (Table 1). Effects of microbial activity on the pore size distribution of aggregates cannot be ruled out and were not studied in this work. For example Preston et al. (1999) showed that the stimulated microbial activity produces less heterogeneous and less connected soil cracks, suggesting that polysaccharides and fungal hyphae not only contribute to aggregate stabilization through a bonding action, but also change the heterogeneity and connectivity of cracks, diminishing the negative breakdown of microcraking.

4.2. Microbial agents of aggregate stability

It is well established that when fresh organic matter is added to soil, bacterial and fungal populations increase

and stabilize aggregates by (i) direct enmeshment of aggregates and particles by fungal hyphae (Degens, 1997), (ii) production of extracellular polysaccharides by bacteria and fungi which glue mineral particles (Chenu, 1995), and (iii) production of hydrophobic substances (Capriel et al., 1990).

All measured microbial agents of aggregate stability (microbial polysaccharides, fungal biomass and hydrophobic compounds by measuring HWEC, ERG content and WDPT, respectively) increased after addition of organic matter and were highly correlated with the slow and fast wetting tests (Table 1).

We used the ERG content as an indicator of fungi. It was better correlated with aggregate stability than the microbial biomass. This is in agreement with several studies, based either on the use of bactericides and/or fungicides (Hu et al., 1995; Bossuyt et al., 2001), or on correlations (Chantigny et al., 1997). This prominent role of fungi compared to that of bacteria can be explained by the contribution of fungi to the three mechanisms cited above: physical entanglement, production of extracellular polysaccharides and production of hydrophobic substances. Here, high-correlation coefficients between ERG and MWD for fast-wetting and slow-wetting aggregate stability tests were consistent with occurrence of the three roles of fungi. Furthermore, the close correlation of ERG with WDPT is consistent with an increased hydrophobicity due to fungi.

HWEC are a fraction relatively enriched in polysaccharides from microbial origin (Haynes and Francis, 1993) and are thus assumed to represent extracellular polysaccharides. HWEC is a pool of C involved in changes in aggregate stability occurring over relatively short time-periods (Puget et al., 1999). In our experiments HWEC was significantly correlated with aggregate stability. The test the more closely related was the slow wetting test (Table 1) and the time patterns of both variables were surprisingly similar (Figs. 4 and 5a). Thus, in agreement with Kiem and Kandeler (1997), aggregate stability depended more on the production of binding substances by microorganisms, than on microbial numbers.

4.3. Dry–wet cycles net effects

Dry–wet cycles had strong effects on microbially derived variables over the first cycle: soil respiration was stimulated, MBC decreased, and ERG content slightly increased. The following cycles had no effect on respiration, no additional one on MBC, none on ERG and HWEC decreased. Magid et al. (1999) stated that in most papers showing a significant increase in respiration, the drying–rewetting process was accompanied by other physical changes: manual perturbation or increased temperature. We carefully manipulated the samples in such a way that no structural degradation and minimal temperature change occurred. The drying was done in only ~10 h. In addition, we rewetted the samples slowly to field capacity (−10 kPa). We assume, therefore, that previously protected organic matter was not de-protected by dry–wet cycles. Thus, as only the first dry–wet cycle significantly affected the respiration rate in the +ST treatment (Fig. 1), we attribute it to the decomposition of the added straw which was not decomposed during drying (Magid et al., 1999) and, as microbial biomass decreased by 15–19%, to the death of microorganisms. Subsequent dry–wet cycles did not affect respiration rates probably because the substrate became less available. Sorensen (1974) indicated that the effect of dry–wet cycles on the decomposition of organic matter decreases the longer the organic material is incubated in the soil. This suggests, according to Degens and Sparling (1995), that the dry–wet cycles have a stronger effect on mineralization of labile pools of organic matter.

Literature about the effects of dry–wet cycles on fungal and bacterial communities is not clear. Shipton (1986) reports that fungi can remain active in soils at very low water potential, contrarily to bacteria. Hattori (1988) suggested that fungi are more sensitive to drying and wetting because of their location on the outer surfaces of aggregates. West et al. (1987) and Scheu and Parkinson (1994) found no consistent trends in the fungal and bacterial biomass after air-drying soils, indicating that the susceptibility of bacteria and fungi to drying is not generally different, and would rather depend on the bacterial and fungal communities growing in different soil materials. In our experiment, the ERG content was unaffected by dry–wet cycles, whereas total microbial biomass was. Hence fungi appeared to be more resistant than bacteria to dry–wet cycles, perhaps because of the short period of drying and the water potential applied ($\Psi \ll -1.5$ MPa).

HWEC were also affected by dry–wet cycles, especially in the +ST treatment, however, the decline in HWEC occurred later than that of microbial biomass and increased with time. Both bacteria and fungi can synthesize and exude extracellular polysaccharides and one study reported an increase in extracellular polysaccharides production by bacteria with drying (Roberson and Firestone, 1992). Here, we suggest that the decrease in HWEC with dry–wet cycles, is due to bacterial death and that HWEC have a longer residence time that microbial biomass.

Several experiments report that dry–wet cycles disrupt aggregates and thus diminish the proportion of stable aggregates (Utomo and Dexter, 1982; Denef et al., 2001). In most of these experiments, the wetting is sudden and slakes the aggregates. In our case, we slowly rewetted the aggregates by capillarity. We incubated 3–5 mm aggregates and most of them kept their initial size over the dry–wet cycles (results not shown). However, their stability varied. The dry–wet cycles had no effect on the stability of aggregates to slaking, decreased their stability to slow rewetting and increased their stability to the stirring after prewetting test (Fig. 5). Microcracks are created when rewetting slowly aggregates, because of differential swelling

(Le Bissonnais, 1996). Here, we propose that the differential swelling and shrinkage during dry–wet cycles created microfissures that became failure zones when the aggregates were exposed to the slow wetting. It created stable aggregates of small size and this effect of dry–wet cycles was more pronounced with straw than without, showing an interaction with microbially mediated aggregation. This interaction was probably due to the detrimental effect of dry–wet cycles on bacteria and on extracellular polysaccharides production, which resulted in less binding agents in aggregates that are subjected to more stresses.

Dry–wet cycles had no impact on MWD after the rapid wetting test because this test is very aggressive and disrupts all aggregates to small macroaggregates (0.2–2 mm) and microaggregates (0.05–0.2 mm).

The stirring after prewetting test assesses aggregate interparticle cohesion. Dry–wet cycles increased the cohesion of aggregates incubated with straw, and had no such effects on reference samples. Drying probably created additional intermolecular associations between organic macromolecules, such as extracellular polysaccharides, and mineral surfaces (Haynes and Swift, 1990), and thus increased interparticle cohesion within aggregates, at the scale of small macroaggregates. In fact, the lower contents of measured HWEC after dry–wet cycles may be due either to a lower production in soil by microorganisms as suggested earlier, or to a stronger adhesion to mineral surfaces, decreasing their solubilization by hot water.

Microorganisms decomposing straw, located at the outer surfaces of 3–5 mm aggregates were then responsible for the stabilization, within these aggregates, (i) of zones 0.2–2 mm which were stable to slaking and to mechanical breakdown, i.e. with enhanced cohesion and hydrophobicity, and (ii) of larger zones, >2 mm in diameter, which were stable to the smaller stresses of slow rewetting. This hierarchy of aggregate stabilization by microbial activity was revealed by the combination of tests. Straw located between 3–5 mm aggregates, hence stimulated microbial binding agents well within the aggregates, in agreement with results from Gaillard et al. (1999) on the detritusphere of wheat straw. Dry–wet cycles, characterized by fast drying and slow rewetting, consolidated the stabilized 0.2–2 mm zones, and created failure zones between them. We observed no effect of dry–wet cycles on microbially mediated hydrophobicity of aggregates.

4.4. Conclusions

With an incubation experiment, we confirmed that straw addition stimulated microbial activity and showed that it stabilized aggregates both by increasing their cohesion and hydrophobicity. Microorganisms produced carbohydrates and water-repellent substances, which were more persistent or had more persistent effects than the microorganisms themselves. The net effects of dry–wet cycles were less important than those due to straw addition, but were more pronounced in the presence of an important microbial activity, demonstrating an interaction. Prediction of short-term variations in aggregate stability should take into account the complex interactions between drying rewetting events, and the dynamics of microbial populations and their binding and aggregating agents. An analysis at the scale of elementary mechanisms, i.e. increasing cohesion and hydrophobicity of aggregates appears necessary, given the complex nature of aggregate stability.

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References

Amézketa, E., 1999. Soil aggregate stability: a review. Journal of Sustainable Agriculture 14, 83–151.

Angers, D.A., Edwards, L.M., Sanderson, J.B., Bissonnette, N., 1999. Soil organic matter quality and aggregate stability under eight potato cropping sequences in a fine sandy loam of Prince Edward Island. Canadian Journal of Soil Science 79, 411–417.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16, 195–208.

Capriel, P., Beck, T., Borchert, H., Härter, P., 1990. Relationships between soil aliphatic fraction extracted with supercritical hexane, soil microbial biomass, and soil aggregate stability. Soil Science Society of America Journal 54, 415–420.

Caron, J., Kay, B.D., Stone, J.A., Kachanoski, R.G., 1992. Modeling temporal changes in structural stability of a clay loam soil. Soil Science Society of America Journal 56, 1597–1604.

Chantigny, M.H., Angers, D.A., Prevost, D., Vezina, L.P., Chalifour, F.P., 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Science Society of America Journal 61, 262–267.

Chenu, C., 1995. Extracellular polysaccharides: an interface between microorganisms and soil constituents. In: Huang, P.M. (Ed.), Environmental Impact of Soil Component Interactions. Natural and Anthropogenics Organics. Lewis Publishers, CRC Press, Boca Raton, FL, p. 217.

Chenu, C., Guérif, J., 1991. Mechanical strength of clay minerals as influenced by an adsorbed polysaccharide. Soil Science Society of America Journal 55, 1076–1080.

Chenu, C., Le Bissonnais, Y., Arrouays, D., 2000. Organic matter influence on clay wettability and soil aggregate stability. Soil Science Society of America Journal 64, 1479–1486.

De Gryze, S., Six, J., Brits, C., Merckx, R., 2005. A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. Soil Biology & Biochemistry 37, 55–66.

Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Australian Journal of Soil Research 35, 431–459.

Degens, B.P., Sparling, G.P., 1995. Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil. Plant and Soil 175, 197–203.

Degens, B.P., Sparling, G.P., Abbott, L.K., 1996. Increasing the length of hyphae in a sandy soil increases the amount of water-stable aggregates. Applied Soil Ecology 3, 149–159.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology & Biochemistry 33, 1599–1611.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biology and Fertility of Soils 22, 299–304.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350–356.

Gaillard, V., Chenu, C., Recous, S., Richard, G., 1999. Carbon, nitrogen and microbial gradients induced by plant residues decomposing in soil. European Journal of Soil Science 50, 567–578.

Gong, P., Guan, X., Witter, E., 2001. A rapid method to extract ergosterol from soil by physical disruption. Applied Soil Ecology 17, 285–289.

Hallett, P.D., Young, I.M., 1999. Changes to water repellence of soil aggregates caused by substrate-induced microbial activity. European Journal of Soil Science 50, 35–40.

Hattori, T., 1988. Soil Aggregates in Microhabitats of Microorganisms. Institute for Agricultural Research, Tohoku University, pp. 37, 23–36.

Haynes, R.J., 2000. Interactions between soil organic matter status, cropping history, method of quantification and sample pretreatment and their effects on measured aggregate stability. Biology & Fertility of Soils 30, 270–275.

Haynes, R.J., Francis, G.S., 1993. Changes in microbial biomass-C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. Journal of Soil Science 44, 665–675.

Haynes, R.J., Swift, R.S., 1990. Stability of soil aggregates in relation to organic constituents and soil water content. Journal of Soil Science 41, 73–83.

Hu, H., Coleman, D.C., Beare, M.H., Hendrix, P.F., 1995. Soil carbohydrates in aggrading and degrading agroecosystems—Influences of fungi and aggregates. Agriculture Ecosystems & Environment 54, 77–88.

Kiem, R., Kandeler, E., 1997. Stabilization of aggregates by the microbial biomass as affected by soil texture and type. Applied Soil Ecology 5, 221–230.

Le Bissonnais, Y., 1996. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 47, 425–437.

Magid, J., Kjaergaard, C., Gorissen, A., Kuikman, P.J., 1999. Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added 14C-labelled plant material. Soil Biology & Biochemistry 31, 595–602.

Panabokke, C.R., Quirk, J.P., 1957. Effect of initial water content on stability of soil aggregates in water. Soil Science 83, 185–195.

Perfect, E., Kay, B.D., Loon, W.K.P.v., Sheard, R.W., Pojasok, T., 1990. Factors influencing soil structural stability within a growing season. Soil Science Society of America Journal 54, 173–179.

Preston, S., Griffiths, B.S., Young, I.M., 1999. Links between substrate additions, native microbes, and the structural complexity and stability of soils. Soil Biology & Biochemistry 31, 1541–1547.

Puget, P., Angers, D.A., Chenu, C., 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. Soil Biology & Biochemistry 31, 55–63.

Recous, S., Robin, D., Darwis, D., Mary, B., 1995. Soil inorganic N availability: effect on maize residue decomposition. Soil Biology & Biochemistry 27, 1529–1538.

Roberson, E., Firestone, M., 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Applied and Environmental Microbiology 58, 1284–1291.

Robert, M., Chenu, C., 1992. Interactions between microorganisms and soil minerals. In: Stotzky, G., Bollag, J.M. (Eds.), Soil Biochemistry. Marcel Dekker, New York, pp. 307–404.

Scheu, S., Parkinson, D., 1994. Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. Soil Biology & Biochemistry 26, 1515–1525.

Shipton, P.J., 1986. Infection by foot and root rot pathogens and subsequent damage. In: Wood, R.K.S., Jellis, G.K. (Eds.), Plant Diseases, Infection, Damage and Loss. Blackwell Scientific, Oxford, p. 139.

Sorensen, L.H., 1974. Rate of decomposition of organic matter in soil as influenced by repeated air drying–rewetting and repeated additions of organic material. Soil Biology & Biochemistry 6, 287–292.

Suwardji, P., Eberbach, P.L., 1998. Seasonal changes of physical properties of an Oxic Paleustalf (Red Kandosol) after 16 years of direct drilling or conventional cultivation. Soil & Tillage Research 49, 65–77.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soil. Journal of Soil Science 62, 141–163.

Utomo, W.H., Dexter, A.R., 1982. Changes in soil aggregate water stability induced by wetting and drying cycles in non-saturated soil. Journal of Soil Science 33.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.

West, A.W., Sparling, G.P., Grant, W.D., 1987. Relationships between mycelial and bacterial populations in stored, air-dried and glucose-amended arable and grassland soils. Soil Biology & Biochemistry 19, 599–605.

Yang, X.M., Wander, M.M., 1998. Temporal changes in dry aggregate size and stability: tillage and crop effects on a silty loam Mollisol in Illinois. Soil & Tillage Research 49, 173–183.