

Role of a Saprophytic Basidiomycete Soil Fungus in Aggregate Stabilization

T. C. Caesar-TonThat* and V.L. Cochran

ABSTRACT

Loss of water stable aggregates due to excessive tillage has increased the potential for soil erosion in semi-arid farming-systems. Fungi can be responsible for the resistance of soil aggregates to breakdown upon wetting. We studied the effects of a saprophytic lignin decomposer basidiomycete isolated from plant litter on soil aggregation and stabilization. The basidiomycete produces large quantities of non water-soluble extracellular materials that bind soil particles into aggregates. Water stability of aggregates amended with the fungal mycelia and the degree of biodegradation of the binding agents by native soil microorganisms was determined by the wet sieving method. Data demonstrated that fungal amended aggregates supplemented with a source of carbon (millet or lentil straw) were much more water stable and resisted microbial degradation longer than when they were prepared with fungal homogenates alone.

INTRODUCTION

Increased public concern over the environment necessitates the identification of the key mechanisms involved in soil structure formation. A clear understanding of these processes will help in decreasing soil erosion and the loss of organic matter. Among soil microorganisms, fungi are important in both the formation and stabilization of soil aggregates (Lynch and Brach, 1985).

Basidiomycete fungi include ectomycorrhizae of forest trees and decay organisms of plant residues. Their existence depends on the formation of an extensive network of hyphae that is very persistent and most do not produce asexual spores or survival structures in soil or plant residues. They produce extracellular materials composed of polysaccharides, glycolipids or glycoproteins that are known to have numerous biological functions including antimicrobial or pharmaceutical activities (Lorenzen and Anke 1998). Little is known however, whether or not some of these products play a role in soil aggregation and stabilization. Other kinds of endomycorrhizal fungi such as arbuscular mycorrhizal (AM) fungi play an important role in soil aggregate stabilization (Sutton and Sheppard 1976; Tisdall and Oades 1979; Forster and Nicolson 1981; Miller and Jastrow 1990; Thomas et al. 1986; Wright and Upadhyaya 1996). Other authors also reported the role of non-AM mycorrhizal fungi other than basidiomycetes (Martin 1945; Harris et al. 1964; Aspiras et al. 1971; Kinsbursky et al. 1989; Eash et al. 1994; Toyota et al. 1996; Tisdall et al. 1997) but little is known about the role of

saprophytic basidiomycetes in soil stabilization. Although Tisdall et al. (1997) studied *Rhizoctonia solani*, a soil borne basidiomycete from the Corticiaceae, and found that this fungus could form stable aggregates of slurry clay (equivalent spherical diameter $< 2 \mu\text{m}$) that was separated from soil by sedimentation under gravity, no investigation has been conducted on aggregation of natural soil particles with saprophytic basidiomycetes. Based on preliminary studies, we hypothesize that saprophytic basidiomycetes are important in soil aggregation. The objective of this study was to determine the role a saprophytic lignin decomposing basidiomycete isolated from plant residues in forming and stabilizing artificial soil aggregates, 1-2 mm in size composed of soil particles $< 0.25 \text{ mm}$.

MATERIALS AND METHODS

Fungal isolation from soil and culture conditions

Isolates of basidiomycetes were obtained from non-disturbed rangeland near Sidney Montana and processed using the techniques of Warcup (1950) and Warcup and Talbot (1962). The basidiomycete strain was cultured in potato dextrose broth (PDB) (Difco Laboratories, Detroit, MI) for 4 weeks at room temperature on a shaker with a setting of 150 rpm. A selective agar medium containing lignin, guaiacol, and benomyl (Thorn et al. 1996) was utilized to determine the presence of lignolytic enzymes produced by the basidiomycete.

Preparation and analysis of artificial aggregates

Artificial non-amended and fungal-amended aggregates were prepared from sandy soil deposited along the Yellowstone River bank near Sidney, MT (14% clay, 14% silt, and 72% sand). Soil was collected from the upper 10-15 cm of the soil profile. Soil was allowed to air-dry at room temperature then was passed through 0.25-mm sieve to remove larger soil particles. This material was chosen because of its low clay content and inability to form stable aggregates in its native condition. To prepare fungal-amended aggregates, mycelia of a four week-old culture were filtered through Miracloth (Calbiochem-Novabiochem Corporation, La Jolla, CA) and the wet mycelia were homogenized (Brinkmann Instrument Inc., Westbury, NY) for 1 min until all mycelia were disrupted. Fungal-amended aggregates were prepared with fungal homogenates ranging from 0.003 to 0.5 g per g soil. In most cases, soil aggregates were prepared using 0.33 g per g soil. The soil mixture was uniformly wetted with distilled water then dried for 24 h at 60 °C in a forced-air oven. The soil cake was then manually

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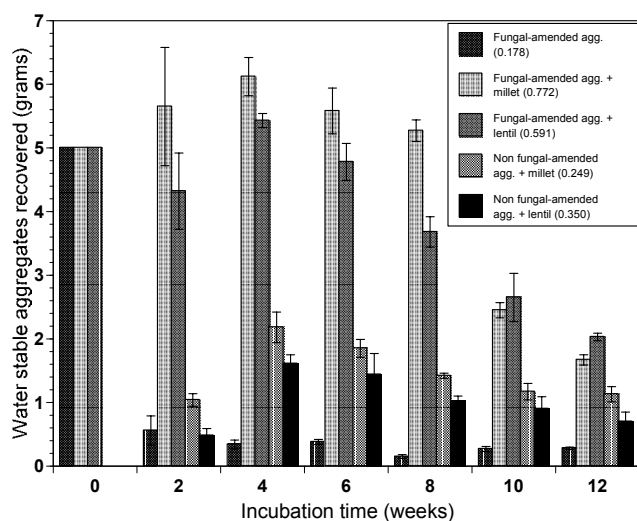


Figure 1. Changes in the amount of recovered water stable aggregates from artificial fungal-amended and non-amended soil aggregates during 12 weeks incubation in non-sterile soil. Vertical bars indicate standard error of mean of three replicates. Values between parentheses indicate least significance difference ($P < 0.05$).

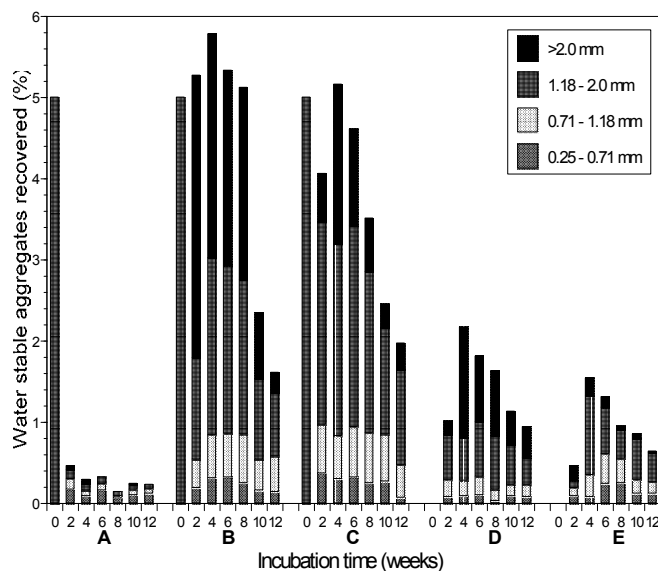


Figure 2. Size distribution of water stable aggregates recovered from non-sterile soil for an incubation period of 12 weeks. A. Fungal-amended aggregates without supplements. B. Fungal-amended aggregates supplemented with ground millet. C. Fungal-amended aggregates supplemented with ground lentil straw. D. Non fungal-amended aggregates supplemented with millet. E. Non fungal-amended aggregates supplemented with lentil straw. Non fungal-amended aggregated without supplements were unstable throughout the incubation. Each date point represents the average of triplicates.

ground with a pestle into 1 to 2 mm size-aggregates. To prepare non-amended aggregates, soil was wetted with distilled water without the presence of the fungus and processed the same way as above. Aggregate stability of artificial aggregates was determined by a modification of the procedure described by Kemper and Rosenau (1986). Four grams of aggregates was added to 0.25-mm sieve (3.7-cm diameter). The aggregates on the sieves were allowed to wet up via capillary movement by placing the sieve in a pan and adding sufficient water to just reach the sieve screen. After the aggregates were visibly moist throughout, water was added to cover the aggregates and the sieves raised and lowered manually for 3 min to wash out fine particles from disrupted aggregates. The aggregates remaining on the screen were then oven dried at 60°C for 16h and weighed. The percentage of water stable aggregates was defined as the portion of the original sample that remained on the sieve. Each determination was made in triplicate.

Experiments to determine the stability of fungal-amended aggregates when exposed to soil microorganisms were conducted using fungal-amended aggregates with a supplemental source of carbon provided by either millet (5% w/w) or lentil straw (5% w/w) that were ground and passed through 0.25 mm sieve. Experiments were carried out in disposable filter units (Falcon™, 115 ml volume, Becton Dickinson, Lincoln Park, New Jersey). Bulk soil used for the experiment was obtained from a commercial bulk supply and listed as topsoil (27% clay, 27% silt and 46% sand). The type of clay was not determined but contained little expanding lattice types of clay. Thirty grams of bulk soil, which had passed through a 0.25 mm sieve and 5 g of artificial fungal-amended soil aggregates supplemented with 5% ground millet or 5% lentil straw were mixed with the soil and placed in the filter units. A control in triplicate was prepared in similar manner except that non-amended aggregates were used instead of fungal-amended aggregates.

Another set of controls was prepared from the sieved soil supplemented only with ground millet or lentil straw. Water potentials of experimental units were adjusted to 0.01 Mpa by a procedure similar to that described by MacKay and Carefoot (1981). Distilled water was added to completely cover the soil surface and then let stand for 10 minutes. A suction of 0.01 Mpa was then applied until water no longer dripped from the filter funnel (about 1 h). Filter funnels with the soil samples in place were incubated at room temperature in the dark. To maintain soil moisture, soil surface was wetted every two days with 2 ml of tap water per funnel delivered from a spray bottle. At two-week intervals, filter units were removed from the dark and the soil samples were allowed to air-dry for 48 hours at room temperature. Aggregate stability was determined in triplicate by wet sieve analysis based on the technique of Low (1954) adapted for soil of poor stability.

Experiments to demonstrate that growing fungi bind soil particles forming water stable aggregates were conducted in sterile conditions. Sterile fungal-amended soil aggregates were obtained by autoclaving at 121°C for 2 hours on each of three successive days. Bulk soil was passed through 0.25 mm-sieve before autoclaving the same way as soil

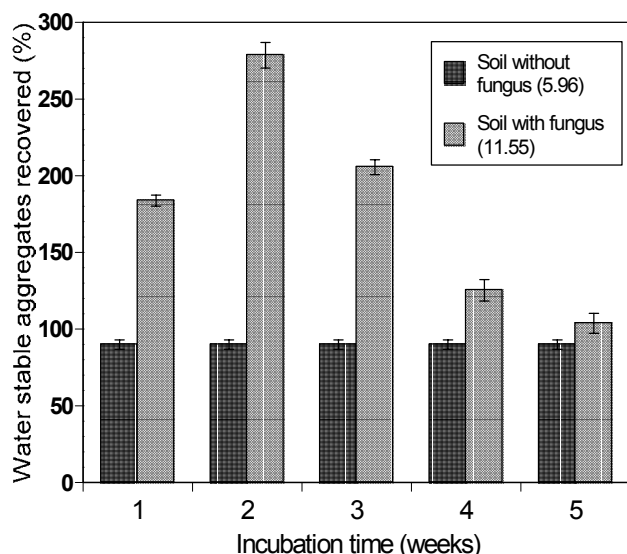


Figure 3. Effect of inoculating with basidiomycete fungus into sterile soil on stability of recovered artificial fungal-amended soil aggregates. Aggregates stability is expressed in percentage of 250- μ m soil particles bound into 1-2 mm aggregates after water stability test. Each date point represents the average of triplicates. Values between parentheses indicate least significance difference throughout the incubation period in non-sterile soil. Results of experimental controls showed that non-amended ($P < 0.05$).

aggregates. Sterility tests were undertaken by incubating samples of bulk soil or soil aggregates on a potato dextrose agar dish for a week at room temperature. Thirty grams of sterile 0.25 mm-sieved bulk soil at about 20% (w/w) water content and 5 grams of fungal-amended aggregates were added aseptically to Magenta tissue culture box (Sigma, Saint Louis, MO). Vents composed of a microporous polypropylene 0.2- μ m pore size-membrane (Sigma, Saint Louis, MO) were used to close the tissue culture boxes to allow proper ventilation in sterile conditions. Except for the controls, soil samples containing soil aggregates were inoculated with four week-old fungal mycelia (0.1 g wet weight). The culture boxes were then incubated in a humidified incubator at 15° C. At one-week intervals, the soil samples in the culture boxes were allowed to air-dry for 48 h. To determine aggregate stability, soil samples were subjected to wet sieving described by Low (1954). The experiments were repeated three times.

The data were analyzed statistically using ANOVA. These analyses were accomplished using Stat View (SAS Institute) (Abacus Concepts 1987).

RESULTS

A selective medium for saprophytic lignin decomposer basidiomycetes based on the incorporation of lignin and a chromogenic substrate, guaiacol, (Thorn et al. 1996) was used to demonstrate that the isolate used in this study produces hydrolytic enzymes or laccases. The fungus caused reddening of the guaiacol indicating presence of activity of the enzymes (data not shown).

The effects of incubation in non-sterile conditions on the stability of artificial soil aggregates amended or not with the

fungus and supplemented with or without 5 % millet or 5 % lentil straw are illustrated in Fig. 1. All aggregates were incubated in non-sterile soil and thus contained indigenous soil microorganisms. Initial amount (5 g) of fungal amended aggregates (1.18-2 mm) supplemented or not with millet or lentil straw was 100% water stable and non fungal-amended aggregates are 100% non water stable after wet sieving. Aggregates prepared from sandy soil without the presence of the fungus possessed no inherent stability. After two weeks of incubation, the amount of water stable aggregates (WSA) retrieved from soil that contains fungal amended aggregates supplemented with millet was 113% compared to the initial amount (100%) of aggregates added at time zero. A maximum was reached after 4 weeks (122 %). Then the amount of WSA decreased to 33.3% by 12 weeks. Fungal amended aggregates supplemented with lentil straw were more sensitive to degradation on incubation than aggregates supplemented with millet. There was a slight degradation of aggregates after 2 weeks (86%), followed by a regain in stability after 4 weeks to 123%. Thereafter the amount of WSA decreased steadily. Fungal amended aggregates without supplement of organic matter (millet or lentil straw) were initially 100% stable to wet sieving but became almost completely unstable in 2 weeks and remained unstable aggregates that were initially 100% unstable in water remained unstable for the duration of the incubation in non-sterile soil. Another set of experimental controls using non amended soil aggregates (100% unstable in water) supplemented with millet or lentil straw was undertaken to determine whether or not available C can induce formation of WSA in presence of endogenous microorganisms. Results showed that WSA were formed after 2 weeks (20.8% WSA retrieved from samples supplemented with millet and 9.6% with lentil straw) and after 4 weeks the amount of WSA reached a peak of 43.7% WSA for samples with millet and 32.1% for samples with lentil straw.

Results of size-fractionation of WSA retrieved from bulk soil are illustrated in Fig. 2. Fractionation of WSA from the controls (fungal-amended aggregates without supplements) showed negligible difference in the size of aggregates in soil containing fungal-amended aggregates without sources of carbon (Fig. 2A). Fungal-amended aggregates supplemented with 5% millet (Fig. 2 B) showed a considerably greater amount of large aggregates (> 2 mm) after 2 weeks compared to the amount of aggregates (1.18-2 mm) initially added in soil at the beginning of the experiment. This amount decreased after 4 weeks but remained for the duration of incubation. In contrast, fractionation of WSA retrieved from soil containing fungal-amended aggregates supplemented with lentil straw (Fig. 2 C) showed a predominance of 1.18-2 mm size aggregates for the whole duration of the incubation. There was a predominance of large aggregates in soil containing non fungal-amended aggregates supplemented with millet (Fig. 2 D) in comparison with soil containing non-amended aggregates supplemented with lentil straw (Fig. 2 E), where those of 1.18-2 mm predominate.

The effects of growth of the basidiomycete on the stability of artificial fungal-amended aggregates are illustrated in Fig. 3. These experiments were carried out in

sterile conditions. Extensive fungal growth resulted in a considerable increase of WSA after 2 weeks of incubation. There was 206% WSA after 2 weeks compared to the initial amount of aggregates, then after 5 weeks the amount of WSA gradually decreased thereafter. The percentage of aggregates in the control is 98.3%.

DISCUSSION

Our study demonstrated that a saprophytic lignin decomposing basidiomycete has the ability to efficiently bind and stabilize soil particles into water stable aggregates. This is because this fungus secretes large amounts of water insoluble extracellular compounds that act as binding agents of soil particles. The binding capability of the fungus to soil particles was not reduced at high temperature (microwave to boiling state for 5 min) indicating that these secreted materials are not composed of large proteins (data not shown).

Cultivation has resulted in loss of organic matter (Beare et al. 1994; Tiessen et al. 1982) due to disruption of soil aggregates (Elliot 1986; Tisdall and Oades 1982), and no till management with plant residues returned has improved soil aggregation (Carter 1992; Weill et al. 1989). We demonstrated that artificial fungal-amended aggregates were significantly more resistant to biodegradation and maintain their integrity much longer when they were supplemented with an organic matter source such as millet or lentil straw than fungal amended aggregates without supplement (Fig. 1). It is very likely that addition of C, an energy source, promoted microbial activity and the production of more soil-binding agents (Aspiras et al. 1971).

We found that both millet and lentil straw improved aggregate stability during incubation but the amounts of larger aggregates (>2 mm) recovered from fungal amended aggregates with millet were higher than those with lentil straw (Fig. 2A and B). Similar results were obtained with non fungal-amended aggregates with millet compared to lentil straw (Fig. 2D and E). The greater amount of soil aggregates in soil with millet compared to that with lentil straw occurred early indicating greater microbial production of binding agents. This is likely due to greater availability of C from the starch in the millet compared to cellulose in lentil straw (Reinertsen et al. 1984; Cochran et al. 1988).

Our results indicated that addition of growing mycelia in sterile soil, which contain sterile artificial fungal-amended aggregates previously autoclaved, provoked a considerable increase in aggregate stability. The amount of WSA recovered was almost triple that of the controls after 2 weeks of incubation, suggesting that the fungus alone bound more soil particles. In this experiment, aggregate size-fractionation could not be undertaken because the mycelial inoculum has a strong tendency to form clumps and cannot be completely disrupted in equal living pieces. Therefore, it was difficult to determine with accuracy whether or not there was an increase in size of the initial added aggregates or formation of new aggregates. It is important to note that artificial non-amended aggregates prepared from sandy soil possessed no inherent stability. This suggests that sandy soil used in our study lacks inherent active aggregating agents.

The possible role of saprophytic lignin decomposer basidiomycetes to aggregate and stabilize soil in plant litter has been almost totally neglected. This now appears to be a fruitful field of inquiry not only in relation to the mechanism by which they stabilize soil in plant litter but also to the wider problems of understanding the importance of this class of fungi and their role in different litter resource ecosystems. Crop residue and tillage management practices have the potential to promote the growth of these organisms; thus promoting increased soil aggregation, increased water infiltration, reduced run-off and reduced soil erosion.

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