Biol Fertil Soils (2000) 32:374–380 © Springer-Verlag 2000

ORIGINAL PAPER

T.-C. Caesar-TonThat · V.L. Cochran

Soil aggregate stabilization by a saprophytic lignin-decomposing basidiomycete fungus
I. Microbiological aspects

Received: 27 September 1999

Abstract We studied the effects of a saprophytic lignin-decomposing basidiomycete isolated from plant litter on soil aggregation and stabilization. The basidiomycete produced large quantities of extracellular materials that bind soil particles into aggregates. These binding agents are water-insoluble and heat-resistant. Water stability of aggregates amended with the fungus and the degrees of biodegradation of the binding agents by native soil microorganisms were determined by the wet-sieving method. The data demonstrated that aggregates supplemented with a source of C (millet or lentil straw) were much more water-stable and resisted microbial decomposition longer than when they were prepared with fungal homogenates alone. Moreover, retrieval of fungal-amended aggregates supplemented with millet during the first 4 weeks of incubation in natural soil exhibited more large aggregate fractions (>2 mm) than the ones supplemented with lentil straw. The possible relationship of the role of basidiomycetes in litter decomposition and soil aggregation is discussed.

Keywords Fungi · Basidiomycete · Saprophytic lignin-decomposing basidiomycete · Soil aggregation · Water-stable aggregate

Introduction

Soil structure is created when physical forces (drying, shrink-swell, freeze-thaw, root growth, animal movement, or compaction) mold the soil into aggregates. As aggregates become larger, the architecture, arrangement, and stability of the aggregates are important because these factors have an impact on pore size. If the soil aggregates are unstable during rainfall, water infiltration decreases due to partial blocking of the pores with dispersed soil particles. As the percentage of water-stable aggregates (WSA) increases, water infiltration increases with decrease in erodibility. Therefore, aggregate size and stability control the soil pore-size distribution, water infiltration rates, and erodibility. Tisdall and Oades (1982) presented a conceptual model of soil structure that describes the binding of primary mineral particles into microaggregates (50–250 μm diameter) and of microaggregates into macroaggregates (>250 μm diameter). It was proposed that soils consist of aggregates held together by organic and precipitated inorganic materials (Emerson et al. 1986; Stotzky 1997). These aggregates retain water that can form 'bridges' with the water of adjacent aggregates. These clusters of aggregates, with their adjacent water, constitute the microhabitat in soils wherein the microbes function and reproduce.

Among soil microorganisms, fungi can play an important role in the formation and stabilization of soil aggregates (Lynch and Bragg 1985). The two main processes involved in the biological improvement of soil aggregate stability are the adhesive effects of microbial metabolic products and entanglement of soil particles by filamentous fungi. Extracellular materials produced by fungi were found to act as cementing agents and to be responsible for the resistance of soil aggregates to breakdown upon wetting; however, mycelial entanglement may play a role in stabilizing aggregates, but to a lesser extent (Rennie et al. 1954; Greenland et al. 1962; Molope and Page 1986; Chenu 1989; Kinsbursky et al. 1989; Robertson et al. 1991; Tisdall 1991; Hu et al. 1995; Tisdall et al. 1997). Wright et al. (1996) have isolated a glycoprotein from vesicular arbuscular mycorrhizal fungi (VAM) that is highly correlated with aggregate stability.

Basidiomycetes inhabit most climatic zones from the arctic to the tropics. They include the majority of ectomycorrhizal fungi, and some significant plant patho-

T.-C. Caesar-TonThat (✉) · V.L. Cochran
USDA/ARS, Northern Plains Agricultural Research Center, Sidney, MT 59270, USA
e-mail: caesart@sidney.ars.usda.gov
Tel.: +1 406 482 9415
Fax: +1 406 482 5038

gens. Many saprophytic basidiomycetes inhabiting soil and fragmentary plant litter have significant ligninolytic abilities. Their existence in litter 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 litter. They produce extracellular products 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, about whether or not some of these products play a role in soil aggregation and stabilization. Many studies have indicated that vesicular arbuscular (VA) mycorrhizal fungi play an important role in soil aggregate stabilization (Sutton and Sheppard 1976; Tisdall and Oades 1979; Forster and Nicolson 1981; Thomas et al. 1986; Miller and Jastrow 1990). Other authors have also reported the role of non-VA 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 at 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 μm) that separated from soil by sedimentation under gravity, no investigation has been conducted on aggregation of natural soil particles.

The objectives of this study were to determine the role of a saprophytic lignin-decomposer basidiomycete isolated from plant residues in forming and stabilizing artificial soil aggregates (1–2 mm in size composed of soil particles <0.25 mm), and to study the microbiological changes in incubated artificial aggregates.

Materials and methods

Fungal isolation from soil and culture conditions

The basidiomycete used in this study was isolated in eastern Montana. The site has been infested with leafy spurge for an undetermined length of time and recently began to exhibit a decline in stand density. Two methods of fungal isolation were used, including those of Warcup (1950) and Warcup and Talbot (1962). Method 1 consisted of picking, screening or sieving of soil containing the desired mycelium, with the mycelium being subjected to various treatments. Chunks or particles of soil ranging from 0.5 to 2 cm in diameter were processed within 24 h. The soil particles were broken into 1.0–1.5 g crumbs, placed in a beaker and a jet of tap water used to further disintegrate the crumbs. After the heavier sediments had settled to the bottom of the beaker, the supernatant was decanted. The process was repeated until the supernatant remained clear. The remaining soil residue, usually containing abundant mycelia, was distributed among several petri dishes, and molten agar cooled to ca 45 °C was poured into the dishes. Various agar media were used in this procedure, including Czapek Dox agar supplemented with 0.5% yeast extract, malt extract agar (MEA), or potato dextrose agar supplemented with 0.5% yeast extract. In method 2, a variation on this method consisted of placing soil crumbs into petri plates and pouring a thin layer of cooled medium over them. Plates were incubated at 10–15 °C and assessed for mycelial growth periodically over 2 months. Mycelia were removed from individual colonies and examined microscopically for clamp connections. Hyphae with clamp connections were subcultured on MEA.

Fungal culturing and growth

The basidiomycete strain was cultured in potato dextrose broth (PDB) (Difco Laboratories, Detroit) for 4 weeks at room temperature on a shaker with a setting of 150 rpm. To test growth pattern and the ability of the fungus to aggregate soil particles, malt yeast extract broth, Trypticase soy broth (Becton Dickinson, Cockeysville, Md.), and corn meal yeast extract broth were also used. A selective agar medium containing lignin, guaiacol, and benomyl (Thorn et al. 1996) was utilized to determine the presence of lignin enzymes produced by the basidiomycete.

Preparation and analysis of artificial aggregates

Artificial non-amended and fungal-amended aggregates were prepared from fine sand (14% clay, 14% silt, and 72% sand). Soil was collected from upper 10–15 cm of the soil profile. Soil was allowed to air-dry then was passed through a 0.25-mm sieve to remove larger soil particles. To prepare fungal-amended aggregates, mycelia of a 4-week-old culture were filtered through Miracloth (Calbiochem-Novabiochem, La Jolla, Calif.) and the wet mycelia were homogenized (Brinkmann Instrument, Westbury, N.Y.) for 1 min at setting no. 4. Fungal homogenates were examined with a microscope (Nikon Microphot FX) using a 40X objective and phase contrast optics to make sure that all mycelia were disrupted. A predetermined amount of fungal homogenate was mixed thoroughly with dry, sieved soil. 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 ground with a pestle into particles of 1–2 mm. To prepare non-amended aggregates, soil was wetted with distilled water without homogenized fungal mycelia 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). A 4 g portion of aggregates was added to a 0.25 mm mesh sieve (3.7 cm diameter). The materials in the sieve was allowed to wet via capillary action 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 were 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 and weighed. The percentage of WSA 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 C provided by either millet (5% w/w) or lentil straw (5% w/w) that were ground and passed through a 0.25 mm sieve. To study the stability of fungal-amended soil aggregates in non-sterile conditions, experiments were carried out in disposable filter units (Falcon; 115 ml volume, Becton Dickinson, Lincoln Park, N.J.). Bulk soil used for the experiment was obtained from a garden supply at Sidney, Montana (27% clay, 27% silt and 46% sand). 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 was prepared in a 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 soils and aggregates were adjusted to 0.01 Mpa by a procedure similar to that described by MacKay and Carefoot (1981). Tap water was added to completely cover the soil surface and was then left to stand for 10 min. 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, the soil surface was wetted every 2 days with one squeeze of tap water per funnel delivered from a spray bottle. At 2-week intervals, filter units were removed from the dark and the soil samples were allowed to air-dry in the funnel for 48 h at room temperature. Aggregate stability was determined by wet sieving analysis based on a modified technique of Low (1954) adapted for soil of poor stability. Samples of air-dried soil were completely immersed with distilled water for 1 min then soil contents of the funnel were carefully poured on a 0.25 mm sieve to avoid any disruption of soil aggregates. The sieve was then subjected to gentle vertical oscillations of 2.5-cm amplitude over a period of 3 min. The contents of each sieve were then removed, the aggregates remaining on the screen were then oven-dried at 60 °C and weighed. Stability to wet sieving was calculated as the amount by mass of retained particles with effective diameter exceeding 0.25 mm. After recording the total weight, the aggregates were placed on a nest of sieves (2.00, 1.18, 0.70, and 0.25 mm openings) and each size-fraction was weighed and recorded.

Experiments to demonstrate that growing fungal mycelia bind soil particles forming WSA were conducted in sterile conditions. Sterile fungal-amended soil aggregates were obtained by autoclaving at 121 °C for 2 h on each of three successive days. Bulk soil was passed through a 0.25 mm mesh sieve before autoclaving the same way as soil aggregates. Sterility tests were undertaken by incubating samples of bulk soil or soil aggregates on a potato dextrose agar dish for 1 week at room temperature. Thirty grams of sterile 0.25 mm-sieved bulk soil at about 20% (w/w) water content and 5 g of fungal-amended aggregates were added aseptically to a Magenta tissue culture box (Sigma, Saint Louis). The vent closure consisted of a polypropylene membrane with 0.2 μm pores (Sigma) to allow proper ventilation in sterile conditions. Except for the controls, soil samples containing soil aggregates were inoculated with 4-week-old fungal mycelia (0.1 g wet weight). The culture boxes were then incubated in a humidified incubator at 15 °C. At 1-week intervals, contents of soil samples in culture boxes were allowed to air-dry for 48 h. To determine aggregate stability, soil samples were subjected to wet sieving described above. The experiments were repeated three times.

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

Low temperature scanning electron microscopy

Samples consisted of soil aggregates supplemented with 5% millet which had been exposed to an inoculum of 4-week-old living mycelia (see protocol above) in sterile conditions. After 2 weeks of culture, soil aggregates were deposited on an aluminum stub on a thin layer of embedding medium (Tissue-Tek, Torrance, Calif.). Another set of samples consisted of living fungal mycelia that had been deposited on a sterile aluminum stub for 2 h on a drop of culture media. The samples were plunged into freshly prepared nitrogen slush (= −210 °C). The frozen-hydrated tissue was transferred under vacuum to a cryopreparation unit (Oxford CT 1500) attached to a scanning electron microscope (SEM) JOEL JSM-6100 equipped with a LaB_6 source and turbomolecular pumping. The materials were then sputter coated with 1–2 nm gold inside the cryopreparation chamber. The samples were then transferred to the main chamber of the SEM to be analyzed. During sample analysis, the pressure in the SEM chamber was $1'10^6$ Torr and the acceleration voltage was 12 kV.

Results

When homogenates of fungal mycelia were mixed with 250 μm sieved sandy soil at a concentration of 0.33 g per g soil and oven-dried at 60 °C, 100% of soil aggregates artificially produced from the mixture was found stable in water. Soil particles start to aggregate at a concentration as little as 0.017 g per g soil but only about 10% was water-stable. Thus, it was decided in this work to use a fungal mycelia concentration of 0.33 g per g soil to investigate the role of the basidiomycete in soil aggregation. Fungal growth also has an effect on the stabilization of soil particles and results show that a 1-week-old basidiomycete culture is already capable of aggregating and stabilizing sandy soil with 90% of the aggregates water-stable (data not shown). Because the fungus grows slowly, we used 4-week-old fungal cultures in order to have enough biomass for all our experiments. WSA were produced by mixing soil particles with intact fungal mycelia, with mycelia that was homogenized to a thick emulsion, or with mycelia boiled for 10 min in a microwave oven. The fungus was cultivated in four different types of media (potato dextrose broth, malt yeast extract broth, trypticase soy broth, and corn meal yeast extract broth) without losing its capability to stabilize soil particles. A selective medium for saprophytic lignin-decomposer basidiomycetes based on the incorporation of lignin and a chromogenic substrate, guaiacol (Thorn et al. 1996), demonstrated that the fungal isolate used in this study produced 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 supplemented or not with organic matter (5% millet or lentil straw) are illustrated in Fig. 1. All aggregates were in-

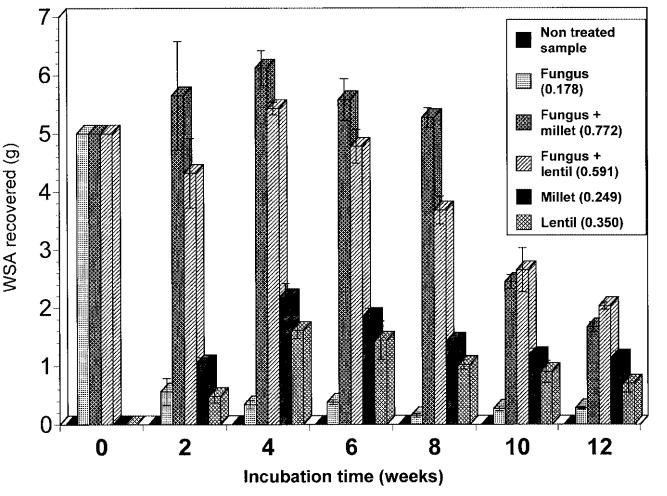

Fig. 1 Response of water stability of artificial fungal-amended and non-amended soil aggregates (*WSA*) for 12 weeks incubation in non-sterile soil. *Vertical bars* indicate standard error of mean for three replicates. Values between *parentheses* indicate least significant difference ($P<0.05$)

cubated in non-sterile soil and thus contained indigenous soil microorganisms. An initial amount (5 g) of fungal-amended aggregates (1.18–2 mm) supplemented or not with 5% millet or 5% lentil straw was 100% water-stable and non-amended aggregates were 100% non-stable in water after wet sieving. After 2 weeks of incubation, the amount of WSA retrieved from soil that contained fungal-amended aggregates supplemented with 5% millet was 1.13 times more than the initial amount (5 g) of aggregates added at time zero. A maximum was reached after 4 weeks of incubation (1.22 times more). Then the amount of WSA decreased to 33.3% by 12 weeks of incubation. Fungal-amended aggregates supplemented with 5% lentil straw were more sensitive to degradation on incubation than aggregates supplemented with 5% millet. There was a slight degradation of aggregates after 2 weeks of incubation, followed by a return to stability after 4 weeks. 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 throughout the incubation period in non-sterile soil. Results of experimental controls showed that non-amended 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 5% millet or 5% lentil straw was tested to determine whether or not organic matter can induce formation of WSA in the 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 natural soil are illustrated in Fig. 2. Fungal-amended aggregates supplemented with 5% millet (Fig. 2A) showed a considerably greater amount of large aggregates (>2 mm) after 2 weeks of incubation compared to the amount of aggregates (1.18–2 mm) initially added to soil at the beginning of the experiment. This amount decreased after 4 weeks of incubation but remained higher than the initial aggregates for the duration of incubation. In contrast, fractionation of WSA retrieved from soil containing fungal-amended aggregates supplemented with 5% lentil straw (Fig. 2B) showed a predominance of 1–2 mm sized aggregates for the whole duration of the incubation. Fractionation of WSA from the controls showed a negligible difference in the size of aggregates in soil containing fungal-amended aggregates without sources of C (Fig. 2C), and a predominance of large aggregates (>2 mm) in soil containing non-amended aggregates supplemented with millet (Fig. 2)D.

In comparison with soil containing non-amended aggregates supplemented with millet (Fig. 2E), the effects

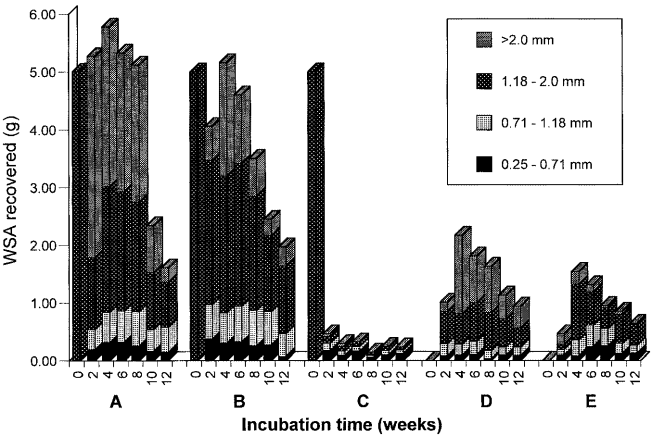

Fig. 2A–E Size distribution of water-stable aggregates (*WSA*) recovered from non-sterile soil for an incubation period of 12 weeks. **A** Fungal-amended aggregates supplemented with ground millet. **B** Fungal-amended aggregates supplemented with ground lentil straw. **C** Fungal-amended aggregates without supplements. **D** Non-amended aggregates supplemented with millet. **E** Non-amended aggregates supplemented with lentil straw. Non-amended aggregates without supplements were unstable throughout the duration of the incubation. Each *data point* represents the average of triplicates

of growth of the basidiomycete on the stability of fungal-amended aggregates supplemented with 5% millet 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 2.1 times more WSA after 2 weeks compared to the initial amount of aggregates at time zero; then the amount of WSA gradually decreased thereafter.

Low temperature scanning electron microscopy involved direct examination of frozen-hydrated material at low temperature in a scanning microscope. This technique results in a superior preservation of the biological specimens because frozen-hydrated materials are rapidly immobilized and stabilized by cryofixation, and

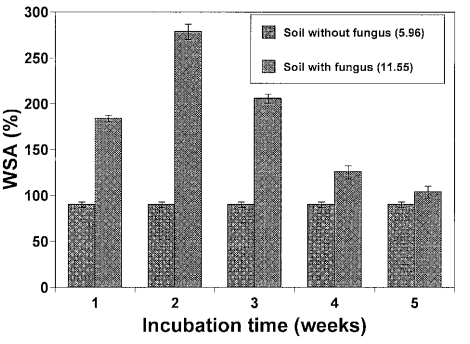

Fig. 3 Effect of inoculating with the basidiomycete fungus into sterile soil on the water stability of soil aggregates (*WSA*). Aggregate stability was expressed as the percentage of 250-μm soil particles bound into 1–2 mm aggregates after water stability test. Each *data point* represents the average of triplicates. Values between *parentheses* indicate least significant difference ($P<0.05$)

are not exposed to fixatives or solvents. The micrograph of soil aggregates that were exposed to the fungus for 2 weeks in sterile conditions demonstrated that the ability of the fungus to aggregate soil particles correlated with the presence of a fibrillar network of extracellular materials. Fibrillar bridges were found to adhere to soil particles (Fig. 4A, arrow). Fig. 4B shows the formation of mucilage (arrow) along individual fungal hyphae.

Discussion

Basidiomycetes causing decay of woody litter have been subject to numerous taxonomic and physiological studies (Clipson et al. 1987; Boidin et al. 1998), but remarkably little is known about the organisms responsible for fine plant litter decay on or in soil. This lack of information may be due to the general inability to isolate cultures of this class of fungi from soil. Recently, Thorn et al. (1996) used a combination of the particle filtration technique and a selective indicator medium to isolate non-sporulating saprophytic basidiomycetes producing ligninolytic abilities from soil and described their diversity and distribution in different ecosystems. Because saprophytic fungi are responsible for decomposition and nutrient-release processes more than bacteria (Beare et al. 1993), and because saprophytic and facultative pathogens with saprophytic phases, including basidiomycetes, are likely to be the dominant recyclers of plant wastes in soil (Boosalis et al. 1967; Ploetz et al. 1985), their role in soil aggregation should be addressed. Our study demonstrated that a saprophytic lignin-decomposer basidiomycete can efficiently bind and stabilize soil particles into WSA. This fungus secretes a large amount of insoluble extracellular compounds (Fig. 4B) that act as binding agents of soil particles. The capability of the fungus to bind soil particles was not reduced at high temperature (microwave to boiling state for 5 min), suggesting that these secreted materials are not composed of large proteins. Moreover, WSA can be produced by mixing soil particles with homogenates of fungal mycelia, indicating that the effect of enmeshing soil particles by fungal hyphae is not a prerequisite for soil aggregation. Characterization of some of the extracellular compounds secreted by the fungus will be reported separately.

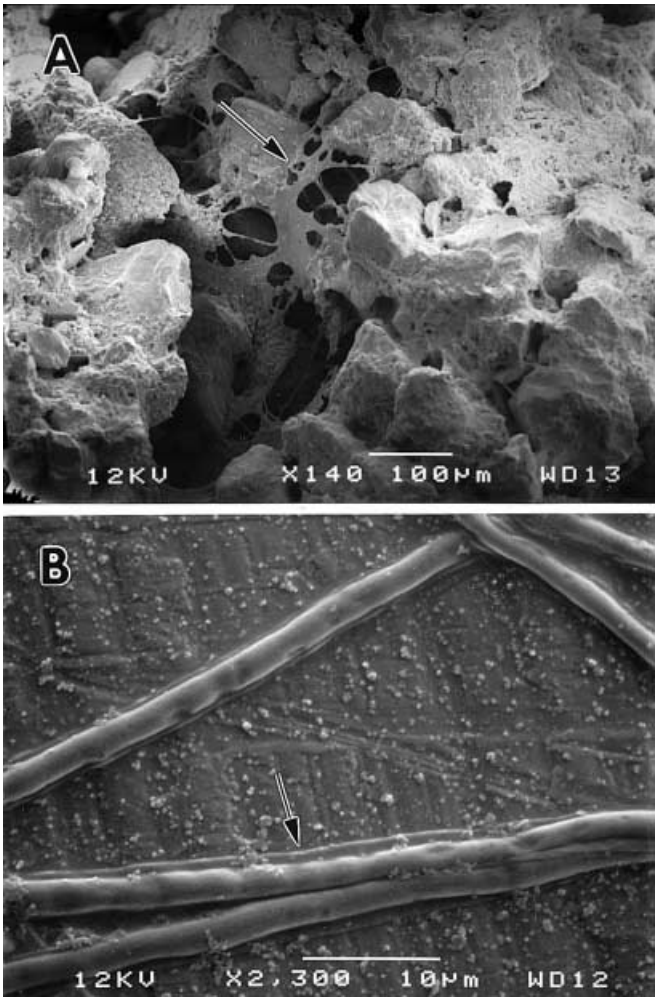

Fig. 4A,B Low-temperaturescanning electron microscopy. **A** A portion of a soil macroaggregate exposed to fungal invasion. An extensive fibrillar material produced by the fungus forms bridges between soil particles (*arrow*). **B** Mucilage produced along fungal hyphae (*arrow*)

In arable systems, litter input and decomposition is often influenced by disturbance (tillage) and the removal of large quantities of organic materials (harvest) as harvested products. Cultivation of soils results in loss of organic matter (Tiessen et al. 1982; Hendrix et al. 1992; Beare et al. 1994) due to disruption of soil aggregates (Van Veen and Paul 1981; Tisdall and Oades 1982; Elliott 1986; Kay 1990), and no-till management when plant residues are not removed, can improve soil aggregation (Weill et al. 1989; Carter 1992). We demonstrated that artificial fungal-amended aggregates supplemented with millet or lentil straw were significantly more resistant to biodegradation and maintain their integrity much longer than fungal-amended aggregates without C supplements (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). Moreover, a gradual loss of WSA was detected after 8 weeks of incubation in natural soil. We attribute this to endogenous microflora consuming the available C in the added substrates and the soil binding materials secreted by the fungus.

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. 2). Similar or identical results were obtained with non-amended aggregates with millet compared with lentil straw. 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 to sterile soil containing WSA supplemented with millet resulted in a considerable increase in water aggregate stability (Fig. 3). The amount of WSA recovered almost tripled after 2 weeks of incubation when compared to the controls, suggesting that more soil particles were bound to the existing aggregates by the fungus. 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 into equal living pieces. Therefore, it was difficult to determine with accuracy whether or not there was an increase in size of the initial 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 the sandy soil used in our study lacks inherent aggregating agents. One of the advantages of using sandy soil for our experiments is that this type of soil provides good experimental controls, whereas the agricultural soils used in most studies in aggregation (Harris et al. 1964; Aspiras et al. 1971; Eash et al. 1994) contain organic matter (an aggregating substance) that cannot be removed completely. Sandy soil was chosen to avoid possible nonspecific aggregation caused by clay particles for which the fungal binding agents were not responsible.

The possible ability 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 but also to the wider problems of understanding the importance of this class of fungi and their role in different litter resource ecosystems. Since under natural conditions much of the available lignin is converted to humus, clarification of the interactions which occur between ligninolytic fungi in the soil environment is vital to our knowledge of soil structure and fertility. The classification of the fungus is currently under study.

Acknowledgements We are grateful to April Verhasselt for technical assistance. We also thank Dr. A.C. Kennedy, Dr. R.J. Kremer and Dr. R.G. Thorn for critically reading the manuscript.

References

Abacus Concepts (1987) State View II. Abacus Concepts, Berkeley, Calif.

Aspiras RB, Allen ON, Harris RF, Chesters G (1971) The role of microorganisms in the stabilization of soil aggregates. Soil Biol Biochem 3:347–353

Beare MH, Pohlad BR, Wright DH, Coleman DC (1993) Residue placement and fungicide effect on fungal communities in conventional and no-tillage soil. Soil Sci Soc Am J 57:392–399

Beare MH, Hendrix PF, Coleman DC (1994) Water-stable aggregates and organic matter fractions in conventional- and no-tillage soils. Soil Sci Soc Am J 58:777–786

Boidin J, Mugnier, J, Canales R (1998) Taxonomie moleculaire des Aphyllophorales. Mycotaxon 66:445–491

Boosalis MG, Sumner DR, Rao AS (1967) Overwinter conidia of *Helminthosporium turcicum* on corn residue and in soil in Nebraska. Phytopathology 57:990–996

Carter MR (1992) Influence of reduced tillage systems on organic matter, microbial biomass, macroaggregate distribution and structural stability of the surface soil in a humid climate. Soil Till Res 23:361–372

Chenu C (1989) Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. Soil Biol Biochem 21:299–305

Clipson NJW, Cairney JWG, Jennings DH (1887) The physiology of basidiomycete linear organs. I. Phosphate uptake by cords and mycelium in the laboratory and in the field. New Phytol 105:449–457

Cochran VL, Horton KA, Cole CV (1988) An estimation of microbial death rate and limitations of N or C during wheat straw decomposition. Soil Biol Biochem 20:293–298

Eash NS, Karlen DL, Parkin TB (1994) Fungal contributions to soil aggregation and soil quality, In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (eds) Defining soil quality for a sustainable environment. SSSA Spec Publ No 35, Madison, WI, pp 221–228

Elliott ET (1986) Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci Soc Am J 50:627–633

Emerson WW, Foster RC, Oades JM (1986) Organo-mineral complexes in relation to soil aggregation and structure. In: Huang PM, Schnitzer M (eds) Interactions of soil minerals with natural organic matter and microbes. (SSSA special publication no. 17) SSSA, Madison, Wis., pp 521–548

Forster SM, Nicolson TH (1981) Aggregation of sand from a maritime embryo sand dune by microorganisms and higher plants. Soil Biol Biochem 13:199–203

Greenland DJ, Lindstrom GR, Quirk JP (1962) Organic materials which stabilize natural soil aggregates. Soil Sci Soc Am Proc 26:366–371

Harris RF, Chesters G, Allen ON, Attoe OJ (1964) Mechanisms involved in soil aggregate stabilization by fungi and bacteria. Soil Sci Soc Am Proc 28:529–532

Hendrix PF, Coleman DC, Crossley DA (1992) Using knowledge of soil nutrient cycling processes to design sustainable agriculture. J Sustain Agric 2:63–82

Hu S, Coleman DC, Beare MH, Hendrix PF (1995) Soil carbohydrates in aggrading and degrading agrosystems: influences of fungi and aggregates. Agric Ecosyst Environ 54:77–88

Kay BD (1990) Rates of change of soil structure under different cropping systems. Adv Soil Sci 12:1–52

Kemper WD, Rosenau RC (1986) Aggregate stability and size distribution. In: Klute A (ed) Methods of soil analysis: physical and mineralogical methods, part 1, 2nd edn. (ASA, SSSA special publication no. 9) ASA, SSSA, Madison, Wis., pp 425–442

Kinsbursky RS, Levanon D, Yaron B (1989) Role of fungi in stabilizing aggregates of sewage sludge amended soils. Soil Sci Soc Am J 53:1086–1091

Lorenzen K, Anke T (1998) Basidiomycetes as a source for new bioactive natural products. Curr Org Chem 2:329–364

Low AJ (1954) The study of soil structure in the field and the laboratory. J Soil Sci 5:57–74

Lynch JM, Bragg E (1985) Microorganisms and soil aggregation stability. In: Stewart BA (ed) Advances in soil science. Springer-Verlag, Berlin Heidelberg New York, pp 135–171

MacKay DC, Carefoot JM (1981) Control of water content in laboratory determination of mineralizable nitrogen in soils. Soil Sci Soc Am J 45:444–446

Martin JP (1945) Microorganisms and soil aggregation. I. Origin and nature of some of the aggregating substances. Soil Sci 59:163–174

Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol Biochem 22:579–584

Molope MB, Page ER (1986) The contributions of fungi, bacteria and physical processes in the development of aggregate stability of a cultivated soil. In: Lopez-Real JM, Hodges RD (ed) The role of microorganisms in a sustainable agriculture. AB Academic Publishers, Berkhamstead, UK

Ploetz RC, Mitchell DJ, Gallaher RN (1985) Population dynamics of soilborne fungi in a field multicropped to rye and soybeans under reduced tillage in Florida. Phytopathology 75:1447–1451

Reinertsen SA, Elliott LF, Cochran VL, Campbell GS (1984) Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. Soil Biol Biochem 16:456–464

Rennie DA, Truong E, Allen ON (1954) Soil aggregation as influenced by microbial gums, level of fertility and kind of crop. Soil Sci Soc Am Proc 18:399–403

Robertson EB, Shlomo S, Firestone MK (1991) Cover crop management of polysaccharide-mediated aggregation in a orchard soil. Soil Sci Soc Am J 55:734–739

Stotzky G (1997) Soil as an environment for microbial life. In: Elsas JD van, Travors J, Wellington EMH (eds) Modern soil microbiology. Marcel Dekker, New York, pp 1–20

Sutton JC, Sheppard BR (1976) Aggregation of sand dune soil by endomycorrhizal fungi. Can J Bot 54:326–333

Thomas RS, Dakessian S, Ames RN, Brown MS, Bethlenfalvay GJ (1986) Aggregation of a silty clay loam soil by mycorrhizal onion roots. Soil Sci Soc Am J 50:1494–1499

Thorn RG, Reddy CA, Harris D, Paul EA (1996) Isolation of saprophytic basidiomycetes from soil. Appl Environ Microbiol 62:4288–4292

Tiessen H, Steward JWB, Bettany JR (1982) Cultivation effects on the amounts and concentration of carbon, nitrogen, and phosphorus in grassland soils. Agron J 74:831–835

Tisdall JM (1991) Fungal hyphae and structural stability of soil. Aust J Soil Res 29:729–743

Tisdall JM, Oades JM (1979) Stabilization of soil aggregates by the root systems of ryegrass. Aust J Soil Res 17:429–441

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. J Soil Sci 33:141–163

Tisdall JM, Smith SE, Rengasamy P (1997) Aggregation of soil by fungal hyphae. Aust J Soil Res 35:55–60

Toyota K, Ritz K, Young IM (1996) Microbiological factors affecting the colonization of soil aggregates by *Fusarium oxysporum* f.sp. Raphani. Soil Biol Biochem 28:1513–1521

Van Veen JA, Paul EA (1981) Organic carbon dynamics in grassland soils. 1. Background information and computer simulations. Can J Soil Sci 61:185–201

Warcup JH (1950) The soil-plate method for isolation of fungi from soil. Nature 175:953–954

Warcup JH, Talbot PHB (1962) Ecology and identity of mycelia isolated from soil. Trans Brit Mycol Soc 45:495–518

Weill AN, De Kimpe CR, McKyes E (1989) Effect of tillage reduction and fertiliser on soil macro- and microaggregates. Can J Soil Sci 68:489–500

Wright SF, Franke-Snyder M, Morton JB, Upadhyaya A (1996) Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181:193–203