Pergamon

PII: S0038-0717(97)00207-1

Soil Biol. Biochem. Vol. 30, No. 7, pp. 905–916, 1998
Published by Elsevier Science Ltd
Printed in Great Britain
0038-0717/98 $19.00 + 0.00

CONTRIBUTIONS OF INTERACTING BIOLOGICAL MECHANISMS TO SOIL AGGREGATE STABILIZATION IN RESTORED PRAIRIE*

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(Accepted 7 August 1997)

Summary—A better understanding of the soil aggregation process is needed to address a variety of concerns, including soil quality and erosion, agricultural sustainability, soil C sequestration, the mobility of hazardous chemicals and remediation of contaminated sites. We used data from a chronosequence of tallgrass prairie restorations and a path analysis approach to evaluate how the lengths of two dia size classes of fibrous roots, the length of external mycorrhizal hyphae, microbial biomass C, hot-water soluble carbohydrate C and soil organic C interact to promote the stabilization of soil aggregates. The measured binding agents accounted for 88% of the variation in macroaggregates >212 μm diameter and goodness-of-fit indexes indicated a good practical fit of the path model to the data. The restoration of macroaggregate structure in this system was apparently driven by the direct and indirect effects of roots and external hyphae, with lesser relative contributions by the three measured C pools. Although the two root size classes had similar direct effects on the percentage of macroaggregates, their indirect contributions differed substantially. Fine roots (0.2–1 mm diameter) exerted considerable indirect effects via their strong influences on external hyphae and microbial biomass C. Very fine roots (<0.2 mm dia) made a stronger contribution to soil organic C than fine roots, but their overall indirect contribution to aggregation was minimal. In addition, the relative importance of each binding agent varied for different size fractions of macroaggregates and generally supported the hypothesis that the effectiveness of various binding mechanisms depends on the physical dimensions of the binding agents relative to the spatial scales of the aggregate planes of weakness being bridged. Published by Elsevier Science Ltd

INTRODUCTION

The importance of soil structure to soil tilth, water relations, root penetration and erosion potential has been studied in an agricultural context for years. However, the role of soil structure in controlling soil ecosystem function and the feedbacks between soil structure and soil organisms are only now being elucidated (Elliott and Coleman, 1988). Consequently, a better understanding of the formation, stabilization and degradation of soil aggregates and the resulting effects on soil structure will be necessary to address a variety of environmental concerns, ranging from the fate and transport of hazardous wastes and the remediation of contaminated sites to the potential C sink strength of terrestrial ecosystems.

Modern approaches to investigating the formation and stabilization of aggregates in soils where organic matter is the major binding agent have been influenced considerably by the hierarchical view of the aggregation process proposed by Tisdall and Oades (1982). In this conceptual model, the mechanisms of aggregate formation and stabilization and the relative importance of these mechanisms change with spatial scale as primary particles and clay microstructures are bound into microaggregates (20–250 μm diameter) and as increasingly larger macroaggregates (>250 μm up to several millimeters diameter) are formed by the binding together of microaggregates and smaller macroaggregates.

Organic binding agents can vary in dimensions over four orders of magnitude, ranging from short-chain organic molecules to plant roots (Kay, 1990). Tisdall and Oades (1982) suggested that organic binding agents can be classified into three broad categories on the basis of their temporal persistence: (1) transient (mainly polysaccharides); (2) temporary (roots, fungal hyphae, bacterial cells, and algae)

*The submitted manuscript has been created by the University of Chicago as operator of Argonne National Laboratory under Contract No. W-31-109-ENG-38 with the U.S. Department of Energy.
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and (3) persistent (humic materials associated with polyvalent metal cations and polymers strongly sorbed to clays). Transient binding agents generally persist on the order of weeks and temporary agents may exist for periods ranging from months to a few years. The long-term existence of persistent agents (perhaps tens to hundreds of years) may be due more to protection from decomposition by their physicochemical associations with inorganic soil components than to any inherent biochemical inertness (Kay, 1990).

A consequence of aggregate hierarchy is the porosity exclusion principle (Dexter, 1988). If aggregate hierarchy exists, then smaller aggregates should have smaller pores, greater contact between particles and higher bulk densities than larger aggregates because the latter also contain larger pores between the smaller aggregates that comprise them (Oades, 1993). As such, the effectiveness of various binding mechanisms will depend on their physical dimensions relative to the dimensions of the pores (i.e. planes of weakness) being bridged (Kay, 1990).

In soils where organic matter is accumulating, some researchers have suggested that roots and fungal hyphae provide the mechanical framework for the formation and initial stabilization of macroaggregates (e.g. Elliott and Coleman, 1988; Gupta and Germida, 1988; Oades and Waters, 1991). Once stable macroaggregates are formed, intense biological activity can occur at sites within them. As a result, substantial amounts of polysaccharides and other organics are deposited and serve to further stabilize both the macroaggregates and the microaggregates that comprise them. In addition, the results of electron microscopy studies suggest that the decomposition process is what leads to the development of an aggregate hierarchy (Tiessen and Stewart, 1988; Oades and Waters, 1991). Particulate organic matter (mostly plant and fungal debris) is often found at the core of microaggregates 90–250 μm in diameter and is protected from rapid decomposition by encrustation with inorganic material. This particulate matter is eventually decomposed, leaving cavities surrounded by smaller microaggregates believed to be stabilized by the metabolic products and bodies of microbes that colonized the debris (Oades and Waters, 1991).

Although cultivation of previously undisturbed soils usually results in the loss of organic C and the degradation of soil aggregates (Tiessen *et al.*, 1982; Dormaar, 1983; Elliott, 1986; Gupta and Germida, 1988), major improvements in stability can occur without significant increases in organic C. Thus, aggregate stability may be more strongly affected by some C pools than others (Kay, 1990). For example, Haynes and Swift (1990) found that aggregate stability was more closely associated with the hot-water extractable carbohydrate pool than with total soil organic C (SOC) or the hydrolyzable carbohydrate pool.

In our initial studies of a chronosequence of prairie restorations, we found that a stable macroaggregate structure developed rapidly once cultivation ceased (Jastrow, 1987). Later, we employed the statistical technique of path analysis to demonstrate how aggregate size distributions at this site are influenced by roots with differing morphologies and by the external hyphae of mycorrhizal fungi (Miller and Jastrow, 1990). Because the restoration chronosequence provides a gradient of aggregate stabilization, along with gradients of root and hyphal proliferation and of various C pools, it may be used to examine how different organic binding mechanisms interact to promote aggregate stabilization. We present here a conceptual model of the potential interrelationships among roots, mycorrhizal hyphae, SOC, microbial biomass C (MBC) and hot-water soluble carbohydrate C (hwsCHO-C) and of their contributions to aggregate stabilization in disturbed mollisols. We use path analysis to test the adequacy of the model and to compare the relative importance of these aggregate binding agents in a system undergoing restoration. We also extend this approach to investigate whether the relative strength of each binding mechanism changes with the spatial scale of the aggregates being stabilized.

MATERIALS AND METHODS

Study area

The study area consisted of four plots in a chronosequence of restored tallgrass prairie and an adjacent agricultural field within the National Environmental Research Park at Fermi National Accelerator Laboratory (Fermilab), Batavia, IL, 48 km west of Chicago. The agricultural field represented a baseline level of soil aggregation for the site and was variously cultivated for over 100 years, most recently in continuous corn (*Zea mays* L.). The prairie restorations had been similarly cultivated for row crops, but in 1969–1970 the area was taken out of cultivation and allowed to revert to an old-field condition until each plot was plowed, disked and planted with prairie species. The four sampled restorations (planted in spring 1975, autumn 1977, spring 1981 and spring 1984) encompassed the range of plot ages within the chronosequence. At the time of sampling (17–27 June 1985), the plots had completed 10, 7, 4 and 1 growing seasons, respectively. Although aggregate size distributions were not measured at the time these plots were planted, when similar plots were planted after longer periods of old field, the initial percentages of water-stable macroaggregates were usually slightly above those reported for the cornfield and were always below those found after one growing season in prairie.

All plots were located primarily on Mundelein silt loam (fine-silty, mixed, mesic Aquic Argiudoll). Some of the restorations also occupied lesser areas of Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquoll) and limited areas of Wauconda silt loam (fine-silty, mixed, mesic Udollic Ochraqualf). More detailed descriptions of the sites, prairie restoration methods, burn histories and plant species composition were reported by Jastrow (1987).

Sample collection and methods

Within each of the sampled plots, 10 sample stations consisting of a 0.5 m^2 circular quadrat were located by using a stratified random design. The aboveground standing crop inside each quadrat was clipped to within 2 cm of the surface and removed. After clipping, four soil cores (4.8-cm dia) were taken from the center of each quadrat to a depth of 10 cm. Two of these cores were used to assay root length, one was used to estimate both the external length of mycorrhizal hyphae and MBC and one was used to determine the size distribution of water-stable aggregates. Three additional cores from equally spaced locations just inside the perimeter of the quadrat were pooled to determine SOC and hwsCHO-C for each quadrat. Bulk density was determined for each quadrat by the core method (Blake and Hartge, 1986). Because of the number of samples, the number of parameters to be measured and the noticeable fungal growth that occurs on these soils within a few months under refrigerated conditions, all soil cores were stored frozen and thawed overnight at 4°C before analysis.

Roots and rhizomes were removed from two separate cores by repeated flotation and sieving (500-μm sieve) in water. Fibrous roots ($\leq$1 mm dia) were separated from rhizomes and coarse roots ($>$1 mm dia) without differentiating between current and previous years' growth. Fibrous root lengths were estimated from 0.25-g fresh subsamples by using the gridline intersect method as detailed in Cook *et al.* (1988). A natural break in root size classes was noticed at 0.2 mm dia; therefore, fine fibrous roots (0.2–1 mm dia) and very fine fibrous roots ($<$0.2 mm dia) were scored separately. Root length estimates for the two soil cores were pooled for each quadrat.

A modified membrane filter technique was used to estimate the length of external mycorrhizal hyphae. The field-moist core was broken apart, most roots and rhizomes were removed and the soil was passed through a 2-mm sieve. Two 10-g subsamples were soaked overnight in separate beakers with 300 ml of sodium hexametaphosphate solution (0.4%), sonicated for 25 s at 120 W and then diluted 1:20. A 20-ml aliquot of the diluted suspension was centrifuged at 1000*g* for 8 min and the pellet was resuspended in 50% glycerol. After centrifugation at 75*g* for 30 s, the supernatant was filtered through a 20-μm-mesh nylon filter. The filter was placed in a staining solution of lactic trypan blue and vortexed to resuspend hyphae. After 1.5 h, the staining solution was filtered quantitatively through a cellulose nitrate membrane filter with a 0.8-μm pore size. Seventy fields of view were scored by a grid intersection method (Olson, 1950) at 160$\times$ by using an eyepiece reticle. Hyphae were identified as mycorrhizal on the basis of morphology (Nicolson, 1959). Hyphal length estimates were corrected for soil moisture and averaged for each quadrat.

Microbial biomass C was estimated by the fumigation–extraction method (Vance *et al.*, 1987) on subsamples of the soil prepared for determination of external hyphal lengths. Field-moist subsamples (10 g) were incubated at room temperature for 27 h, fumigated with alcohol-free chloroform for 24 h and extracted (after removal of the fumigant) for 30 min with 50 ml of 0.5 M potassium sulfate. Nonfumigated controls were incubated concurrently with the fumigated subsamples but were extracted when fumigation commenced. Organic C concentrations in the extracts were determined by ultraviolet degradation in a Sybron Photochem Organic C analyzer. The difference between extracted C in fumigated and nonfumigated soils was multiplied by 2.64 and corrected for soil moisture to estimate MBC (Vance *et al.*, 1987).

The size distribution of water-stable macroaggregates was determined by slaking of air-dry soil followed by wet sieving. A modification of Kemper and Chepil (1965) was used, as detailed in Jastrow (1987). The entire core was broken apart along its natural breaking points to pass a 9.5-mm sieve, air dried and wet-sieved through a nest of sieves with hole widths of 4750, 2000, 1000 and 212 μm. For this study, aggregate quantities retained on the 4750-μm and 2000-μm sieves were evaluated together as one $>$2000-μm fraction.

The pooled cores used to determine SOC and hwsCHO-C were broken apart and most roots and large organic debris were removed. After air-drying, the soil was gently crushed and passed through a 2-mm sieve. Soil organic C was measured colorimetrically on Walkley-Black dichromate digests (Nelson and Sommers, 1982). After further grinding of soil samples to pass a 250-μm sieve, two 1-g subsamples were extracted with 20 ml of deionized water for 16 h at 80°C. Extracts were centrifuged, filtered through 0.45-μm membrane filters (prewashed with 100 ml of deionized water), shaken for 30 min with Ca-saturated cation exchange resin (Deng and Tabatabai, 1994), analyzed for total reducing sugars by the arsenomolybdate method (Nelson, 1944) and averaged for each quadrat.

Data on external hyphal lengths, MBC, SOC and hwsCHO-C per gram of soil were converted to a

soil volume basis by using data on soil bulk density for each quadrat.

Path model development and assumptions

We used path analysis to investigate the causal processes underlying our observational data (e.g. Pedhazur, 1982; Asher, 1983; Mitchell, 1992). Path modeling techniques do not allow determination or testing of causality between variables. Rather, *a priori* knowledge of the system or theoretical grounds are used to construct a conceptual model (path diagram) of the causal and noncausal interrelationships among the measured variables and then the data are used to evaluate the model.

Our conceptual model (Fig. 1) expands on our earlier model examining the relationships among root lengths, the colonized lengths of roots and the lengths of external mycorrhizal hyphae and their effects on soil aggregation (Miller and Jastrow, 1990). Indirect paths involving the functional relationships between roots and external hyphae via colonized root lengths were not included in Fig. 1 because our purpose in this study was to investigate the mechanistic contributions of potential aggregate binding agents. Instead, hypothesized contributions of SOC, MBC and hwsCHO-C to aggregate stabilization were added. Also, we used the percentage of water-stable macroaggregates ($>212\ \mu$m dia) as the measure of soil aggregation because (1) it weights all aggregate size classes equally and (2) it facilitates comparisons of how the relative contributions of the measured variables change for various size fractions of macroaggregates. The following is an account of the assumptions and empirical information used to develop our conceptual model (Fig. 1).

The lengths of fine and very fine roots, external hyphal length, MBC and hwsCHO-C were assumed to have direct effects on the percentage of macroaggregates (e.g. Tisdall and Oades, 1979, 1980, 1982; Gupta and Germida, 1988; Haynes and Swift, 1990; Miller and Jastrow, 1990; Oades and Waters, 1991; Tisdall, 1991). In addition to their direct effects, the two size classes of roots have indirect effects through their associations with external hyphae (Miller and Jastrow, 1990) and through their effects on SOC and MBC and, indirectly, on hwsCHO-C (see below).

We assumed that fine root length was correlated with but not causally linked to very fine root length for two reasons. First, not all very fine roots are derived from the larger fine root size class; rather, the two size classes may be largely associated with different plant species. Second, even if a significant portion of very fine roots were derived from fine roots, a causal linkage would inflate the indirect effects of fine roots on aggregation in a manner that does not reflect a true mechanism associated with the roots that physically occur within the fine root size class.

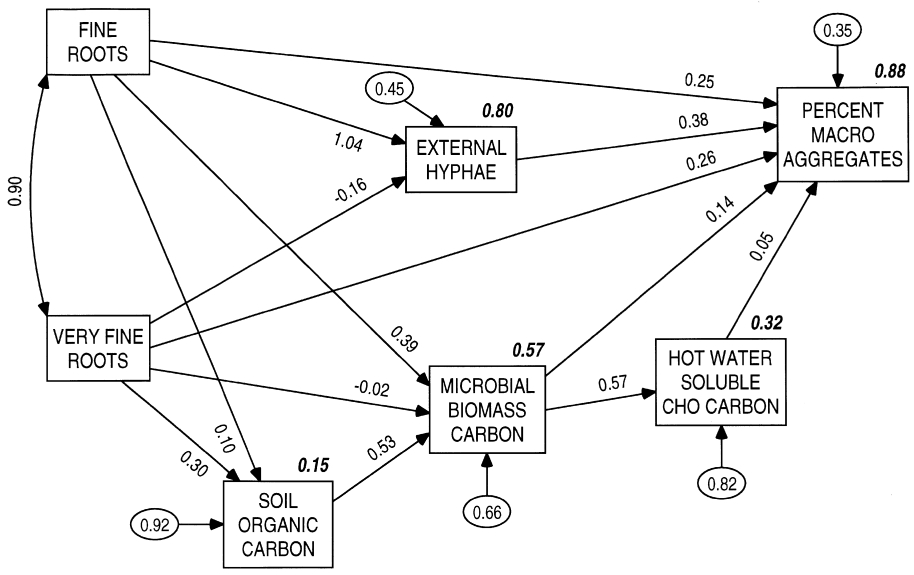

Fig. 1. Path model of hypothesized causal relationships among the lengths of roots and mycorrhizal fungal hyphae, three soil C pools and the percentage of water-stable soil aggregates in a chronosequence of prairie restorations. Fine roots are 0.2–1 mm dia; very fine roots are <0.2 mm dia. Single-headed arrows indicate direct causal relationships and double-headed arrows indicate unanalyzed correlations. Numbers are path coefficients and proportion of total variance explained (r^2; shown in bold italics) for each endogenous (dependent) variable ($n = 49$). The numbers within ellipses represent the proportion of unexplained variance $[(1 - r^2)^{1/2}]$ and, thus, indicate the relative contribution of all unmeasured or unknown factors to each dependent variable.

We also made several assumptions about causal interactions among the measured C pools. First, we assumed SOC is directly affected by inputs from the two size classes of roots. These inputs could include soluble exudates, rhizodeposition from functional roots and dead root tissue (e.g. Newman, 1985). Second, exudates and rhizodeposition from both size classes of roots were assumed to directly affect MBC in the active rhizosphere (Elliott *et al.*, 1984; Foster, 1985; Newman, 1985; Smucker and Safir, 1986). For rhizodeposited materials that become complexed with polyvalent cations or adsorbed to clay surfaces (e.g. Cheshire, 1985) and for dead root tissue, however, we assumed the effect on MBC is indirect via SOC. Third, the contributions of roots and SOC to hwsCHO-C were limited to indirect effects via MBC, because hwsCHO-C is believed to represent a pool of extracellular polysaccharides that are primarily microbial in origin (Redl *et al.*, 1990; Haynes and Francis, 1993). Lastly, the effect of SOC on macroaggregates was restricted to indirect paths through MBC and hwsCHO-C. We included a path from SOC to macroaggregates initially but found very little of the correlation was attributable to a direct causal component. Also, because the chronosequence is an aggrading system, changes in SOC appeared to be more of a response to rather than a driving mechanism of aggregation (Jastrow, 1996). Similarly, we did not include hydrolyzable carbohydrates in the model because preliminary investigations indicated that it was only marginally correlated to the percentage of macroaggregates ($r = -0.27$, $n = 49$, $P = 0.06$) and that its causal contribution was negligible. The exclusion of direct links from SOC or hydrolyzable carbohydrate to aggregation is also supported by other studies (e.g. Baldock and Kay, 1987; Baldock *et al.*, 1987; Haynes and Swift, 1990).

Data handling and path analysis methods

For the calculation of observed correlations and for the path analysis, logarithmic transformations were applied to the data for root and external hyphal lengths to normalize data distributions and linearize relationships with other variables. Multicollinearity among the independent variables was assessed for each dependent variable by using the COLLIN option for PROC REG in SAS (SAS Institute Inc., 1985), which provides an eigenanalysis of the scaled cross-products matrix for the independent variables (Kleinbaum *et al.*, 1988). Fairly strong collinearity was detected between fine root length and external hyphal length and the lengths of fine and very fine roots were marginally collinear. Consequently, all of the variables were centered (i.e. the mean value for each variable was subtracted from each of the individual data values for that variable) to eliminate the collinearity problems.

By using path analysis, the total correlations between independent and dependent variables were decomposed into causal components (direct and indirect effects) and noncausal components (spurious and unanalyzed contributions). In the path diagram (Fig. 1), a straight single-headed arrow indicates a direct causal path. Indirect causal effects occur if a variable is linked to a given dependent variable via one or more intermediary variables (i.e. a compound path) by always moving in the causal direction of the arrows. The path coefficients indicate the relative magnitudes of the direct effects of the independent variables linked to each dependent variable. The total causal effect of one variable on another is the sum of the direct and indirect effects. Spurious noncausal components are similar to indirect effects because another variable(s) links the two variables of interest. However, the effect is not substantively meaningful because the initial link is to a variable that is causally prior to the variable of interest and thus the resulting compound path violates the causal ordering of the variables. Unanalyzed noncausal components result if the two variables of interest are linked by unanalyzed correlations between the variables (i.e. the hypothesized relationship between variables is not zero, but the variables are not considered to be causally related).

Path coefficients and their significance levels were calculated by the structural equation modeling approach (Mitchell, 1992) with the Amos 3.51 program (Arbuckle, 1995), which carries out an analysis of moment structures using a maximum likelihood criterion. Indirect effects, correlations implied by the structure of the path model, and two goodness-of-fit tests (the χ^2 statistic and the Tucker–Lewis index) were also calculated with Amos 3.51.

The goodness of fit of the path model to the observed data was tested because potential paths from some variables to other variables were hypothesized (restricted) to be zero and, thus, were not represented in the path diagram. The specified pattern of restricted and free paths implies a pattern of covariance among the variables that can be compared with the observed covariance structure by a log-likelihood ratio that is distributed approximately as χ^2 (Pedhazur, 1982; Mitchell, 1992). The null hypothesis is that the observed and model-implied covariance matrices are equal. Rejection of the null hypothesis leads to the conclusion that the model does not fit the data. Because the χ^2 statistic is affected by sample size, its usefulness has been questioned (e.g. Pedhazur, 1982; Marsh *et al.*, 1988). Consequently, we also evaluated the model with an index of "practical" fit (Tucker and Lewis, 1973), which compares the fit of the hypothesized model to the fit of a "null" model (i.e. all variables in the model are assumed to be uncorrelated). We used the Tucker–Lewis index because, in compari-

sons to other commonly used indexes of practical fit, it appears to be the least affected by sample size (Marsh *et al*., 1988). The Tucker–Lewis index tends to range from zero to one (it may go higher), with large values suggesting a good fit and values over 0.9 indicating little chance for substantial improvement (Bentler and Bonett, 1980).

RESULTS

The dataset

The data used in the path analysis are presented in Table 1. In general, all variables except the 212–1000 μm aggregate fraction increased across the chronosequence. Observed correlations among all variables were significant ($P \leq 0.05$) except for the relationships of the 212–1000 μm aggregate fraction with SOC, MBC and the 1000–2000 μm aggregate fraction (Table 2). All variables except SOC were highly correlated ($P \leq 0.001$) with the percentage of macroaggregates >212 μm.

Path analysis

Eighty-eight percent of the variation in macroaggregates >212 μm was explained by the five variables hypothesized to have direct effects on aggregation (Fig. 1). The strongest path was from external hyphal length. The paths from fine and very fine roots were essentially equal and were stronger than the path from MBC. The path from hwsCHO-C was the weakest.

The relative contribution of fine roots to external hyphae was quite strong compared to the path from very fine roots, which was negative. Interestingly, very fine roots had a much stronger path to SOC than did fine roots, but the reverse was true for MBC. In fact, the path from very fine roots to MBC was negligible. Soil organic C contributed the strongest path to MBC.

Fine root length had the greatest total effect on macroaggregates due to its strong direct effect and to an even stronger indirect effect through its influences on external hyphal length and the three measured C pools (Table 3). Although the total effect of external hyphal length was entirely direct, it was the second strongest. The total effect of very fine root length followed, even though the summation of its indirect effects through external hyphae and the three C pools was negative. Of the three measured C pools, MBC contributed the strongest total effect.

The indirect effect of very fine roots on MBC via SOC resulted in an overall positive effect, but the total effect was still substantially less than the total effects of fine roots or SOC. Also, fine roots had a stronger indirect effect on hwsCHO-C than very fine roots because of the relationships with MBC. Overall, MBC had the greatest total effect on hwsCHO-C.

The log-likelihood ratio χ^2 for our model was 13.1 (df = 7, $P = 0.070$) and the Tucker–Lewis index was 0.943, suggesting a good practical fit of the model to the data. Consequently, implied correlations calculated from the model deviate very little, in most cases, from observed correlations (Fig. 2). The most extensive deviations occurred in the relationships of fine roots, external hyphae and macroaggregates with hwsCHO-C.

Analyses for separate aggregate size fractions

A separate path analysis was performed for each of three macroaggregate size classes (>2000, 1000–2000 and 212–1000 μm diameter) as summarized in Table 4. The path diagrams were identical to those in Fig. 1, except that each aggregate size class was substituted, in turn, for the percentage of macroaggregates >212 μm. In each case, the path coefficients were also identical to those in Fig. 1, except for the five paths leading directly to aggregate size class. The proportion of variation in each aggregate size class explained by direct effects decreased as aggregate size class decreased. However, Tucker–Lewis index values suggested that the practical fits of the models to the data were relatively good, although the fit for all macroaggregates >212 μm was better.

Table 1. Mean quantities (standard errors in parentheses; $n = 10$) of measured soil aggregate size fractions and of the organic binding agents evaluated by path analysis for a chronosequence of restored tallgrass prairie

	Cornfield	Restored prairie			
	0 gs*	1 gs	4 gs	7 gs†	10 gs
Total macroaggregates >212 μm (% whole soil)	39.2 (2.5)	67.9 (2.0)	85.2 (1.8)	89.1 (1.6)	91.5 (0.9)
Aggregate fraction >2000 μm (% whole soil)	9.5 (1.2)	24.6 (2.0)	49.0 (1.9)	51.9 (2.4)	52.8 (2.7)
Aggregate fraction 1000–2000 μm (% whole soil)	5.4 (0.5)	14.5 (1.3)	16.4 (0.7)	19.7 (1.5)	20.6 (1.2)
Aggregate fraction 212–1000 μm (% whole soil)	24.3 (1.9)	28.8 (1.4)	19.7 (1.2)	17.4 (1.1)	18.1 (1.3)
Fine root length (cm cm^{-3} soil)	0.7 (0.2)	6.6 (1.0)	23.8 (4.7)	23.8 (2.1)	24.9 (2.5)
Very fine root length (cm cm^{-3} soil)	0.6 (0.2)	24.7 (8.7)	64.6 (11.6)	60.0 (14.2)	48.9 (7.4)
External hyphal length (m cm^{-3} soil)	16.9 (0.8)	26.9 (1.3)	39.4 (1.9)	45.0 (2.7)	45.4 (1.7)
Soil organic C (mg cm^{-3} soil)	38.3 (1.0)	48.4 (1.1)	41.9 (2.2)	47.2 (2.8)	49.7 (2.2)
Microbial biomass C (μg cm^{-3} soil)	422 (40)	732 (35)	757 (82)	823 (94)	850 (47)
Hot-water soluble carbohydrate C (μg cm^{-3} soil)	143 (5)	134 (3)	162 (8)	180 (8)	179 (6)

*gs = complete growing seasons since cultivation ceased.
†$n = 9$.

Table 2. Pearson product-moment correlation (r) matrix for root lengths, external hyphal length, measured soil C pools and soil aggregate size fractions ($n = 49$)

	FRL	VRL	EHL	SOC	MBC	CHO	Macro	Large	Med	Small
Fine root length (FRL)†	1.00									
Very fine root length (VRL)†	0.90***	1.00								
External hyphal length (EHL)†	0.89***	0.77***	1.00							
Soil organic C (SOC)	0.36**	0.38**	0.31*	1.00						
Microbial biomass C (MBC)	0.57***	0.54***	0.56***	0.67***	1.00					
Hot-water soluble carbohydrate C (CHO)	0.51***	0.39**	0.54***	0.49***	0.57***	1.00				
Total macroaggregates >212 μm (Macro)	0.91***	0.85***	0.89***	0.43**	0.65***	0.55***	1.00			
Aggregate fraction >2000 μm (Large)	0.90***	0.80***	0.90***	0.28*	0.55***	0.60***	0.92***	1.00		
Aggregate fraction 1000–2000 μm (Med)	0.75***	0.75***	0.74***	0.54***	0.66***	0.51***	0.89***	0.69***	1.00	
Aggregate fraction 212–1000 μm (Small)	−0.50***	−0.37**	−0.55***	0.02	−0.20	−0.54***	−0.39**	−0.69***	−0.18	1.00

†Data were $\log(X + 1)$ transformed.
* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

For the >2000 μm size fraction, the direct effects of fine roots and external hyphae were strongest. The contribution of hwsCHO-C was greater than for macroaggregates as a whole. The largest single total effect was due to fine roots because of their very strong association with external hyphae in addition to their own direct effects.

For the 1000–2000 μm size class, very fine roots had the largest total effect, which was due essentially to direct effects. The importance of external hyphae was still strong but was relatively less than for the >2000 μm size class. However, the relative contributions of MBC increased considerably. Although the direct effect of fine roots was negative, its strong positive indirect effects allowed its total contribution to be essentially equal to that of external hyphae and MBC. The total effect of SOC was relatively greater for this size class than for the others.

The large negative effects of fine roots, external hyphae, and hwsCHO-C on 212–1000 μm aggregates imply that slaking results in relatively few small macroaggregates when these binding agents are present in larger quantities and *vice versa*. The strongest positive effect on this size class was due to very fine root length. The direct effect of MBC was greatest for this size class, but its total effect was reduced considerably by the negative indirect effect resulting from its influence on hwsCHO-C.

DISCUSSION

The strong influences of roots and mycorrhizal hyphae on the stabilization of aggregates in restored prairie has already been demonstrated (Miller and Jastrow, 1990). Yet, various soil C pools are also often proposed as important aggregate stabilizing mechanisms. At the Fermilab site, almost all of the potential organic binding agents that we evaluated were strongly correlated with the amounts of stable aggregates. Because of the underlying interactions and covariance among binding agents, a simple comparison of correlations could lead to erroneous or indecisive conclusions about the relative importance of each agent in this system. By using path analysis, however, we were able to separate the direct and indirect causal components from the noncausal components of these strong correlations and, thereby, to evaluate the relative contributions of various aggregate binding agents in a grassland system recovering from long-term disturbance.

Total causal effects on aggregation

Although C is accumulating in this system (Jastrow, 1996), the restoration of a stable macroaggregate structure is apparently driven by the direct and indirect effects of roots and external hyphae, rather than by the C pools often considered important to aggregation. This finding supports Tisdall

Table 3. Decomposition of correlations with macroaggregate percentage, external hyphal length, soil organic C, microbial biomass C and hot-water soluble carbohydrate C into direct and indirect effects

Variables in correlations	Direct effect*	Indirect effect*	Total effect*
	Macroaggregate percentage		
Fine root length	0.25	0.47	0.72
Very fine root length	0.26	–0.04	0.22
External hyphal length	0.38	0	0.38
Soil organic C	0	0.09	0.09
Microbial biomass C	0.14	0.03	0.17
Hot-water soluble carbohydrate C	0.05	0	0.05
	External hyphal length		
Fine root length	1.04	0	1.04
Very fine root length	–0.16	0	–0.16
	Soil organic C		
Fine root length	0.10	0	0.10
Very fine root length	0.30	0	0.30
	Microbial biomass C		
Fine root length	0.39	0.05	0.44
Very fine root length	–0.02	0.16	0.14
Soil organic C	0.53	0	0.53
	Hot-water soluble carbohydrate C		
Fine root length	0	0.25	0.25
Very fine root length	0	0.08	0.08
Soil organic C	0	0.30	0.30
Microbial biomass C	0.57	0	0.57

*Direct effects are simple paths and are equal to the path coefficients in Fig. 1. Indirect effects are the sum of the products of the chain of path coefficients for all compound paths for which the independent variable is connected to the dependent variable while maintaining the causal direction of the arrows. Total effects are the sum of direct and indirect effects.

and Oades (1982) conceptual model of macroaggregate stabilization via temporary binding agents. The strong effects of roots and hyphae, coupled with lesser total effects for the three C pools, lend support to the view that in systems where C is aggrading, roots and hyphae may provide the mechanical framework for the formation and initial stabilization of macroaggregates, with further stabilization occurring as the result of a variety of processes and activities within the macroaggregate (Elliott and Coleman, 1988; Gupta and Germida, 1988; Kay, 1990; Oades and Waters, 1991; Tisdall, 1991; Jastrow and Miller, 1988). Gupta and Germida (1988) suggested that microbial biomass and mucigels are a major component of the relatively labile organic matter believed to be important in the binding of microaggregates into macroaggregates (Dormaar, 1984; Elliott, 1986). Although hwsCHO-C was strongly correlated with macroaggregates, the results of the path analysis suggested that its importance relative to other mechanisms was minimal. This is probably because a large share of its correlation with macroaggregates was related to MBC and roots, which overall were better predictors in this system. Also, because of the lability of this pool, it may be turned over quickly wherever microbes are active, making the pool too dynamic and patchy to be a strong predictor unless it is considered in the absence of other mechanisms.

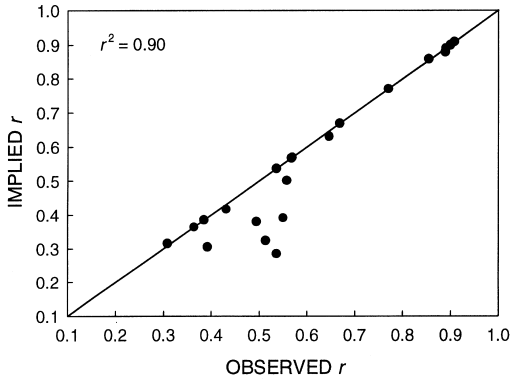

Fig. 2. Comparison of observed correlations among measured variables with implied correlations calculated from the path model. The line ($y = x$) indicates where points would fall in a perfectly fit model.

Direct effects of roots and hyphae on aggregation

The direct effects of fine and very fine roots were essentially equal, in contrast to our earlier model in which the relative effect of fine roots was much greater (Miller and Jastrow, 1990). A major reason for this was our switch from aggregate geometric mean diameter (and its heavy weighting on the amounts of large macroaggregates) to the percentage of macroaggregates (see the direct effects of each root size class on each aggregate size fraction in Table 4). Another factor was inclusion of the three C pools. In addition to changes caused by adding direct paths from hwsCHO-C and MBC to macroaggregates, the indirect effects of roots

Table 4. Decomposition of correlations with separate macroaggregate size fractions into direct and indirect effects from separate path analyses for each size fraction

Variable in correlation	Aggregate fraction >2000 μm*			Aggregate fraction 1000–2000 μm†			Aggregate fraction 212–1000 μm‡		
	Direct effect	Indirect effect	Total effect	Direct effect	Indirect effect	Total effect	Direct effect	Indirect effect	Total effect
Fine root length	0.41	0.48	0.89	−0.13	0.47	0.34	−0.27	−0.38	−0.65
Very fine root length	0.08	−0.06	0.02	0.45	−0.01	0.44	0.16	0.08	0.24
External hyphal length	0.44	0	0.44	0.32	0	0.32	−0.40	0	−0.40
Soil organic C	0	0.03	0.03	0	0.17	0.17	0	0.04	0.04
Microbial biomass C	−0.05	0.10	0.05	0.28	0.04	0.32	0.35	−0.27	0.08
Hot-water soluble carbohydrate C	0.18	0	0.18	0.07	0	0.07	−0.47	0	−0.47

*$r^2 = 0.87$; log-likelihood ratio $\chi^2 = 17.3$, df = 7, $P = 0.015$; Tucker and Lewis (1973) index = 0.903.
†$r^2 = 0.69$; log-likelihood ratio $\chi^2 = 16.0$, df = 7, $P = 0.025$; Tucker and Lewis (1973) index = 0.902.
‡$r^2 = 0.39$; log-likelihood ratio $\chi^2 = 18.9$, df = 7, $P = 0.009$; Tucker and Lewis (1973) index = 0.857.

through the three C pools represented a more realistic partitioning of root effects. The contributions of these indirect mechanisms were included in the direct effects of roots on aggregates in the earlier model.

Much of the direct effects of roots and hyphae may be conceptualized by viewing the physical entanglement mechanism as a "sticky string bag" (Oades and Waters, 1991). Roots and hyphae not only form a network that can serve as a framework for macroaggregate formation, but extracellular mucilage coatings on root and hyphal surfaces can strongly sorb to inorganic materials, thereby helping to stabilize aggregates (Tisdall and Oades, 1979; Gupta and Germida, 1988; Tisdall, 1991; Dorioz *et al.*, 1993). In addition, encrustation of roots and hyphae with inorganics is believed to physically slow decomposition thereby preserving aggregate structure for a time even after the roots and hyphae senesce (Oades and Waters, 1991). Furthermore, the pressures exerted by growing roots and by localized drying caused by water uptake are physical forces that promote both aggregate formation and degradation (Kay, 1990). Root density and distribution can thus influence aggregate size.

The importance of roots and rhizosphere activities to aggregation was summarized by Allison (1968). He suggested that nearly ideal conditions for aggregate formation and stabilization exist simultaneously in the rhizosphere of grasses. Fine roots influence aggregation by (1) exerting pressures that help to form aggregates; (2) continually removing water, causing localized drying that produces stresses and strains; (3) producing exudates and rhizodeposition that either directly or indirectly, through microbial decomposition, play a role in aggregation and (4) serving as a continual source of particulate organic matter and, eventually, humus.

Root morphology and indirect effects of roots on aggregation

Miller and Jastrow (1990) called attention to the importance of root morphology to aggregation. Although the two root size classes had similar direct effects on the percentage of macroaggregates, their indirect contributions differed substantially. Thus, different types of roots may vary in their relationships with mycorrhizal fungi, soil C pools and below-ground function. Any effects associated with root size class may be a function of root morphology *per se* or of plant species or lifeform. In restored prairie at the Fermilab site, prairie grasses and perennial Compositae were the best predictors of fine root length, whereas cool-season C3 grasses were more strongly associated with very fine roots (Miller and Jastrow, 1990).

Fine roots had a stronger influence on external hyphae than did very fine roots. In fact, without intermediary paths through colonized root lengths, the direct effect of very fine roots on external hyphae was negative. Although not strong, this relationship suggests that hyphal lengths were generally reduced in areas with high densities of very fine roots, perhaps because species with heavy investments in extensive, very fine fibrous root systems are not usually very dependent upon mycorrhizal fungi to obtain needed nutrients (Hetrick *et al.*, 1988, 1992). In fact, in other studies at the Fermilab site, an ungrazed pasture of cool-season C3 grasses was found to have greater lengths of very fine roots but lower external hyphal lengths compared to the prairie (Reinhardt and Miller, 1990; Miller *et al.*, 1995). Thus, a large share of the indirect effects of fine roots on macroaggregates was due to their strong positive effect on external hyphae, whereas the negative relationship between very fine roots and external hyphae was largely responsible for the negative indirect effect of very fine roots on macroaggregates.

Similarly, fine roots also exhibited a strong direct effect on MBC in contrast to the negligible effect of very fine roots. This finding suggests that fine roots may foster more microbial growth and activity in their rhizospheres than do very fine roots. Because fine roots may often have more cortex than very fine roots, rhizodeposition of sloughed cells may be greater around fine roots. Similarly, exudation rates, amounts, or quality may be higher for fine roots, perhaps because of (1) inherent differences among plant species or lifeforms, (2) differences in

lateral meristem activity, or (3) greater associations with mycorrhizal fungi (Merckx *et al.*, 1985; Azcón-Aguilar and Barea, 1992; Finlay and Söderström, 1992). Also, because interroot distances are smaller at the higher root densities associated with a proliferation of very fine roots or because of differences related to the types of plant species that produce very fine roots, competition for available nutrients may be greater and, thus, more limiting to microbial growth and activity around living roots in this size class (Van Veen *et al.*, 1989).

In contrast, very fine roots made a stronger contribution to SOC than did fine roots. The roots of warm-season prairie grasses are generally coarser, longer lived and more resistant to decay than the roots of cool-season grasses (Weaver and Zink, 1946; Weaver, 1947). Although the quality of roots can influence the rate of their decomposition, finer roots within the same species generally decompose at a slower rate than coarser roots (Weaver, 1947; Davenport *et al.*, 1988; Dormaar and Willms, 1993). Most of the increases in SOC may be accumulations of mineral-associated organic matter that is physically protected from decomposition within stable aggregates (Jastrow, 1996) and much of this accumulation may be related to the increasing lengths of very fine roots across the chronosequence (Cook *et al.*, 1988).

Aggregate hierarchy and organic binding mechanisms

The results of separate analyses for the three macroaggregate size fractions lend additional support to the role of the porosity exclusion principle as a factor controlling the types of organic binding agents that function at different spatial scales within aggregates (Tisdall and Oades, 1982; Oades and Waters, 1991; Dorioz *et al.*, 1993). In general, the relative importance of the binding mechanisms for each aggregate size fraction was related to the physical size of the mechanism.

For example, fine roots had their largest direct and total effects on aggregates >2000 μm diameter. In contrast, very fine roots had little effect on this size fraction but exerted their strongest direct and total effects on aggregates in the 1000–2000 and 212–1000 μm size fractions. Because of the physical forces exerted by root growth and by localized drying (Kay, 1990; Oades, 1993), root density plays a role in determining aggregate size. When a proliferation of very fine roots results in higher root densities, the extensive development of larger macroaggregates may be physically prohibited.

Although the physical size of external hyphae suggests they might be more important for the binding of smaller macroaggregates, hyphae were most strongly associated with fine roots and, consequently, were of greatest relative importance in the two largest aggregate size fractions. The negative effects of fine roots and external hyphae on the smaller macroaggregate fractions occurred because samples with the greatest lengths also exhibited the greatest stability to rapid wetting. Hence, few of the larger macroaggregates in these samples slaked into smaller macroaggregates or microaggregates.

The relative importance of MBC can be explained by spatial scale; MBC contributed little to aggregates >2000 μm in diameter but had its strongest direct effect on the 212–1000 μm size fraction. The indirect effects of SOC were dependent on the effects of MBC and hwsCHO-C, but hwsCHO-C did not appear to be functioning in relation to its spatial scale. However, this role for hwsCHO-C may not be unusual; Dormaar (1984) reported relatively higher proportions of monosaccharides in larger macroaggregates compared to smaller macroaggregates and microaggregates.

With its most positive effect on the largest aggregate size fraction and a strong negative effect on the smallest, hwsCHO-C appeared to be associated more with fine roots and external hyphae than with MBC. Indeed, modification indexes (Arbuckle, 1995) generated for diagnostic purposes (see Mitchell (1992) for a brief explanation) indicated that addition of a path from external hyphae to hwsCHO-C would cause the greatest improvement of model fit to the data (Tucker–Lewis index = 0.979; log-likelihood ratio $\chi^2 = 7.91$, df = 6, $P = 0.245$; path coefficients for paths from external hyphae and MBC to hwsCHO-C = 0.32 and 0.40, respectively; r^2 for hwsCHO-C = 0.38).

It is possible that significant amounts of C may be lost or exuded by the external hyphae of mycorrhizal fungi or by mycorrhizal roots (Finlay and Söderström, 1992). The extracellular mucilages of saprophytic fungi are often water soluble (Chenu, 1989) and the mucilages produced by mycorrhizal fungi could also be soluble, especially in hot water. Furthermore, because mycorrhizal hyphae can extend significant distances from the root (Friese and Allen, 1991; Jakobsen *et al.*, 1992), much of the mucilage produced by these fungi may be deposited beyond the rhizospheric zone of increased microbial activity and, thus, may persist for relatively long periods (compared to root mucilages) before being decomposed.

CONCLUSIONS

The processes of soil aggregate stabilization are complex and involve a variety of binding mechanisms interacting at a range of spatial scales. By using path analysis to evaluate the roles of several organic binding agents in soil aggregation, we were able to confirm the importance of roots and mycorrhizal hyphae as driving factors for macroaggregate stabilization in a system recovering from disturbance. In addition, we obtained a better understanding of the underlying mechanisms associated with

the various binding agents in this system. In particular, very fine roots appeared to be involved primarily in direct effects such as physical enmeshment; whereas, the effects of fine roots were largely indirect, through their strong associations with mycorrhizal fungi and their influences on microbial activity. Furthermore, analyses for three size classes of macroaggregates support the hypothesis that the effectiveness of various binding mechanisms depends on the physical dimensions of the binding agents relative to the spatial scales of the aggregate planes of weakness being bridged.

Acknowledgements—This work was supported by the U.S. Department of Energy, Office of Energy Research, Office of Health and Environmental Research, Environmental Sciences Division, Global Change Research. We are grateful to R. Cronn, M. Fink and B. Fox for help with field sampling and in the laboratory and to M. Arroyo, B. Cook, M. Mondecar, D. Sherman, K. Spokas and K. von der Heide for technical assistance in the laboratory. We thank the National Environmental Research Park at Fermilab for permission to collect samples.

REFERENCES

Allison F. E. (1968) Soil aggregation: Some facts and fallacies as seen by a microbiologist. *Soil Science* **106,** 136–143.

Arbuckle J. L. (1995) *Amos 3.51 for Windows User's Guide*. SmallWaters Corporation, Chicago.

Asher H. B. (1983) *Causal Modeling*. Sage University Paper Series on Quantitative Applications in the Social Sciences, 07-003. Sage Publications, Beverly Hills.

Azcón-Aguilar C. and Barea J. M. (1992) Interactions between mycorrhizal fungi and other rhizosphere microorganisms. In *Mycorrhizal Functioning: An Integrative Plant-Fungal Process*, ed. M. F. Allen, pp. 163–198. Chapman and Hall, New York.

Baldock J. A. and Kay B. D. (1987) Influence of cropping history and chemical treatments on the water-stable aggregation of a silt-loam soil. *Canadian Journal of Soil Science* **67,** 501–511.

Baldock J. A., Kay B. D. and Schnitzer M. (1987) Influence of cropping treatments on the monosaccharide content of the hydrolysates of a soil and its aggregate fractions. *Canadian Journal of Soil Science* **67,** 489–499.

Bentler P. M. and Bonett D. G. (1980) Significance tests and goodness of fit in the analysis of covariance structures. *Psychological Bulletin* **88,** 588–606.

Blake G. R. and Hartge K. H. (1986) Bulk density. In *Methods of Soil Analysis. Part 1*, ed. A. Klute, 2nd edn, pp. 363–375. American Society of Agronomy, Madison.

Chenu C. (1989) Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. *Soil Biology & Biochemistry* **21,** 299–305.

Cheshire M. V. (1985) Carbohydrates in relation to soil fertility. In *Soil Organic Matter and Biological Activity*, eds. D. Vaughan and R. E. Malcolm, pp. 263–288. Martinus Nijhoff/Dr W. Junk, Dordrecht.

Cook B. D., Jastrow J. D. and Miller R. M. (1988) Root and mycorrhizal endophyte development in a chronosequence of restored tallgrass prairie. *New Phytologist* **110,** 355–362.

Davenport J. R., Thomas R. L. and Mott S. C. (1988) Carbon mineralization of corn (*Zea mays* L.) and bromegrass (*Bromus inermis* Leyss.) components with an emphasis on the below-ground carbon. *Soil Biology & Biochemistry* **20,** 471–476.

Deng S. P. and Tabatabai M. A. (1994) Colorimetric determination of reducing sugars in soils. *Soil Biology & Biochemistry* **26,** 473–477.

Dexter A. R. (1988) Advances in characterization of soil structure. *Soil and Tillage Research* **11,** 199–238.

Dorioz J. M., Robert M. and Chenu C. (1993) The role of roots, fungi and bacteria on clay particle organization. An experimental approach. *Geoderma* **56,** 179–194.

Dormaar J. F. (1983) Chemical properties of soil and water-stable aggregates after sixty-seven years of cropping to spring wheat. *Plant and Soil* **75,** 51–61.

Dormaar J. F. (1984) Monosaccharides in hydrolysates of water-stable aggregates after 67 years of cropping to spring wheat as determined by capillary gas chromatography. *Canadian Journal of Soil Science* **64,** 647–656.

Dormaar J. F. and Willms W. D. (1993) Decomposition of blue grama and rough fescue roots in prairie soils. *Journal of Range Management* **46,** 207–213.

Elliott E. T. (1986) Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils. *Soil Science Society of America Journal* **50,** 627–633.

Elliott E. T. and Coleman D. C. (1988) Let the soil work for us. *Ecological Bulletins* **39,** 23–32.

Elliott E. T., Coleman D. C., Ingham R. E. and Trofymow J. A. (1984) Carbon and energy flow through microflora and microfauna in the soil subsystem of terrestrial ecosystems. In *Current Perspectives in Microbial Ecology, Proceedings 3rd International Symposium on Microbial Ecology, Michigan State University, 7–12 August 1983*, eds. M. J. Klug and C. A. Reddy, pp. 424–433. American Society for Microbiology, Washington, D.C.

Finlay R. and Söderström B. (1992) Mycorrhiza and carbon flow to the soil. In *Mycorrhizal Functioning: An Integrative Plant-Fungal Process*, ed. M. F. Allen, pp. 134–160. Chapman and Hall, New York.

Foster R. C. (1985) *In situ* localization of organic matter in soils. *Quaestiones Entomologicae* **21,** 609–633.

Friese C. F. and Allen M. F. (1991) The spread of VA mycorrhizal hyphae in the soil: Inoculum types and external hyphal architecture. *Mycologia* **83,** 409–418.

Gupta V. V. S. R. and Germida J. J. (1988) Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biology & Biochemistry* **20,** 777–786.

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

Hetrick B. A. D., Kitt D. G. and Wilson G. T. (1988) Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. *Canadian Journal of Botany* **66,** 1376–1380.

Hetrick B. A. D., Wilson G. W. T. and Todd T. C. (1992) Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. *Canadian Journal of Botany* **70,** 1521–1528.

Jakobsen I., Abbott L. K. and Robson A. D. (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120,** 371–380.

Jastrow J. D. (1987) Changes in soil aggregation associated with tallgrass prairie restoration. *American Journal of Botany* **74,** 1656–1664.

Jastrow J. D. (1996) Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. *Soil Biology & Biochemistry* **28,** 665–676.

Jastrow J. D. and Miller R. M. (1998) Soil aggregate stabilization and carbon sequestration: Feedbacks through organomineral associations. In *Soil Processes and the Carbon Cycle*, eds. R. Lal, J. Kimble, R. Follett and B. A. Stewart, pp. 207–223, CRC Press, Boca Raton.

Kay B. D. (1990) Rates of change of soil structure under different cropping systems. *Advances in Soil Science* **12,** 1–52.

Kemper W. D. and Chepil W. S. (1965) Size distribution of aggregates. In *Methods of Soil Analysis. Part 1*, ed. C. A. Black, pp. 499–510. American Society of Agronomy, Madison.

Kleinbaum D. G., Kupper L. L. and Muller K. E. (1988) *Applied Regression Analysis and Other Multivariable Methods*, 2nd edn. PWS-KENT, Boston.

Marsh H. W., Balla J. R. and McDonald R. P. (1988) Goodness-of-fit indexes in confirmatory factor analysis: The effect of sample size. *Psychological Bulletin* **103,** 391–410.

Merckx R., Den Hartog A. and Van Veen J. A. (1985) Turnover of root-derived material and related microbial biomass formation in soils of different texture. *Soil Biology & Biochemistry* **17,** 565–569.

Miller R. M. and Jastrow J. D. (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology & Biochemistry* **22,** 579–584.

Miller R. M., Reinhardt D. R. and Jastrow J. D. (1995) External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* **103,** 17–23.

Mitchell R. J. (1992) Testing evolutionary and ecological hypotheses using path analysis and structural equation modeling. *Functional Ecology* **6,** 123–129.

Nelson N. (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *The Journal of Biological Chemistry* **153,** 375–380.

Nelson D. W. and Sommers L. E. (1982) Total carbon, organic carbon and organic matter. In *Methods of Soil Analysis. Part 2*, eds. A. L. Page, R. H. Miller and D. R. Keeney, 2nd edn, pp. 539–579. American Society of Agronomy, Madison.

Newman E. I. (1985) The rhizosphere: Carbon sources and microbial populations. In *Ecological Interactions in Soil: Plants, Microbes and Animals*, eds. A. H. Fitter, D. Atkinson, D. J. Read and M. B. Usher, Special Publication of the British Ecological Society No. 4, pp. 107–121. Blackwell Scientific, Oxford.

Nicolson T. H. (1959) Mycorrhiza in the Gramineae. I. Vesicular–arbuscular endophytes with special reference to the external phase. *Transactions of the British Mycological Society* **42,** 421–438.

Oades J. M. (1993) The role of biology in the formation, stabilization and degradation of soil structure. *Geoderma* **56,** 377–400.

Oades J. M. and Waters A. G. (1991) Aggregate hierarchy in soils. *Australian Journal of Soil Research* **29,** 815–828.

Olson F. C. W. (1950) Quantitative estimates of filamentous algae. *Transactions of the American Microscopy Society* **59,** 272–279.

Pedhazur E. J. (1982) *Multiple Regression in Behavioral Research*, 2nd edn. Holt, Rinehart and Winston, New York.

Redl G., Hubner C. and Wurst F. (1990) Changes in hot water soil extracts brought about by nitrogen immobilization and mineralization processes during incubation of amended soils. *Biology and Fertility of Soils* **10,** 45–49.

Reinhardt D. R. and Miller R. M. (1990) Size classes of root diameter and mycorrhizal fungal colonization in two temperate grassland communities. *New Phytologist* **116,** 129–136.

SAS Institute Inc. (1985) *SAS/STAT™ Guide for Personal Computers*, Version 6 Edn. SAS Institute Inc., Cary.

Smucker A. J. M. and Safir G. R. (1986) Root and soil microbial interactions which influence the availability of photoassimilate carbon to the rhizosphere. In *Microfloral and Faunal Interactions in Natural and Agro-Ecosystems*, eds. M. J. Mitchell and J. P. Nakas, pp. 203–244. Martinus Nijhoff/Dr W. Junk, Dordrecht.

Tiessen H. and Stewart J. W. B. (1988) Light and electron microscopy of stained microaggregates: The role of organic matter and microbes in soil aggregation. *Biogeochemistry* **5,** 312–322.

Tiessen H., Stewart J. W. B. and Bettany J. R. (1982) Cultivation effects on the amounts and concentration of carbon, nitrogen, and phosphorus in grassland soils. *Agronomy Journal* **74,** 831–835.

Tisdall J. M. (1991) Fungal hyphae and structural stability of soil. *Australian Journal of Soil Research* **29,** 729–743.

Tisdall J. M. and Oades J. M. (1979) Stabilization of soil aggregates by the root system of ryegrass. *Australian Journal of Soil Research* **17,** 429–441.

Tisdall J. M. and Oades J. M. (1980) The effect of crop rotation on aggregation in a red–brown earth. *Australian Journal of Soil Research* **18,** 423–433.

Tisdall J. M. and Oades J. M. (1982) Organic matter and water-stable aggregates in soils. *Journal of Soil Science* **33,** 141–163.

Tucker L. R. and Lewis C. (1973) A reliability coefficient for maximum likelihood factor analysis. *Psychometrika* **38,** 1–10.

Van Veen J. A., Merckx R. and Van De Geijn S. C. (1989) Plant- and soil-related controls of the flow of carbon from roots through the soil microbial biomass. *Plant and Soil* **115,** 179–188.

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

Weaver J. E. (1947) Rate of decomposition of roots and rhizomes of certain range grasses in undisturbed prairie soil. *Ecology* **28,** 221–240.

Weaver J. E. and Zink E. (1946) Length of life of roots of ten species of perennial range and pasture grasses. *Plant Physiology* **21,** 201–217.