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ENHANCEMENT OF SOIL AGGREGATION BY THE COMBINED INFLUENCES OF SOIL WETTING AND DRYING AND ROOT-MICROBIAL ASSOCIATIONS

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ABSTRACT

Plant root modifications of soil aggregates in the rhizosphere have been reported frequently. However, there are conflicting reports of soil aggregate stabilization by multiple cycles of root exudation and soil water removal. Stimulation of soil microorganisms by root exudates was studied in soils collected to depths of 20 cm in conventionally tilled (CT), no-tilled (NT), and native grassland (NG) at the Kellogg Biological Station in the southwestern Michigan. This study presents a method for measuring the combined affects of root infusions of a mixture of 7 carbohydrates, 4 organic acids, and 12 amino acids compounds and root extractions of soil water with multiple soil wetting and drying cycles. Polyvinyl chloride (PVC) cylinders, 10 cm diameter x 12 cm length, prepared by drilling a longitudinal row of 11 holes at 1 cm intervals, were used as containers for soil samples. In this study, Rhizos soil solution samplers (SSS) have been used to simulate plant roots. Soil microbial biomass and mean weight diameter were estimated at the end of the wetting and drying cycles. Root exudate compounds increased soil aggregate stability in macroaggregate fractions by 5 times compared to the control after nine wetting and drying cycles. Additions of root exudate compounds increased microbial activities of soils adjacent to artificial roots for all management practices. Aggregate stabilities of soils exposed to long-term tillage management, responded more to additions of C and N compounds than did soil aggregates from native grasslands.

To my wife Magnine Sidibe and daughters Ouriba and Sedinte

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INTRODUCTION

Soil structure, defined as the arrangement of primary soil particles into secondary particles or peds (Soil Sci. Soc. Am., 1984), controls and indirectly describes the distribution of soil pore space. The development of a stable soil structure is important for controlling several soil characteristics such as water retention, gaseous diffusion, hydraulic conductivity, mechanical resistance, and erodibility.

Processes controlling the formation of stable soil aggregates are complex and include many biological, chemical, and physical components of the soil system. Combinations of textural units, ions, water, organic matter, microbial and fauna activities determine the various degrees of aggregate stability (Tisdall, 1991). The development and stability of soil aggregates provide multiple foundations for soil productivity and sustainable crop production. Therefore, it is important to understand the roles of as many soil components involved in soil aggregation as possible.

Individual factors, controlling the formation of soil aggregates, Fig 1 , have not been completely separated to determine their relative importance. Soil structure is greatly affected by clay content and mineralogy because of cohesive and adhesive properties associated with the clay and organic matter (Kemper and Rosenau, 1986).

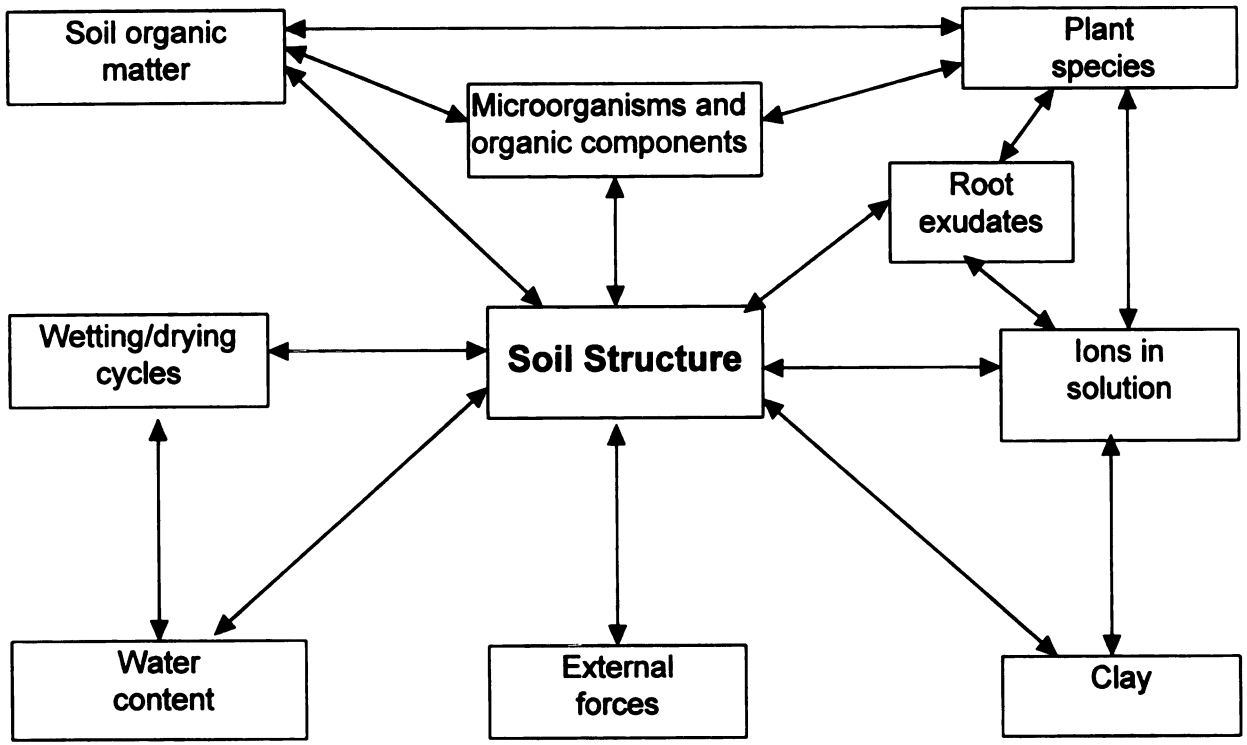

Fig.1. Interactions among soil structural components and multiple compounds of the soil. Adapted from Tisdall, 1991; Kemper and Rosenau, 1986.

Climate is highly variable and an independent factor. Temperature has an important affect on the rate of chemical reactions including those bonds of biological metabolism, e.g. microbial biomass (Pawluk, 1988; Formanek et al., 1984). Soil drying improves contact of particles by bringing them in closer proximity. The strength of a single aggregate depends on the number of mineral contact points (Horn, 1990). Utomo and Dexter (1982) investigated the effects of wetting and drying on sterile and nonsterile soils which have been tilled, non-tilled, and remolded. Their work demonstrated that variation in soil structural stability was also affected by microbial activity.

Improving soil structure, particularly in agricultural production systems, is an essential component to consider, when developing the best management practice. Best management practices which augment or maintain soil organic matter (SOM) content appear to have the most dramatic effect on soil structure (Tisdall and Oades, 1982). Agricultural activities, such as spreading manure, adding imported plant residue, and rotating cultivation seasons with periods of legume-based green manure crops, may counteract losses of SOM. However, the level of SOM is seldom maintained by conventional agricultural production practices. The influence of a cropping system on soil aggregation is a function of the varied and combined effects of diverse physical, biological, and chemical agents on the formation and degradation of soil aggregates (Harris, et al., 1966).

Microbial populations in the soil are responsible for the degradation of plant tissue to produce humidified SOM. Microorganisms and plant roots exude

organic compounds into the soil which directly bind soil particles together. Root exudates also serve as a rich source of carbon (C) and nitrogen (N) for many microorganisms. Microorganisms prefer metabolizing fresh exudes to dead tissue (Reid and Goss, 1982).

Fine roots have been also reported to physically bind soil particles together (Tisdall and Oades, 1982). They constitute a major source of organic material for the soil and affect its structure, aeration, and biological activity. Difficulties associated with past root measurements should not detract one from the tremendous need to quantify numerous morphological and physiological components of roots. This lesser known area of the plant-soil-atmosphere continuum must be quantified before we can predict the fixation and utilization of carbon at the plant, landscape, or at the global level by either functional or mechanistic models (Smucker, 1990). Rovira (1973) found that the major exudation in plant roots occurs in the elongation zone. Root cap cells and polysaccharide exuded at the apex are the major components of root contributions to the rhizosphere. Baldock et al. (1987) studied bromegrass and corn rotation effect on soil structural stability. No significant differences in total carbohydrates were found. In their study, eight sugars were found in all soils. Corn roots had more galactose and glucose. Only very small aggregates (0.5 mm diameter) were found to have significant differences in total carbohydrates. Angers and Muhuys (1989) found that continuous cropping of corn for 2 years decreased carbohydrate content by 30% and organic matter by 9 %. They found

a strong correlation between carbohydrate contents and mean weight diameter of soil aggregates. When wetting and drying cycles are associated with root releases of organic compounds (exudates), soil structure is usually improved (Fig. 2). During the wetting and drying cycles, parallel clay crystals (about 5μm diameter) are grouped together closely enough to behave in water as a unit (Emerson, 1959, 1977).

The objectives and hypotheses of this study were:

1) To evaluate artificial corn root exudate contributions to soil aggregation in the rhizosphere.

Hypothesis: Root exudates increase soil aggregate stability more in the rhizosphere than in bulk soils.

2) To correlate soil microbial biomass with soil aggregate stability in the rhizosphere.

Hypothesis: Higher microbial populations in the rhizosphere promote soil aggregate stability.

3) To correlate soil aggregate stability with the frequency and duration of wetting and drying cycles.

Hypothesis: Wetting and drying cycles improve soil aggregate stability.

4) To determine the relationship between soil management history and the effect of C and N additions on aggregate stability.

Hypothesis: Soil aggregates are more stable in grassland than in conventional and no tillage management systems.

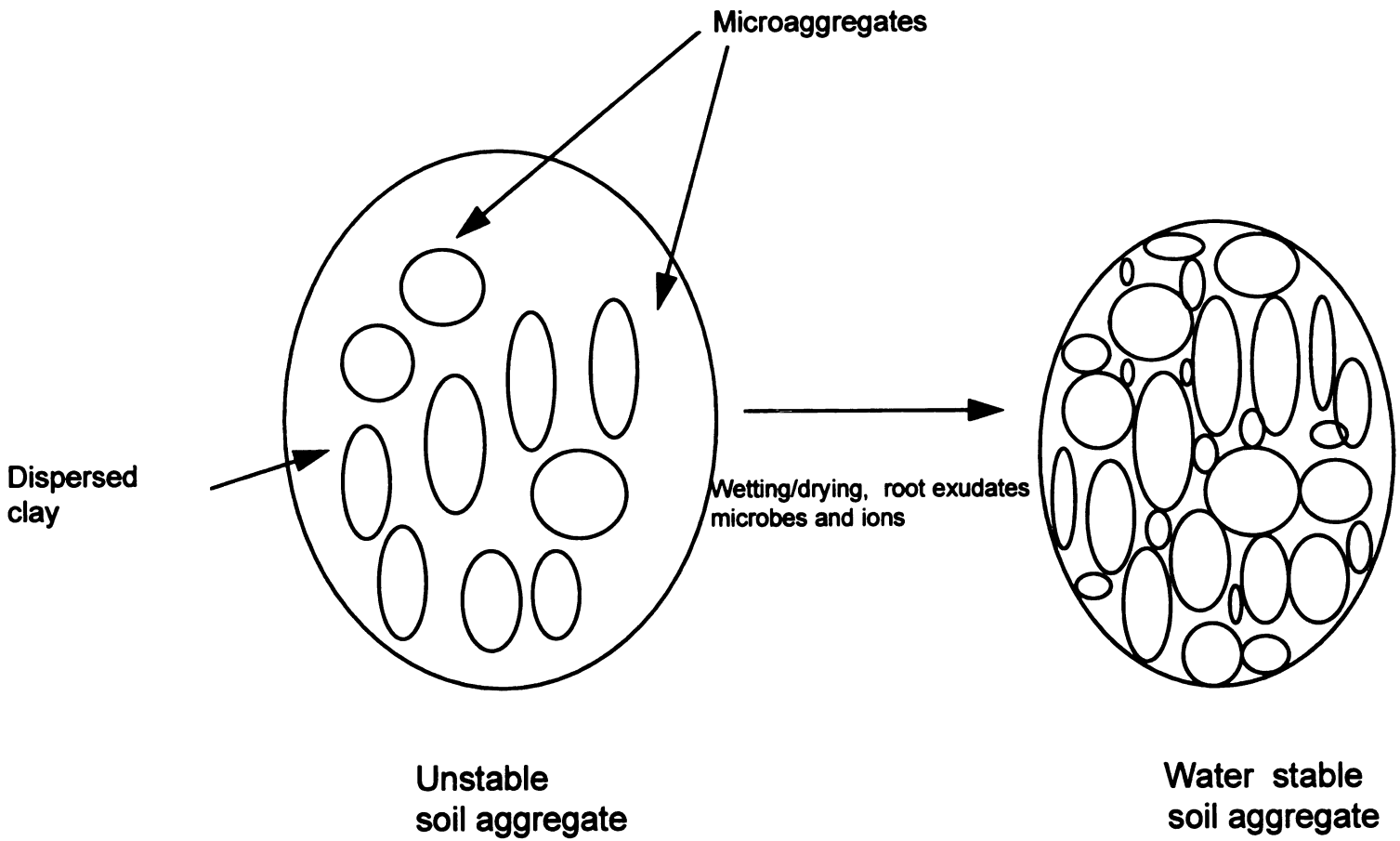

Fig. 2. Wetting/drying, root exudations, microbes and ions modifications of soil aggregate stability.

2.0 LITERATURE REVIEW

Soil structure

Soil aggregates, composed of primary particles and binding agents, are the basic units of soil structure. Good structure for crop growth depends on the presence of aggregates of soil particles 1 to 10 mm across which remain stable when wetted (Tisdall and Oades, 1982). Roots and hyphae stabilize larger soil aggregates (macroaggregates) defined as greater than 250 μm across. Consequently, macroaggregation is controlled by crop and soil management systems, as management generally influences the growth of plant roots (Tisdall and Oades, 1982). The importance of soil aggregation in crop production is related to water and air relationships within the soil. A productive agricultural soil must have a wide range of pore sizes (Lynch and Bragg, 1985), which allow rapid infiltration and drainage (Tisdall and Oades, 1982). The deterioration of soil structural stability is a major factor contributing to increased rates of soil degradation by processes such as erosion, and compaction (Coote et al., 1988; Wall et al., 1988), with consequent effects on productivity and the environment.

Aggregates having diameters of 20-250 μm are very stable, partly because they are small, but also because they contain several types of binding agents whose effects are additive. Water-stable aggregates 2-20 μm across consists of particles bonded together so strongly by persistent organic bonds that they are not disrupted by agricultural practices (Tisdall and Oades, 1982). Clay particles in these aggregate particles are oriented tangentially to the

bacterial surface. However in soils, perfect alignment of clay plates occurs rarely. Small aggregates produced by slaking, remove clay particles and block some pores (Arnold, 1978). In the field, dispersed clay blocks pores which transmit or store water. Slaking and dispersion together produce undesirable structures such as surface crusts (Tisdall and Oades, 1979). The stability of surface aggregates is most important because aggregates below the surface are protected from rapid wetting (Lynch and Bragg, 1985). When surface aggregates are unstable, crusts can be formed and inhibit the movement of water and air into the soil. Organic binding agents involved in stabilizing soil aggregates can be considered in three main groups.

Transient binding agents are organic materials (polysaccharides) which decompose rapidly and are associated with large aggregates. Readily available substrates (glucose) increase water-stable aggregation which is transient (several weeks) because the glues are decomposed readily (Tisdall and Oades, 1982). The significance of polysaccharides as glues in soil aggregates was reviewed by several authors (Martin, 1971)

Temporary binding agents include roots and fungal hyphae, particularly vesicular-arbuscular (VA) mycorrhizal hyphae (Tisdall and Oades, 1979). These binding agents build up in the soil within a few weeks or months as root systems and associated hyphae grow, and are probably associated with young macroaggregates (Tisdall and Oades, 1982). Stabilization of aggregates by fungi in the field is limited to periods when readily decomposable material are

available. Most of the microbial filaments which have been reported to stabilize aggregates in the field in the presence of plants may have been VA mycorrhizal fungi (Tisdall and Oades, 1978).

Persistent binding agents consist of partially degraded, aromatic humic material associated with amorphous iron, aluminum and iron- aluminosilicates. These form large organo-mineral fractions of temperate and tropical soil (Turchenek and Oades, 1979) and are thought to be at the centers of highly stable aggregates.

Inorganic binding compounds may be regarded as cementing agents. If they are dominant, the presence of organic glues may be of little extra benefit. Regular cultivation may reduce the content of organic matter and the chemical fertility, with little influence on the physical properties of such soils. However, in the surface layers of many agricultural soils, it appears that organic matter plays a major role on binding aggregates because of the rapid wetting and raindrop impacts on aggregates at the soil surface.

Two forces holding particles together in aggregates in moist soils are the surface tension of the air to water interface and cohesive tensions associated with the liquid phase (Kemper and Rosenau, 1986). As soil dries, the water phase recedes into capillary wedges surrounding particle-to-particle contacts and films between closely adjacent platelets. The interfacial tension and internal cohesive tension pull adjacent particles together with great force as soil dries. As the highly adhesive liquid phase of thin moisture films pulls adjacent mineral

particles into closer proximity, there are also more opportunities for hydrogen and other cationic bonding between adjacent oxygen and hydroxyl groups and other mineral surfaces.

Much of the work on the stability of hydrated aggregates utilized elutriation through various sized tubes to separate the aggregates into several sizes. Yoder (1936) pointed out the deficiencies of this method. It is difficult to avoid some turbulence in water flowing in wide columns, and some aggregates stay in the tube if only laminar flow occurred. Therefore, he developed a wet-sieving procedure that used a nest of sieves which oscillate vertically and rhythmically, so that water is made to flow up and down through the sieves. Kemper and Koch (1966) concluded that aggregate stability can be best determined by using a single sieve. This involves much less investment and it can be well correlated with important field phenomena. It is generally considered that weight of large aggregates is more indicative of good structure for most agricultural purposes than is a weight of small aggregates (Tisdall and Oades, 1982). Van Bavel's (1949) concept of the mean weight diameter (MWD), based on weighing the masses of aggregates of the various size classes, has been used widely.

The MWD is an index of the stabilities of soil aggregates of different size-fractions. It can be calculated by equation (1).

$$MWD = \sum_{i=1}^{n} x_i w_i \qquad (1)$$

where x_i is the size fraction of each sieve and w_i is the proportion of the total sample weight. The equation generally overestimates the original MWD where only five separate size-fractions are used. The use of Van Bavel's concept is time consuming. However, the correlation using five size-fractions is excellent. The geometric mean diameter (GMD) is an index of aggregate-size distribution. It is calculated by the equation (2).

$$GMD = \exp\left[\sum_{i=1}^{n} w_i \log x_i / \sum_{i=1}^{n} w_i\right] \qquad (2)$$

where wi is the weight of aggregates in a size class with an average diameter xi and Σ wi is the total weight of the sample. Gardner (1956) found that the aggregate size distribution in most soils is approximately log-normal rather than normal. This approach describes aggregate-size distributions of most soils, using two parameters, the GMD and the log standard deviation. The main disadvantage of expressing data in terms of GMD and log standard deviation is the extensive work involved in obtaining them. The log standard deviation must be obtained by either graphical or differential interpolation from the data. The best methods for presenting aggregate size distribution data seemed to be MWD or GMD and the log standard deviation. The correlation coefficient between MWD and GMD was about 0.9. The GMD and the log standard deviation give a more complete description of the size distribution than the MWD. However, the MWD used for this study is easy to calculate and easy for most individuals to

visualize. Both can be used to represent aggregate size distribution for statistical analysis.

Wetting and drying forces on soil aggregation

Wetting processes can be highly disruptive to soil aggregates. Ion hydration and osmotic swelling forces pull water between clay platelets, pushing the clay platelets apart and causing swelling of the aggregates (Kemper and Rosenau, 1986). If soil aggregates are wetted slowly at atmospheric pressure, the binding is still sufficiently strong to hold most of the primary particles together in aggregates. Drying of soil allows particles to come in contact or closer proximity, and the strength of a single aggregate depends on the number of contact points or the forces that can be transmitted at each contact point (Horn, 1990).

Wet-dry cycles can increase the mineralization of organic C and N in soil (Birch and Friend, 1961; Seneviratne and Wild, 1985). A proportion of the increase in mineralization can be attributed to the death of significant numbers of microorganisms during soil drying (Van Gestel, et al., 1991), but most of the increase in mineralization is due to increased availability of soil organic C. Wet-dry cycles increase the amounts of organic C available to microorganisms because of the additional exposure of organic C sequestered within the interiors of soil aggregates. Disruption of micro-aggregates exposes C if located at the interiors of broken soil aggregates, during drying and re-wetting (Powlson and

Jenkinson, 1976; Van Gestel et al., 1991). Sorensen (1974) showed that the wet-dry cycles have a larger effect on the mineralization of more recently accumulated, and possible more labile, pools of organic matter. Utomo and Dexter (1982) found that 6 wetting and drying episodes of a fine sandy loam soil increased the stability of remolded aggregates by up to 4 times. Jager and Bruins (1975) reported that soils can become completely structureless after 60 cycles of wetting and drying. Air-drying of clay and loam soils before wet-sieving increased the stability of aggregates because of increased particle-to-particle contact which favors the formation and adsorption of inorganic and organic compounds (Haynes and Swift, 1990; Reid and Goss, 1982).

Soil water content

The variable amount of water contained in a unit mass or volume of soil, and the energy state of water in the soil are important factors affecting the growth of plants (Hillel, 1982). Many soil properties depend on soil water content (swelling and shrinkage, bulk density, air content, and gas exchange of the soil). Soil water content affects the growth and respiration of roots, the activity of microorganisms, and the chemical state of the soil (oxidation-reduction potential).

Volumetric water content: The volume wetness (often termed the volumetric water content or volume fraction of soil water) is generally computed as a percentage of the total volume of the soil rather than on the basis of the

volume of particles alone (Hillel, 1982). This author has shown that the volumetric water contents of sandy soils ranges from 40 to 50 %; for medium-textured soils it is approximately 50 %; and for clayey soils it can approach 60 %. The Time Domain Reflectometry (TDR) technique has been used to measure volumetric water contents (Dalton, 1992).

Root exudates

Plant root systems anchor plants, absorb ions and water for them. They also produce quantities of C and N compounds by exudation and root decomposition. For many years the quantity of organic compounds released from roots were underestimated primarily because the results were based upon measurements of plant roots grown in nutrient solutions and/or axenic conditions (Smucker, 1984). This author showed also that root turnover or the death and decomposition of fine roots, may account for a large loss of organic compounds into soil. Coleman (1976) demonstrated that root production is often the largest C input to the ecosystem as 54 % of grass root tips appear to survive for less than 1 month. Healthy roots also release many organic compounds (Rovira et al., 1978). Roots of many species have been shown to exude organic compounds of varying composition and complexity. Organic materials lost from plant roots have been classified by Rovira et al. (1978).

Root exudates: Root exudates are substances (sugars, amino acids, organic acids, hormones and vitamins) leaking (passively) from intact cells along

a concentration gradient. There are low-molecular-weight compounds, including water-soluble and volatile compounds (Rovira, 1969, 1973; Pearson and Parkinson, 1961). Root exudates diffuse through soil and are used by microorganisms. The loss of root exudates appears to be proportional to the concentration of these organic compounds at the root surface since removal of the compounds increases exudation (Smucker, 1984). Secretions: Compounds of low or high molecular weight (polymeric carbohydrates and enzymes) which are actively released by root tissues. Lysates: Compounds released by autolysis of epidermal cell walls and increase from increasing distance from the root apex.

Mucilages:are secreted by Golgi vesicles primarily in the root cap area. They also originate from hydrolysates of the polysaccharide of the primary cell wall, between the epidermal cells and sloughed root cap cells. Root-cap mucilage, which accumulates in the corn rhizosphere as the tip extends through the soil, is composed of polysaccharide molecules with many complex oligosaccharide branches which have neutral sugars at their terminals (Watt et al. 1993).

Rovira (1969) listed several environmental conditions which affecte root exudation. These include plant species, plant age, temperature, light, plant nutrition, soil moisture, root damage, and foliar applications. Physiological age of plants also affect root exudation composition and quantity (Martinez-Toledo et al., 1988). Tyrosine is only released by tomato roots during the fruit formation and is absent during the purely vegetative phases of growth. Rovira (1969) showed that substantially smaller quantities of sugars, organic acids, and some

amino acids are released by plant roots during fruiting than in the earlier vegetative phases of growth. He also suggested that soil microbes may affect root exudation either by altering root metabolism or the permeability of cells, or by the microbial assimilation of certain constituents of the root exudate. Rovira (1969) showed that a wide range of organic materials, including sugars, amino acids, and organic acids, are lost to the soil in the exudate, secretion, lysate fractions. Many of these compounds are readily used by a wide range of soil microorganisms, and they are largely responsible for the types and numbers of microorganisms in the rhizosphere. Cheshire and Mundie (1990) showed that the release of large amounts of water-soluble carbohydrates from corn roots was due to cell lysis. Root exudate solutions contained mainly glucose but also galactose and mannose. Darbyshire and Greaves (1973) released the chemical composition of wheat root exudate (12 organic acids, 12 sugars, and 25 amino acids). Barber and Martin (1976) reported that gaseous and soluble C released by root systems growing in soils or solution culture amounted to 20 to 39 % of the C translocated to the roots. Carbon transfer from the root to the rhizosphere results in a net loss of photosynthates from plant root systems (Smucker, 1984).

The rhizosphere is defined as the volume of soil that is adjacent to and influenced by the plant root (Hiltner, 1904). In another words, the rhizosphere is the physical location in soil where plants and microorganisms interact. The work of Lochhead et al. (1947) helped to understand the qualitative as well as the quantitative differences between rhizosphere soil and bulk soil. Clark (1949)

demonstrated that the ratio of organisms in rhizosphere is different from the organisms in the bulk soil. He demonstrated that not only the plant species and soil water contents influence rhizosphere microorganisms but also the quantity of soil adhering to roots from which suspensions were prepared for counting. He proposed the term "rhizoplane" for the microbiology of the root surface. The rhizoplane is defined as the contact at the root-soil interface.

Polysaccharides defined as high molecular weight carbohydrates containing many (hundreds or thousands) of monomeric units connected to one another by a type of covalent bond, referred to as a glycosidic bond (Brock and Madigan, 1988). Cellulose is the simplest and most frequently occurring structural polysaccharide of the cell wall. Tillage disrupts a variety of bonding mechanisms in soil and reduces soil stability. It exposes SOM to microbial attack and decreases the fungal biomass (Rovira and Greacen;1957; Gupta and Germida, 1988). Most of the temporary increases in porosity, following cultivation, is the result of a flush of biological activity of soil microorganisms, resulting in a temporary improvement in aggregate stability (Molope et al., 1987).

Root exudates of corn consist of 61 % of carbohydrates, 8 % of proteins, 2 % of amino acids and 29 % of organic acids (Guckert et al., 1991; Buyanovsky and Wagner, 1997). Glucose, arabinose, galactose, and mannose were the predominant sugars. Brock and Madigan (1988) showed that amino acids are monomeric units of proteins. Rovira and Greacen (1957) showed that the low solubility of some amino acids (glutamic, glutamine, cystine, lysine, histidine,

arginine) influenced the relative abundance of these elements in the extracted materials, giving an incorrect impression of the balance of amino acids in the root exudate. The largest quantity of amino acids is synthesized by the root hair zone, older parts of the root contain smaller amounts. Wallace and Lochhead (1950) showed that the growth requirements of 80 % of the amino acid dependent rhizosphere bacteria could be satisfied by supplying methionine as the sole amino acid. They suggested that the methionine may be one of the major amino acids excreted into the rhizosphere.

Simulated root studies

Information on the water and nutrient dynamics in the root is essential for appropriate soil management and fertilizer application, since the root zone is the area where soil and plant interact. Richards (1941) grouped the methods of soil sampling into five categories: suction, displacement, compaction, centrifugation, molecular adsorption. The only practical method to collect the soil solution nondestructively is the suction method. A porous ceramic cup has been used as a suction material (Debyle et al., 1989). This device has some limitation due to the heterogeneous medium as root zone. Yanai et al. (1993) used a hollow fiber, instead of a porous cup, as a soil solution sampler. Hollow fiber is a semitransparent fiber used for hemo dialysis and water purification, with internal diameter of 475 μm and external diameter of 900 μm. It operates as a micro-sieve, which blocks 90 % of particles 40 nm in diameter but allows solutes to

pass in spite of the elimination of suspension and colloid materials as well as bacteria. To evaluate the performance of the sampler, Yanai et al. (1993) examined ion adsorption-desorption. They found that the effect of the sampler was too small to affect the composition and concentration of the soil solution. Bouldin (1989) suggested that soil solution composition changes and ion concentrations are interrelated. The looped hollow fiber sampler has outstanding properties such as flexibility, small size, and negligible ion exchange.

A Rhizos soil solution sampler (SSS), another device, was used to simulate roots. It is produced from a hydrophilic porous polymer. This material is ideal for greenhouses, laboratories, waste water sampling, and forestry studies. Sampling by rhizos SSS is appropriate when successive samples form one position in soil are needed.

Ions and water uptake by plant roots

Fresh plant matter is about 80 to 95 % water. Initial entry of water into the root is mainly through that region which extends for a few centimeters behind the tip (Epstein, 1972). The vascular tissue is the pathway of water in the root, through the stem, and into the petioles and the veins of leaves. When water moves through the soil to the root surface, it brings with it ions and other solutes. If these arrive at the root surface faster than they are taken up, they accumulate in the rhizosphere. Esptein (1972) showed that water uptake depends on root length distribution in soil, phenomenological stage of the plant, air content, water

content and water conductivity of the soil, soil temperature and potential transpiration. Osmotic component of soil water potential has little effect on water. Water migrates throughout the mesophyll much as it does through the cortex of the root.

Extensive root proliferation within the rooted zone result in rapid soil water depletion. Meyer et al., (1990) showed that during a drying period, wheat roots removed from an undisturbed soil 154 mm of water in 49 days (3.1 mm day^{-1}), while in repacked soil they removed 174 mm in 57 days (3.1 mm day^{-1}). The rate of root extension remained reasonably constant in undisturbed soil, but generally increased with time in the repacked soil. The production of new roots enables the exploration of new soil volume.

Soils contain considerable quantities of inorganic ions which are bound to the charged surfaces of clay and organic soil particles. The gradual release of these reservoirs of bound ions provides a continuous supply of nutrients for plant absorption. Soil particles, particularly the silicates have an overall negative charge and are capable of binding or absorbing cations added to solution. Aluminosilicates are negatively charged because some of the O valencies remain free.

The inorganic ions released by the root are in exchange for the cations and ions absorbed into the root. Significant release occurs when cations and anions absorption are unequal. The degree of disparity between cation and anion uptake varies with nitrogen, plant species, and stage of plant growth

(Esptein, 1972). Plant roots release organic materials as well as inorganic ions. The effect of organic ions released by roots on diffusion or mass-flow is related to their effect on ion solubility. The increased concentration influence the availability of other nutrients that reach the root mainly by diffusion.

At least 16 chemical elements (C, H, O, N, S, P, K, Ca, Mg, Fe, B, Mn, Cu, Zn, Mo, and Cl) are required for plant growth. Eight of these elements are absorbed in the form of oxides (C, H, O, N, S, P, Mo, B) while the other eight are taken up as metallic ions. Traditionally, the elements have been divided into two groups, the macronutrients and micronutrients. Macronutrients (C, H, O, and N) make up more than 96 % of plant dry weight at ear leaf sample of corn, the nitrogen content is 2.76 - 3.5 %, phosphorus 0.25 - 0.50 %, potassium 1.71 - 2.5 %, calcium 0.21 - 1.00 %, magnesium 0.16 - 0.60 % and sulfur 0.16 - 0.50 %. Micronutrient contents are generally reported as $\mu g\ g^{-1}$. Quantities of these compounds range from: manganese 20 -150, iron 21 - 250, boron 4 -20, copper 6 -20, zinc 20 -70 and molybdenum 0.1 -2.0 (Vitosh et al., 1994). Nutrients in soil solution arrive at the surface of the root as a result of two kinds of movement: bulk flow and diffusion. High ion concentrations of the outer solution seem to favor passive uptake processes (influx). At low concentrations, net efflux may occur through the pores. One third of the C fixed by higher plants is converted to cellulose, and massive amounts of lignin and aromatic derivatives are also formed. Both NH_4^+ and NO_3^- are commonly present in soil solution, and both are readily taken up by roots. Potassium is absorbed in great amounts even when its

concentration in soil solution is low (Kolek and Kozinka, 1992). Potassium uptake by root tissues is an active process (taken up against the electrochemical potential gradient) (Ussing, 1949). Phosphate occurs in plants in its most highly oxidized form. Phosphate absorption by roots takes place in epidermal cells, but the main site of its uptake is the cortical tissue.

Soil organic matter

Organic matter consists of partially decayed plant residues that are no longer recognizable as plant material, the microorganisms, the small fauna involved in decomposition, and the by products of decomposition that undergo a process called humification to form the material known as humus (Paul and Clark, 1996). The process of formation of soil organic matter or humus is primarily a biological one, in which nearly all of the flora and the fauna living in or on the soil play a direct or indirect role. The microorganisms that are so active in the decomposition of plant and animal residues are using a portion of these for the building of their own bodies which become a considerable portion of soil organic matter (Allison, 1973). Paul and Clark (1996) showed that an amino acid (glucine) reacting with a phenol (catechol) derived either from the partial degradation of lignin or from microbial pigments such as those produced by fungus, form aminoquinone and can condense to form brown high molecular-weight nitrogenous humates. The polyphenol reaction is considered important in forming SOM from lignin or melanin-degradation products. The browning

reaction involves sugars (glucose) reacting with an amino compound such as glucine to form three intermediates compounds (3-C aldehydes and ketones, reductones, and furfurals). All of those react with amino compounds to form dark colored end products. Nitrosation, a mechanism in which oxidized N reacts with phenols, is often associated with N loss from soils. It is also believed to participate in the formation of SOM (Paul and Clark, 1996). They listed the factors affecting the decomposition of soil organic matter (composition and particle size, microorganisms, available nutrients, water, temperature, pH, and aeration).

The organic fraction of the soil consists of a complex system of substances (Kononova et al., 1961). Components of decompositing plant and animal residues, consist of various nitrogenous and non-nitrogenous organic compounds (carbohydrates, organic acids, fats, waxes, resins etc.). These compounds form 10 -15 % of the total amount of SOM. In developed soils, humic substances form up to 85 - 95 % of the humus (Kononova, 1961). Humic acids contain 50 - 58 % C and 5.0 - 5.5 % N. Soil humic materials generally have more C than carbohydrates and amino acids (45 - 48 %). Ninety-five percent or more of the nitrogen in soil surface is usually present in organic form (Allison, 1973).

Continuous cultivation increases the mineralization rates of SOM and tends to exert a detrimental effect on soil tilth. These responses are enhanced when soil physical conditions are suboptimal for mechanical manipulations (Lynch and Bragg, 1985). Tillage affects soil structure in significant ways.

Comparisons of virgin to cultivated soils have shown that cultivation profoundly reduces the stability of soil structure (Elliot, 1986), such deterioration can occur after a few years. Haynes and Swift (1990) found that long-term pasture samples had a greater aggregate stability than long-term arable samples. They also found that the aggregate stability of a regrassed site (13 years of arable plus 2 years of pasture) was marvedly higher than that of a corresponding site from 15 years of arable cropping. Mytton et al. (1993) suggest that clover is more effective than ryegrass in developing rapid improvements in soil structure.

Microbial biomass

Aggregation within the surface horizons of many soils is predominantly a function of the microbiological production and decomposition of soil binding agents. In the presence of a suitable energy source, diverse fungi, streptomycetes, and bacteria bind soil particles into water-stable aggregates. The temporary increase in aggregation was frequently observed following incubation of soils amended with organic materials, and is related closely to microbial activity. Microbial metabolism of soil organic amendments is accompanied by the production of organic aggregating materials which are metabolized by the soil microflora in the absence of a more readily available energy source. Microbial polysaccharides and fungal mycelia play major roles as soil-binding materials. Fungal biomass in soil is the main component of the microbial biomass.

Microbial biomass is defined as the dry weight, volume, or other quantitative estimation of organisms; the total mass of living organisms in an ecosystem. The amount of biomass in soil can be assessed by the quantification of a particular cell constituent of the microorganisms. Jenkinson and Ladd (1981) mentioned the basic requirements for this approach: the substance used to estimate microbial biomass must be present in all organisms in the same known concentration at all times. The microbial biomass itself may represent a labile pool of C and nutrient elements. In agricultural soils 200-1000 μg biomass C g^{-1} soil is often found. This cell mass fixes 100-600 kg N and 50-300 kg P ha^{-1} in the upper 30 cm of soil (Martens, 1993). These amounts often exceed the annual application of nutrients supplied as fertilizer to soils in agricultural practice.

Soil microbial biomass is an important component of the soil organic matter, and therefore, has an important impact on soil structure. It is a labile component of the soil organic fraction containing 1 to 3 % of the total soil C and up to 5 % of the total soil N (Smith and Paul, 1990). These materials are converted by microorganisms in order to generate energy and to produce new cellular metabolites to support their maintenance and growth (Martens, 1993). Microbial biomass has been shown to be a sensitive indicator of differences in sustainable cropping systems (Anderson and Domsch, 1989). The size and activity of the soil microbial biomass must be assessed to understand nutrient fluxes in managed and natural ecosystems.

Comparative studies have generally measured temporal fluctuations in microbial biomass of natural and perturbed agroecosystems. The change in microbial biomass values due to single effects such as tillage, soil type, climate, and crops has been the focus of much research (Smith and Paul, 1990). Soil microbial biomass has been used in studies of degradation of added organic chemicals, residue decomposition, and polluted soils. The toxicity of pollutants and the degradation of organic compounds can be monitored by following changes in the soil microbial biomass (Horwath and Paul, 1994). These biomass measurements when combined with tracer techniques will help answer questions concerning soil processes of importance to agricultural management and understanding the ecosystem functioning (Smith and Paul, 1990). We now seem to understand the significance of microbial biomass values, but the question remains, "how does microbial biomass influence soil structure?".

Among the methods to measure soil microbial biomass carbon, the four most commonly used are; (1) chloroform fumigation (CO_2-C flush), (Jenkinson and Powlson, 1976); (2) substrate-induced respiration (SIR), (Anderson and Domsch, 1978); (3) soil adenosine triphosphate content (ATP), (Jenkinson and Oades, 1979); and (4) microbial biovolume derived by microscopy, (Jenkinson et al., 1976). It is important to underline that none of these methods directly enumerates the C content of the microbial biomass needing to be converted.

Chloroform fumigation incubation method (CFI)

The most common methods to measure soil microbial biomass are based on chloroform-fumigation. Fumigation of soil with chloroform ($CHCl_3$) increases the amount of C extractable with 0.5 M of K_2SO_4 (Jenkinson 1966). Fumigation of soil samples with chloroform vapor causes a flush of decomposition during a subsequent 10 days incubation at 25° C compared with an unfumigated soil (Jenkinson 1966). Chloroform fumigation may dissolve waxy films protecting decomposable substrates. The application CFI method is confounded by the difficulty in ascertaining the contribution of non-microbial C to the fumigation flush (F_c) (Horwath and Paul 1994).

The chloroform fumigation extraction (CFE) method

CFE has few problems. It is rapid, although requiring additional analytical procedures (i.e., C digestion followed by titration, Kjeldahl procedures for organic N, etc.) for the determination of C and N which can lead to inconsistent results as compared to CFI.

To obtain the microbial biomass C, the proportion (P) of the control (UF_c) should be subtracted from the fumigated flush (F_c) and would vary as a function of the ratio F_c/UF_c. A linear function was used to determine the fraction of the control to subtract from the fumigated flush as described in equation (3):

$$P = K_1 (F_c / UF_c) + K_2 \qquad (3)$$

where P is the fraction of UF_c to subtract from F_c, K_1 and K_2 were estimated by minimizing the sum of squares of the difference between MBC and the

microscopic biovolume. Therefore, microbial biomass C (MBC) can be calculated by equation (4):

$$MBC = (F_c - UF_c)\, P \;/\; 0.41 \qquad (4)$$

Combining the two equations gives the formula in equation (5).

$$MBC = [F_c - (UF_c\,(K_1\,(F_c / UF_c) + K_2))] / 0.41 \qquad (5)$$

where K_1= 0.224, K_2= 0.33, F_c= fumigated and UF_C= unfimugated. The CFI is simple to perform, the estimation of CO_2 is easy. A critical concern is that chloroform kills all microorganisms in soil which makes difficult to estimate the original microbial biomass. Also the direct quantification of soil microorganisms by other techniques does not correspond to the results of the fumigation-incubation method. The fumigation with $CHCl_3$ makes some of the humic fraction of soils more available for degradation. The control problem when doing fumigation is one of the most controversial, some authors try to estimate microbial biomass without control but the result they obtain is twice as big as when they use a control. Voroney and Paul (1984) advised caution in omitting a control in measuring biomass in fumigation incubation.

The CFE method is rapid. It presents a good index for respiration. During the process no ammonium immobilization or denitrification activity is noted and also there is a low interference from nonmicrobial labile C and N substrates that can be used during the incubation. CFE requires additional instruments. It is also difficult to measure small amounts of soil C.

The substrate-induced respiration (SIR) method was introduced by Anderson and Domsch (1978) to rapidly estimate the amount of C held in living, non-resting microorganisms in soil samples. Biomass estimate in amended soils are of special interest under agriculture practice. In order to test the reliability and limitations of the CFI, CFE, and SIR methods, agricultural soils were amended with 1 % dried sewage sludge (Martens, 1985). Biomass C estimates were carried out 3, 7, 17, 21, and 28 days after the addition of the organic material. Three days after the amendment the CFI and SIR methods gave undoubtedly erratic results. The CFI method showed the typical underestimation caused by the problem with a large value for the control. The results of the SIR method confirmed the expected overestimation, indicating a shift in the physiological ages of the microbial cells (Martens, 1985). With the SIR method, amendments affected the biomass C estimates even after 4 weeks. Their results showed that the SIR technique requires long pre-incubation times before reliable estimates can be expected after the addition of a C source. Compared with the results obtained at later sampling dates, the chloroform fumigation extraction method obviously gave overestimates in two soils 3 days after the amendment (Martens, 1985).

West et al (1986) pointed out that conversion factors obtained with a relatively narrow range of soils may not be universally applicable. In studies on New Zealand grassland soils they found a wide range of soil dependent conversion factors between some of these methods. They evaluated and

recalculated equations and common conversion factors given in the literature by an application of statistical methods. For the k_C factor of the fumigation-incubation method they reviewed the published data and calculated a high degree of variability. The relation between this method and the SIR method was found to be uncertain.

The ATP extraction method was devised for the extraction and measurement of adenosine 5'-triphosphate (ATP) in soil. ATP is a universal cell constituent. It is present in all living cells and can be estimated with great sensitivity by the luciferin-luciferase system. ATP extraction is rapid. One disadvantage of the ATP method is that extraction of ATP from the cells is usually incomplete and it is decomposed by enzymatic or chemical hydrolysis during the extraction process. After extraction, ATP is strongly adsorbed by soil constituents (West and Sparling, 1986).

Microscopic observations provide direct estimates of biovolume. However, biovolume to weight , C, N conversions have remained problematic (Paul and Clark 1996; Bottomley, 1994). Microscopy measurements are applicable to direct field studies. Microscopes are expensive and require care. Discrete organisms with volumes greater than these categories were not included in volume calculations. When required, volume was converted to biomass C by assuming buoyant densities of 1.09 g ml^{-1} and dry matter contents (w/w) of 21 and 30 % for mycelial organisms and bacteria respectively (West and Sparling, 1986).

Summary

This literature review is an overview of the interactions among plant roots and their exudates, soil water, soil ions, and microbial biomass with a myriad of activities involving soil aggregate development, function, and stability. Carbon and nitrogen compounds from plant roots stimulate rhizosphere microorganisms which contribute to soil aggregation processes. Frequent soil wetting and drying cycles contribute directly to the positioning of soil particles. Interactions of root exudates, soil ion and water contents, and augmented soil cohesion improve soil aggregate stability. The best methods of measuring soil aggregate stability and microbial biomass were reviewed and compared.

3.0 SIMULATED ROOT EXUDATES ENHANCED SOIL AGGREGATION DURING WETTING-DRYING CYCLES

ABSTRACT

Plant root modifications of soil aggregates in the rhizosphere have been reported frequently. However, there are conflicting reports of soil aggregate stabilization by multiple cycles of root exudation and soil water removal. This study presents a method for measuring the combined affects of root infusions of carbon and nitrogen compounds and root extractions of soil water with multiple soil wetting and drying cycles. Polyvinyl chloride (PVC) cylinders, 10 cm diameter x 12 cm length, prepared by drilling a longitudinal row of 11 holes at 1 cm intervals, were used as containers for soil samples. Several methods have been used to collect soil solution nondestructively. In this study, a Rhizos soil solution sampler (SSS) has been used to simulate plant roots. These small tubes, 2.5 mm x 100 mm with an inside diameter of 1.4 mm, are constructed of hydrophilic polymers, having an average pore diameter of 0.1 μm. These root-like tubes were used to extract soil water and exude carbon (C) and nitrogen (N) compounds, during multiple cycles at one position in the soil. This study was designed to examine the effects of C and N incorporation during wetting/drying cycles on soil aggregation. A mixture of 7 carbohydrates, 4 organic acids, and 12 amino acids was injected into soil by five porous Rhizos Soil Solution Samplers (SSS), and submitted to 3 days incubation during each cycle. Soil water contents were estimates by time domain reflectometry (TDR) during the wettint and drying

cycles. Soil microbial biomass and mean weight diameter were estimated at the end the wetting and drying cycles.

INTRODUCTION

Several methods have been used to collect soil solutions nondestructively. A large porous ceramic cup has been used to extract soil solutions by suction (Debyle et al., 1989). This device has some limitations due to the heterogeneous medium of the root zone. Yanai et al. (1993) used a hollow fiber instead of a porous cup, as a soil solution sampler.

In this study a Rhizos soil solution sampler (SSS) was used to simulate roots. This hydrophilic porous polymer is small, root-like, and can be used to sample water contents in numerous soils. Extraction of soil solution and injection of C and N compounds by Rhizos SSS seems appropriate for rhizosphere measurements, especiallly when successive samples are injected into one position of soil a volume.

Several methods for measuring the stability of soil aggregates have been reported. The aim for aggregate stability tests is to describe and rank soil behavior under rainfall in a way that can be related to real environments (Le Bissonnais, 1996). Because of the range in field conditions, under which the samples are collected, the physical condition of samples needs to be standardized before testing so that different soils can be compared. Major factors in this standardization of methods includes soil moisture and structure of the

original sample. Numerous studies have shown that initial moisture content has a large influence on soil aggregate stability when soil aggregate stability was quantified by sieving (Haynes et al., 1990).

The activity of soil microorganisms and their influence on plants is largely dependent on the utilization of substrates which originate either as root exudates or plant residues. In this study, corn root exudates were simulated in the laboratory to better understand the stimulatory contributions exudates have on microorganism activities in soil aggregates during several wetting and drying cycles. The chloroform fumigation incubation method was used to estimate microorganism activities of the microbial biomass.

Specific objectives of the study were: 1) to evaluate artificial corn root exudate contributions to microbial biomass by using Rhizos SSS microtubes, 2) to evaluate the "exudation " and "absorption" properties of Rhizos SSS microtubes upon rhizosphere distances.

MATERIALS AND METHODS

Soil samples for this study were collected at Kellogg Biological Station in three different soil management groups. The conventional tillage (CT) which is plowed, the no tilled (NT), and the 40 years native grassland (NG). The percentage of sand, silt and clay for the Kalamazoo loam soil samples used in this study are listed in Table 1. The carbon and nitrogen contents for NG are high compare to

Table 1. Selected characteristics of the Agroecosystem site soils at Kellogg Biological Station (KBS), 1994, 1997.

	pH	Sand	Silt	Clay	Org C	N
					1997	1997
		----------	----------	%----------		
CT	6.3	42.1	43.1	14.7	1.24 (0.03)	0.11 (0.01)
NT	5.5	42.2	44.3	13.5	1.13 (0.10)	0.10 (0.01)
NG	6.7	39.1	46.8	14.1	1.82 (0.09)	0.16 (0.01)

Values in parentheses are standard deviations.
CT conventional tillage, NT no tilled, NG native grassland.

CT and NT. The ratios C/N for the three management groups are 11 for CT, 10 for NT, and 12 for NG.

Soil column construction

Polyvinyl chloride (PVC) cylinders, 10 cm diameter x 12 cm length, were prepared by drilling a longitudinal row of 11 holes, at 1 cm intervals, along one side of the cylinder Fig. 3. Cylinders were then sawed in half, lengthwise through the row of holes. Cylinders were reassembled and secured using duct tape (Nashua, Watervliet, NY 12189). Four layers of cheese cloth, secured with duct tape, were used to close one end of the cylinder. Soil aggregates 2 - 4 mm in diameter, obtained by sieving air-dried soil samples, from each field plot, were uniformly packed into the PVC cylinders. The soil aggregates were saturated from the underside by placing cylinders inside a container partially filled with distilled water. Enclosed soil columns were saturated to the surface for 16 hours. Following complete soil saturation, the second end of the cylinder was also closed with four layers of cheese cloth and secured to the PVC tube by plastic duct tape.

Simulated plant roots

Five Rhizos Soil Solution Samplers (SSS) (Ben Meadows Company. P.O Box 80549, Atlanta (Chamblee), GA 30366) were inserted into the saturated soils at spacings of 2 cm, Fig. 4. An internal stainless steel wire, Fig. 5, with 0.8

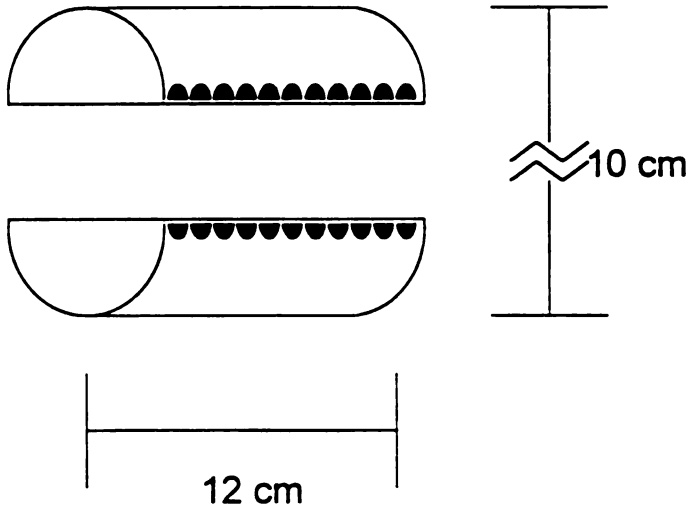

Fig. 3. Split PVC cylinder with holes on a longitudinal row.

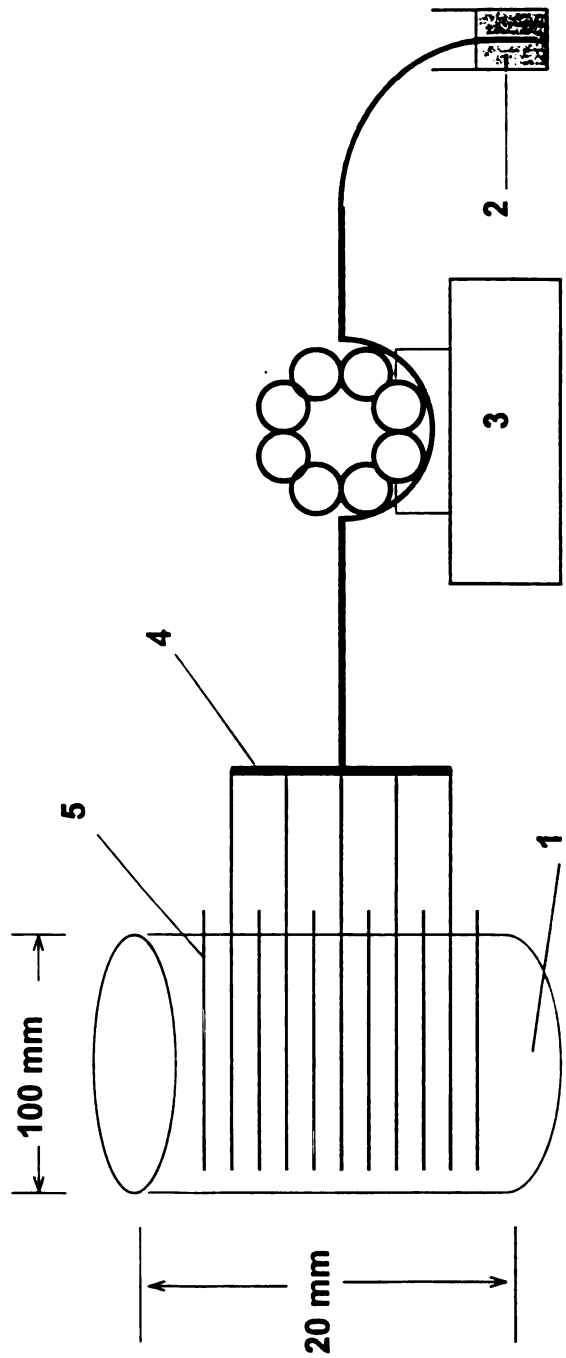

Fig. 4. Laboratory configuration for introducing carbon and nitrogen compounds to "rhizosphere" regions of soil: 1) Split PVC cylinder containing soil, 2) Simulated root exudate solution, 3) Peristaltic pump, 4) Manifold of 5 Rhizos SSS tubes, and 5) TDR probe.

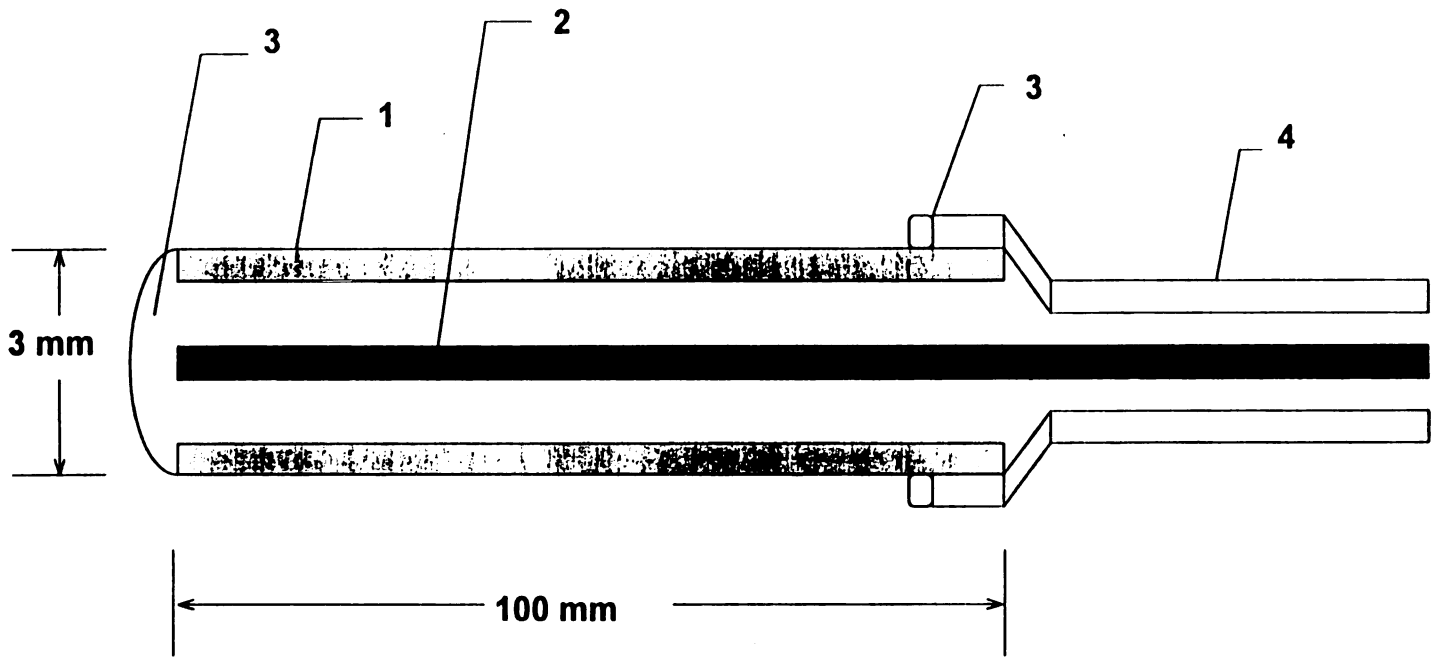

Fig. 5. Schematic drawing of laboratory Rhizo SSS system for introducing carbon and nitrogen compounds to "rhizosphere" regions of soil: 1) Porous teflon hydrophilic tube (average porosity of 0.1 μm), 2) Stainless steel wire support, 3) Adhesives, and 4) Polyethylene tube.

mm diameter and 150 mm length connected to the closed end of this porous polymer tube, gives the sampler enough rigidity to be inserted it into the soil.Stainless rods (3 mm x 100mm), sharpened on one end, were inserted, parallel to each other above and below each artificial root for measuring volumetric water contents of the rhizosphere by Time Domain Reflectometry (TDR). Porous Rhizos SSS tubes were used for injecting simulated artificial corn root exudate solutions. Rhizos tubes (2.5 mm x 100 mm) with an inside diameter of 1.4 mm, were constructed of hydrophilic polymers which had an average pore diameter of 0.1 μm. Solutions were added to the soil via the Rhizos tubes which were connected to a manifold with PVC tubing (Fig. 4). A multiple channel peristaltic pump (Cole-Parmer Instrument Company. Chicago, IL 60648) was used for adding and withdrawing simulated corn root exudates and soil solutions.

Artificial root exudate solutions

Analyses of organic materials found on, in, or near corn roots reveal a wide assortment of aliphatic amino acids, aromatic acids, amides, and sugar compounds (Guckert et al., 1991; Paul and Clark, 1996; Buyanovsky and Wagner, 1997). We simulated these corn root exudates by dissolving 12 amino acids, 7 carbohydrates, and 4 organic acids at concentrations reported in Table 2. Carbohydrates constituted 63.35 %, amino acids 9.36 %, and organic acids 27.29 %. The pH of the solution was 2.2. In the mixture solution C content within the solution was 40.74%, N 1.36%, H 6.69%, O 51.17%, and S 0.04%. The C/N

Table 2. Carbon and nitrogen compounds and quantities of simulated corn root exudates used in laboratory injections into soil columns of a Kalamazoo loam soil.

Carbohydrates	gL^{-1}	Amino acids	gL^{-1}	Organic acids	gL^{-1}
Arabinose	0.50	Alanine	0.02	Acetic acid	0.76
Galactose	1.45	Asparagine	0.16	Butyric acid	0.76
Glucose	1.31	Glutamine	0.16	Malonic acid	0.76
Mannose	1.04	Glycine	0.06	Succinic acid	0.76
Ribose	0.73	Isoleucine	0.02		
Sucrose	1.48	Leucine	0.03		
Xylose	0.58	Lysine	0.03		
		Methionine	0.15		
		Phenylalanine	0.02		
		Proline	0.33		
		Tyrosine	0.16		
		Valine	0.11		
Totals	**7.06**		**1.25**		**3.04**

Adapted from Guckert et al. (1991); Buyanosvsky and Wagner (1997); Paul and Clark (1996).

ratio was 30:1.

Many other materials have been added to soils, such as straw and sawdust, which contain C:N ratios from 80 and 400, respectively (Foth and Ellis, 1988). During the cycle, there is a decomposition and a continual loss of C as respiratory CO_2, with an accompanying increase in the N percentage and adecrease in the C:N ratio (Foth and Ellis, 1988). These added C and N compounds were metabolized by the soil microorganisms during three day incubation periods.

Wetting and drying cycles

The soil aggregates packed inside a PVC container were saturated for 16 hours. After saturation water was first removed from the soil columns in a manner which simulated root withdrawal of soil water by vacuum extraction through the five Rhizos tubes, at the rate of 8.8 ml/min for two hours until air entry and then at a minimum rate of 0.9 ml/min for 22 hours. After 24 hours of water removal by the pump, water contents approximated field capacity (22 to 25 %). A pulse of 5 ml of root exudate solutions plus a chase of 25 ml of distilled water were added to soils adjacent to the five Rhizos SSS tubes in each cyclinder at the rate of 6.3 ml/min. When the injection was completed, the samples were incubated at 22° C for three days. During the incubation, soil microorganisms metabolized the C and N added to the soil. After three days of incubation, the cyclinders were air-dried by placing them on their sides with both

ends exposed to a high, 3.40 m^3/min, flow rate of air under the ventillation hood (Kewaunee Scientific Equipment Corp. Adrian Michigan 49221), for four days. When soil moistures approached air-dry water contents ranging from 2 - 5 % (v/v), the cyclinders were removed for another cycle of saturation, vacuum extraction pulse-chase, injection of root exudates, incubation and air drying. At the end of the designated wetting and drying cycles, soil air-dry samples were collected from the rhizosphere and bulk soil. A knife and saw were used to separate rhizosphere and bulk soil from each cylinder as diagramed in Fig. 6. Soils collected 1 cm around the Rhizos tubes were designated as rhizosphere soil. The next 1 cm was collected as border soil to avoid the mixture of rhizosphere and bulk soil. The adjacent 2 cm was collected as buk soil, and the outlayer 1 cm , the portion in contact with the PVC was discarded to avoid the effect of PVC.

Air-dried soil samples were prepared for wet sieving by saturating with a nebulizer (Sunbeam Ultrasonic Humidifier, Oster Household Products). A humidifier nebulizer was used to avoid rapid wetting of dry aggregates, causing them to slake into smaller aggregates (Kemper and Rosenau, 1986). Four preweighed and presieved samples, uniformly distributed on the top 15.2 cm diameter sieve with openings of 4 mm were saturated by the humidifier nebulizer connected to four PVC plastic containers, Fig. 7. Uniform saturation of soil aggregates by the humidifier system was completed after 12 hours (Santos et al, unpublished).

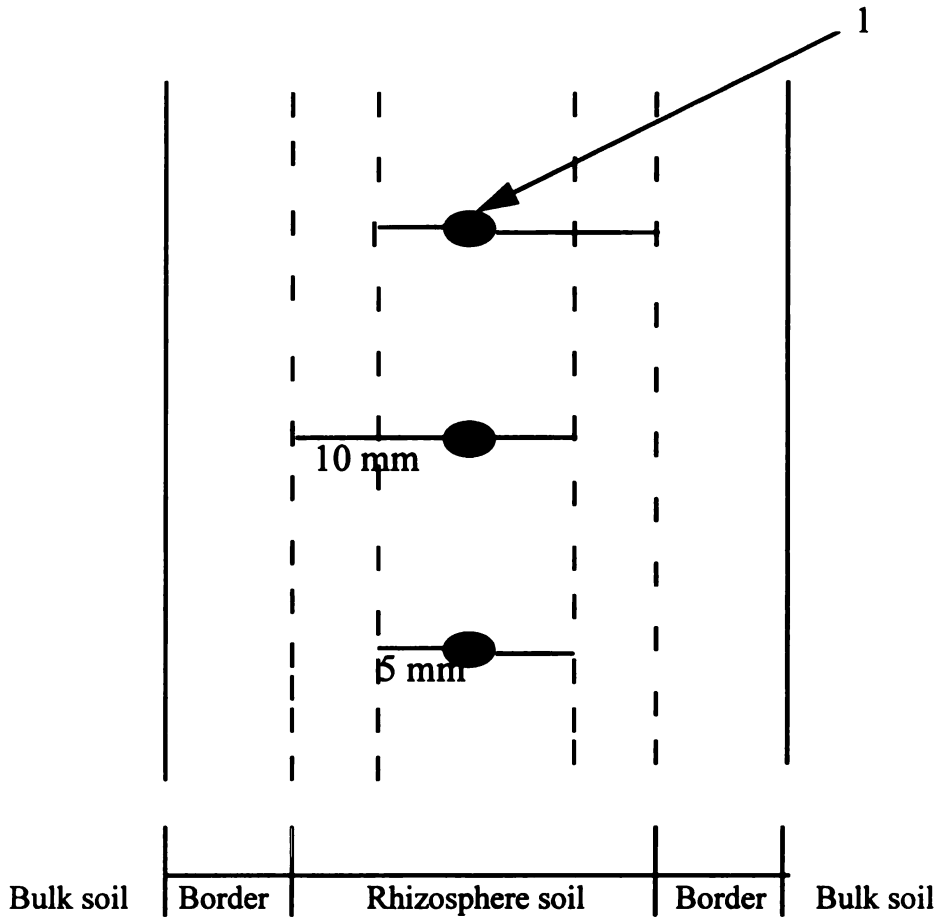

Fig.6. Diagrammatic representation of sample collected from rhizosphere and bulk soil when cylinders were opened after multiple wetting and drying cycles.
(1) Rhizos SSS microtubes.

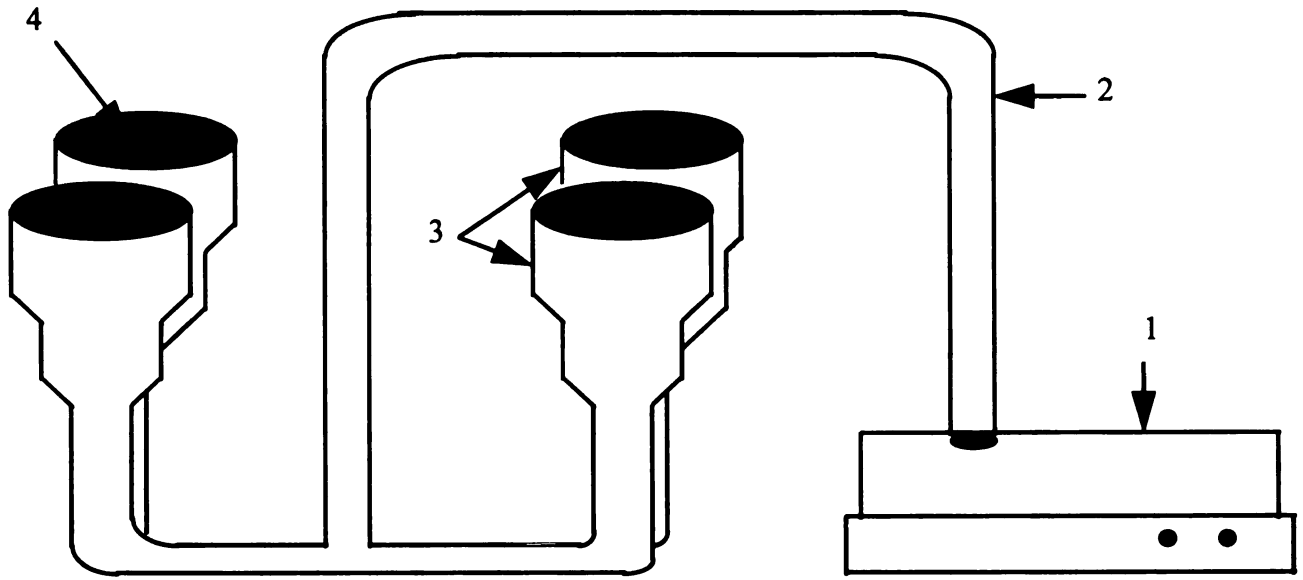

Fig. 7. Nebulization system used to pre-saturate soil aggregates before wet sieving. 1) Nebulizer system, 2) Mist conduent, 3) Manifold of PVC tubes supporting 4 mm sieve, and 4) Screen supporting soil aggregates.

Aggregate stability

Soil aggregate stability was determined by using the wet sieving method here after referred to as the Yoder method which is fully described by Kemper and Rosenau (1986). Twenty grams of air-dry aggregates, subsampled for water content, were saturated by nebulization, as described above, before placing on the uppermost sieves of four nests. The sieving machine had an oscillation speed of 33 rpm. Four nests containing 6 sieves (4, 2, 1, 0.5, 0.25, and 0.106 mm) were oscillated vertically for 20 min, so that water flowed through the screens containing the aggregates as described by Hillel, (1982).

After wet sieving, the nest of sieves were removed and the content of each sieve transfered to a 50 ml beaker and oven dried for 24 hours. The content of each sieve was weighed and thoroughly dispersed by adding 50 ml sodium hexamethaphosphate (5 %), and dispersing in a machine (Multimixer. Sterling Multi-products, Inc) by mixing for 20 min. The primary particles were oven dried for 24 h and weighed. The mean weight diameter equation (1) was used to estimate the aggregate stability, after correction for the coarse sands.

The delta value of the MWD was used for the statistical analysis. The delta value is given by equation (6):

$$\Delta_{MWD} = ((MWD_t - MWD_c)/MWD_c)*100 \qquad (6)$$

where MWD_t is the mean weight diameter of the treatment (root water and root C and N added), and MWD_c is the mean weight diameter for water control.

Microbial biomass

The chloroform ($CHCl_3$) used in the chloroform fumigation incubation estimations of microbial biomass was triple distilled at 55° C with 5 % H_2SO_4 according to the method described by Jenkinson and Powlson (1976) to remove ethanol and 10 g of anhydrous K_2CO_3 were added to the dry chloroform to remove water.

Six subsamples of 25 g each, were weighed for microbial biomass estimation. Three replicates were fumigated and three were unfumigated. Vacuum desiccators containing these samples were lined with freshly moistened paper towels prior to fumigation. For the fumigation treatment, the glass sample bottles containing the soil were placed into a desiccator along with a 100 ml beaker containing 50 ml $CHCl_3$. The desiccator was sealed and connected to the vacuum pump, and evacuated until the chloroform boiled (four times). The desiccator was sealed under vacuum and placed in the dark at 25 °C for 24 hours. The evacuation was repeated after 24 h and the samples were prepared for incubation. The fumigated and control soil samples were placed in jars with an open 20 ml scintillation vial containing 2 ml 2 M NaOH to trap CO_2. Three black jars with no soil samples containing scintillation vials were placed to trap the atmospheric CO_2. All the jars were tightly closed and incubated for 10 days at 25 °C. Direct analysis of CO_2 was achieved by the double endpoint titration method. An automatic titrator (Schott Geräte titrator, model TR 156, Yonkers, NY 10701) was standardized using 0.1 N HCl. Two ml of 2 M $SrCl_2$ were added to

the solution to precipitate the trapped CO_2 to strontium carbonate and titrated to a pH of 7.0.

Soil water contents

Volumetric water contents were estimated by the Time Domain Reflectometer (TDR) method (Topp et al., 1982; Campbell, 1990). Topp et al., (1980) proposed a polynomial which relates an empirical relationship between volumetric soil moisture content and the apparent dielectric constant of soil (ε). They assumed that the dielectric constant of soil, for all practical purposes, was insensitive to variations in bulk density, temperature, salinity, and mineral composition.

Soil volume water contents were calculated by using the Topp, et al., (1982) equation (7).

$$\theta_v = [-5.3*10^{-2} +(2.92*10^{-2}*K_a)-(5.5*10^{-4}*K_a^2)+(4.3*10\text{-}6*K_a^3)] *100 \qquad (7)$$

where K_a is the apparent dielectric constant and $K_a = (c*t/L^2)$; t is the signal travel time in nanoseconds and $t = (B-A)/(V_p*c)$; c is the propagation velocity of an electromagnetic wave in free space and c = 30 cm/nsec; Vp=0.99; L is the length of the transmission line (9.5 cm) which was the length of the TDR rod used in these studies, A is the minimum and A the maximum. For this application, this equation could be reduced to equation (8)

$$\theta_v = - 0.053 + 0.0292\varepsilon - 0.00055\varepsilon^2 + 0.0000043\varepsilon^3 \quad (8)$$

where ε is the electromagnetic wave in a medium of dielectric constant,

$\varepsilon = (c^2 * t^2)/4L^2$

A critical step in TDR application to soil moisture determination is the identification of the second inflection point. The first inflection point, which is the junction of the probe and the transmission line, is unaffected by the soil water content or salinity (Dalton et al., 1984). The second inflection point, which occurs at the end of the probe, may be very weakly defined in problem waves due to noises in the circuitry (Dalton, 1992).

RESULTS AND DISCUSSION

Root extraction and infusion

Five Rhizos SSS tubes, connected to the peristaltic pump Fig.4, were used to vacuum extract up to 8.8 ml per minute through the 5 Rhizos root-like resulting in a specific absorption rate of 2.24 μl per mm^2 of root surface tubes. Soil water contents were reduced enough to permit air to enter the root-like tubes while being pumped at this high rate for periods of two hours. Then tubes were pumped at the rate of 0.9 ml/min (eg., 0.23 μl per mm^2 of root surface) removing both air and soil water for an additional period of 22 hours. Following this 24 hours "root extraction" period volumetric soil water contents, were reduced from saturation 38.42 ± 1.30, to 25.59 ± 3.77%, Fig. 8. Therefore, approximately 134 cc of soil water were removed from each soil column by the artificial roots during each of the nine wetting and drying cycles, Fig. 9.

Following root water extraction, simulated corn root exudates were injected into rhizosphere regions of the soil columns. Five ml of solution consisting of 12 amino acids, 7 carbohydrates, and 4 organic acids, Table 2, were injected at concentrations similar to root exudates (Guckert et al., 1991; Paul and Clark, 1996; and Buyanosky and Wagner, 1997) assuming uniform distribution among all five Rhizos tubes and uniform leakage flow along the surfaces of each tubes, then each Rhizos SSS injection of 56.75 μg of C and N resulted in "specific root exudation" rates of 1.45 μg of C and N exudates per cc of root surface. These C and N exudates were washed through the connecting

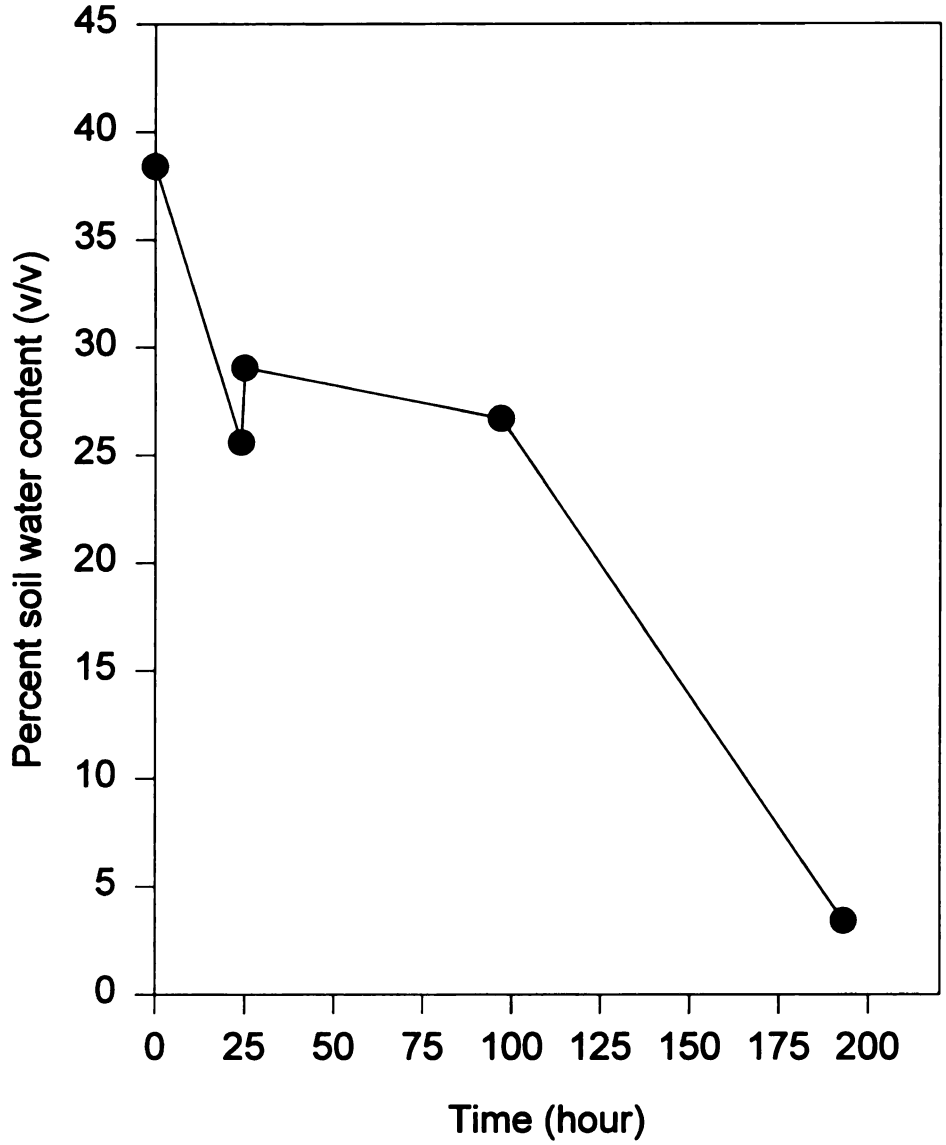

Fig. 8. An example of the change in soil water content of the rhizosphere region of a Kalamazoo loam soil contained in the PVC column and fitted with 6 TDR probes and 5 Rhizos SSS microtubes during each wetting and drying cycle.

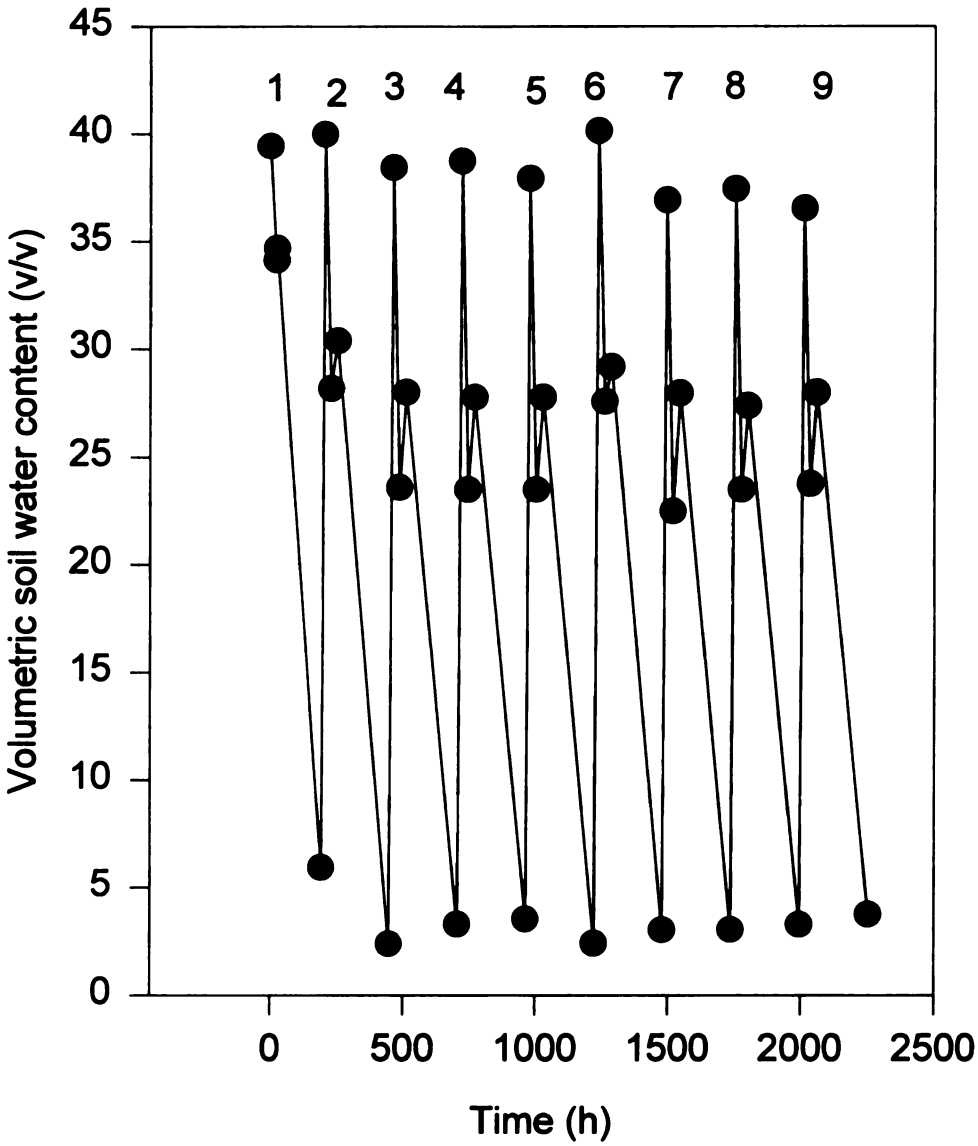

Fig.9. Volumetric soil water contents for samples collected in CT of Kalamazoo loam soil subjected to nine wetting and drying cycles during a 94-day period.

lines and Rhizos tubes by 25 ml of distilled water. Each root injection and subsequent washing resulted in a 3 - 4 % increase in soil water content , primarily in the rhizosphere regions of the PVC soil columns, Fig. 8. Microbes in these soil columns metabolized these simulated root exudates for 3 days then columns were air dried to approximately 3 % volumetric soil water contents, Fig. 8.

Soil aggregate stabilization in response to root exudates and multiple soil wetting, to saturation, and air drying were evaluated by repeating the processes outlined above for 9 cycles, Fig. 9. There more similar volumetric soil water contents of 38.42 ± 1.30 % for the 9 saturation events and 3.42 ± 1.06 % for the 9 air dry events. Although soil water contents following root extraction 25.59 ± 3.77 %, and root injection 29.04 ± 2.32 % varied somewhat, all soil water contents returned to nearly similar values during the 94-day period which encompassed the 9 complete wetting and drying cycles, Fig. 9.

Soil microbial biomass

The distances which root C and N compounds moved beyond the surfaces of the Rhizos tubes were evaluated by measuring microbial activities at 0 - 5 and 0 - 10 mm distances from the root surfaces as estimated by the microbial biomass assay after 9 cycles of wetting and drying. Similar microbial biomass values in experiments 0 - 5 and 0 - 10 mm, Table 3, suggest the Rhizos

Table 3. Influence of rhizosphere distances from root of 0 -5 and 0 - 10 mm on microbial biomass estimates following simulated C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil subjected to conventional tillage (CT), n=3.

Root treatments	Rhizosphere distance - mm		Rhizosphere distance - mm	
	0 - 5	> 5	0 - 10	> 10
	μg C g^{-1} of soil			
Root water	231 (36)†	173 (29)	209 (57)	201 (31)
Root C and N added	297 (27)	262 (23)	258 (19)	217 (21)

† The numbers in parentheses are standard deviation.

system is a repeatable estimate of root activities. Values for each soil position and treatment are similar even though soil in the 0 - 5 and 0 - 10 mm experiments were prepacked to different bulk densities of 1.30 g/cc and 1.15 g/cc. However, rhizospheres less than 5 mm appear to be too small for these Rhizos experiments as significant increases in the microbial biomass values occurred between the C and N exudate treatments and the water controls of the bulk soils, suggesting root exudates migrated into soil regions > 5 mm beyond the root , Table 3. When rhizosphere soil samples were extended to 10 mm beyond the root, then significant differences in the microbial biomass values could be detected between rhizosphere soils (0 - 10 mm) and bulk soils (> 10 mm) from the root surface. However, there were no significant differences in the microbial biomass values between the bulk soils of the water control and the C and N exudates for the 0 - 10 mm experiment, Table 3.

Aggregate stability

Soil aggregate responses to simulated root exudation and root extraction of soil water can be verified by measuring the mean weight diameter (MWD) of soil aggregates adjacent to the Rhizos tubes (ie., rhizosphere) and in bulk soil at distances > 10 mm from the artificial root microtubes. The water stability of soil aggregates, 4.75 - 6.30 mm across, removed from the rhizosphere region (ie., 0 10 mm) after 9 cycles of wetting and drying was always greater than for aggregates removed from the bulk soil region of the soil column, Fig. 10. In tilled (CT) and non tilled (NT) soils, additions and extractions of water and C and N

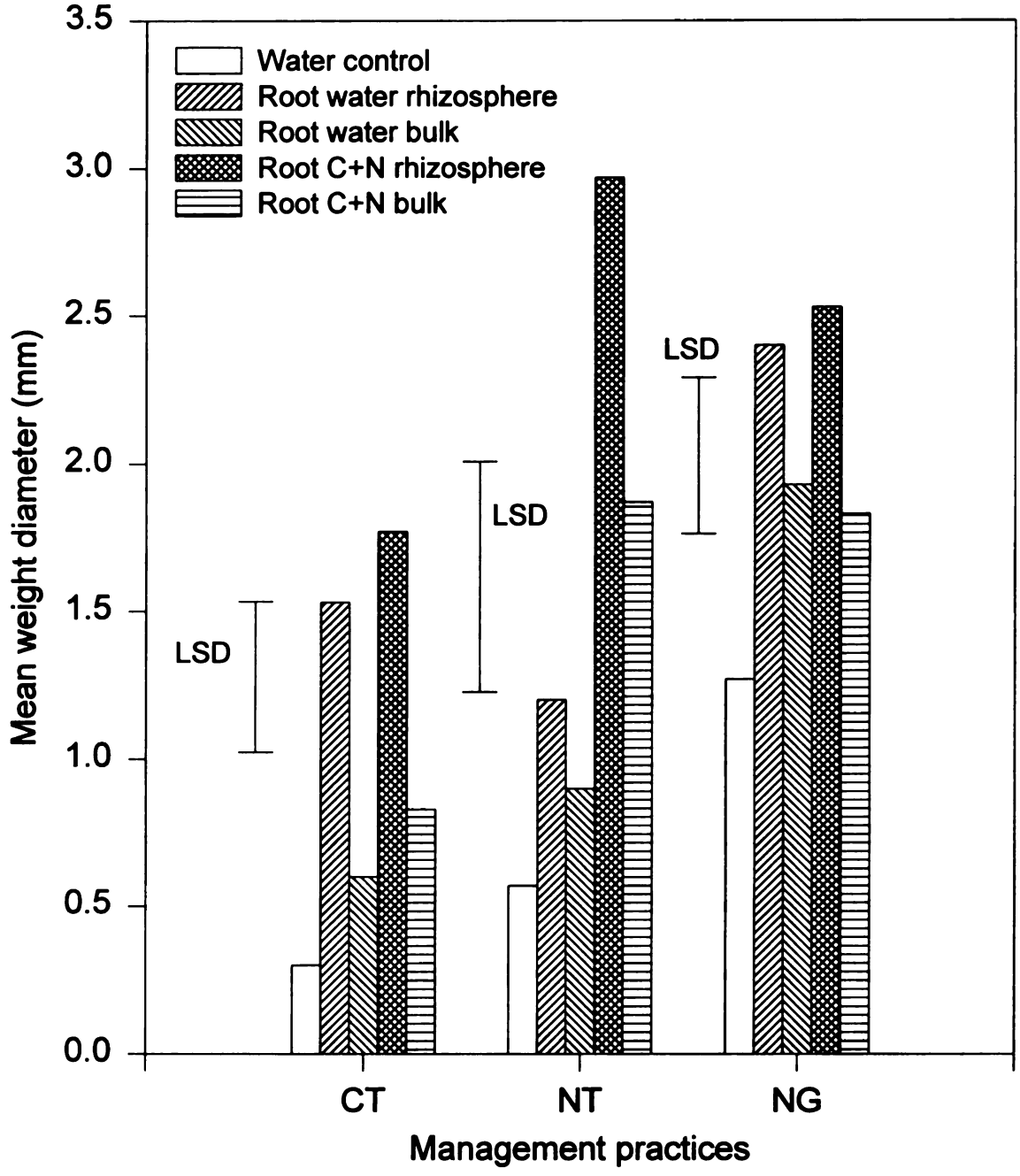

Fig.10 Mean weight diameter (MWD) for soil aggregates 4.75-6.30 mm across, following simulated root C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil subjected to 10 years of CT and NT and 40 of NG, n=3.

exudates by the simulated roots increased MWD of aggregates in the rhizosphere 2 to 5 fold more than the water control without simulated roots. Although this trend was also measured for soils taken from native grasslands (NG), the contrast between rhizosphere and bulk soils were smaller. Aggregates in the bulk region of the soil (eg., > 10 mm from roots) were less stable, lower MWD, for soils subjected to all three management practices, Fig. 10.

These data suggest that wetting and drying partially disperse clay and other fine soil fractions from the surfaces of soil aggregates within the soil column as observed by soil losses during the saturation phase of each wetting and drying cycles (unpublished observations). Apparently water removal by the nine vacuum extractions through the Rhizos tubes accumulated some of the dispersed clay on soil aggregates located in the rhizosphere regions adjacent to the simulated root (Reid and Goss, 1980). Subsequent additions of C and N compounds stimulated microbial growth in the rhizosphere regions during the 3 day incubation periods. The resultant polysaccharides and other microbial products (Lynch and Bragg, 1985) combined with the dispersed clay developed more water-stable aggregates during the drying phase of each wetting and drying cycle.

Summary

This experiment demonstrated the influence of artificial roots, root exudates, wetting and drying modifications on microbial biomass and soil aggregate stability. The presence of artificial roots used to removed water from the soil during the wetting and drying cycles has an impact on the reorganization of clay particles and microbial activity inside the cyclinder. Application of root C and N as exudates improved aggregate stability by increasing the number of microorganisms in the rhizosphere soil, and the quantity of larger aggregates and their mean weight diameters. Aggregate stability increases in the rhizospheres were 4 to 5 times greater compared to the baseline. The number of wetting and drying cycles affect the aggregate stability. One of the powerfull component of soil aggregate formation is the orientation of clay particles. The further experiments can look the mechanisms of clay movement around the Rhizos SSS, and the concentration of specific bacteria in the rhizosphere soil which act as glues in soil aggregate formation.

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4.0 Root Modifications of Soil Microbial and Aggregation Processes in the Rhizosphere

ABSTRACT

The aim of this study was to examine the effects of C and N incorporation and wetting/drying cycles on soil aggregation. A mixture of 7 carbohydrates, 4 organic acids, and 12 amino acids was injected into soil by five porous Rhizos Soil Solution Samplers (SSS), 10 cm long, 3 mm in diameter, and submitted to 3 days incubation periods during each cycle. Soils were subjected to three and nine wetting and drying cycles to simulate natural soil conditions.The three wetting and drying cycles did not generate the formation of aggregates 4.75 - 6.30 mm when a 2 - 4 mm aggregates were packed and submitted to wetting and drying cycles. Carbon and N compounds accompanied by nine wetting and drying cycles increased the mean weight diameter (MWD) in the rhizosphere 4.9 times in aggregates 4.75 - 6.30 mm. MWD increases in NT aggregates 1 - 2 mm across, was 53 % when sampled from the root exudate rhizospheres. The volumetric water contents were constant at saturation 38.05 %, after incubation and 7 days air drying the water content was 2.94 %. Microbial biomass of the samples collected from the three different management practices varied depending on the season of sampling. Additions of root exudate compounds increased microbial biomass of soil adjacent to artificial roots for all management practices by approximately 50 % for NT soils. The application of C and N compounds along with 9 cycles of wetting and drying caused significant

increases in the microbial biomass and stabilities of soil aggregates in rhizosphere adjacent to artificial roots.

Introduction

Soil aggregate stability, as determined by the wet sieving method, is influenced by the number of wetting and drying cycles and the content of microbial biomass carbon. The number of repeated wetting and drying cycles have a great impact on the mean weight diameter (MWD) and the percentage of water stable aggregates (WSA). When an unstable air-dried aggregate is wetted rapidly, it slakes into smaller sub-units (Emerson, 1977). Rovira and Greacen (1957) and Utomo and Dexter (1982) found that aggregates collected from field soils and submitted to wet-dry treatments reduced aggregation by 14 to 48%. Wet-dry treatments had significantly reduced total macroaggregation of soil. Total macroaggregation declined rapidly from 35 to less than 25% after two cycles (Degens and Sparling, 1995). The first wet-dry cycle resulted in large declines in the proportions of aggregates from 0.25 - 0.5 mm, 1 - 2 mm, and > 2 mm by 54-65, 22-30, and 27-48%. When microorganisms were present, soil aggregation increased in a few weeks, due primarily to microbial decomposition of added organic material with the production of aggregating materials (McCalla et al., 1957; Lynch and Bragg, 1985).

Wetting and drying cycles have been suggested to increase the amounts of organic C available to microrganisms due to the greater exposure of organic C

during the disruption of microaggregates or soil pores during drying and re-wetting (Powlson and Jenkinson, 1976; Van Gestel et al., 1991). The effect of wetting and drying cycles on subsequent decomposition of organic matter decreases with longer incubation of the organic material (Sorensen,1974). This suggests that wetting and drying cycles have a larger effect on the mineralization of more recently accumulated pools of organic matter (Degens and Sparling, 1995). Changes in aggregate stability in the rhizosphere have often been attributed to the presence of root exudates (Oades, 1984; Reid et al., 1982; Turcheneck and Oades, 1979). Due to their polysaccharide nature, root exudates play a significant role in the "cementation" of soil particles (Turcheneck and Oades, 1979). The use of intact root mucilage, collected from maize plants, produced additional evidence of the adsorption of exudates on clay minerals (Habib et al., 1990; Morel et al., 1987).

The global objective of this study was to evaluate the separate and combined affects of both root exudates and soil wetting-drying on soil aggregate stability in the rhizosphere. Specific objectives were: 1) to evaluate the contributions of artificial corn root exudates on microbial biomass activities; 2) to evaluate soil aggregate stability responses to multiple wetting and drying cycles; and 3) to estimate soil aggregate stability responses to both root exudates and soil wetting and drying cycles.

Materials and Methods

Study site description and experimental design

Soils used for this study, collected at Kellogg Biological Station (KBS), are classified as a Kalamazoo loam (Fine loamy, mixed, mesic, Typic Hapludalf: Soil Conservation Service, 1990) near Hickory Corners, Michigan. Soil samples were collected from plots subjected to three management practices. Two were from agricultural sites which received 10 years of conventional moldboard plowing and secondary tillage (CT), or no-tillage (NT), and the third was from a nearby native grassland (NG), for the past 40 years.

Soil sampling

Soil samples from the Ap horizons of the three management practices were taken to depths of 20 cm on November 25, 1995; May 2, 1996; and November, 22 1996. Composite samples were then transported to the laboratory and split into thirds. One third of each composite sample was sieved as moist samples through nested sieves with 2 and 4 mm mesh sieves and was used to estimate moisture content and microbial biomass within 24 h of sampling. A second fraction was stored at 4° C, for estimating soil aggregate stability. A third fraction was spread evenly on a greenhouse bench to air dry for 10 days. The composite sample was then sieved to obtain a fraction from 2 - 4 mm.

Soil column construction and soil preparation

Polyvinyl chloride (PVC) cylinders, 10 cm diameter x 12 cm length, were prepared by drilling a longitudinal row of 11 holes, at 1 cm intervals, along one side of the cylinder (Sissoko and Smucker, 1997). Soil aggregates 2 - 4 mm in diameter, obtained by sieving air-dried soil samples, from each field management system, were uniformly packed into the PVC cylinders at a bulk density of 1.30 g/cc for the first 9-cycle experiment and 1.15 g/cc for the second 9-cycle experiment. The soil columns of aggregates were saturated from the underside by placing cylinders inside a container partially filled with distilled water. Enclosed soil columns were saturated to their surfaces for 16 hours.

Simulated plant roots

Five Rhizos soil solution samplers (SSS) (Ben Meadows Company. P.O Box 80549, Atlanta, GA 30366) were inserted into the saturated soils at spacings of 2 cm (Sissoko and Smucker, 1997). In this study, Rhizos SSS tubes were used to simulate roots. Rhizos microtubes consist of a hydrophilic porous polymer which is constructed into a small root-like tube which can be used to sample soil solutions. Extraction of soil solution and the injection of C and N compounds by Rhizos SSS are most appropriate when successive samples are extracted or added at one position within a soil.

Analyses of organic materials found on, in, or near corn roots reveal a wide assortment of aliphatic amino acids, aromatic acids, amides, and sugar compounds (Guckert, et al., 1991; Paul and Clark, 1996; and Buyanovsky and

Wagner, 1997). Corn root exudates were simulated by dissolving 12 amino acids (9%), 7 carbohydrates (63%), and 7 organic acids (28%) in distilled water at concentrations which composed an artificial root exudate containing 56.8 mg of C and N compounds per injection (Sissoko and Smucker, 1997).

Wetting and drying cycles

Soil aggregates packed inside the PVC container were saturated for 16 hours. Water was first removed from the soil column in a manner which simulated root withdrawal of soil water, by vacuum extraction through the five Rhizos microtubes until mostly air was withdrawn. At the end of each designated number of wetting and drying cycles, air-dry samples were collected from the rhizosphere and bulk soils (Sissoko and Smucker, 1997).

Air-dried soil samples were prepared for wet sieving by saturating with a nebulizer (Sunbeam Ultrasonic Humidifier, Oster Household Products). The nebulizer was successfully used to avoid rapid wetting of dry aggregates, which would have caused them to slake into smaller aggregates as reported by Tisdall and Oades (1982). Four preweighed and presieved samples, uniformly distributed on the top 15.2 cm diameter sieve, with openings of 4 mm, were saturated by a nebulizer connected to four PVC plastic containers (Sissoko and Smucker, 1997). Uniform saturation of soil aggregates by the nebulizer system was completed after 12 hours (Santos et al, unpublished).

Soil microbial biomass

The chloroform ($CHCl_3$) used in the chloroform fumigation incubation estimations of microbial biomass was triple distilled at 55 °C with 5 % H_2SO_4 according to the method described by Jenkinson and Powlson (1976) to remove ethanol and ten g of anhydrous K_2CO_3 were added to the chloroform to remove water.

Six subsamples of 25 g each were weighed for microbial biomass estimation. Three replicates were fumigated and three were unfumigated. Fumigated samples were lined with freshly moistened paper towels. For the fumigation treatment, the glass sample bottles containing the soil were placed into a desiccator along with a 100 ml beaker containing 50 ml $CHCl_3$. The desiccator was sealed and connected to the vacuum pump, and evacuated until the chloroform boiled (four times). The desiccator was sealed under vacuum and placed in the dark at 25 °C for 24 hours. The evacuation was repeated after 24 h and the samples were prepared for incubation. The fumigated and control soil samples were placed in jars with an open 20 ml scintillation vial containing 2 ml 2 M NaOH to trap CO_2. Three black jars with no soil samples containing scintillation vials were also established as controls to trap the atmospheric CO_2. All the jars were tightly closed and incubated for 10 days at 25 °C. Direct analysis of CO_2 was achieved by the double endpoint titration method. An automatic titrator (Schott Geräte titrator, model TR 156, Yonkers, NY 10701) was standardized using 0.1 N HCl. Two ml of 2 M $SrCl_2$ were added to thesolution to precipitate the trapped CO_2 to strontium carbonate and titrated to a pH of 7.0.

Mean weight diameter (MWD)

Soil aggregate stability was determined by using the wet sieving method here after referred to the Yoder method as described by Kemper and Rosenau (1986). Twenty grams of air-dry aggregates, subsampled for water content, were saturated by nebulization, as described above, before placing on the uppermost sieves of four nests. The sieving machine had an oscillation speed of 33 rpm. Each nest containing 6 sieves (4, 2, 1, 0.5, 0.25, 0.106 mm) was oscillated vertically for 20 min, so that water flowed through the screens containing the aggregates as described by Hillel, (1982).

After wet sieving the nest sieves were removed and the content of each sieve transfered to a 50 ml beaker and oven dried for 24 h. The contents of each sieve were weighed and thoroughly dispersed by adding 50 ml sodium hexamethaphosphate (5 %), and dispersing in a machine (Multimixer. Sterling Multi-products, Inc) was run for 20 min. The primary particles were oven dried for 24 h and weighed. The mean weight diameter equation (1) was used to estimate aggregate stability, after correction for the coarse sands. The delta value of the MWD was used for the statistical analyses. The delta value is given by equation (6)

Percentage of water stable aggregates (WSA)

The air dry aggregates were placed on the uppermost sieve of the nest of sieves and immersed in water. The nest of sieves were then oscillated vertically forcing water to flow up and down through the screens. After 20 min the nest of sieves was removed from the water and the material left on each sieve was oven-dried and weighed. The contents of each sieve were dispersed by adding 50 ml sodium hexamethaphosphate (5 %), and completely dispersed by mixing in a dispersing machine for 20 min. The weight of sand retained after the second sieving was then subtracted from the total weight of undispersed material retained after the first sieving. The percentage of water stable aggregates was calculated by equation (9)

$$WAS = \frac{\text{(weight retained) - (weight of sand)}}{\text{(total sample weight) - (weight of sand)}} \times 100 \qquad (9)$$

Soil water contents

Time domain reflectometry (TDR) is a viable method for determining soil water contents in small uniform sections of soils ranging from near saturation to air-dry. The relationship between the dielectric constant and the volumetric water content was found to be essentially independent of soil type, density, salt content , and temperature (Topp et al., 1982). In this experiment volumetric water contents in the rhizosphere region of the soil cores were monitored by TDR to determine soil drying rates and uniformity of rewetting. Soil volume water contents were calculated by using the Topp, et al., (1982) equation (7).

A critical step in TDR application to soil moisture determination is the identification of the second inflection point. The first inflection point, which is the junction of the probe and the transmission line, is unaffected by the soil water content or salinity (Dalton et al., 1984). The second inflection point, which occurs at the end of the probe, may be very weakly defined in problem waves due to noises in the circuitry (Dalton,1992).

RESULTS AND DISCUSSION

Aggregate stability

The mean weight diameter (MWD) values of field samples were different for samples collected at different times of the year, Table 4. Samples collected in November had higher MWD values compared to samples collected in May. Lower MWD values for soil aggregates in May, after the frost had disappeared from the soil, may have been the result of dilution and/or leaching of ions from soils by frequent spring rains or could have resulted from lower microbial activity, due to colder soil temperatures. Less stable soil structural units, during the Spring of each year, could be one reason why some soil types are more vulnerable to compaction by tillage in the Spring.

Three wetting and drying (w/d) cycles

Three w/d cycles had little affect upon the stability of soil aggregates > 2 mm. No soil aggregates larger than 4.75 mm, could be extracted from NG, after 3 w/d cycles. The MWD of soil aggregates, 2 - 4.75 mm, were the largest for NG soils and smallest for CT soils, Fig. 11. MWD for water controls without Rhizos roots appeared to be similar to or greater than water and C plus N exudate treatments from the Rhizos microtubes. C and N additions to the rhizospheres of Rhizos microtubes significantly increased the MWD of aggregates 2 - 4.75 mm across, from CT soils, but not from NT nor from NG soils Fig. 11 and Table 5. Three w/d cycles significantly increased (positive delta MWD values) the

Table 4. Mean weight diameter for Kalamazoo loam soil collected from three management practices which received 10 years of conventional tillage (CT) and no tillage (NT), and from native grassland (NG). Samples were air-dried then sieved to aggregate sizes from 2 - 4 mm across.

	Mean weight diameter (MWD)		
Date	CT	NT	NG
	---------- mm ----------		
November 1995	1.47	2.08	2.19
May 1996	1.17 (0.09)	1.20 (0.05)	1.53 (0.06)
November 1996	1.38 (0.26)	1.90 (0.06)	2.14 (0.15)

Table 5. Influences of simulated root C and N compounds added during three wetting and drying cycles of a Kalamazoo loam soil, sampled from CT, NT, NG fields on the delta MWD estimations of aggregate stability for sieved aggregates ranging from 2 - 4.75 mm across, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- % ----------					
Root water	-48a	-42a	-5a	-18a	-7a	-2a
Root C+N added	-18a	-43a	-1a	-10a	-1a	-7a

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD.

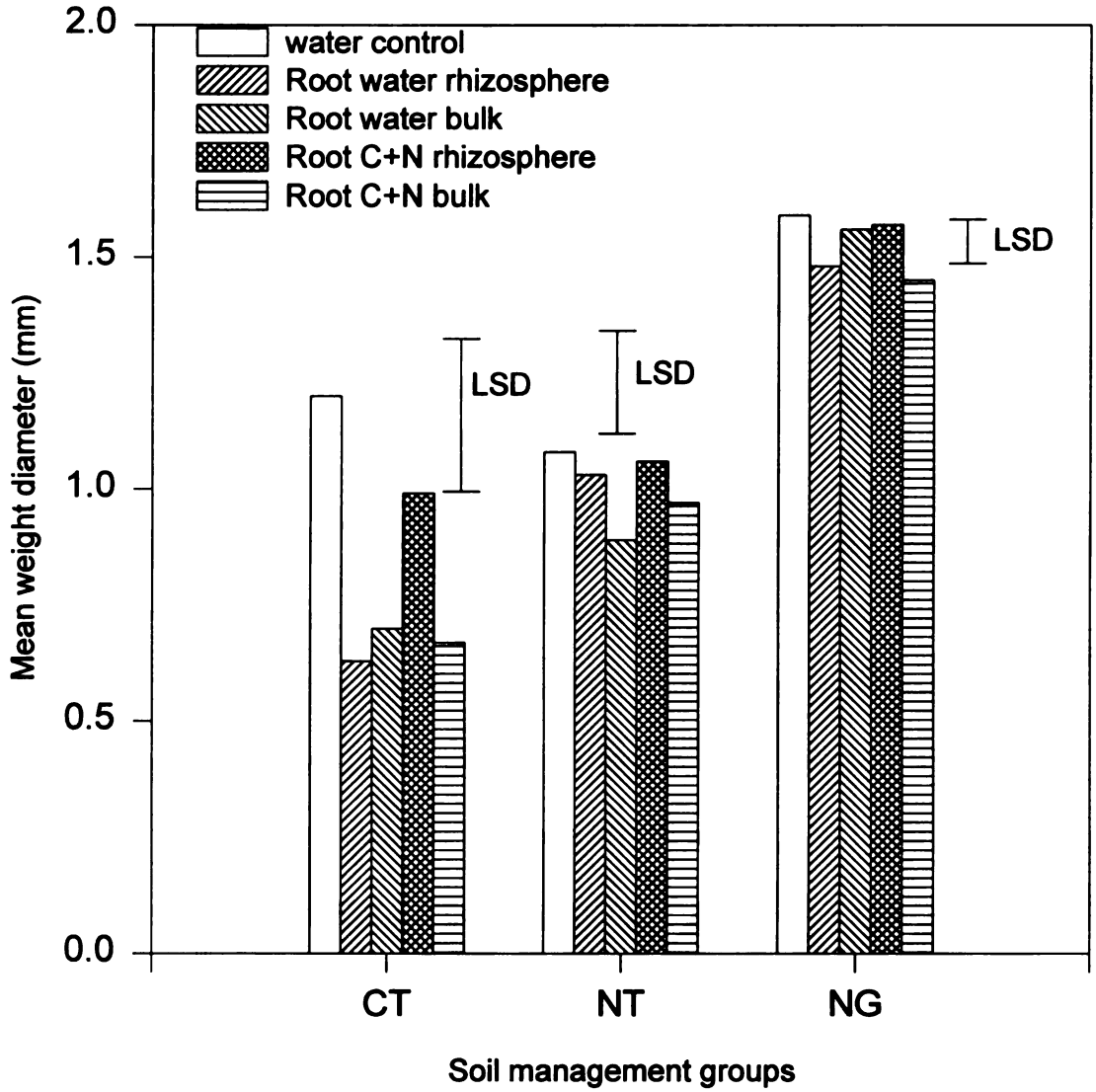

Fig. 11. Mean weight diameter for soil aggregates 2-4.75 mm across following simulated root C and N compounds and three wetting and drying cycles of a Kalamazoo loam soil subjected to 10 years of CT and NT and 40 of NG.

stabilities of soil aggregates 1 - 2 mm, for both CT and NT soils, Fig 12 and Table 6. Increased (positive) delta MWD in the NT and CT treatments but not the NG treatment, where aggregates were more stable initially, suggest that C and N compounds were great enough in the NG treatment as to not respond to three cycles of wetting and drying. Greater increases in the delta MWD values for the C and N treatments of NT soils, Table 6, also verifies that C and N compounds may be lower in the tilled (ie., CT and NT) than the NG soils.

When C and N were added during each of the three wetting and drying cycles, delta MWD values for aggregates, 2 - 4.75 mm across, from CT soils decreased, Table 5. Additions of water caused soil aggregates to be less stable than additions of C and N. Additions of C and N caused aggregates in the rhizosphere to become more stable than those in bulk soil Fig. 11.

The percentages of water stable aggregates (WSA), 2 - 4.75 mm across, for bulk soils were significantly greater for NT soils subjected to three cycles of wetting and drying when water and C and N compounds were added by the Rhizos microtubes, Fig. 13. Additions of only C and N to the rhizosphere caused significant increase in the WSA of rhizosphere aggregates from CT soils. Stabilities of this sized soil aggregates, extracted from NG soils, > 10 mm from the Rhizos microtubes, declined significantly. WSA for 2 - 4.75 mm in all regions of NG soils treated with Rhizos solutions actually declined when compared to the water controls, Fig. 13. Although aggregates, 1 - 2 mm across, were more water stable in NG soils, Rhizos treatments resulted in no significant changes from the

Table 6 Influences of simulated root C and N compounds added during three wetting and drying cycles

Table 6 Influences of simulated root C and N compounds added during three wetting and drying cycles of a Kalamazoo loam soil, sampled from CT, NT, NG fields on the delta MWD estimations of aggregate stability for sieved aggregates ranging from 1 - 2 mm across, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- % ----------					
Root water	15a	12a	29b	37ba	-3a	-3a
Root C+N added	-1a	-2a	53a	42ba	-3a	-5a

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD.

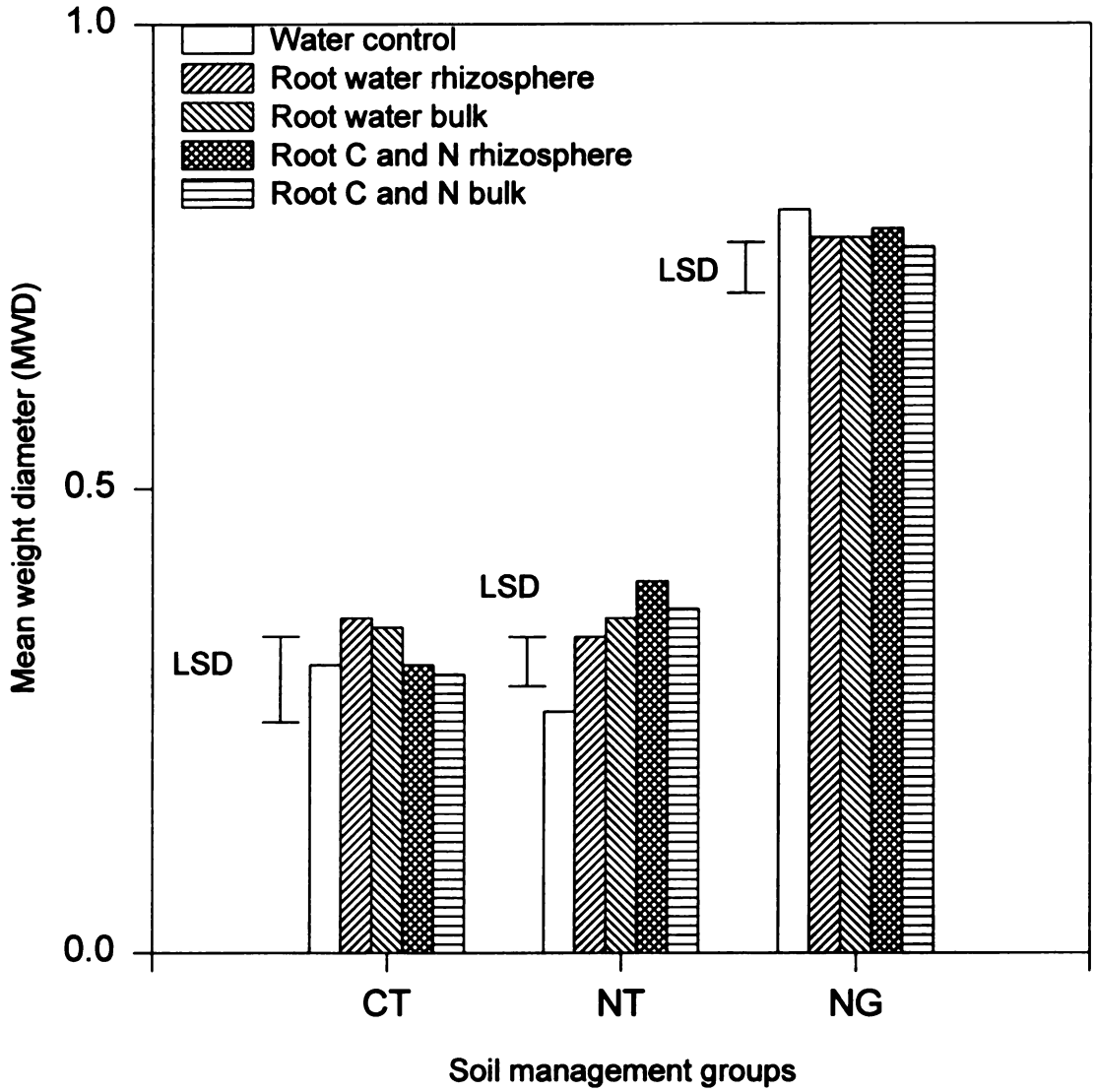

Fig.12. Mean weight diameter (MWD) for soil aggregates 1-2 mm across, following simulated root C and N compounds and three wetting and drying cycles of a Kalamazoo loam soil subjected to 10 years of CT and NT and 40 years of NG, n=3.

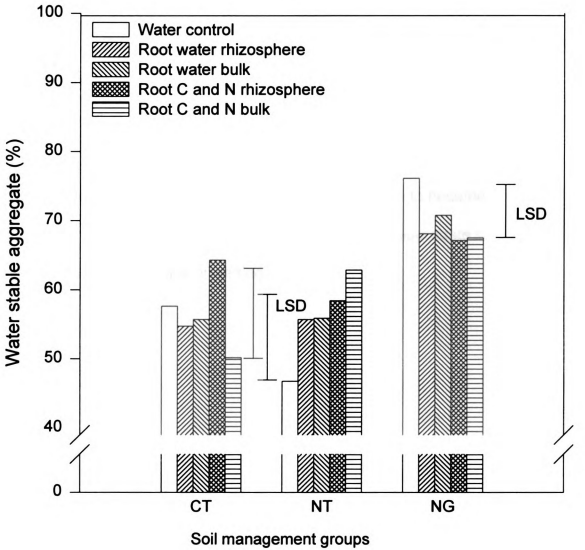

Fig.13. Percentage water stable aggregates (2- 4.75 mm) of a Kalamazoo loam after three wetting and drying cycles.

water control among the water and C + N treatments during three cycles of wetting and drying, Fig. 14. The absence of increased soil aggregation stabilities among tillage treatments of these soils, agrees with Degens and Sparling (1995) who reported that two w/d cycles reduced aggregate stabilities by 25 - 35%. These data suggest that movement of clay, cations, and other soil components, associated with soil aggregation processes, did not seem to become effectively organized in the tilled soils to a degree which enhances aggregate stability during the first three cycles of wetting and drying.

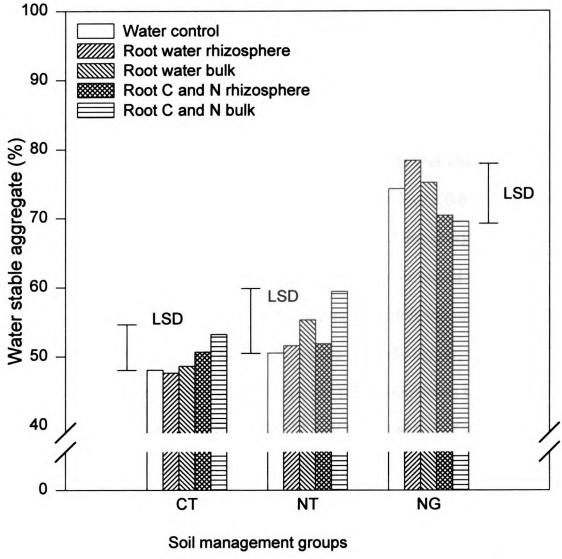

Fig.14. Percentage water stable aggregates (1- 2 mm) of a Kalamazoo loam after three wetting and drying cycles.

Nine wetting and drying (w/d) cycles

Experiment one of the two nine-w/d cycles was completed without a water control, which contained no Rhizos tubes nor TDR probes. The bulk densities of the Kalamazoo loam soils for experiment one and two were 1.35 and 1.15 g/cc. Another difference between the two 9-w/d cycle experiments was that the "rhizospheres" of these experiments were defined as soil regions 0-5 and 0-10 mm from the Rhizos microtubes. Nine w/d cycles produced larger aggregates (ie., 4.75 - 6.30 mm) than did three w/d cycles for all three managed soils. MWD of aggregates, 4.75 - 6.30 mm, were significantly increased by the nine cycles of wetting and drying when C and N compounds were naturally higher, as in the NG soils, or when C and N compounds were added to the rhizospheres by simulated roots in CT, NT, and NG soils, Table 7. Root C and N stimulation of aggregate stabilities appeared to diminish as aggregate sizes decreased from 4. 75 - 6.30 to 2 - 4.75 and 1 - 2 mm across, Tables 7, 8 and 9.

Experiment two of the 9-cycle w/d treatment demonstrated drammatic and significant increases in the stabilities of aggregates, 4.75 - 6.30 mm across, especially in the rhizospheres of soils when water was removed and either water and/or C plus N compounds were injected into soils from CT management programs, Fig. 15 and Table 7. C and N treatments also caused substantial, 5-fold, increase in the stability of aggregates from rhizospheres of NT soils. Greater, 2-fold, MWDs of aggregates in the rhizospheres of root water treatments suggest that the withdrawal of water by roots may concentrate clay

Table 7. Influences of simulated root C and N compounds added during nine wetting and drying cycles of a Kalamazoo loam soil, sampled from CT, NT, NG fields on the delta MWD estimations of aggregate stability for sieved aggregates ranging from 4.75 - 6.30 mm across, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	%					
Root water	411ab	100c	110ab	58b	89a	52a
Root C and N added	489a	178bc	420a	227ab	99a	44a

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD.

Table 8. Influences of simulated root C and N compounds added during nine wetting and drying cycles of a Kalamazoo loam soil, sampled from CT, NT, NG fields on the delta MWD estimations of aggregate stability for sieved aggregates ranging from 2 - 4.75 mm across, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- % ----------					
Root water	121a	28a	24ab	2b	10a	10a
Root C and N added	132a	26a	45a	11b	14a	8a

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD.

Table 9. Influences of simulated root C and N compounds added during nine wetting and drying cycles

Table 9. Influences of simulated root C and N compounds added during nine wetting and drying cycles of a Kalamazoo loam soil, sampled from CT, NT, NG fields on the delta MWD estimations of aggregate stability for sieved aggregates ranging from 1 - 2 mm across, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- % ----------					
Root water	1a	-37b	-4a	-10a	17a	6a
Root C and N added	-11ab	-37b	31a	11a	50a	32a

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD.

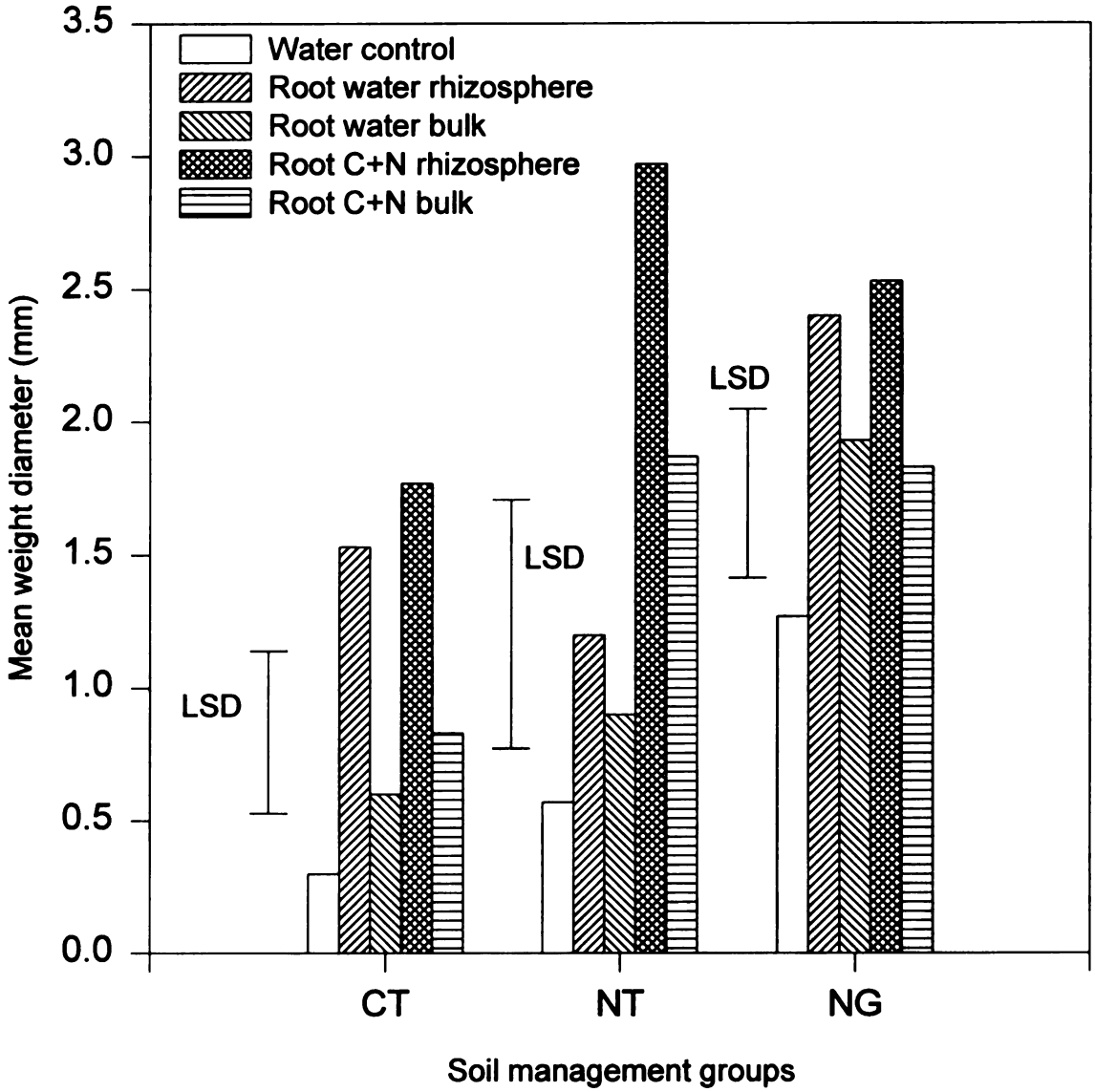

Fig.15. Mean weight diameter for soil aggregates 4.75-6.30 mm across, following simulated root C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil subjected to 10 years of CT and NT and 40 of NG, n=3

and other fine textured soil particles in regions surrounding plant root systems. Additions of C and N compounds by root systems futher augment stabilization processes of macroaggregates adjacent to roots. Although MWDs were greater for all treatments of the NG soils, additions of C and N compounds still demonstrated significant increases in MWDs of rhizosphere soils, located within 10 mm of the root, Fig. 15 and Table 7. Similar trends of increased aggregate stabilization by root-based C and N compounds continued among smaller soil aggregates from tilled soils, Figs. 16 and 17. Withdrawal of water and injections of C and N compounds by roots caused significant, 120 to 130%, increases in the MWDs of aggregates, 2-4.75 mm, taken from rhizosphere regions of CT soils, Fig. 16. Similar trends for increased values in rhizosphere MWDs of CT soils, over those from bulk soils of the same treatment, were also observed for smaller, 1-2 mm aggregates, Fig. 17. MWDs were the greatest in the rhizosphere and bulk soil regions of NG for all three aggregate sizes ranging from 1 - 6.30 mm, Figs. 15, 16, and 17. Values for the delta MWDs of treatments containing simulated C and N exudations for aggregates 2 - 4.75 and 4.75 - 6.30 mm, Tables 7, 8, and 9, suggest that macroaggregates are more responsive to root modifications of soil aggregates than are smaller, 1 - 2 mm, aggregates.

Soil aggregate stabilities in this study appear to be greater in soils from native grasslands (NG). This information is supported by Haynes and Swift (1990). The injection or withdrawal of water and C plus N compounds from the Rhizos SSS simulations of roots significantly increased aggregate stabilities of

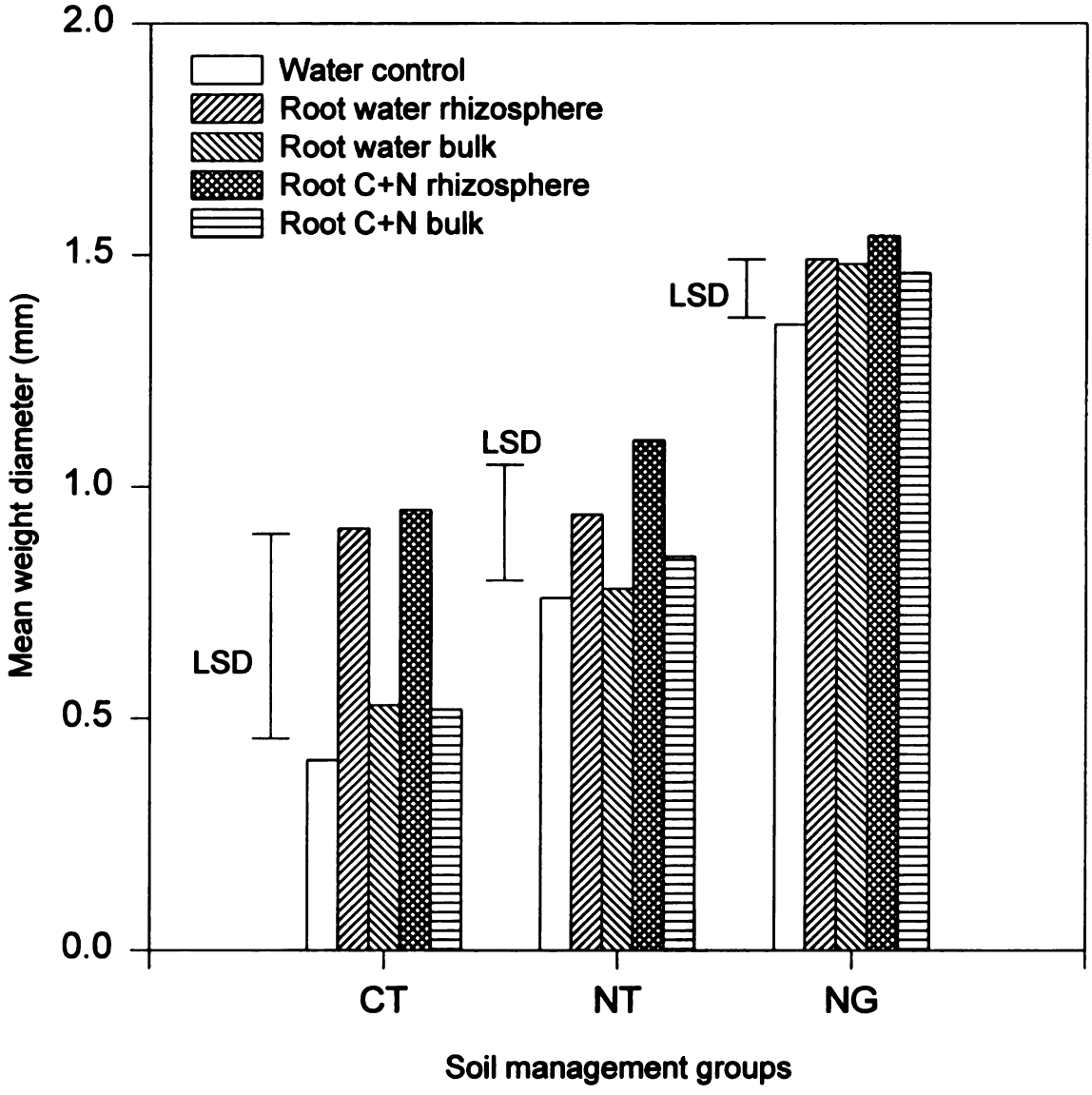

Fig.16. Mean weight diameter (MWD) for soil aggregates 2-4.75 mm across, following simulated root C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil subjected to 10 years of CT and NT and 40 of NG, n=3

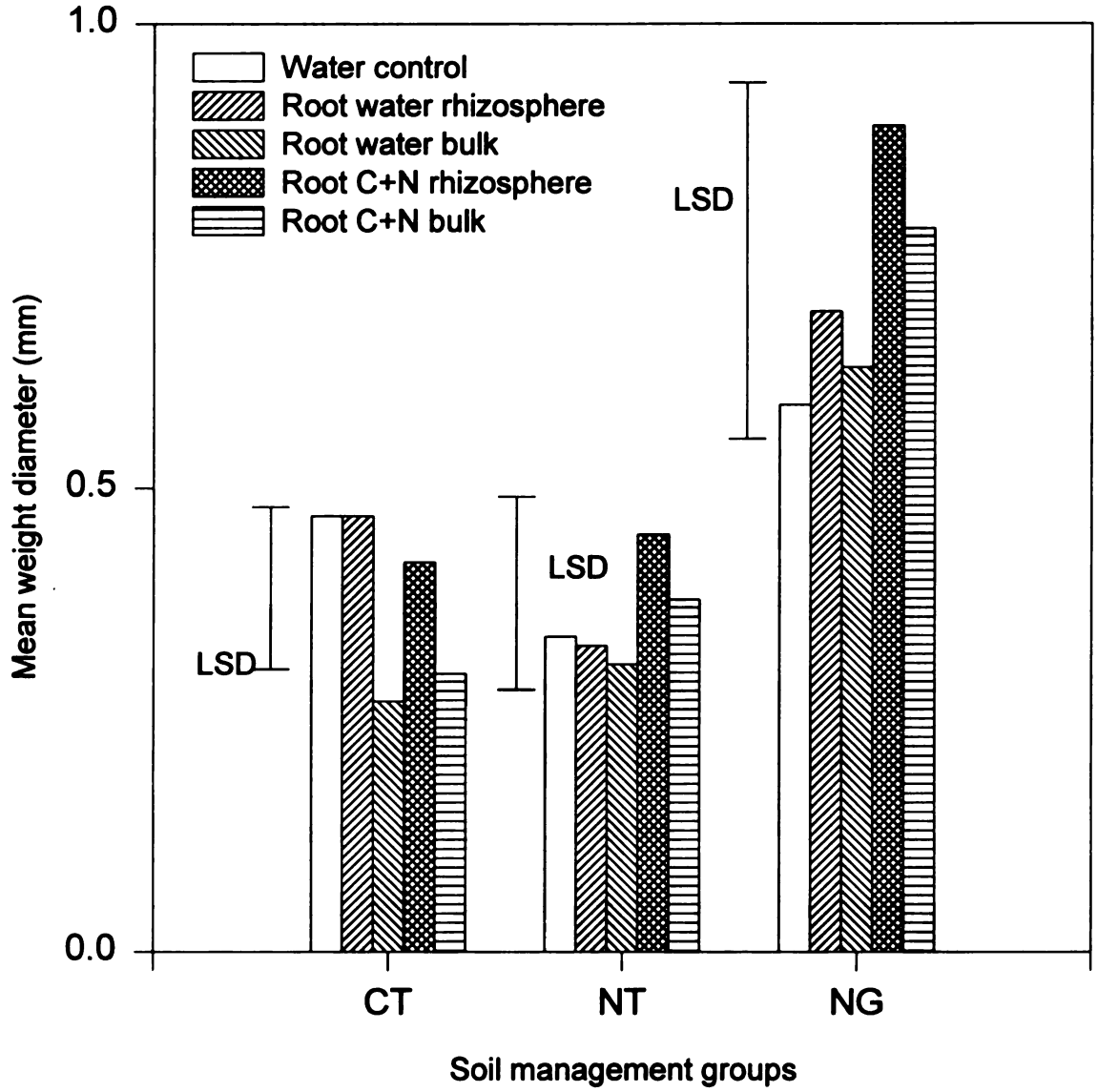

Fig.17. Mean weight diameter for soil aggregates 1- 2 mm across, following simulated root C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil subjected to 10 years of CT and NT and 40 years of NG, n=3

CT and NT soils more than for NG soils. Nine wetting and drying cycles increased the stability of macroaggregates, 4.75 - 6.30 mm across, in the rhizospheres of CT soils nearly 5-fold above the background water control while MWD of the bulk soils approximately doubled. MWD of NT aggregates in the rhizosphere increased more than 4-fold while aggregates in the bulk soils increased 60%. Nine w/d cycles slightly decreased the MWD of smaller macroaggregates, 1-2 mm across, for CT soils. These data suggest ways in which water and solutes move in aggregated soils may depend on the mode of saturation of the pore space which is made up of the micropore region within the aggregates and the macropores surrounding them (Youngs and Leeds-Harrison, 1990). The nine w/d cycles of this study enhanced soil particle aggregation which formed larger and stronger soil aggregates. Utomo and Dexter (1982) reported that six wetting and drying cycles of a fine sandy loam soil increased the stability of remoulded aggregates by up to 4 times. Others have noted that soils become completely structureless after 60 w/d cycles (Jager and Bruins, 1975) suggesting that the mode of w/d cycling as well as the losses of fine particles and associated important cations associated with soil aggregation processes may cause reductions in soil aggregation sizes and strengths of different macroaggregates.

Soil microbial activities

Dates of sampling

Soil microbial biomass estimations from three management practices on three different dates varied considerably, Table 10. Generally, microbial biomass values for CT and NT soils were lower than those for the native grassland. In November 1996 the soil was frozen and the top 3 to 5 cm were removed during sampling. This may be one of the reason the soil microbial biomass is low compared to November 1995.

Three wetting and drying cycles

Three wetting and drying cyclesincreased the microbial biomass in the rhizosphere soils of NT when root exudates were added. Increases of microbial biomass were 15 % when root water was added and 51 % when root exudates were added, Table 11. Root additions of water or C plus N had little affect on microbial biomass values of the bulk soil. Neither root exudates nor additions or withdrawals of water had any affects on the microbial biomass activities in NG soils, Table 12.

Nine wetting and drying cycles

In contrast to bulk soils, additions of root C and N compounds significantly increased the microbial biomass values for rhizosphere soils from all management practices tested in this study, Table 13. Nine wetting and drying cycles of soils receiving simulated root exudates increased, the microbial biomass values for rhizosphere soils over the control values of NG soils.

Table 10. Background microbial biomass from samples collected from 0 - 20 cm in the three management practices in Kalamozoo loam soil subjected to 10 years CT, NT and at least 40 years of NG.

	Microbial biomass		
Date	CT	NT	NG
	---------- $\mu g\ C\ g^{-1}$ soil ----------		
November 1995	269	277	412
May 1996	275 (68)	296 (81)	357 (8)
November 1996	175 (22)	210 (12)	310 (16)

Values in parentheses are standard deviations of the means, n= 3.

Table 11. Microbial biomass estimates of rhizosphere and bulk soils following simulated C and N compounds and three wetting and drying cycles of a Kalamazoo loam soil collected in May 1996, subjected to two levels of tillage and native grassland, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- μg C g^{-1} of soil ----------					
Control	-	267	-	218	-	312
	-	(28)	-	(53)	-	(35)
Root water	285 (19)†	283 (43)	251 (72)	207 (69)	301 (34)	300 (91)
Root C and N added	299 (22)	282 (10)	329 (28)	231 (27)	323 (22)	290 (31)

† Values in parentheses are the standard deviations of the mean, n=3.
R= Rhizosphere soil.
B= Bulk soil.

Table 13. Delta values of soil mixtures

Table 12. Delta values of soil microbial biomass in rhizosphere and bulk soils of aggregates, from a Kalamazoo loam <2 mm after three wetting and drying cycles of the Ap horizon, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- % ----------					
Root water	7a	6a	15ab	-5b	-3a	-4a
Root C and N added	12a	5a	51a	6ab	3b	-7a

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD. Control CT= 267 ± 28; Control NT= 218 ± 53; Control NG= 312 ± 35.

Table 13. Microbial biomass estimates of rhizosphere and bulk soils following simulated C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil collected in November 1996 subjected to two levels of tillage and a native grassland, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	μg C g^{-1} of soil					
Control	-	222	-	222	-	268
	-	(43)	-	(39)	-	(27)
Root water	209	201	230	188	265	272
	(57)†	(31)	(31)	(21)	(7)	(19)
Root C and N added	258	217	253	182	454	341
	(19)	(21)	(23)	(37)	(30)	(12)

† Values in parentheses are the standard deviations of the mean, n=3.
R= Rhizosphere soil.
B= Bulk soil.

These data suggest that rhizosphere of 0 - 10 mm, certainly less than 20 mm, were established by the Rhizos SSS microtubes of this study. Root exudates increased microbial activities by 16 % in CT, 14 % in NT, and 69 % in NG for rhizosphere soils above control values, Table 14. Greater microbial biomass in the rhizospheres of NG soils may be due to greater quantities of microorganisms in this soil, Table 13. The negligible and negative affects of 9 wetting and drying cycles, without additions of root C and N compounds, on microbial biomass estimates of soil microbial activities, Table14, suggest that soil wetting and drying of these soils does not liberate more C and N for mineralization and contradicts the conclusions by Degens and Sparling (1995).

Decreases in the microbial biomass of bulk soils is expected after nine wetting and drying cycles because most ions, including C and N are diluted with each w/d cycles resulting in greater competition for the limited C and N compounds. As available C and N compounds in the soil are used up microorganism numbers decline. Microbial biomass after nine wetting and drying cycles for the samples collected on three different dates are variable with consistently higher treatment responses observed for soils sampled on November 1995, Table 11, 13 and 14. One of the reason is because the soil was frozen and the top 3 to 5 cm were removed when samples were collected. Also microbes are more active when it starts to be warm. Microbial biomass values, after 9 w/d cycles, for samples collected on November 1995, Table 15, had higher values than those collected on November 1996, Table 13, because the surface 3 - 5 cm were removed due

Table 14. Delta values of soil microbial biomass in rhizosphere and bulk soils, of aggregates, from a Kalamazoo loam <2 mm after nine wetting and drying cycles of the Ap horizon, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	---------- % ----------					
Root water	-6b	-9b	4ba	-15b	-1b	1b
Root C and N added	16a	-2ab	14a	-18b	69a	27ab

Values followed by the same letter in the rhizosphere (R) and bulk (B) soil aggregates for each plant and soil management group are not significantly different at the 0.05 level of probability according to the Fishers LSD. Control CT= 222 ± 43.21; Control NT= 222 ± 38.76; Control NG= 268 ± 27.46 μg C g-1 of soil.

Table 15. Microbial biomass estimates of rhizosphere and bulk soils following simulated C and N compounds and nine wetting and drying cycles of a Kalamazoo loam soil collected in November 1995, subjected to two levels of tillage and a native grassland, n=3.

	Conventional tillage		No tillage		Native grassland	
	R	B	R	B	R	B
	μg C g^{-1} of soil					
Root water	231 (36)†	173 (29)	215 (7)	289 (16)	377 (12)	370 (13)
Root C and N added	297 (27)	262 (23)	368 (35)	283 (22)	598 (17)	408 (22)

† Values in parentheses are the standard deviations of the mean, n=3.
R= Rhizosphere soil.
B= Bulk soil.

to their frozen condition at the time of sampling. This suggests higher concentration of microorganisms in the top o - 5 cm than in the 5 - 20 cm.

In conclusion, microbial biomass in rhizosphere soil increased with additions of root exudates. The increase was significant of the nine wetting and drying cycles in CT, NT, and NG. However, there were no significant differences in bulk soil. In three wetting and drying cycles, only root exudates in rhizosphere of NT shows a significant difference. These differences show that few of the C and N compounds added, actually migrated into the bulk soil. No significant increases in microbial biomass occurred during the 9 w/d cycles, for the root water controls. These data also contradict Van Gestel et al., (1990) who suggested that greater amounts of organic C becomes available to microorganisms as a result of the disruption of micro-aggregates or soil pores during drying and re-rewetting. This phenomenon may have occurred in NG soil, but not in historically tilled agroecosystems (eg., CT, NT). Changes in aggregate stability in the rhizosphere have often been attributed to the presence of root exudates (Oades, 1984; Reid et al., 1982; Turcheneck and Oades, 1978). Due to its polysaccharidic nature, root exudates play a significant role in the 'cementation' of soil particles (Turcheneck and Oades, 1978). It was shown to bind with the surface of minerals present in natural or artificial soils (Guckert et al., 1975; Tisdall and Oades, 1982). The use of intact root mucilage, collected from maize plants , produced evidence of the adsorption of exudates on clay minerals(Habib et al., 1990; Morel et al., 1987).

Summary

The results obtained during this experiment demonstrated the influence of artificial roots, root exudates, wetting and drying modifications on soil aggregate stability, previously subjected to different management practices. The presence of artificial roots used to removed water from the soil during the wetting and drying cycles has an impact on the reorganization of clay particles inside the cyclinder. The presence of artificial roots with no C and N increased aggregate stability by 2.3 times. Application of root C and N as exudates improved aggregate stability by increasing the quantity of larger aggregates and their mean weight diameter. Aggregate stability increased in the rhizosphere 4.9 times greater when compared to the baseline. The number of wetting and drying cycles affect aggregate stability. More stable aggregates were expected after three wetting and drying cycles. Soil management practices significantly affected the formation and stabilization of soil aggregates when subjected to root C and N. Larger and more stable aggregates were formed in treated NG soils. The greatest improvements in soil structure were obtained through the application of root exudates in NT, and CT. This means that aggregates in tilled plots are more responsive to improvements when changes are made in plant and soil management practices.

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5.0 CONCLUSIONS

The interactions among plant roots and their exudates, soil water, soil ions, and microbial biomass activities affect soil aggregate development, function, and stability. Carbon and nitrogen compounds from plant roots stimulate rhizosphere microorganisms which contribute to soil aggregation processes. Frequent soil wetting and drying cycles appeared to contribute directly to the positioning of soil particles. Interactions of root exudates, soil ion and water contents, and augmented soil cohesion improved soil aggregate stability. This experiment has demonstrated the influence of artificial roots, root exudates, wetting and drying modifications on microbial biomass and soil aggregate stability. The presence of artificial roots used to remove water from the soil during the wetting and drying cycles has an impact on the reorganization of clay particles and microbial activity inside the cyclinder. Application of root C and N compounds which simulate exudates (composed of different organic acids, carbohydrates, and amino acids) by Rhizos SSS microtubes improved aggregate stability by increasing the number of microorganisms in the rhizosphere soil, and the quantity of larger aggregates as well as their mean weight diameters. Microbial biomass decreased when samples were collected far from the Rhizos SSS microtubes. Aggregate stability increases in the rhizospheres were 4 to 5 times greater when compared to the water controls for the aggregate sizes 4.75 - 6.30. The number of wetting and drying cycles

affected the aggregate stability. The three wetting and drying cycle has no significant effect on the improvement of soil aggregate stability. However, the nine wetting and drying cycles has a great impact on the formation and stabilization of soil aggregates. The management history has a very significant affect on the formation and stabilization of soil aggregates. The addition of root exudates accompanied by an adequate number of wetting and drying cycles improved soil aggregates in no tilled (NT) and conventionally tilled (CT) more than the native grassland (NG) which was already well aggregated.

One of the powerful components of soil aggregate formation is the soil clay content. It's orientation has a direct impact on soil aggregate formation. In this study, it appeared that removal of water, by Rhizos vacuum extractions accumulated some of the dispersed clay on soil aggregates located in the rhizosphere regions adjacent to the simulated roots. Additional experiments need to be conducted to better understand the mechanisms associated with clay movement around the Rhizos SSS, and the interactions of bacteria in the rhizosphere soil which combined with other C and N compounds act as a cementing agents which form stable soil aggregate formation.

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