Short-Rotation Woody Crops
Program
at
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College of Environmental Science & Forestry

Biomass Power for Rural Development
Technical Report:

SOIL SUSTAINABILITY AND PRODUCTIVITY IN SHORT ROTATION INTENSIVE CULTURE

Interim Program Report

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EXECUTIVE SUMMARY

Interest in biomass energy from short rotation intensive culture (SRIC) systems is growing and cogent. This is because (a) carbon (C) released during combustion and produced from other related activities is equivalent to C sequestered in harvested biomass and (b) carbon sequestered in litter, roots and soil organic matter (SOM), not utilized for bio-energy, can constitute potential C sinks. Biomass energy has excellent potential to (a) reduce fossil energy emissions which are increasing atmospheric carbon dioxide levels and (b) abate the specter of global warming. Impact of SRIC on SOM is important because SOM reflects and controls many soil properties and influences site productivity. Soil C may be a good indicator of the stability of terrestrial ecosystems. Knowledge of soil C sequestration and turnover would aid our understanding of soil productivity and sustainability in SRIC systems and the potential as C sink.

Studies specific to soil C storage for energy crops are sparse with values ranging from a loss of 0.64 to a gain of 5.76 Mg/ha/yr (Mann and Spath 1997). Soil C is lost within the upper 30cm during establishment and tending. With plantation age, soil C sequestration increase with 12-18 year-old plantations sequestering 1.63 Mg/ha/yr (Hansen 1993). Soil C accumulation in six and seven-year-old willow and hybrid poplar plots was not effected by fertilization, spacing and cutting cycle (Park 1996).

Most SOM studies report total SOM. Soil organic matter is composed of a variety of fractions or pools with different decomposition rates. These include a microbial biomass fraction (Sparling 1991); a labile, or actively cycling fraction; and a stable, or recalcitrant fraction (Tate 1987; Theng 1989; Ellert and Gregorich 1995). These different fractions of SOM lead to a variety of biological and physical methods for studying SOM dynamics. The microbial biomass fraction is a more sensitive indicator of organic matter dynamics than total SOM (Sparling 1991). Total SOM is not a sensitive or accurate indicator of short-term changes in SOM. The actively cycling fraction is more sensitive to management, necessitating a distinction between the actively cycling and more stable fractions of SOM (Ellert and Gregorich 1995).

Soil particle size fractionation isolates SOM into organo-mineral size fractions containing organic matter in various stages of decomposition and stability (Tiessen et al. 1984). Fractionation of soil according to particle size and subsequent chemical characterization of the organo-mineral size fractions may advance our understanding of organic matter dynamics (Christensen 1987). Soil organic matter dynamics may be inferred from soil microbial biomass turnover or activity. Soil microbial biomass carbon (SMBC) may indicate potential microbial activity because C contained within microbial biomass is stored energy for microbial processes (Rice et al. 1996). Microbial biomass C provided a better indicator of long-term effects of cropping and management practices on SOM in agricultural fields (Jordan et al. 1995). The ratio of microbial biomass C to total organic C has been used to measure and monitor of SOM dynamics (Sparling 1992; Rice et al. 1996).

This research focuses on SOM because it influences many soil properties and is a key determinant of soil fertility and productivity. The goal of this project is to evaluate soil sustainability and productivity in SRIC by studying SOM fractions over time. Soil samples will be collected from plots of willow ranging in age from 1 to 10 years and SOM determined using physical and biological approaches. Physical approaches involve determining C in organic matter pools associated with sand, silt and clay size fractions within 0-60 cm soil depth. Biological approaches involve determining SMBC as an actively cycling organic matter pool within 0-20 cm soil depth. Willow clone SV1 and hybrid poplar clone NM6 in SRIC of different root stock ages (0, 4, 7, and 10 years), located on a number of sites (Tully, Lafayette, Milleken, Kintigh, and Massena), and spanning a wide range of treatments (fertilization, irrigation and cutting cycles) in New York are being studied.

RESEARCH PROPOSAL

ABSTRACT

Soil sustainability and productivity are important criteria that can be used to assess the long-term productivity of Short Rotation Intensive Culture Systems (SRIC) of hybrid poplars and willows. Evidence from natural forests, plantations, and SRIC suggest that harvest nutrient removals can potentially deplete soil nutrient reserves and affect long-term productivity. There is also evidence that trees increase soil fertility by increasing soil organic matter content and concentrating nutrients at the surface in litter. This research focuses on soil organic matter because it influences many soil properties and is a key determinant to soil fertility and productivity. The goal is to evaluate soil sustainability and productivity in SRIC by studying soil organic matter changes over time. Hybrid poplar clone NM6 and willow clone SV1 in SRIC of different root stock ages (0, 4, 7, and 10y) located on a number of sites (Tully, Lafayette, Milliken, Kintigh, and Massena) in New York are being studied. The influence of fertilization and irrigation on soil organic matter will be assessed. Soil samples will be collected from the various sites during May and October. Organic matter will be evaluated using biological and physical approaches. Biological approaches involve determining carbon in microbial biomass as an actively cycling organic matter pool. Physical approaches involve determining carbon in organic matter pools associated with sand, silt, and clay size fractions. The duration of the study is from July 1997 to December 2001. Soil samples collected between July 1997 and December 1998 will be analyzed and reported in my thesis. [1]

INTRODUCTION and BACKGROUND

The State University of New York College of Environmental Science and Forestry (SUNY-ESF) at Syracuse has since 1986 been a center for research and development in short-rotation intensive culture systems (SRIC) involving hybrid poplars and willows. Initial research endeavors concentrated on clonal selections, effects of spacing, harvest cycle, fertilization and irrigation on biomass production. Results are published in a number of reports, student theses, and scientific journals. The published literature presents some information on nutrient contents of foliage and woody biomass, litter, and soil physical and chemical properties. Soil fertility and its maintenance is important for the long-term productivity of SRIC. However, research has not yet attempted to assess changes in soil fertility over time in SRIC.

The potential for soil fertility depletion and its effects on long-term productivity in SRIC should be of concern. Evidence from natural forest stands and plantations suggests that harvest nutrient removals can potentially deplete nutrient pools in forest ecosystems and affect the long-term productivity of forests (Tritton et al. 1989; Federer et al. 1989; Hornbeck et al. 1990). There is also evidence that higher rates of biomass production and harvest nutrient removals in SRIC of fast-growing hardwoods (Ericsson 1994) can potentially deplete soil nutrient reserves. Repeated harvests require fertilization inputs to replenish nutrient removals (Ulzen-Appiah 1987). Unfortunately, even though the long-term sustainability and productivity of these systems depend on how they affect the soil, research on effects of SRIC on soil has generally lagged behind attempts to promote these systems.

Soil fertility depletion can be studied by fertilizer trials and soil analysis. From SRIC research conducted at Tully, New York, Kopp et al. (1993) and (1996) reported that annual fertilization with N, P, and K significantly increased the rate at which willow clones reached their maximum annual wood biomass production. However, annual maximum attained was not influenced by fertilization (Kopp et al. 1993). Also, except soil pH, which decreased, harvest nutrient removal did not noticeably affect soil properties in fertilized willow bioenergy plots due to litter inputs (Adegbidi 1994). These results suggest that annual fertilization probably replenished harvest nutrient removal. Fertilization can be

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[1] Revised November 1998. First proposal submitted July 1997.

avoided in favor of increased rotation length. Organic matter inputs (through litter) can maintain soil properties over time through the ability of the trees to re-cycle nutrients contained in the organic matter inputs.

The focus of this research is on soil organic matter dynamics in SRIC. The broad goal is to evaluate soil fertility sustenance and hence, soil sustainability and productivity in SRIC by studying changes in soil organic matter over time. Soil organic matter has been chosen as an index of soil sustainability and productivity because it contributes significantly to soil fertility and influences many soil properties: acts as a source and sink for nutrient elements; has charge properties and provides sites for ion exchange; modifies soil physical structure and influences soil water regimes and influences many biological processes in soil (Swift and Woomer 1993). Therefore, a decrease or increase of soil organic matter could affect soil fertility.

The research paradigm is that maintenance or storage of soil organic matter in SRIC maintains or improves soil fertility over time. Soil sustainability and productivity is thus achieved. Alternatively, the loss of soil organic matter in SRIC over time lowers soil fertility and thus soil sustainability and productivity. The concept of soil sustainability and productivity as used here refers to the long-term use of soil that would not result in soil fertility decline. Hence, knowledge of soil organic matter loss and storage in SRIC would aid our understanding and enhance our ability to predict soil sustainability and productivity of these systems.

Unfortunately, there are very few reported studies specific to soil carbon storage for energy crops. Mann and Spath (1997) described data in the literature as sparse and contradictory and reported storage values ranging from a loss of 0.64 to a gain of 5.76 Mg/ha/yr. In hybrid poplar plantations in the North Central States, Hansen (1993) reported soil carbon loss during establishment and tending within the upper 30cm surface soil. However, with plantation age, soil carbon sequestration increased with 12-18 year old plantations sequestering 1.63 Mg/ha/yr. At Tully, New York, Park (1996) reported that fertilizer, spacing and cutting cycle had no effects on soil carbon accumulation in 6 and 7 year old willow and poplar plots.

Hansen (1993), Park (1996) and Mann and Spath (1997) reported on total soil organic matter. There is evidence that soil organic matter is composed of a variety of fractions or pools with different decomposition rates. These include a microbial biomass fraction (Sparling 1991); a labile, or actively cycling fraction; and a stable, humic or biodegradation resistant fraction (Tate 1987; Theng 1989; Ellert and Gregorich 1995). These different fractions of soil organic matter lead to a variety of biological and physical methods for studying soil organic matter dynamics. Sparling (1991) suggested that soil microbial biomass fraction is a more sensitive indicator of organic matter dynamics than total carbon. Ellert and Gregorich (1995) have suggested that total soil organic matter is not a sensitive or accurate indicator of short-term changes although it is a good indicator of long-term changes. The actively cycling fraction is more sensitive to management, necessitating a distinction between the actively cycling and more stable fractions of soil organic matter.

Tiessen et al. (1984) also suggested that fractionation of soil into particle sizes distinguishes soil organic matter into organo-mineral size fractions containing organic matter in various stages of decomposition, stability and loss in the soil system. Christensen (1987) reported that the decomposability of organic matter increased in the order: silt, whole soil, clay and sand. He explained that soil mineral particles, especially clay minerals, play an important role in the stabilization and turnover of soil organic matter. He suggested that physical fractionation of soil according to particle size and the chemical characterization of the organo-mineral size fractions provide another area of soil organic matter research.

This research focuses on soil microbial biomass and organic matter associated with organo-mineral size fractions in assessing the impacts of SRIC on soil organic matter dynamics and hence, soil sustainability and productivity. Studying these organic matter fractions would provide a better understanding of the distribution and turnover of soil organic matter.

STUDY OBJECTIVES

The specific objectives of this research are to determine over time, the impacts of SRIC on:

a) microbial biomass carbon in surface soil (0-20 cm depth);
b) organic matter content of whole soil and distribution in sand, silt, and clay fractions within 0-60 cm depth of profile; and
c) the effects of irrigation and fertilization on (a) and (b) above.
d) Also to determine amongst (a) and (b), which is more sensitive at detecting the impacts of SRIC on soil organic matter.

RESEARCH QUESTIONS AND HYPOTHESES

In order to meet the specific objectives and assess the impacts of SRIC on soil organic matter over time, this research asks three questions and tests three hypotheses. The questions are:

a) Do microbial biomass and organic matter in whole soil, sand, silt and clay fraction increase, decrease, or remain constant with increasing plantation age?
b) Are the increases or decreases in microbial biomass and organic matter of whole soil, sand, silt, and clay fractions influenced by fertilization and irrigation?
c) Which of these organic matter pools, microbial biomass, whole soil, sand, silt, and clay fractions, is more sensitive to changes in organic matter?

The specific research hypotheses are that with increasing plantation age in SRIC:

a) Microbial biomass and organic matter in whole soil, sand, silt, and clay fractions will increase over time.
b) Fertilization and irrigation will increase microbial biomass and organic matter in whole soil, sand, silt, and clay fractions over time.
c) The sensitivity of the organic matter pools will increase in order: silt, whole soil, clay, sand, and microbial biomass.

LITERATURE

Microbial Biomass

This organic matter fraction includes bacteria, fungi, actinomycetes, algae, protozoa, and microfauna and is the living component of soil organic matter. Microbial biomass is normally estimated as microbial carbon and typically comprises 1-4% of total organic carbon in soil. Microbial biomass serves as a store of labile organic matter, is responsible for the decomposition, mineralization, and nutrient cycling through decomposition of organic matter. Microbial carbon fraction has a faster turnover rate of 0.4-4 y, many times faster than the bulk of organic matter. In addition, microbial biomass reacts to change more quickly, shows a greater proportional change, and is a more sensitive indicator of organic matter dynamics than total organic matter. Therefore, it serves as a sensitive indicator of change and future trends in organic matter levels and equilibrium (Sparling 1991; Gregorich et al. 1994).

Three main biochemical methods provide indirect measurements of microbial biomass C. These are chloroform fumigation incubation (FI) (Jenkinson and Powlson 1976), substrate-induced respiration (SIR) (Anderson and Domsch 1978), and extractable biomass adenosine 5’-triphosphate (ATP) (Jenkinson and Oades 1979). The FI involves fumigating soil with chloroform vapor, removing the chloroform and incubating the soil. Microbial biomass is calculated from the difference between amounts of CO_2 evolved during incubation by fumigated and unfumigated soil.

An improvement of the FI, the fumigation extraction method (FE) directly extracts biomass constituents after removing the fumigant for measuring microbial biomass C (Vance et al. 1987; Veroney et al. 1993; Rice et al. 1996). The FE has the following advantages: no requirement for a microbial mineralization stage; avoids problems associated with incubation methods such as selecting appropriate controls; can be applied to soils of low moisture content, and is generally more reliable than the fumigation incubation method (Sparling and Ross 1993). The FE will be used to determine microbial biomass C in this study.

Gupta and Germida (1988) used soil microbial biomass measurements to study soil organic matter changes in aggregate size classes resulting from cultivation. Insam et al. (1991) used microbial biomass to demonstrate changes in soil fertility caused by the addition of various organic and nitrogen fertilizer amendments resulting in increased soil organic matter content. Also, Powlson et al. (1987) and Ocio et al. (1991) used microbial biomass to demonstrate early indication of small changes in soil organic matter.

Sparling (1992) found microbial biomass C and ratio to soil organic C useful measures to monitor soil organic matter. Both also provided a more sensitive index than organic carbon measured alone in soil under pasture, native soils, exotic forests and arable cropping. Gregorich et al. (1994) reported that microbial biomass has to be compared to a related soil parameter to indicate whether soil organic matter is increasing or decreasing. For example, the ratio of microbial biomass C to total organic carbon or the ratio of CO_2-C respired to microbial biomass C provides a measure of organic matter dynamics. Rice et al. (1996) have also suggested expressing microbial biomass C to total soil organic C to provide a measure of soil organic matter dynamics.

Physical Fractionation

Physical fractionation of soil according to particle size is based on the concept that soil organic matter associated with particles of different sizes differs in structure and function and therefore, plays different roles in soil organic matter (Tiessen et al. 1984). Size fractionation may be applied to whole soil samples or to heavy fractions of soil. The procedure involves dispersing soil in water. The fraction >63 µm is recovered and further separated by wet and dry sieving. Organo-mineral fractions <63 µm are isolated by gravity sedimentation in water and the <2 µm fraction subdivided by centrifugation (Christensen 1992).

Literature examples of the application physical fractionation in soil organic matter include: the isolation of particle size fractions (Genrich and Bremner 1974); cultivation effects on organic matter composition in size fractions (Tiessen and Stewart 1983); physical separation of soil organic matter (Elliot and Cambardella 1991); physical fractionation of soil and organic matter in primary size and density separates (Christensen 1992), and carbon turnover in soil physical fractions (Buyanovsky et al. 1994).

Christensen (1992) reported on the distribution, mineralization, and stabilization of organic matter associated with primary particle size in an extensive review of the literature. Most of the soil organic matter in arable soils is associated with clay and silt. Clay size separates often accounted for more than 50% of whole soil organic matter. For example, in a range of Danish Ap-horizons, 48-69% of soil organic matter was associated with clay, 21-43% with silt and 2-10% with sand. In a number of soils, silt size separates appeared to contain the highest proportion of whole soil organic matter. The distribution of soil organic matter among particle size separates, however, depends on the degree of soil disaggregation achieved during dispersion. Limited soil dispersion results in significant accumulations of soil organic matter in silt and sand separates.

The potential bioavailability of soil organic matter increases as particle size decreases. Generally, the mineralization rate of clay soil organic matter is twice that of observed for silt. Organic matter associated with clay accounts for most of the mineralization in soils (about 60%), silt contributes about 30% and sand 10%. The greater mineralization rate from clay than from silt-associated soil organic matter may result from differences in the bioavailability of the particle-bound soil organic matter (Christensen 1992).

Evidence suggests a relative enrichment of newly formed soil organic matter in clay. However, as decomposition progresses, silt size fraction accumulates an increasing proportion of the more stable soil organic matter. Lower decomposition rate of silt-associated soil organic matter relative to clay and the transfer to silt of stabilized decomposition products from other size separates is responsible for this. Thus, soil organic matter formed during decomposition is distributed among most size separates, but newly formed soil organic matter in clay is readily transferred or decomposed. In the long-term, soil organic matter will decline rapidly in the clay size fraction and from microbial biomass, whereas silt will tend to accumulate higher proportions of the more stable residual soil organic matter (Christensen 1992).

Carbon Enrichment Factors

Christensen (1992) and Amelung et al. (1998) used "enrichment factors" or carbon enrichment ratios to characterize carbon pools. The concentrations of organic matter associated with various particle sizes isolated from different soils can be compared using enrichment factors or ratios. The enrichment factor, E, relates the content of some component in a particular size fraction to the content of that component in the whole soil, as for example clay carbon enrichment, $E_{C\text{-clay}}$=(g C kg^{-1} clay)/(g C kg^{-1} whole soil). Enrichment of a component in a size fraction results in $E>1$, whereas $E<1$ indicates a depletion. The use of enrichment factors excludes the effects of different soil organic matter levels in whole soils.

METHODOLOGY

Study Plots and Sites

To assess the impacts of SRIC on soil organic matter over time, soil samples are to be collected from SRIC demonstration and study plots located at sites in Tully, Lafayette, Milliken, Kintigh, and Massena in New York. These plots provide an array of different root stock ages 0, 4, 7, and 10 y. In addition, these plots span a wide range of treatments; fertilization, irrigation, clones, spacing and cutting cycles.

Clone-Fertilizer Study

Located at Tully, this study was planted in 1987 to determine biomass production potential of willow clones SV1, SH3, SA2, SAM3, SA22 and hybrid poplar clone NM5. Plots were planted at 0.3 m x 0.3 m based on the wood grass concept and harvested annually. The experimental design is a split plot design (SPD) with three replications. Fertilization is the whole-plot factor and clone is the sub-plot factor. Plots are fertilized and irrigated annually. In 1994, willow clone SA22 and SAM3 were removed from the study due to poor survival. In 1997, one non-fertilized replication was destroyed and all the remaining replications were fertilized in 1998. Clone SV1 is to be studied in the fertilized whole-plot and compared to no-tree control plots.

Irrigation Study

This study is located at Tully and involves willow clones SV1, SA22 and SH3 planted at 0.9 m x 0.3 m in 1990. The objective is to determine the effect of irrigation on willow clones harvested on a three-year cycle with fertilization. The experimental design is a SPD with irrigation as whole-plot factor and clone as sub-plot factor. In 1990, clone SH3 was removed from the study due to poor survival. Clone SV1 is to be studied and compared to no-tree, non-irrigated control plots.

Spacing and Cutting Cycle

Located at Tully, this study involves willow clones SV1, SA22, and SH3 planted in 1990. The experimental design is a SPD with cutting-cycle as whole-plot factor and spacing as sub-plot factor. It involves three spacings, 0.3 m x 0.3 m, 0.3 m x 0.9 m, and 0.6 m x 1.05 m and three cutting-cycles, 1, 2, and 3 y. Plots are fertilized and irrigated annually. Clone SH3 and the 1-y cutting-cycle plots were removed from the study. Clone SV1 planted at 0.3 m x 0.9 m with 2 and 3-y cutting-cycles is to be studied and compared to no-tree and non-fertilized and irrigated control plots. The original objective was to determine the effects of cutting-cycle, spacing and clone on biomass production with irrigation and fertilization.

Willow Clone-Site Studies

These studies planted in 1993 involve 19 and 14 willow clones replicated at Tully and Massena, respectively. The clones include SV1, S25, S301, S546, and S365 planted using the double-row planting arrangement (double-row spaced at 1.5 m with 0.70 m inter-row x 0.60 m intra-row spacing). The experimental design is a randomized complete block design (RCBD). Plots are fertilized and harvested on a three-year cutting cycle. At Massena, the first cycle harvest was extended by one year. Clone SV1 is to be studied at both sites and compared to no-tree control plots.

Demo-95 Planting and Clone-Site Studies

Located at Tully (Demo Planting), Milliken, and Kintigh (Clone-Site) these studies involve willow clones SV1, S546, SA2, S35, and S301 and hybrid poplar clone NM6 planted using the double-row spacing in 1995. Each site has four non-replicated treatments of 0, 100, 200, and 300 kg N/ha applied as urea. The experimental design is a completely randomized design (CRD) with the sites as replications. Willow clone SV1 and hybrid poplar clone NM6 with 0 and 100 kg/N are being studied.

Demo-97 Plantings

These are production demonstration plots of willow clones SV1, S25, S301, S365, and S546 and hybrid poplar clone NM6 planted in 1997 at Tully and Lafayette using the double-row planting arrangement. For this study, a SPD with clones as whole-plot factors and fertilization with 0 and 100 kg/ha N applied as urea as sub-plot factors have been superimposed on each site as replications. Clone's SV1 and NM6 are being studied.

FIELD METHODS

Soil Sampling

To test the hypotheses identified in the previous section, soil samples will be collected from the study sites described above during May and October at the beginning and end of the growing season, respectively from July 1997- 2001. On each plot, soil samples will be collected at four sampling points across the rows leaving guard rows. On plots planted using the double row planting arrangement, sampling points will be located within rows and between rows. Soil will be sampled by depth (0-20, 20-40, 40-60 cm) and bulked for each depth. Soil samples will be collected at plot centers or at randomly chosen starting points across rows depending on plot sizes. Given in Appendix Table I is a summary of soil samples collected during October/ November 97, May 98 and October/ November 98.

LABORATORY ANALYSIS

Microbial Biomass

Soil samples collected within 0-20 cm depth will be sieved through a 6mm screen within 24 hrs. Portions will be kept frozen for microbial biomass extraction according to the methods described by

Veroney et al. (1993) and Rice et al. (1996) described in Appendix II. Microbial biomass carbon (C_{mic}) will be determined from microbial biomass extracts using a Dohrmann DC-190 High Temperature TOC Analyzer as dissolved organic carbon.

Physical Fractionation

The remainder of the 0-20 cm, the 20-40, and 40-60 cm depth samples (DS) will be air-dried and sieved through a 2 mm sieve. Depth samples will be fractionated into primary particle sizes: sand-size (>53 µm) as macroorganic matter or particulate organic matter (POM), silt-size (2-53 µm), and clay-size (<2 µm) according to procedures described by Jackson (1956), Christensen (1992), and Gregorich and Ellert (1993) as described in Appendix III**.** Organic carbon will be determined as follows using a Perkin Elmer 2400 CHN Analyzer.

a)In unfractionated or whole soil DS samples as:
C_{WS-20}, C_{WS-40}, and C_{WS-60}.

b) In particle sizes of DS as follows: for
0-20 cm, $C_{sand-20}$, $C_{silt-20}$, and $C_{clay-20}$;
20-40 cm, $C_{sand-40}$, $C_{silt-40}$, and $C_{clay-40}$; and

40-60 cm, $C_{sand-60}$, $C_{silt-60}$, and $C_{clay-60}$.

DATA ANALYSIS

Microbial Biomass Carbon

For each study described above, laboratory analysis will yield data on microbial biomass carbon (C_{mic}). In addition, the ratio of microbial biomass carbon to total carbon (C_{mic}/C_{WS-20}) will be computed. To test that microbial biomass will increase in hypothesis (a), regression analysis will be used to determine the relationship between root stock age (age of plantation) and C_{mic} and ratio of C_{mic}/C_{WS-20}. To test that microbial biomass will increase with fertilization and irrigation in hypothesis (b), analysis of variance will be performed for each study using its experimental design and data on C_{mic} and ratio of C_{mic}/C_{WS-20}.

Physical Fractionation

Similarly, for each study described above, physical fractionation will yield data on C in DS as follows for:
0-20 cm, C_{WS-20}, $C_{sand-20}$, $C_{silt-20}$, $C_{clay-20}$;
20-40 cm, C_{WS-40}, $C_{sand-40}$, $C_{silt-40}$, $C_{clay-40}$; and
40-60 cm, C_{WS-60}, $C_{sand-60}$, $C_{silt-60}$, $C_{clay-60}$.

These data will be used to determine the distribution of organic matter in sand, silt, and clay with depth. In addition, carbon enrichment factors for sand (E_{C-sand}), silt (E_{C-silt}) and clay (E_{C-clay}) of depth samples (0-20, 20-40, 40-60 cm) will be computed and used to determine carbon enrichment in sand, silt, and clay with depth.

a) To test that whole soil organic matter will increase in hypothesis (a), regression analysis will be used to determine the relationship between root stock age and C_{WS-TD}, C_{WS-20}, C_{WS-40}, and C_{WS-60}.

b) To test that organic matter in sand, silt, and clay will increase in hypothesis (a), regression analysis will be used to determine the relationship between root stock age and carbon enrichment of sand (E_{C-sand}), silt (E_{C-silt}) and clay (E_{C-clay}) of depth samples (0-20, 20-40, 40-60 cm).

c) To test hypothesis (b) that organic matter in sand, silt, and clay will increase with fertilization and irrigation, analysis of variance will be performed for each study using its experimental design and data on ($E_{C\text{-sand}}$), ($E_{C\text{-silt}}$) and ($E_{C\text{-clay}}$) of depth samples (0-20, 20-40, 40-60 cm).

d) The coefficient of variation between treatment replicates (CV) and the variation between treatment means (CTV) (Mead et al. 1993; Barrios et al. 1996) will be used to test hypothesis (c) the sensitivity of organic matter pools in silt, whole soil, clay, sand, and microbial biomass. The CV and CTV of whole soil carbon (C_{WS}), sand carbon enrichment ($E_{C\text{-sand}}$), silt carbon enrichment ($E_{C\text{-silt}}$), clay carbon enrichment ($E_{C\text{-clay}}$), and microbial biomass (C_{mic}) will be used to assess whether a significant effect of SRIC on these organic matter pools resulted from low random error (low CV) or a large separation of treatment means (high TCV).

STUDY DURATION

This research covers the period July 1997 to December 2001. Data collected and analyzed during the period July 1997 to December 1999 will be used for my Ph.D. thesis research.

PROGRESS REPORT

This report covers field and laboratory work undertaken during the period July 1997 to December 1998.

FIELD WORK: July-December 1997

Soil Sampling

In the first proposal soil samples (0-20, 20-40, 40-60 cm) were to be collected from all sites in May and October. At Tully and Lafayette, in the Demo-95 and Clone-Site, and Demo-97 plantings, samples were to be collected at six-week intervals beginning in May within 0-20 cm. In the revised proposal soil samples are to be collected from all study sites in May and October.

Demo-97 Plantings

During July plots were demarcated on the Demo-97 Plantings at Tully and Lafayette. Six plots each of 15m length with 10m buffer plots were laid out on the fields. Soil samples (0-20, 20-40, 40-60 cm) were collected from the Demo Plantings-97 in August for both microbial biomass and physical fractionation according to particle size. Surface soil samples (0-20 cm) were also collected in September for microbial biomass. End of growing season soil samples could not be collected because of a late start and a heavy snowstorm in November.

Demo-95 and Clone-Site

At the Tully Demo-95, soil samples were collected in August, and November for both microbial biomass and physical fractionation according to particle size. In September, samples were collected for microbial biomass. At Milliken and Kintigh, samples were collected in November for both microbial biomass and physical fractionation according to particle size.

Other Study Sites

Samples were collected from the Massena clone-site study in November. Soil samples could not be collected from the irrigation, clone-fertilizer, clone-site, and spacing-cutting cycle studies located at Tully as explained above.

FIELD WORK: January-December 1998

Soil Sampling

The beginning of season soil sampling commenced in mid May, and was completed on all study sites in the first week in June. End of season soil sampling commenced in mid October, and was completed on all study sites in the first week in November.

Appendix Table 1 provides a summary of samples collected by sites for analysis. A total of 996 soil samples have been collected, 223 for microbial biomass and 848 for physical fractionation.

Laboratory Analysis

This section of the report covers soil sample preparation, literature search, development of analytical procedures, and sample analysis carried out during the period.

Sample Preparation

All samples collected for physical fractionation (848) have been air-dried sieved through a 2 mm sieve and representative sub-samples stored in white plastic containers for fractionation and determination organic carbon. Samples collected for microbial biomass (223) were sieved through a 6 mm sieve within 24 hrs. Representative sub-samples have been kept frozen in white plastic containers for microbial biomass extraction and determination of dissolved organic carbon.

Literature Search

Considerable time and effort was allocated to understanding the literature on pools of soil organic matter during the period. Current biological and physical approaches used to measure pools of soil organic matter were used to develop laboratory analytical procedures (Appendix II and III).

Sample Analysis

During the period physical fractionation was completed on 100 samples from Massena, Kintigh, Milliken, and Tully. The fractionated samples from Massena were submitted in September 1998 to another laboratory for the determination of organic carbon. Results obtained will be analyzed to determine the success of the procedure and will be presented in a special report. However, the procedure is very slow. The sedimentation procedure that separates silt from clay requires on the average two weeks and sometimes four weeks for soils with high amounts of fine silt. This step in the procedure will be reviewed.

After several unsuccessful attempts, microbial biomass was successfully extracted from 15 samples from Massena and analyzed for dissolved organic carbon. The results showed wide variation between replicated samples suggesting poor lysis of microbes in one of the replicates due probably to a loss of vacuum and chloroform vapor from the dessicator. This problem has been solved for microbial biomass measurements to continue in earnest from mid January 1999.

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Appendix I. Summary of microbial biomass (MB) and physical fraction (PF) soil samples collected from October/November 97 to October/November 98.

STUDY	SITE/YEAR	MB SAMPLES (NUMBER)	PF SAMPLES (NUMBER)	TOTAL
Clone-Fertilizer	Tully-87	9	36	45
Spacing-Cutting-2y	Tully-90	9	36	45
Spacing-Cutting-3y	Tully-90	9	36	45
Demo-97 Planting	Tully-97	48	192	240
Demo-95/Clone-Site	Tully-95	32	84	116
Demo-97 Planting	Lafayette-97	48	192	240
Clone-Site	Massena	21	84	105
Clone-Site	Kintigh	16	64	80
Clone-Site	Milliken	16	64	80
Clone-site	Tully-93	6	26	30
Irrigation	Tully-90	9	36	45
TOTAL		**223**	**848**	**1071**

Appendix II. Analytical Procedure For Soil Microbial Biomass Carbon

A) MATERIALS AND REAGENTS

a) Ethanol-free chloroform: Commercial chloroform purified, dried, distilled in glass and stabilized with heptachlor epoxide.

b) Dessicator: should be inert to chloroform vapor, be of dry seal type and be able to withstand a high vacuum without implosion.

c) Miscellaneous materials and regents:
Fumehood
Boiling chips
125-mL Erlenmeyer flasks
Weighing bottles: for water content determination
0.5 M K_2SO_4 extraction solution
Whatman No. 5 filter papers for filtration
50-mL vials for filtrate

B) DESCRIPTION OF PROCEDURE

a) Preparation of soil

a) Sieve soil through a 6mm sieve and mix thoroughly.
b) Weigh three sub-samples of soil, 25-50g each, one into a weighing bottle for the determination of water content, and two into 125ml flasks, one to be fumigated for 48h and then extracted, and one the control, to be extracted immediately.
c) Dry soil in weighing bottle (with lids off) in an oven at 105°C to constant weight. Cool in a dessicator, reweigh and determine water content of the sample.

b) Fumigation Treatment

a) Line the dessicator with freshly moistened paper towels to prevent drying of the sample during fumigation.
b) Place flasks containing soil samples into the dessicator with 100ml beaker containing 50ml chloroform and a few boiling chips (antibumping granules).
c) Seal and evacuate the dessicator (Take care to vent the fumes released by the vacuum pump) until the chloroform boils vigorously for approximately 30 seconds.
d) Repeat the process three times, after the fourth evacuation, allow the chloroform to boil for two minutes, and close the valve.
e) Seal the dessicator under vacuum and place it in the dark at 25°C for 48h.
f) Release the vacuum, open the dessicator and remove the beaker of chloroform and moistened paper towels. (Keep waste chloroform in a sealed bottle and dispose as hazardous waste, keep paper towels in sealed bags and dispose in regular waste stream).
g) Evacuate the dessicator eight times for three minutes each, letting air pass into the dessicator after each evacuation.

c) Extraction Procedure

a) Add 100ml of 0.5M K_2SO_4 to the flasks containing the nonfumigated control and the fumigated sub-samples.
b) Cap the flasks and place on a shaker for 30mins to 1h.

c) After shaking, filter the solutions through pre-leached or acid-washed Whatman No. 5 or 42 filter paper. Avoid excessive evaporation during the filtration procedure.
d) Collect the filtrate for the measurement of organic C and total N. Cap and store the filtrate for not more than 2-3d, otherwise, freeze until ready for analysis.

(After Veroney et al 1993; Rice et al. 1996).

Appendix III. Analytical Procedure For Physical Frctionation Of Soil Organo-Mineral Complexes

A) SOIL PREPARATION

a) Pass air-dried soil through a 2mm sieve, discard residues retained on sieve.
Weigh 20g sub-samples to determine moisture content at 40 and 65°C.

b) Weigh 25-50g sub-samples into 250ml flasks (with leakproof closures). Dispense 100ml of sodium hexametaphosphate (5g L^{-1}), cap the flasks, and shake for 60mins on a reciprocating or rotary shaker.

B) SEPARATION OF SAND FRACTION (AT 53μm)

a) Pour soil suspension onto one side of a 53μm sieve supported on a glass funnel, and rinse particles from the flask using aliquots of water onto the sieve.

b) Wash silt and clay fraction, through the sieve into a 600ml beaker using a fine jet of water from a wash bottle. Use a rubber policeman to gently brush aggregates as the sand is displaced from one side of the sieve to another. Continue to wash until the sands are washed clean of silt and clay, as indicated by a clear washing solution coming out through the sieve and funnel. The sand + macroorganic matter fraction (SaF) is retained on the sieve. Combine suspension of silt and clay from 600ml beakers into 1L beakers.

c) Carefully transfer SaF from sieve to pre-weighed drying tins in the oven at 40°C to obtain dry weights of the SaF.

C) SEPARATION OF SILT AND CLAY FRACTION (AT 2μm)

a) Allow suspension of silt and clay in 1L beakers to stand for about twelve hours for each 10cm (4ins) depth. Decant the supernatant suspensions into 1L beakers and label as clay fraction (ClF), that is particles less than 2μm. Combine the sediments, that is particles from 2μm to 53μm, the silt fraction (SiF) into 1L beakers. Stir and bring sediments into suspension and transfer into 100ml beaker(s) to a height of 5cm (from the bottom inside measurements).

Allow suspension to settle for three and half-hours, the exact settling time for particles 2 to 53μm to settle at the suspension temperature. Decant the supernatant suspension and add to the clay fraction in the 1L beaker. Exercise care to prevent the decantation of any of the settled silt particles.

b) Add more water to the sediments in the 100ml beaker to the height of 5cm and allow settling for three and half-hours. Decant the supernatant suspension and add to the CiF in 1L beaker. Repeat procedure a number of times to obtain a clear supernatant.

c) Carefully transfer the SiF remaining in the beakers into pre-weighed drying tins and dry in an oven at 40°C to obtain dry weight of SiF.

d) Flocculate the ClF in the supernatant suspension in 1L beakers with $CaCl_2$. Carefully, decant the clear supernatant and centrifuge the sediment at 2400 r.p.m. for thirty minutes (the exact speed and settling time for particles less than 2μm at the suspension temperature) and discard the clear supernatant. Carefully transfer the settled ClF from the centrifuge tube into pre-weighed drying tins and oven dry at 40°C to obtain the dry weight.

e) Use a mortar to grind the SaF, SiF, and ClF. Do the same with a sub-sample of whole soil. Determine the concentration of carbon in the fractions.

(After Jackson 1956; Christensen 1992; Gregorich and Elliot 1993).