BLACK LIQUOR FROM CROP STRAW PULPING AS A POTASSIUM SOURCE AND SOIL AMENDMENT

By
CANMING XIAO

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CANMING XIAO

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of CANMING XIAO find it satisfactory and recommend that it be accepted.

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Chair

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Abstract

by Canming Xiao, Ph.D.
Washington State University
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Chair: William L. Pan

Crop straw represents an abundant, inexpensive and renewable fiber source for papermaking. Use of straw as a papermaking material may reduce deforestation through adopting alternative pulping materials. However, current reliance on Na-based pulping technology often generates black liquor that produces undesirable effects when applied to soil. Pulping of crop straw by substitution of NaOH with KOH should produce black liquor that may be a potential K source and soil amendment while offering an environmentally friendly and economically viable disposal option. Laboratory, growth chamber, and field trials were conducted to examine whether black liquor from wheat and bluegrass straw pulping with KOH could be land applied as a beneficial K and soil amendment. Black liquors generated by bluegrass straw pulping with 100% NaOH, 100% KOH or the 50% NaOH and 50% KOH slightly increased soil pH, indicating that they had potential as mild liming materials. Soil electrical conductivity (EC) increased slightly, but within low levels (< 4 dS m^{-1}), suggesting that these three black liquors would have no soil salinity concern when applied at 5 to 20 mL kg^{-1} soil. Soil

exchangeable sodium percentage (ESP) increased with 100% NaOH or 50% KOH and 50% NaOH based black liquor rates, suggesting that Na-based black liquor may potentially increase soil ESP to levels impacting soil physical conditions. Sweet or field corn did not respond to KCl or KOH-based black liquor application under field conditions, which was likely related to high initial available soil K levels. The KOH-based black liquor had the same effect as KCl in increasing available soil K. It also increased soil respiration, soil microbial biomass C, soil dehydrogenase, β-glucosidase, and arylsulfatase activities, and wet stable macroaggregates compared to non-amended control or comparable KCl rates, suggesting that KOH based-black liquor had potential as a beneficial K source and soil amendment. Polysaccharides and lignin contained in KOH-based black liquor were likely responsible for these beneficial effects.

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Chapter 1

Introduction

Black liquor generated during the chemical pulping process for papermaking has been an environmental concern and disposal problem for the pulp and paper industry due to its high biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, inorganic nutrients along with slowly degradable lignin and its derivatives (Grover et al., 1999). Rangan (1987) reported that black liquor accounts for only 10 to 15% of the total waste effluent, but contributes nearly 95% of the total pollution load of pulp and paper mill effluent. The more stringent regulations limit mill waste effluent entering waters. As a result, the pulp and paper industry has been challenged in pursuing environmentally safe and cost-effective disposal alternatives.

Generally, disposal approach is the chemical recovery process in which the black liquor is concentrated, burned for producing energy, and recycled for recovering inorganic chemicals (Grover et al., 1999). However, combustion not only requires high capital and operational costs, but also causes potential environmental problems during combustion process (Girovich, 1996). The pulp and paper industry has a growing interest in finding disposal alternatives.

Black liquor is rich in organic components and beneficial nutrients, but is rarely considered hazardous due to relatively lower levels of heavy metals (Cox et al., 1997). Land use of such an amendment may help maintain soil fertility by improving physical, chemical and biological properties and thus improving crop yields. Thus, utilization of black liquor as a soil amendment may be an environmentally friendly and economically

viable alternative. For example, Cox et al. (1997) found that application of black liquor increased plant nutrients, soil organic matter, cation exchange capacity (CEC), water holding capacity, and tilth. Increased soil enzyme activities of amylase, phosphatase and dehydrogenase were reported in fields irrigated with paper mill effluents, and this increase was attributable to more enzyme producing microorganisms in amended soil (Kannan and Oblisami, 1990).

Non-wood crop straw fibers have been widely used in pulp and pulp industry in many countries with a shortage of wood fibers such as China and India (Oinonen and Koskivirta, 1999) due to the increasing demand for pulp and paper products and population growth. As a result, the actual available forest source for papermaking has declined rapidly (Hagler, 1996). Such changes have led to a growing interest in using non-wood fiber resources as fiber alternatives.

Wheat straw represents an excellent fiber alternative because it is abundant in quantity. For example, in the middle 1990's, more than 75 million tons of wheat straw residue were available for pulping use in the U.S. (McKean et al., 1995). In 2003, about 214,650 of ha of wheat were grown in WA (Washington Agricultural Statistics Service, 2004), producing an estimated 1.6 million tons of wheat straw assuming 7400 kg straw residue per hectare (McKean, 1995). Timmer (personal communication, 1993) estimated that processing of nearly 10% of the available Pacific Northwest wheat straw would increase the fiber supply in that region by 10%. Wheat straw also has better quality for papermaking compared to the other non-wood fibers such as olive tree fallings, sunflower stalks, vine shoots and cotton stalks (Alcaide et al., 1993) because of its stronger breaking length of paper hand sheets.

Excessive quantities of crop straws are a handling concern for many growers in the world. Open field burning was employed to dispose of excessive straws, improve seeding conditions (Campbell et al., 1991), accelerate vegetative growth, and reduce pest and diseases or weed competition or prevent microbial immobilization of N (Rasmussen and Rohode, 1988). In the middle 1990's, approximately 80,940 ha of wheat straw were burned each year in Washington, releasing 45 million kg of particulates and volatile carbon compounds into atmosphere, and causing a serious environmental problem and health and safety risks (McKean et al., 1995). In many states in the U.S., crop residue burning is not an acceptable practice. As a result, use of crop straws for pulp and papermaking may provide a disposal alternative and add economical return for growers.

Crop straw is usually pulped by Kraft pulping process, which uses an aqueous solution of NaOH and Na_2S at an elevated pressure and temperature (170 ˚C) to release cellulose fibers (Yoon et al., 2001). This pulping process has long been an environmental and public relation issues due to mill odor caused predominately by malodorous reduced sulfur or total reduced sulfur (S) compounds originating from sulfide during pulping process. Black liquor produced in this way is rich in Na. It can be recovered using the Kraft recovery process resulting in minimum environment emission. Pulping processes without the use of NaOH are advantageous (Paul, 1995), especially when considering land disposal of the waste product since Na enhances soil dispersion (Brady, 1990). Soil erodibility increased with the addition of 2% Na in the exchange complex (Singer et al., 1982). Vegetation damage and reduced soil permeability are related to high contents of Na (Blosser and Caron, 1965; Guerri, 1973; Jorgensen, 1970).

Although many benefits could be obtained from the utilization of non-wood fibers in pulp and papermaking, the most common non-wood pulping alkaline process causes many problems, one of which is that Si interferes with alkali recovery and Si removal is costly (Rousu et al., 2002). Thus, in most cases, it makes this operation unprofitable.

Another problem is that high Na in mill byproducts limits its use in agriculture. A modified universal pulping process may offer a new approach, in which concentrated HNO_3 and NaOH are combined for reaction with the raw material (M. Jackson, Consultant, Tolovana Park, OR, personal communication, 2000). The non-wood crop straws are especially amenable to pulping with a HNO_3 system. The node of wheat straw is mechanically removed to reduce Si content before pulping. Pulping on a K basis without use of NaOH or Na_2S compounds may offer an environmentally friendly and economically viable alternative (Wong et al., 1989). H'ng (1997) found that a satisfactory pulp as well as a high value K based fertilizer could be obtained when the Na is substituted with K in Kraft pulping. Black liquor from this pulping process originates from non-Na based chemistry.

Recycling black liquor from pulping may enrich organic substances in black liquor such as polysaccharides and lignin and inorganic nutrients, especially potassium. High pH in black liquor is expected due to alkaline KOH used for pulping. As a result, black liquor may have potential as a K source and soil amendment. However, the beneficial effects on soil properties and crop growth must be demonstrated via experiments. Soil amendments must not be toxic to plants or interfere with soil microbiological metabolism (Abrosimova, 1988). Knowledge about the quality of black liquor is necessary for the black liquor to be efficiently used.

Our hypotheses were: (i) black liquor generated from KOH in place of NaOH for straw pulping may be a potassium source and soil amendment, and (ii) land use of this black liquor may have beneficial effects on soil and crop production.

Literature review

Non-wood fibers and utilization potential for papermaking

Non-wood crop straw represents an abundant, inexpensive, and renewable resource for papermaking, and has been the major available source of fibrous raw material in some countries (Oinonen and Koskivirta, 1999). For example, approximately 70 % of pulp production is obtained from non-wood raw materials in China, with nearly 40% being wheat straw based (Xin, 1998). Non-wood pulp production has doubled in South America, and has tripled in Africa and Middle East over the past 20 years (Jiménez et al., 2000). In the world about 10% of overall pulp production is obtained from non-wood raw material (Thykesson et al., 1997). In the U.S., despite large surpluses of timber materials, the environmental pressure has caused a reduction in available forest land for harvest (Pan et al., 1997). Use of non-wood fibers may help solve the fiber shortage problem and serve as an alternative to wood fibers while lessening deforestation. Wheat straw is the most promising agricultural residue for papermaking with good quality, e.g., stronger breaking length of paper hand sheets when compared to the other non-wood fibers such as olive tree fallings, sunflower stalks, vine shoots and cotton stalks (Alcaide et al., 1993).

Currently, excessive quantities of crop residues are a handling problem in some countries. Excess quantities of crop straw left on the soil surface impede planting

operations. The large quantities of straw are generally tilled into the soil, left on the soil surface in a direct seeding system, or burned before the next crop can be planted. In the middle 1990s, it was reported that 80,940 ha of wheat straw were burned each year in Washington State (McKean et al., 1995), which released 45 million kg of particulates and volatile C compounds into the atmosphere, causing serious air pollution (McKean et al., 1995). Field burning of grass seed crop residues is banned by the Washington State Department of Ecology. Therefore, the use of crop straws for pulp and papermaking may offer a disposal alternative and economical return for growers.

Understanding of the special quality of non-wood fibers is a key to using crop straws for pulp and papermaking. The specific physical and chemical characteristics of non-wood materials play an important role in the pulping technical aspects. Crop straws are considered natural pulping materials because they have similar components with wood fibers, which consist of polysaccharides (cellulose and hemicelluloses) and lignin (Xiao et al., 2001). The composition of non-wood materials varies depending on the non-wood species and the local conditions, such as soil and climates (Jacobs et al., 1999), but usually the inorganic nutrient, silica and hemicellulose contents of non-wood materials are much higher than that of wood fibers (Hurter, 1988). Pan et al. (1999) studied the wheat straw composition and of those factors influencing straw chemistry and morphology. They found great variation in the fiber length of wheat straw. Wheat straw has higher silica and non-fibrous cell content than hardwood (Misra, 1987; Utne and Hegbom, 1992; Cheng et al., 1994). The nodes of the straw have a lower average fiber length than the internodes (Zhang et al., 1990). The lower bulk density, short fiber length

and high content of Si are important physical features of non-wood materials compared to wood materials (Oinonen and Koskivirta, 1999).

Despite the many benefits, utilization of crop straws for papermaking has some problems, one of which is their low bulk density, generally restricting the production rate of the pulp mills. Improved material handling technology may solve this problem. For example, the bulk density of reed canary grass may be increased from 60 to 80 to 115 to 165 kg m^{-3} by using cylindrical presses, and to 190 kg m^{-3} by applying square baling presses (Rousu et al., 2002). Another major problem is the difficulty in recovery of chemicals due to high Si dissolved in black liquor during delignification process. Si recovery from alkaline liquor increases cost, which makes installation unprofitable for small non-wood mills (Rousu et al., 2002). This problem may be solved by removal of leaves and non-fibrous Si-rich materials to reduce Si content in black liquor, or by omitting the recovery process. However, this insufficient recovery and consequent loss of dissolved organic material can lead to serious environmental loads on receiving waters (Rousu et al., 2002). Therefore, land use of black liquor as soil amendment that omits the recovery process may offer a cost-effective and environmentally friendly disposal alternative.

Non-wood fiber pulping processes

Pulping is one of five basic steps involved in papermaking including barking, bleaching, washing and final paper and paper product processing (Ali and Sreekrshnan, 2001). The objective of pulping is to separate lignin and hemicelluloses from the raw material (Mimms et al., 1989) and to produce a cellulose rich pulp (Ali and Srekrishnan, 2001). Pulping can be carried out by several different methods including mechanical,

semi-chemical, and chemical (e.g., Kraft, sulfite pulping). Non-wood fibers are usually pulped by chemical pulping processes, which utilize chemicals to break down the fibers in the presence of heat and pressure.

The Kraft process is one example of chemical pulping processes, which uses $(Na_2)_2SO_4$ (Rydholom, 1965), NaOH and Na_2S (Mimms et al., 1989). Sulfite pulping process, another example of chemical pulping process, uses SO_2, and an oxide of Ca, Mg, Na, or NH_4 (Rydholm, 1965). Ammonium sulfite pulping is considered an alternative to the Kraft process (Akhtar and Young, 1998). Na is considered a concern in water or land disposal of the pulping byproducts. Pulping processes that do not use NaOH, are advantageous (Paul, 1995), especially when considering land disposal of the waste product since Na enhances soil dispersion (Brady, 1990).

Many alternative pulping methods including organic solvents, such as acids, or alcohol as cooking chemicals have been proposed to solve the problems involved in non-wood fiber pulping technology and to satisfy the increasing strict environmental regulations (Rousu et al., 2002). The universal pulping process was proposed (Jackson, 2000, personal communication), in which HNO_3 and NaOH are combined for reaction with the raw material. The non-wood crop straws are especially amenable to pulping with HNO_3 systems. Pulping by substituting NaOH with KOH may offer an environmentally friendly and economically viable alternative (Wong et al., 1989). Black liquor generated in this process should contain relatively high K and low Na concentrations that may be potentially beneficial K source and soil amendment. H'ng (1997) found that a satisfactory pulp as well as a high value K fertilizer could be obtained when the NaOH is substituted with KOH in Kraft pulping.

Environmental concerns

Large quantities of waste effluents including black liquor are produced during papermaking, which creates substantial disposal issues and environmental concerns for the pulp and paper industry. It is reported that on average, one ton of paper production produces 10 – 50 m^3 waste effluent depending on the nature of the raw material, finished product and extent of water use (European Commission, 2001). Black liquor is the second contributor to the biological oxygen demand (BOD) and color load of effluents for papermaking. These effluents have high biological oxygen demand (BOD), chemical oxygen demand (COD), lignin and lignin derivatives, total suspended solids (TSS), color, and high pH (Hanmer, 1988, Juwarker and Subrahmanyam, 1987). They are very toxic in the environment unless treated by secondary processes. Owing to the more restrictive regulations for discharging pulping wastewater directly to waters (Hanmer, 1988; Ali and Sreekrishnan, 2001), the possibilities of the discharge of such untreated waste effluents have become severely limited. As a result, the pulp and paper industry has a growing concern regarding cost-effective methods for disposal of waste effluents. The treatment of black liquor has become an essential prerequisite prior to their discharge to receiving waters.

The color of waste effluents has not been considered an environmental concern until recently (Ali and Sreekrishnan, 2001). Lignin, lignin derivatives and polymerized tannins, are responsible for the color, and are mostly discharged from the alkaline pulping stage, bleaching and recovery stages (Bajpai et al., 1994). Discharge of colored effluents from pulp and paper mills is not only a serous aesthetic problem, but also interferes with algal and aquatic plant productivity by limiting light transmittance (Panchapakesan,

1991). The C-to-C biphenyl linkage structure of lignin or lignin derivatives renders them resistant to microbial degradation, which poses a risk of environmental pollution. Investigations have also found that lignin and its derivatives are very toxic to fish (Owens, 1991).

The Na compounds are utilized as pulping chemicals in Kraft, soda, and sulfite and semi-chemical pulping. As a result, waste effluents including black liquor could be high in both Na and dissolved solids. High contents of Na^+ can reduce water permeability, especially in clay soils, and inhibit plant root development (Blosser and Caron, 1965; Guerri, 1973; Jorgensen, 1970).

Salinity may also be an important concern in land application of waste effluents, and is one of the most important factors reducing soil quality and productivity. High soil salinity reduces crop photosynthesis capacity, which often leads to a decline in growth and yield (Letey et al., 1985; Bresler, 1987; Maas, 1990). Salinity inhibits plant growth by osmotic stress, nutrient imbalance, and specific ion toxicity (Jacoby, 1994; Gunes et al., 1996; Cornillon and Palloix, 1997). Blosser and Owens (1964) suggested that sodium adsorption ratio (SAR) of mill effluents should be less than 8 for irrigation of permeable soil, and less than 8 for soils high in clay to avoid either a severe reduction in soil permeability or the need for gypsum addition to the soil. Kannan and Oblisam (1990) also indicated that effluent irrigation might pose a potential hazard to crops as a result of higher alkalinity.

The pH of waste effluent tends to be mild to strong alkalinity depending on the chemical pulping process. The pH of waste effluent may be a concern relative to vegetation or soil (Thacker, 1986). Application of waste effluents is expected to raise or

decrease soil pH, depending on their properties. Soil pH affects availability of nutrients, controls the composition and diversity of the microbial community, and changes the equilibrium solid phase and impacts plant response. Increased acidity has been shown to decrease effective soil cation exchange capacity (Stumpe and Vlek, 1991; Hetrick and Schwab, 1990). Benefits of liming an acidified soil include reduced H^+, reduced Al and Mn toxicity, increased P and Mo solubility, and increased growth of crops. Application of high pH black liquor is expected to increase soil pH, thereby acting as a mild liming material.

Disposal approaches

The black liquor originating from the pulping processes is usually concentrated first, and then burned to recover pulping chemicals and produce energy. However, full utilization of the liquor is impossible due to huge capital investment (Grover et al., 1999). The operation of a recovery plant is not economically feasible, for smaller mills (Grover et al., 1999). On the other hand, burning process causes air pollution. Alternative disposal approaches of black liquor have been investigated. Studies have been proposed to use it directly as a potassium fertilizer and soil amendment when KOH is substituted for NaOH for pulping process. H'ng (1997) used KOH substitution for NaOH in Kraft pulping, leading to the recovery of a high-value K fertilizer. M. Jackson (Consultant, Tolovana Park, OR, personal communication, , 2000) proposed a universal pulping process, in which HNO_3 and NaOH are combined for reaction with the raw materials. The non-wood straw and agricultural crops are especially amenable to pulping with a HNO_3 system.

Lignin is a natural, complex, heterogenous, and phenylpropanoid polymer consisting of 25 to 30% of plant biomass. The composition and amount of lignin in black liquor depends on the type of pulping process (Smook, 1992). For example, lignin is converted to thio and alkali-lignin in the Kraft process and to lignosulfates in the sulfite process. White-rot fungi have been well known for biodelignification of wheat straw (Arora, 1995). Lignin is comparably resistant to microbial decomposition, and only a limited group of fungi (white-rot fungi) are able to completely decompose lignin to CO_2 (Kirk and Farrell, 1987). During biodegradation, lignin undergoes a gradual oxidative transformation process that introduces carboxyl groups in the molecule (Shevchenko and Bailey, 1996). Compared to other plant compounds, lignin degradation needs carbohydrate as a readily metabolic co-substrate because lignin does not offer a source of energy or of carbon for lignolytic organisms (Haider, 1992). Lignin is considered a major precursor of soil humic substances (Hatakka et al., 2000). Reductions in lignocellulosic materials during transformation in soil or during composting are known to generate humic substances, which are derived from various fractions having lignin as the main core (Burges et al., 1963). Lopez et al. (2002) found that the humification ratio was correlated with lignin degradation. Tuomela et al. (2001) found that 12 to 14% of the radiolabeled lignin (^{14}C-DHP) was bound to humic and fulvic acids and 30 to 39% to humin by the end of the composting experiment. Therefore, lignin contained in black liquor has potential as a soil amendment. Use of lignin or lignin derivatives from black liquor as a soil amendment or slowly released N fertilizer has been reported. For example, Xie et al. (1994) found that NH_4-lignosulfonate, a soluble product of the sulfite pulping process increased contents of soil organic carbon and humic substance, leading to

improved soil aggregation and reduced soil erosion (Abrosimova, 1988). Other investigations have been carried out to convert lignin in black liquor at a high pressure and oxygen levels to be a slowly released nitrogen fertilizer with NH_4^+ compounds (Meier et al., 1994). However, this chemical process has not proved commercially successful (Mahoney, 1998).

Crop irrigation of waste paper mill effluents may be a promising alternative. Land application approach is not only relatively economical viable (Hansen et al., 1990), but also has beneficial effects on yields of many crops (Juwarkar and Subrahamanyam, 1986). Kannan and Oblisami (1990) found that increases in CO_2 evolution, pH, cation exchange capacity (CEC), organic C, and exchangeable Na content in soils irrigated with paper mill effluent over a period of 15 years in India. Howe and Wagner (1996) reported that cottonwood irrigated with paper mill waste effluent for six months did not reduce biomass. Although many beneficial effects have been reported, irrigation of waste effluents may have negative effects on crop yields and soil structure (Bernstein, 1975; Hansen et al., 1980). For example, Rajannan and Oblisami (1979) found undiluted effluent reduced the germination of rice, blackgram, and tomato, and growth as well as the vigor index of seedlings. They attributed the decreased emergence of seeds and growth of seedlings to the high presence of soluble ions and solid materials in the effluents. Their results also suggested that effluent irrigation might pose a potential hazard to crops as a result of higher alkalinity and Na accumulation. Thus, understanding of their characteristics is a key for their efficient use.

Soil quality

Soil quality is determined by the physical, chemical and biological components of the soil (Johansson et al., 1999). Use of organic byproducts from papermaking can return nutrients and organic matter to soil, which could be beneficial to soil quality. Soil structure is an important indicator of soil quality (Low, 1973; Allison, 1973; Dick, 1997). Soil microbial activities may also provide a useful indicator of soil quality (Dick, 1992). Soil microbial activity in terms of soil enzyme activity, soil respiration and microbial biomass can be used as an early and sensitive indicator of changes in soil quality (Dick and Tabatabai, 1993).

(1) Soil aggregation

The role of organic matter in the formation of soil aggregates has been well documented. Lynch and Bragg (1985) concluded that aggregate formation and stabilization are influenced by several factors including the type and amount of organic material, clay content, and iron and aluminum oxides. Three groups of organic binding agents are involved in stabilizing aggregates, namely temporary, transient and permanent binding agents (Tisdall and Oades, 1979). Temporary binding agents are plant roots and fungal hyphae (Tisdall and Oades, 1979). Tisdall and Oades (1982) suggested that temporary agents bind microaggregates into macroaggregates and are probably associated with young macroaggregates. The temporary agents persist for months or years in soil and are affected by management of the soil (Tisdall and Oades, 1979); Transient binding agents are organic materials, which are decomposed rapidly by microorganisms. The most important transient binding agents are the polysaccharides from microbial sources and those from roots in the rhizosphere. Transient binding agents are associated with

macroaggregates (Tisdall, and Oades, 1982); persistent binding agents consist of degraded, aromatic humic material associated with amorphous Fe, Al and aluminosilicates and they are associated with the microaggregates (Tisdall and Oades, 1982). Fungi are believed to be involved in binding together larger soil particles (Tisdall and Oades, 1982; Oades and Waters, 1991), whereas bacteria mainly influence stabilization of clay and silt-sized particles (Lynch and Bragg, 1985; Dorioz et al., 1993; Tisdall, 1994). The mechanical action of microorganisms in stabilizing soil aggregates is thought to be through the secretion of the cementing substances (Lynch and Bragg, 1985). The cementing substances are assumed to be microbial polysaccharides (Metzger et al., 1987) and the spread of hyphae between aggregates (Lynch and Bragg, 1985; Oades and Waters, 1991).

Macroaggregates are defined as >0.25 mm in diameter and microaggregates as <0.25 mm in diameter (Edwards and Bremner, 1967; Tisdall and Oades, 1982). The formation of aggregates appears to be hierachical in that primary particles and clay microstructures are bound into microaggregates, which, in turn, are bound into larger macroaggregates (Tisdall and Oades, 1982; Oades and Waters, 1991).

Macroaggregation depends primarily on temporary binding agents such as fine roots, saprophytic mycelium and fungi hyphae, and is considered to be sensitive to changes in soil organic matter levels induced by management (Tisdall and Oades, 1982). Microaggregates show a relatively high stability against physical disruption (Edwards and Bremner, 1967) and they have low susceptibility to changes in soil organic matter contents affected by management (Tisdall and Oades, 1982).

Soil organic matter is one of the key components of soil structural stability (Low, 1973; Allison, 1973). Decreasing soil organic matter content usually leads to the degradation of soil physical properties, especially soil structural stability. Good soil structure promotes favorable water relations, root environment, and the buildup of organic matter and reduces susceptibility to erosion (Bossuyt et al., 2001). Soils susceptible to water erosion have in common properties including low organic matter content, poor structure and weak aggregate stability (Singer, 1991).

Cultivation causes the disruption of soil aggregates and a decline in the content of organic matter in relation to native sod and pasture soils (Tisdall and Oades, 1982; Elliot, 1986; Kay, 1990). This decline is aggravated if fallow is included in the rotation where crop residues are removed (Juo and Lal, 1977). The most common approach to enhance soil aggregate stability is to apply organic residues (e.g., green manures, farmyard manures, crop residues and organic wastes) to increase the organic matter (OM) contents (Metzger and Yaron, 1987). For example, application of paper mill sludge improved soil structural stability (Chantigny et al., 1999). Ferguson (1956) observed that aggregate stability increased due to the application of black liquor. Application of lignin increases soil aggregate stability and reduces soil erosion (Abrosimova, 1988). Mahoney (1998) also showed that increased water-stable aggregation was due to addition of ammoxidized lignin.

(2) Soil microbial activity

Chemical and physical properties were historically used as measures of soil productivity, but they usually responded more slowly to changes in soil conditions than microbiological component of soils (Anderson and Domsch, 1989; Powlson, 1994).

Microorganisms are very sensitive indicators of changes in soil quality (Kennedy and Papendick, 1995). Soil enzymes play an important role in the cycling of nutrients, fertilizer use efficiency, and their measurement has often been used to indicate changes in soil quality (Dick, 1997). Dehydrogenase is present in all microorganisms, and is an accurate measure of the soil microbial activity (Taylor et al., 2002). Furthermore, β-glucosidase is involved in the degradation of cellulose in soils, and has potential for monitoring biological soil quality (Turner et al., 2002). Fungi are considered the primary source of β-glucosidase in agricultural soils (Hayano and Tubak, 1985). Their synthesis in such organisms is induced by the products of cellulose breakdown, including cellobiose, glucose, and their metabolites (Stewart and Leatherwood, 1976). Arylsulfatase, the enzyme involved in the hydrolysis of arylsulfate by cleaving O-S bonds (Spencer, 1958), is believed to be involved in mineralization of ester sulfate in soil (Tabatabai, 1994).

Soil respiration is an index for organic matter turnover (Debosz et al., 2002) and an indicator of the effect of organic waste amendments on soil microbial activity (Anderson, 1982). The metabolic quotient (qCO_2, the ratio of microbial respiration CO_2 – C rate to microbial biomass C) has been suggested a physiological characteristic of the microbial community in its environment (Anderson and Domsch, 1990; Anderson, 1994). Higher qCO_2 values indicate reduced microbial efficiency, disturbance to an ecosystem or stress on the microorganisms from steady – state conditions, or indicate a shift in the bacterial to fungi ratio in soils (Wardle and Ghani, 1995). Higher qCO_2 values also indicate stress such as low soil pH (Wolters and Jorgensen, 1991), or long-term heavy metal contamination (Flieβbach et al., 1994). Kazunori and Oba (1994) found that

bacteria are less efficient in converting substrate carbon into microbial biomass carbon than fungi, which results in a relative increase in soil respiration and an increase in the metabolic quotient (qCO_2). Disturbances including substrate addition (Ocio and Brookes, 1990) and acidification (Wolters, 1991) result in enhanced qCO_2. In contrast, other studies found that higher qCO_2 reflect increased input of easily decomposable substrates in limed soils (Persson et al., 1989; Bääth and Arnebrant, 1994). The increased substrate availability, which could favor the growth of a certain microorganism, causing changes in soil microbial community structure (Bääth et al., 1995).

Objectives

The overall objectives of this study were to assess whether pulping byproducts from crop straw pulping could be land applied as nutrient sources and soil amendments. The specific objectives were to: (i) evaluate if the lignin obtained from rice straw Kraft black liquor had beneficial effects on soil properties; (ii) examine if KOH based black liquor from bluegrass straw pulping was advantageous over NaOH based or NaOH/ KOH based black liquor in promoting soil quality and corn growth; (iii) examine if KOH based black liquor from wheat straw had beneficial effects on soil microbial activities; (vi) evaluate the aggregate binding effect of KOH based black liquor from bluegrass straw pulping; and (v) examine whether KOH based black liquor from wheat could be land applied as a K source and soil amendment.

Organization and outline of dissertation

This dissertation is organized into six chapters with chapters two through six written in the form of technical manuscripts. Chapter one as a general introduction to the research topic and literature review describes an over all background regarding the problems and research points studied in this dissertation. The second chapter describes soil effects of lignin, a major organic component of pulping black liquor. The third chapter describes soil and crop growth effects of three types of black liquor, which were produced by bluegrass straw pulping with NaOH, KOH or the mixture of NaOH and KOH. The fourth chapter describes soil microbial responses to KOH based black liquor from wheat straw pulping. The fifth chapter describes soil aggregate binding effects of KOH based black liquor from bluegrass straw pulping. The relative importance of fungi and bacteria in macro-aggregation was also discussed in this chapter. The sixth chapter describes the responses of corn to KOH based black liquor from wheat straw pulping as a K source under field conditions. Soil microbial activities and soil aggregation were also presented in this chapter.

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Chapter 2

Lignin from Rice Straw Kraft Pulping: Effects on Soil Properties

Abstract

Lignin and lignin derivatives produced in the papermaking process are an environmental concern for the pulp and paper industry. However, use of rice straw-derived lignin as a soil amendment may have potential to improve soil quality. Sulfuric acid-precipitated lignin from rice straw pulping black liquor at a rate of 3.06 and 6.12 g kg^{-1} (corresponding to 1.67 and 3.34 g C kg^{-1} soil) soil was incubated to evaluate its effects on soil properties. Soil pH decreased by 0.30 and 0.53 units, respectively for the two rates over 8 weeks of incubation, suggesting the lignin from black liquor may have potential as an acidifying agent for alkaline soils. Soil electrical conductivity (EC) only increased by 0.08 and 0.36 dS m^{-1}, indicating application of acidic lignin at these rates would not cause salinity problems. Total soil C increased by 1.46 and 3.13 g C kg^{-1}, and total soil N increased by 0.07 and 0.17 g N kg^{-1} at 8 weeks of incubation. There were no consistent changes in soil NO_3^--N and NH_4^+-N over the incubation period. Lignin improved the macro-aggregation of >2 mm size fraction. Wet micro-aggregate stability of >2mm and 0.5-0.25 mm aggregates increased at the end of incubation compared to control, suggesting lignin may offer a beneficial soil amendment for improving soil aggregation.

Key words: lignin, black liquor, soil organic matter, soil aggregation.

1. Introduction

Lignin and lignin derivatives are produced in great quantities during the pulping process in papermaking (Haider and Kladivko, 1980). Due to their resistance to microbial degradation as well as their water solubility, lignin and lignin derivatives have been an environmental concern for the pulp and paper industry. The composition and amount of lignin depend on the type of pulping process (Smook, 1992). For example, lignin is converted to thio- and alkali-lignin in the Kraft process and to lignosulfates in the sulfite process (Ali and Sreekrishnan, 2001). Lignin and its derivatives are responsible for the color of paper mill waste effluents (Bajpai et al., 1993; Bajpai and Bajpai, 1994; Ganjidoust et al., 1997). Discharge of colored effluents from pulp and paper mills is not only a serous aesthetic problem, but also interferes with algal and aquatic plant productivity by limiting light transmittance (Panchapakesan, 1991). Lignin and its derivatives are very toxic to fish (Owens, 1991; Carey et al., 1993;). Environmental protection regulations are limiting the discharge of colored waste effluents into receiving waters. Consequently, the pulp and paper industry has a growing interest in alternatives for disposal of lignin and its derivative produced in pulping process for papermaking.

Generally, disposal of lignin and lignin derivatives in black liquor is through combustion, in which black liquor is concentrated, and then burned, but this method is costly, and also causes air pollution (Buzzini and Pires, 2002). Various methods also have been widely attempted to remove color by physical, chemical, biological means (Lee et al., 1978; Bhattacharya and Sarma, 1997; Ganjidoust et al., 1997; Diez et al.,

1999; Thakur et al., 2004), but none have been considered to be successful due to high costs involved and partly due to the relatively lower efficiency for color removal.

Lignin is a natural, complex, heterogenous, and phenylpropanoid polymer that comprises 25% to 30% of plant biomass. Lignin is considered the most recalcitrant fraction of plant residues (Alber et al., 1990; Ryan et al., 1990) and is a precursor of soil humic substances. Reductions in lignocellulosic materials during transformation in soil or during composting are known to generate humic acids. Lopez et al. (2002) also found that the humification ratio was correlated with lignin degradation. The amount of humic substances formed from the decomposition of a given organic residue is proportional to its initial lignin content (Melillo et al., 1982). Xie et al. (1993, 1994) found that NH_4-lignosulfonate, a soluble product of the sulfite pulping process increased contents of soil organic C and humic substance. Meier et al. (1994) found that lignin from black liquor could be converted under high pressure and oxygen with NH_4^+ compounds to a slowly released N fertilizer. However, only a limited report is available on the use of lignin from Kraft pulping process as a soil amendment.

Soil structure has been considered an important indicator of soil quality (Low, 1973; Dick, 1997). Loss of structural stability of soils is one of the most important indicators of soil degradation in semiarid areas. Macroaggregates are defined as aggregates larger than 250 μm in diameter, and microaggregates as aggregates smaller than 250 μm in diameter (Edwards and Bremner, 1967). Macroagregates larger than 2 mm consist of 20 to 250 μm microaggregates held together mainly by transient binding agents only in soils low organic C (<1%). The stability of macroaggregates larger than 2 mm is related to the growth of roots and hyphae (Tisdall and Oades, 1982). The

microbial population that develops after addition of organic matter is initially responsible for aggregation formation and stabilization (Metzger et al., 1987). Microaggregates 20 to 250 μm diameter consist largely of particles 2 to 20 μm diameter bonded together by various cements including persistent organic materials and crystalline oxides and highly disordered aluminosilicates (Tisdall and Oades, 1982). Persistent binding agents include degraded, aromatic humic material associated with amorphous Fe, Al and aluminosilicates (Greenland, 1965). Many factors such as texture, organic matter content and iron and aluminum oxides influence the structural stability of a soil (Goldberg et al., 1990). Several correlations have been established between the improvements observed when organic matter is added to soil as an aggregate stabilizing agent (Christensen, 1986). The stability of macroaggregates has been correlated with soil organic matter content (Hamblin and Davies, 1977; Douglas and Goss, 1982). Fortun et al. (1990) concluded that fulvic and humic fractions of organic matter were most important for soil structural stability.

Various waste organic materials including manures, compost and paper mill sludges have been used to improve soil quality (Ravie et al., 1986). Lignin and its derivatives have potential to increase soil humic substances, thus improving soil aggregation. Their land use may provide an alternative for disposal of lignin and its derivative produced in pulping for papermaking.

The objectives of this study were to determine if lignin obtained from rice straw Kraft pulping improves soil aggregation and soil fertility properties.

2. Materials and methods

2.1. Soil and lignin

A surface soil (0-15 cm) was taken from the Irrigated Agriculture Research and Extension Center at Washington State University at Prosser, WA. The soil (fine-silty, mixed, mesic Xeric Torripsamments) had the following characteristics: pH 6.38, organic C 6.44 g kg^{-1}, total N 0.70 g kg^{-1}, Olsen P 10 mg kg^{-1}, Olsen K 163 mg kg^{-1}, and soil water holding capacity 160 mL kg^{-1}.

The rice straw-derived lignin was precipitated from Kraft pulping black liquor. Briefly, rice straw was pulped with NaOH according to Kraft method (Mimms et al., 1989), pulping black liquor was separated from the treated rice straw by draining after cook, and then black liquor was acidified to a pH of 2-3 by adding diluted H_2SO_4, supernatant solution was siphoned, and then precipitated mixture of lignin, polysaccharides and inorganic nutrients (referred as lignin) were obtained (Sun and Tomkinson, 2001). Selected chemical characteristics of lignin are shown in Table 1.

2.2. Experimental setup

The precipitated lignin derived from Kraft pulping of rice straw was oven dried at 60°C for 2 days and then ground to pass through a 0.5 mm sieve. Air-dried soil was passed through a 2-mm sieve and 250 g samples were mixed thoroughly with lignin at the rate of 0, 3.06, 6.12 g kg^{-1} soil (equivalent to 0, 1.67 and 3.34 g C kg^{-1} soil). The treated soils were incubated in a 350 mL plastic containers without cover in a constant temperature chamber at 24 °C for 8 weeks. The experimental design was a randomized block design with 4 replicates for each treatment. Water was added to keep the soil moisture content at 70% of the field water holding capacity every 2 days over the

incubation period. The treated soils were destructively sampled at 0, 2, 4, 6, and 8 weeks of incubation for determination of total C, N, pH and electrical conductivity (EC), and destructively sampled at 2, 4, 6, and 8 weeks of incubation for the determination of NH_4^+-N, NO_3^--N, aggregate size distribution and wet aggregate stability.

2.3. Lignin and soil sample analysis

Total organic C and N concentrations of lignin and soil were determined with a dry combustion (LECO, CNS2000, St. Joseph, MI). Organic N in lignin was calculated by subtraction of inorganic N (NH_4^+-N + NO_3^-- N) from total N.

The apparent total soil C losses over the incubation period were calculated based on the following equation:

C losses (%)= [Total C loss in treated soil (g kg^{-1}) /total C added (g kg^{-1})] x100, where:

Total C loss in the treated soil (g kg^{-1}) = Initial soil C content– soil C content after incubation (g kg^{-1}).

The pH and electrical conductivity (EC) of lignin were measured on a 1:5 ratio (lignin : water) with a pH meter (211/digital pH meter, Orion Research Inc., Boston Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH), respectively (Jansen, 1993). A 0.5 g sample of lignin was digested using a HNO_3-H_2O_2 method (Jones and Case, 1990), and the digestion solution was determined for Na, K, Ca, Mg, and P concentrations by inductively coupled agron plasma spectrometer 61 model (Thermo Jarrell Ash, Franklin, MA). The moisture of lignin was determined by oven drying at 85°C. A soil-saturated paste was equilibrated for one hour for the determination of pH by an Orion Research model digital pH meter and electrical

conductivity (EC) by digital EC meter (Jansen, 1993). Soil NH_4^+-N and NO_3^--N contents were determined in 1 M KCl extracts (1:10 soil : extractant) and measured colorimetrically with a Technicon flow-injection auto-analyzer (Lachat Quickchem. FIA 8000 series, Lachat Instruments, Milwaukee, WI).

Soil aggregate size distribution was measured on a mechanical flat sieve shaker (Moodle TX-86, Serial 10457, Mentor OH) with a 2-minute shaking according to method of Kemper and Rosenau (1986). A 150 g soil sample was sieved into the following size fractions: >2 mm, 2 to 1 mm, 1 to 0.5 mm , 0.5 to 0.25 mm, 0.25 to 0.15 mm, 0.15 to 0.053 mm, and < 0.053 mm, and the weight of each fraction was recorded and reported as a fraction of the total sample weight. Water stable soil aggregation was determined according to the method of Kemper and Rosenau (1986) with modification (Kennedy, personal communication, 2000) using the size fractions: >2 mm, 2 to 1 mm, 1 to 0.5 mm, 0.5 to 0.25 mm obtained from the mechanical flat sieve shaker. A 5 g subsample of each size fraction was weighed into separate aggregate holders (PVC pipes, approximately 6.3 cm in diameter and 3.8 cm in height with cloth attached at one end). The aggregate holders were exposed to 1 cm tension of water (negative potential), allowed to equilibrate for at least 3 hours, transferred to 250 mL beakers using approximately 50 ml water, and placed on an orbital shaker set at 60 rpm for 10 minutes. The contents of the beakers were poured through a 250 μm mesh size sieve and then through a 53 μm sieve. A large plastic weigh boat was placed under the sieves to catch the soil and water material passing through the 53 μm sieve, called the fines fraction. Soil remaining on the 250 μm and 53 μm sieves was removed with water, transferred to a large plastic weighing boat, dried at 85 °C, weighed, and reported as a fraction of the sample weight for each

aggregate size evaluated. Macroaggregate stability was defined as the percent of water-treated aggregates that remained on a 250 μm sieve after 1 cm of tension water treatment and the microaggregate stability as the percent remaining on a 53 μm sieve (Kemper and Rosenau, 1986).

2.4. Statistical analysis

Analyses of variance (ANOVA) were conducted on every measured parameter (SAS Institute, 2002). We found that there were interactions between treatments and sampling times for all measured parameters, therefore, separate analyses were carried out for each measured parameter at each sampling time. Mean separation was performed at the 0.05 significant levels using Bonferroni-Dunn test statistics.

3. Results and discussion

3.1. Soil pH and electrical conductivity (EC)

Lignin additions caused significant reductions in soil pH over the incubation period (Fig. 1a), attributable to the residual H_2SO_4 used in lignin precipition (pH 3.2). Soil pH decreased with increasing lignin application rates ($p \leq 0.05$), and this effect lasted for the duration of experiment. For example, compared to non-amended control, addition of lignin at a rate of 1.67 and 3.34 g C kg^{-1} resulted in immediate reduction of soil pH by 0.24 and 0.45 units, respectively, within the first day of incubation. These pH effects were sustained, with corresponding reduction of soil pH by 0.30 and 0.53 units by 8 weeks of incubation. Soil pH tended to decrease with incubation time (Fig. 1a), and this continuous decrease of soil pH in amended soils over incubation period may be partly related to the nitrification of NH_4^+-N to NO_3^{-1}-N (Fig. 3c) by microbial organisms (Heterick and Schwab, 1990; Rode and Runge, 1991) and partly related to the

decomposition of organic materials in treated soils. Reductions in soil pH with addition of lignin suggest that acidic lignin obtained from black liquor may have potential as an acidifying agent for alkaline soils.

There was a consistent increase in soil electrical conductivity (EC) with increasing rates of applied lignin (Fig. 1b). The ions in the lignin were likely responsible for this increase. There was a general trend that soil EC increased with incubation period as soil pH decreased. Highest electrical conductivity level (1.46 dS m^{-1}) with addition of lignin at a rate of 3.34 g C kg^{-1} was less than 4 dS m^{-1}, suggesting that application of lignin at these rates would not cause soil salinity concerns.

3.2. Total soil C and N

Total soil C increased with increasing lignin rates, and this trend maintained during the entire incubation (Fig. 2a). Lignin addition at rates of 3.06 and 6.12 g lignin kg^{-1} soil increased total soil C by 1.67 to 3.44 g C kg^{-1} soil over unamended control at 0 day of incubation, and 1.46 and 3.13 g C kg^{-1} soil by 8 weeks of incubation, respectively. The decrease in soil C in all treatments over incubation period (Fig. 2a) was likely attributed to soil respiration.

The most soil C losses occurred during the first 2 weeks of incubation for both lignin application rates, then from 4 to 8 weeks of incubation, but with slower rates (Table 2). For example, addition of lignin at 1.67 g C kg^{-1} resulted in about 12.0% of C lost by 2 weeks of incubation and to an additional 4.8% lost between 2 and 8 weeks. Similarly, addition of lignin at 3.34 g C kg^{-1} resulted in 9.9% of C losses by 2 weeks of incubation, and an additional 4.8% of C loss between 2 and 8 weeks of incubation (Table 2). These trends could be attributed to labile carbohydrates such as polysaccharides

contained in the amendment used, which stimulated the microbial activity during the first 2 weeks of incubation, with the slower decomposition during the 2 to 8 weeks of incubation due to more recalcitrant lignin (Berg et al., 1984; Melillo et al., 1989).

Total soil N increased with increasing lignin application rates (Fig. 2b). For example, compared to the non-amended control, the total N of soils receiving 1.67 and 3.34 g lignin derived C kg^{-1} soil was increased by 0.10 g kg^{-1} and 0.18 g kg^{-1} soil respectively within the first day of incubation. The corresponding total N was increased by 0.07 g kg^{-1} and 0.17 g kg^{-1}, respectively, by 8 weeks of incubation. There was a trend for soil N content to decrease over the incubation period (Fig. 2b), suggesting there were N losses during the incubation. Gaseous NH_3 volatilization was not likely responsible for the total N losses in our study, because the addition of lignin reduced soil pH by 0.1 – 0.6 units, in the range of soil pH of 5.4 to 5.7 during 8 weeks of incubation. Nelson (1982) found a positive relationship between NH_3-N loss and pH. In our study, treated soils were unsaturated with soil moisture being 70% of water holding capacity. Under these conditions, nitrification likely predominated, but denitrification could also have occurred (Wolf and Russow, 2000). Both nitrification and denitrification can produce N_2O and NO as well as N_2 emissions (Nelson, 1982), which may be a contributing factor for N losses in our study.

3.3. Nitrogen mineralization

Soil NO_3^--N increased significantly with increasing lignin rates at 6 weeks of incubation, although no significant differences in NO_3^--N were noted at 2, 4 and 8 weeks (Fig. 3a),. There were significant differences in soil $NH4^+$-N concentrations among treated soil only at 2 weeks of incubation (Fig. 3b).

Several reports showed that a lignin derivative, lignosulphonate (LS), from sulfite pulping processing, high in phenyl groups (Sarkanen and Ludwig 1971), inhibited or stimulated microbial activity depending on the amounts of lignin used (Meier et al., 1993; Xie et al., 1993). Low amounts of lignosulfonate stimulated nitrification (Meier et al., 1993; Xie et al., 1993). In our experiment, lignin obtained from the Kraft pulping process, containing phenyl groups, may have stimulated nitrification at the low application rates (Fig. 3a).

3.4. Soil aggregation

Addition of lignin significantly increased aggregates > 2 mm at 6 and 8 weeks of incubation (Fig. 4a), but at the expense of microaggregates from 0.25 to 0.15 mm (Fig. 4b). Significant reductions were noted in the wet macro-aggregate stability of >2mm aggregates (Fig. 5a), and significant increases in the wet micro-aggregate stability of >2mm aggregates ere formed (Fig. 5b).

Microbial polysaccharides produced by various organic materials added to soil are an important transient binding agents (Tisdall and Oades, 1982). Fungi have been recognized as major microbial groups responsible for lignin degradation and main dominate microorganisms in soil macro-aggregation (Singh and Singh, 1996). In our study, the increased macro-aggregates in size of >2 mm of macro-aggregates at 8 weeks of incubation may be partly due to the growth of fungal hyphae. The fungal hyphae are considered a temporary binding agents (Tisdall and Oades, 1979), which may explain the lower stability of macroaggregates. The addition of lignin may increase the content of aromatic humic substances such as fulvic and humic substances (Xie et al., 1994), which

may also be responsible for the increased wet microaggregate stability of > 2 mm aggregates after 8 weeks of incubation.

4. Conclusions

Addition of acidified lignin from Kraft rice straw pulping black liquor at a rate of 1.67 and 3.34 g lignin derived C kg^{-1} soil resulted in significant increases in soil total C and N and soil macroaggregation and wet soil micro-aggregate during 8 weeks of incubation, and also caused a significant reduction in soil pH over 8 weeks of incubation, suggesting this waste material may have potential as an acidifying agent for alkaline soils, but can exacerbate soil acidity of acid soils. Increased soil electrical conductivity over 8 weeks of incubation also was due to the inorganic nutrients contained in acidified lignin, but within low levels, indicating that application of acidic lignin at these rates would have no soil salinity concern. The results suggest that acidified lignin precipitated from rice straw pulping black liquor could be a beneficial soil amendment for improving organic matter and soil aggregation.

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Table 1. Selected properties of precipitated lignin (n = 3, on a wet basis)

Parameter	Concentrations
pH	3.24
EC (ds m^{-1})	19.8
Moisture (%)	85.4
NH_4^+- N (mg kg^{-1})	350
NO_3^- - N (mg kg^{-1})	1.70
Organic N (g kg^{-1})	4.51
Total N (g kg^{-1})	4.86
Total C (g kg^{-1})	79.5
C/N	16.3
Total S (g kg^{-1})	3.09
Na (mg kg^{-1})	640
K (mg kg^{-1})	131
Ca (mg kg^{-1})	246
Mg (mg kg^{-1})	33.0
P (mg kg^{-1})	99.0

Table 2. Percentage of apparent C loss from lignin derived C

Lignin rate ($g\ kg^{-1}$)	Incubation weeks			
	2	4	6	8
	%			
3.06	12.0	14.6	15.6	16.8
6.12	11.5	12.8	13.8	14.7

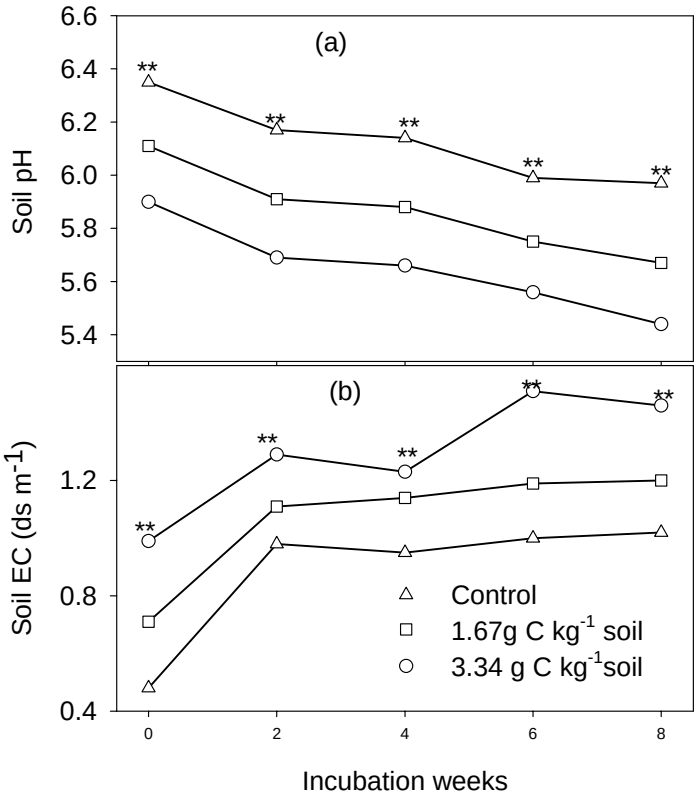

Fig. 1. (a) Soil pH; and (b) electrical conductivity (EC) as influenced by lignin C derived rates. **: significant differences at $p \leq 0.01$ among treatments at each sampling date.

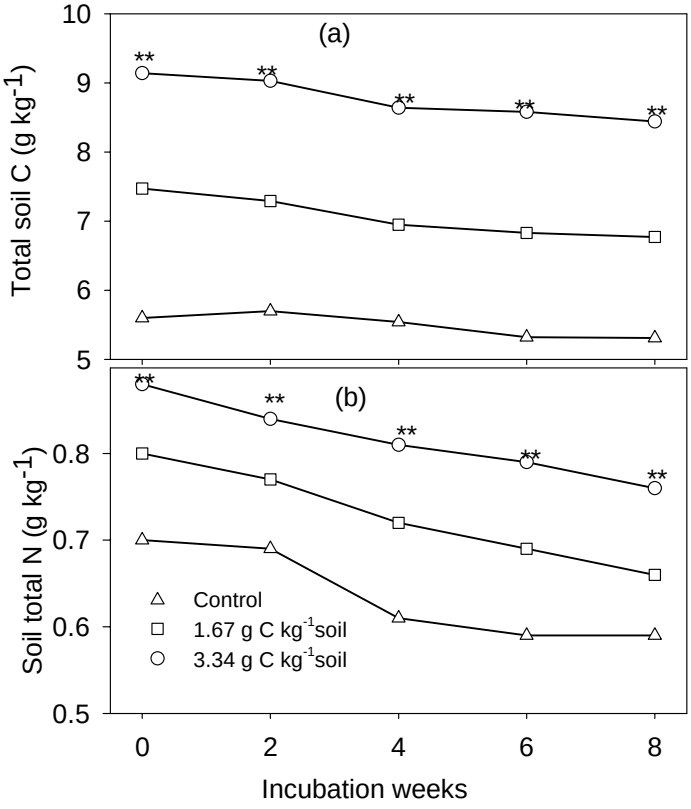

Fig. 2. (a) Total soil C; and (b) soil total N as influenced by lignin derived C rates . **: significant differences $p \leq 0.01$ among treatments at each sampling date.

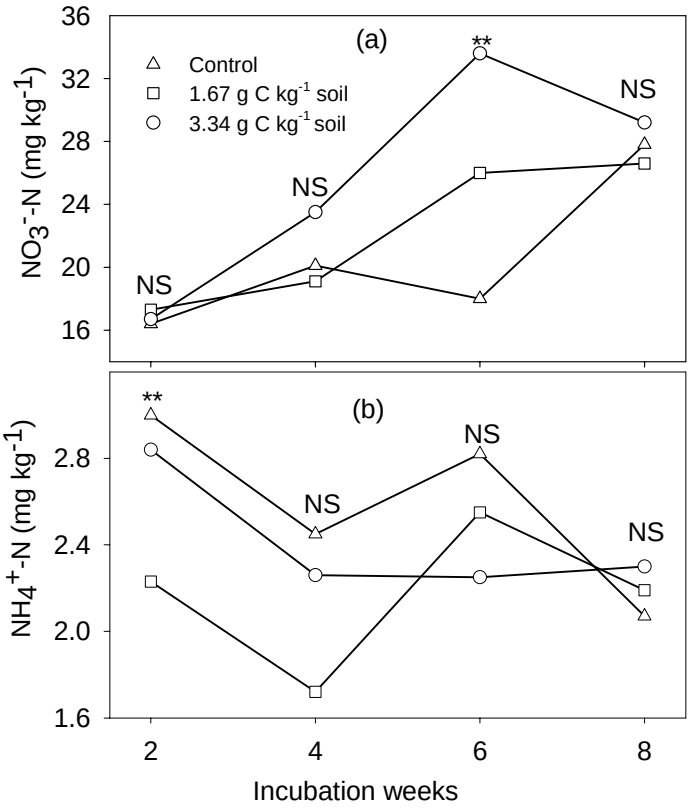

Fig. 3. (a) Soil NO_3^- - N; and (b) soil NH_4^+-N as influnced by lignin derived C rates. NS: no significant differences among treatments at each sampling date, and **: significant differences at $p \leq 0.01$.

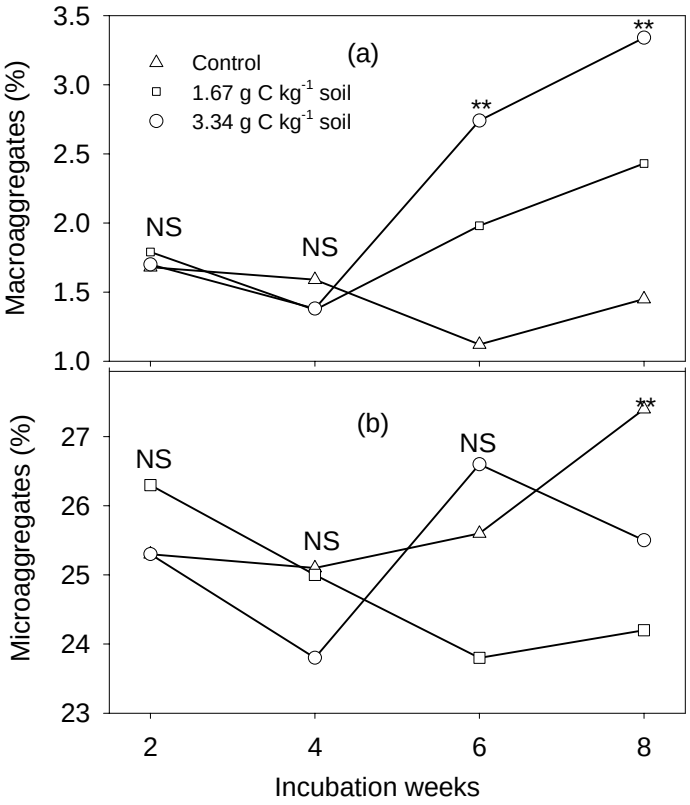

Fig. 4. (a) Macroaggregates > 2 mm; and (b) microaggregates 0.25 to 0.15 mm as influenced by lignin derived C rates. NS: no significant differences among treatments at each sampling date, and **: significatn differences at $p \leq 0.01$.

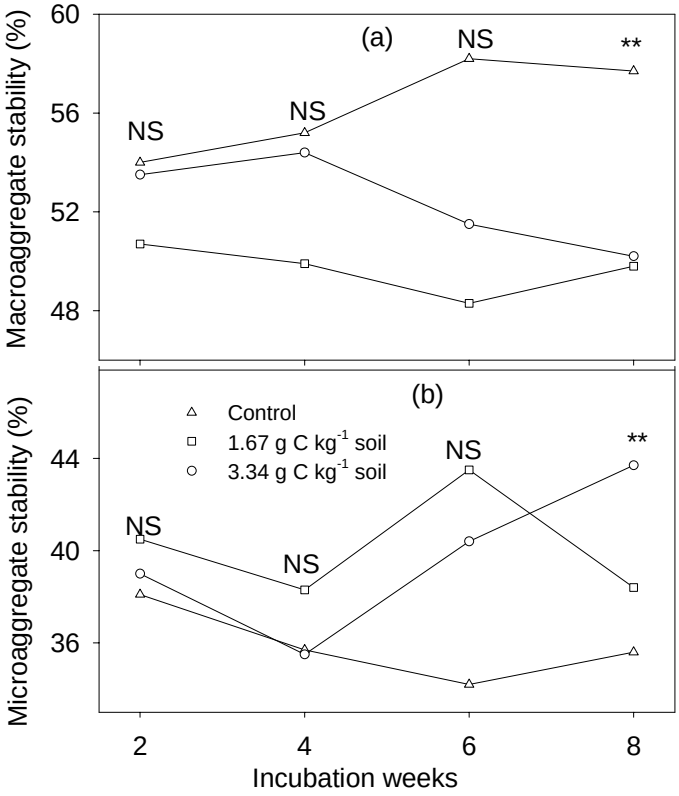

Fig. 5. (a) Wet macroaggregate stabilty; and (b) wet microaggregate stability of >2 mm macroaggregate as influenced by lignin derived C rates. NS: no significant differences among treatments at each sampling date, and ** significant differences at $p \leq 0.01$.

Chapter 3

Effects of Black Liquor on Growth of Corn and Soil Properties

Abstract

Soil applications of black liquor from sodium based pulping decreased soil quality. Substituting sodium hydroxide with potassium hydroxide should generate black liquor that may be land applied as a potassium source and beneficial soil amendment. A growth chamber experiment examined whether black liquor from bluegrass pulping with 100% KOH (KOH based) could be an effective K source, and had beneficial effects over other two black liquors from bluegrass straw pulping with 50% NaOH and 50% KOH (KOH/NaOH-based), or 100% NaOH (NaOH based) on soil properties and the growth of corn (*Zea Mays L.*). There were no differences in shoot and root dry biomass and root length between both K sources, KCl vs. KOH based black liquor, but compared to non-amended control, increased corn biomass was observed in soils with addition of K at a low rate (62.5 mg K kg^{-1}) only from KOH based black liquor, suggesting that other nutrients rather than K contained in black liquor were beneficial to corn growth. No differences were observed in corn biomass among three black liquors, KOH based, KOH/NaOH based or NaOH based applied at 5 to 20 mL kg^{-1}. Corn dry biomass decreased with increasing NaOH based black liquor rates, which may be related to high Na additions. Soil pH increased by 0.1 to 0.2 units, and soil electrical conductivity (EC) increased by 0.07 to 0.17 dS m^{-1}, indicating that these three black liquors may have potential as a mild fluid liming material, and would not cause a soil salinity concern. Soil

exchangeable sodium percentage (ESP) increased only with NaOH or KOH/NaOH based black liquor rates, suggesting that Na-based black liquor potentially increased soil ESP to detrimental levels to soil physical conditions.

Key words: black liquor, potassium hydroxide, sodium hydroxide pulping, soil properties, corn.

1. Introduction

Black liquor generated by the pulp and paper industry has been considered an important pollution source due to its high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), color and alkaline nature (Hanmer, 1988, Juwarker and Subrahmanyam, 1987; Grover et al., 1999). Due to more stringent regulations on the quality of effluent entering receiving waters, the pulp and paper industry has an interest in finding cost-effective and environmentally friendly alternatives for disposal.

Black liquor contains organic substances such as polysaccharides and lignin, beneficial inorganic nutrients such as K, Ca, Mg, Zn and Cu etc., and relatively low or undetectable concentration of heavy metals (U.S. EPA 1982). Land use of it as soil amendment may improve soil fertility and crop production. However, salinity may also be an important concern in land application of black liquor due to its high electrical conductivity (EC) level. High soil salinity reduces crop photosynthesis capacity, which often leads to a decline in growth and yield (Letey et al., 1985; Bresler, 1987; Maas, 1990). Salinity inhibits plant growth by osmotic stress, nutrient imbalance, and specific ion toxicity (Jacoby, 1994; Gunes et al., 1996; Cornillon and Palloix, 1997). The alkaline nature of black liquor may be another concern relative to vegetation or soil (Thacker,

1986). It has been known that soil pH affects availability of nutrients, controls the composition and diversity of the microbial community, and changes the equilibrium solid phase and impacts plant response. Alkaline Na compounds are utilized in pulping process, producing black liquor containing Na. Na is also an important concern in land application of waste black liquor because Na can lead to reduced permeability. Substituting NaOH with KOH or using less NaOH should generate black liquor high in K and low in Na concentrations that may be land applied as a beneficial soil amendment. However, beneficial effects on soils and crop gro wth should be demonstrated for efficient use of this waste product.

The objectives of this study were to determine if: (i) black liquor generated from bluegrass straw pulping with KOH had beneficial effects on corn (*Zea Mays* L.) growth over KCl, (ii) substitution of K for Na in alkaline pulping improves soil and crop responses, and (iii) KOH based black liquor is an effective K source.

2. Materials and methods

2.1. Soil and three types of black liquor

A growth chamber experiment was conducted on a soil (fine-silty, mixed, mesic Xeric Torripsamments) collected from the Washington State University Experiment Station at Othello, WA. The soil had pH 7.5; electrical conductivity (EC) 0.26 dS m^{-1}; organic C 6.44 g kg^{-1}; total N 0.70 g kg^{-1}; Olsen K 125 mg kg^{-1}; cation exchange capacity (CEC) 15.1 cmol kg^{-1}, and water holding capacity 160 mL kg^{-1} soil.

Three types of black liquor were generated according to the modified Universal Pulping process (M. Jackson, Consultant, Tolovana Park, OR, personal communication, 2001). Briefly, alkaline substances, NaOH and KOH were separately used for pulping at

three proportions: (i) 100% NaOH, (ii) 100% KOH, and (iii) 50% NaOH and 50% KOH. Bluegrass straw was mixed with three proportions of NaOH to KOH, concentrated HNO_3, alum, ($Al_2(SO_4)_3.14H_2O$), and water at a ratio of 100 : 10 : 1 : 0.1 : 600 on a weight basis. The mixtures were cooked at ambient pressure and 90 ˚C for 1 hour. After cooling, black liquors were separated by draining from pulped bluegrass. The black liquors generated by 100% KOH, 50% NaOH and 50% KOH, or 100% KOH were referred to as KOH, NaOH/KOH, or NaOH based black liquors, respectively. Their chemical characteristics are shown in Table 1.

2.2. Experimental setup

The experiment design was a randomized complete block with 13 treatments and four replicates. The 13 treatments consisted of a non-amended control, and 4 amendments: KCl, KOH based, KOH/NaOH based, and NaOH based black liquor, and each amendment had 3 application rates (Table 2). Both K sources, KCl or KOH based black liquor were applied at 62.5, 125, 250 mg kg^{-1} (corresponding to 140, 280, 560 kg K ha^{-1}, assuming soil with a bulk density of 1.19 g cm^{-3} and with a depth of 15 cm). The corresponding application rates of KOH based black liquor were calculated as 5, 10, and 20 mL kg^{-1} soil based on its K concentration. The NaOH/KOH and NaOH based black liquors were applied at the same rates as those used for KOH based black liquor (5, 10 and 20 mL kg^{-1}), which had similar total N additions as KOH based black liquor, but had different total K, Na or C (Table 2). Nitrogen applied at 100 mg N kg^{-1} soil as $CO(NH_2)_2$ and phosphorus at 50 mg P kg^{-1} as $Ca(H_2PO_4)_2.H_2O$ were added to each treatment. Soil was passed through a 2-mm sieve and 3 kg of soil were treated with the above 13 treatments. The treated soils were manually mixed thoroughly in plastic bags, and put in

pots lined with plastic bags to prevent nutrient losses.

All pots were placed into a growth chamber under 16 hours of light with light intensity of 570 μmol photons m^{-2} s^{-1} per day. Six corn (*zea mays* L.) seeds were sown into each pot and thinned to 3 seedlings per pot after emergence of corn. Distilled water was added to maintain field water holding capacity each day during the period of experiment. After 3 weeks of corn growth, the corn shoots were harvested and oven-dried for the determination of dry biomass. The corn roots were carefully removed and washed from the soil for the determination of root length. After measurement of corn root length, corn roots were oven dried for the determination of dry root biomass. Soil subsamples were taken after the root removal for the determination soil pH, electrical conductivity (EC), sodium exchangeable percentage (ESP), and available soil K. The dried corn shoot was ground to pass a 20-mesh screen for the determination of K, Na, Ca, and Mg concentrations.

2.3. Soil, black liquor and plant sample analysis

Corn root length was determined by the method as described as Pan et al. (1991). The pH and electrical conductivity (EC) of black liquor and soil were determined by a pH meter (211/digital pH meter, Orion Research Inc., Boston, Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH), respectively (Jansen, 1993). The total residue, total suspended residue and total dissolved residue of black liquor were determined by the methods of Richard et al. (1984). A 5 mL subsample of black liquor was oven-dried at 85 °C for 24 hours for determination of solid content. A 2.0 mL subsample of black liquor and corn shoot subsamples of 0.5 g were digested using a HNO_3-H_2O_2 method (Jones and Case, 1990). The digestion solution was

analyzed for K, Ca, Mg, Si, Mn, Cu, Zn, and Cr concentrations by inductively coupled argon plasma spectrometer 61 (Thermo Jarrell Ash, Franklin, MA). Total C and N concentrations were measured by a Leco analyzer (LECO, CNS2000, St. Joseph, MI). Subsamples of 1 mL of black liquor were diluted to 200 times by 1 M KCl for the determination of NH_4^+-N and NO_3^--N concentrations by a flow-injection autoanalyzer (Technicon Industrial Systems, Tarrytown, NY). Soil exchangeable cations Na, K, Ca and Mg and cation exchange capacity was measured by the method of Hendershot and Lalande (2000). Exchangeable Na and cation exchange capacity were used to calculate soil exchangeable sodium percentage (ESP).

2.4. Statistical analysis

Data were analyzed using a SAS statistical package (SAS Institute, 2002). One way analysis of variance (ANVOA) was conducted to compare the means of different treatments. Treatment effects were considered significant using the least significance difference at the 0.05 significance level. Contrasts were used to compare one treatment to a group of treatments, or between grouped treatments. Contrasts for linear KCl applied at 62.5 to 250 mg K kg^{-1}, or KOH, KOH/NaOH and NaOH based black liquors applied at 5, 10, 20 mL kg^{-1} were conducted to detect the effects of increasing application rates.

3. Results and discussion

3.1. Characteristics of three types of black liquor

Three types of black liquor were strongly alkaline with a value of pH ranging from 10.0 to 12.1, with NaOH based black liquor being the highest pH of 12 (Table 2). Electrical conductivity (EC) ranged from 23.5 to 26.7 dS m^{-1}, indicative of high soluble salts in black liquor, which may represent a salinity concern when considering land use.

The K and Na supplied by the pulping chemicals and other inorganic nutrients such as Ca, Mg, Zn, Mo, Cu, Mn and Fe released from the bluegrass straw during the pulping process contributed to the soluble salts in the black liquors.

Substituting NaOH with KOH for pulping reduced Na and increased K concentrations in black liquor (Table 2). H'ng (1997) found that a satisfactory pulp as well as a high value potassium based fertilizer could be obtained when the sodium is substituted by potassium in Kraft pulping.

In addition to beneficial inorganic macro- and micronutrients, the black liquors contained organic C, which was mostly derived from polysaccharides and lignin in black liquor (Fukuzumi et al., 1980; Ali and Sreekrishnan, 2001; Puértolas et al., 2001), suggesting that black liquor may have potential as a soil amendment. Alum, ($Al_2(SO_4)_3.14H_2O$) was used for pulping. As a result, Al was found in these black liquors with concentrations ranging from 21 to 62 mg L^{-1}. Heavy metals such as Cr and V (Table 1) in three black liquors were undectable or very low compared to sewage sludges (Camberato et al 1997). Therefore, heavy metals should not be a concern when black liquor is land applied.

3.2. Corn shoot, root biomass and root length

No differences were observed in corn biomass (shoot, root or total shoot and root dry weight) in soils receiving KCl at low rate of 62.5 mg K kg^{-1} compared to non-amended control. However, addition of KOH based black liquor at the same K rate increased shoot or total biomass ($p \leq 0.10$), and increased corn biomass was observed in soils treated with three types of black liquor at the low rate of 5 mL kg^{-1} (Table 3), suggesting that corn growth had no responses to K supply, but the other nutrients

contained in black liquor were beneficial to corn growth.

No differences were observed in corn dry biomass between both K sources, KCl vs. KOH based black liquor, or among the three black liquors, KOH based vs. KOH/NaOH based, or vs. NaOH based (Table 3). There was a negative linear relationship in corn shoot or total biomass existed between KOH based black liquor application rates ($p \leq 0.05$), which we cannot explain based on this experiment. No significant linear relationship was found in KOH/NaOH based black application rates from 5mL to 20 mL kg^{-1} soil in corn biomass. Linear contrasts showed that decreased weight of corn shoot, and shoot and root were observed in soils treated with increasing NaOH based black liquor rates (Table 3), which may be related to its high Na additions of 47 to 188 mg kg^{-1} soil (Table1). High concentrations of Na in paper mill effluent inhibited crop root development (Jorgensen, 1970). Forest damage could be seen with high sodium addition of Kraft pulping waste effluent from papermaking for irrigation (Blosser and Caron, 1965; Guerri, 1973).

No differences were observed in corn root length among any treatments, grouped treatments, or linear contrasts (Table 3).

3.3. Corn shoot nutrient concentrations

Amendments significantly decreased corn shoot Ca and Mg and increased shoot K and Na concentrations over non-amended control (Table 4). Contrasts showed that there were no significant differences in shoot Ca, Mg, K and Na concentrations between both K sources, KCl vs. KOH based black liquor. No consistent effects on corn shoot Ca, Mg, K and Na concentrations were observed among three black liquors, KOH based vs. KOH/NaOH based, or vs. NaOH based.

Linear contrasts showed that shoot Ca and Mg concentrations generally decreased with increasing KCl, KOH based, KOH/NaOH based or NaOH based black liquor application rates, which proportionally increased K and Na additions to soil (Table 2). This may be explained by the fact there are antagonism relationships between K^+ or Na^+ and Ca^{2+} or Mg^{2+}. Linear contrasts showed that shoot K concentrations increased with KCl, KOH based, KOH/NaOH based or NaOH based black liquor. Increased shoot Na concentrations were observed only in soils applied with KOH/NaOH based and NaOH based black liquor (Table 4).

3.4. Soil pH, electrical conductivity (EC), exchangeable sodium percentage, and available soil K

Amendments had significant effects on soil pH, electrical conductivity (EC), sodium exchangeable percentage (ESP) and available soil K (Table 5). Soil pH ranged from 7.7 to 8.1, with only 0.1 to 0.2 unit increases due to application of these three black liquors, suggesting that black liquors applied at these rates offered a mild liming material. Contrasts showed that there were significant differences in soil pH between both K sources. KCl vs. KOH based, and three black liquors, KOH based vs KOH/NaOH based, or NaOH. Linear contrast showed that soil pH increased with increasing application rates of three black liquors.

KCl resulted in the maximum electrical conductivity (EC) of 1.42 dS m^{-1} among amendments (Table 5). Soil EC ranged from 0.43 to 0.53 dS m^{-1} in soils treated with three black liquors, with only 0.07 to 0.17 dS m^{-1} increases over non-amended control, suggesting that these three black liquors applied at these rates would not cause a salinity problem. It has been reported that no detrimental effects on crop growth were found in

soils with EC levels below 2 dS m^{-1} (Jansen, 1993). Linear contrasts showed no differences in EC among four amendments except for KCl application rates.

Soil amendments had significant effects on soil exchangeable sodium percentage (ESP) ranging from 0.37 to 2.25% (Table 5). Contrasts showed that significant differences were observed in ESP between both K sources, KOH based black liquor vs. KCl, or among three black liquors, KOH based vs. KOH/NaOH based, or vs. NaOH based. NaOH based black liquor resulted in the maximum ESP levels among these three black liquors applied at 5 to 20 mL kg^{-1} with an average of ESP of 1.40%, followed by KOH/NaOH based with an average ESP of 0.76%, and KOH based had the least EC level of 0.58% on average. Contrasts for linear application rates showed that soil ESP increased with increasing application rates of KOH/NaOH based and NaOH based black liquors, but no such relationships were observed in KOH based black liquor. Soil ESP is a criterion for evaluating whether soil sodium is high enough to affect soil physical condition. When ESP is less than 15%, it will not generally cause detrimental impacts on soil physical conditions or the growth of crops (Hawkes et al., 1985). Thus, these three black liquors applied at 5 to 20 mL kg^{-1} soil would not have detrimental impacts on soil physical conditions, but repeated use of NaOH, or KOH/NaOH based liquor or use of them at higher rates may have potential to increase ESP to detrimental levels (>15%).

Amendments had significant effects on available soil K (Table 5). KCl and KOH based black liquor resulted in the maximum available soil K compared to non-amended control. Contrasts showed no significant differences in available soil K between KCl vs. KOH based black liquor, suggesting that KOH based black liquor could be an effective K source. Linear contrasts found available soil K increased with increasing KCl, KOH

based, KOH/NaOH based or NaOH based black liquor.

4. Conclusions

Addition of KOH based black liquor at a low rate (62.5 mg K kg^{-1}) increased corn biomass, but applied KCl at this rate did not result in significant differences in corn biomass compared to non-amended control, suggest that other nutrients contained in KOH based black liquor were beneficial to corn growth. Corn had no responses to K additions from KCl or KOH based black liquor, which may be related to high initial available soil K level. Linear contrasts showed that corn shoot weight decreased with only NaOH based black liquor application rates, suggesting that high sodium additions may inhibit corn growth. These three black liquors increased soil pH by 0.1 to 0.2 units and soil electrical conductivity by 0.07 to 0.17 units, when applied 5 to 20 mL kg^{-1}, suggesting that they have a potential mild fluid liming material and would have no soil salinity concern. Soil exchangeable sodium percentage (ESP) ranged from 0.46 to 2.25% in soil, and increased with application rates of KOH/NaOH based, or NaOH based black liquor only, suggesting that Na-based black liquor potentially increased soil ESP to hazardous levels to soil physical conditions. No differences were observed in available soil K between both K sources, KOH based black liquor KCl, suggesting that KOH based black liquor could be an effective K source.

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Table 1. Selected characteristics of three types of black liquor (n = 3)

Parameters	KOH BL[§]	KOH/NaOH BL	NaOH BL
pH	10	11	12
EC (ds/m)	25	24	27
Total residue (g L^{-1})	75	72	91
Total suspended residue (g L^{-1})	11	6.1	8.4
Total dissolved residue (g L^{-1})	59	61	77
Total C (g L^{-1})	20	20	25
Total N (g L^{-1})	2.2	2.2	2.4
K (g L^{-1})	12.5	7.8	4.3
Na (g L^{-1})	0.02	4.07	9.4
NH_4^+-N (mg L^{-1})	75	80	92
NO_3^--N (mg L^{-1})	451	465	432
Ca (mg L^{-1})	184	145	158
Mg (mg L^{-1})	66	47	55
P (mg L^{-1})	240	208	225
Fe (mg L^{-1})	52	18	51
Mn (mg L^{-1})	4.8	3.50	4.63
Cu (mg L^{-1})	5.0	5.0	0.70
Mo (mg L^{-1})	Undetectable	Undetectable	Undetectable
Zn (mg L^{-1})	1.3	1.0	1.6
Al (mg L^{-1})	51	21	62
Cr (mg L^{-1})	Undetectable	Undetectable	0.44
V (mg L^{-1})	Undetectable	Undetectable	0.12

§: BL: black liquor

Table 2. Treatment combinations and total K, Na, C and N additions in each treatment

Treatment ID	Treatment	BL rates (mL kg^{-1})	K	Na	C	N
			---------- mg kg –1 soil --------			
1	Control	-	0.0	0.0	0.0	0.0
2	KCl (R1)	-	62.5	0.0	0.0	0.0
3	KCl (R2)	-	125	0.0	0.0	0.0
4	KCl (R3)	-	250	0.0	0.0	0.0
5	KOH BL (R1)	5.0	62.5	0.1	100	11
6	KOH BL (R2)	10.0	125	0.2	200	22
7	KOH BL (R3)	20.0	250	0.4	400	44
8	KOH/NaOH BL (R1)	5.0	38.8	20.4	100	11
9	KOH/NaOH BL (R2)	10.0	77.5	40.8	200	22
10	KOH/NaOH BL (R3)	20.0	155	81.6	400	44
11	NaOH BL (R1)	5.0	21.6	47	125	12
12	NaOH BL (R2)	10.0	43.2	94	250	24
13	NaOH BL (R3)	20.0	86.3	188	500	48

§: BL: black liquor; R1, 2, 3: application rates; KCl (R1, R2 and R3): 62.5, 125 and 250 mg K kg^{-1} soil; three BL (R1, R2 and R3): 5, 10 and 20 mL kg^{-1} soil, corresponding to 11 to 44 mg N kg^{-1} soil.

Table 3. Corn growth as affected by the application of KCl or black liquors[£]

Treatment	Shoot	Root	Shoot + root	Root length
	---------- g pot^{-1} ---------			- m pot^{-1} -
Control	7.2	1.7	8.8	136
KCl (R1)	7.3	1.9	9.2	147
KCl (R2)	6.7	1.7	8.4	136
KCl (R3)	6.7	1.6	8.3	127
KOH BL (R1)	8.1	2.0	10.1	149
KOH BL (R2)	7.3	1.6	8.9	134
KOH BL (R3)	7.0	1.5	8.5	128
KOH/NaOH BL (R1)	7.7	2.0	9.7	148
KOH/NaOH BL (R2)	7.3	1.8	9.1	137
KOH/NaOH BL (R3)	7.2	1.7	8.9	127
NaOH BL (R1)	8.3	1.9	10.2	153
NaOH BL (R2)	6.6	1.5	8.1	130
NaOH BL (R3)	6.6	1.5	8.1	119
---------- Contrast --------	---------- p values ----------			
Control vs. KCl (R1)	NS	NS	NS	NS
Control vs. KOH BL (R1)	0.09	NS	0.08	NS
Control vs. three BLs (R1)	0.048	0.086	0.036	NS
KCl vs. KOH BL	NS	NS	NS	NS
KOH based BL vs. KOH/NaOH BL	NS	NS	NS	NS
KOH BL vs. NaOH BL	NS	NS	NS	NS
KOH/NaOH BL vs. NaOH BL	NS	NS	NS	NS
Linear KCl rates	NS	NS	NS	NS
Linear KOH BL rates	0.065	0.094	0.047	NS
Linear KOH/NaOH BL rates	NS	NS	NS	NS
Linear NaOH BL rates	0.012	0.091	0.012	NS

§: BL: black liquor; R1, 2, 3: application rates; KCl (R1, R2 and R3): 62.5, 125 and 250 mg K kg^{-1} soil; three BL (R1, R2 and R3): 5, 10 and 20 mL kg^{-1} soil, corresponding to 11 to 44 mg N kg^{-1} soil.

[£] Means with the same letter are not significant differences ($p \leq 0.05$).

Table 4. Shoot Ca, Mg, K and Na concentrations as affected by the application of KCl or black liquors[£]

Treatment	Ca	Mg	K	Na
	---------- g kg $^{-1}$ ----------			-- mg kg^{-1}
Control	6.87 a	3.70 a	32.0 h	17.0 cde
KCl (R1)	6.32 bcd	3.02 bc	36.2 fg	18.3 cde
KCl (R2)	6.37 abc	2.67 def	42.1 abc	16.6 cde
KCl (R3)	6.62 ab	2.50 efg	43.3 abc	15.3 de
KOH BL (R1)	5.72 ef	2.85 cd	37.5 ef	13.7 e
KOH BL (R2)	5.62 cde	2.70 de	45.0 ab	14.4 de
KOH BL (R3)	5.32 ef	2.25 g	45.6 a	17.0 de
KOH/NaOH BL (R1)	5.77 e	3.12 b	37.9 edf	18.3 cd
KOH/NaOH BL (R2)	5.62 de	2.77 cd	41.6 bcd	18.7 ede
KOH/NaOH BL (R3)	5.20 fg	2.42 fg	45.7 a	23.7 b
NaOH BL (R1)	5.80 ef	3.25 b	33.0 gh	19.2 c
NaOH BL (R2)	5.75 e	3.15 b	40.3 cde	21.2 bc
NaOH BL (R3)	5.20 g	2.80 cd	44.2 abc	35.6 a
---------- Contrast -------	---------- p values ----------			
KCl vs. KOH BL	NS	NS	NS	NS
KOH BL vs. KOH/NaOH BL	NS	0.022	NS	< 0.0001
KOH BL vs. NaOH BL	NS	< 0.0001	0.003	< 0.001
KOH/NaOH BL vs. NaOH BL	NS	0.0003	0.028	0.015
Linear KCl rates	NS	0.0004	0.0024	NS
Linear KOH BL rates	0.003	< 0.0001	0.0007	NS
Linear KOH/NaOH BL rates	0.025	< 0.0001	0.0003	0.022
Linear NaOH BL rates	0.0089	0.0007	< 0.0001	< 0.0001

BL: black liquor; R1, 2, 3: application rates; KCl (R1, R2 and R3): 62.5, 125 and 250 mg K kg^{-1} soil; three BL (R1, R2 and R3): 5, 10 and 20 mL kg^{-1} soil, corresponding to 11 to 44 mg N kg^{-1} soil.

[£] Means with the same letter are not significant differences ($p \leq 0.05$).

§: BL: black liquor; R1, 2, 3: application rates; KCl (R1, R2 and R3): 62.5, 125 and 250 mg K kg^{-1} soil; three BL (R1, R2 and R3): 5, 10 and 20 mL kg^{-1} soil, corresponding to 11 to 44 mg N kg^{-1} soil.

[£] Means with the same letter are not significant differences ($p \leq 0.05$).

Table 5. Selected soil properties as affected by application of black liquors and KCl[£]

Treatment	pH	EC ($dS\ m^{-1}$)	ESP (%)	Available soil K ($mg\ kg^{-1}$)
Control	7.7 e	0.36 f	0.44 gh	95 fg
KCl (R1)	7.7 e	0.56 c	0.44 gh	107 efg
KCl (R2)	7.6 f	0.86 b	0.49 fg	131 cd
KCl (R3)	7.6 g	1.42 a	0.37 h	190 a
KOH BL (R1)	7.8 d	0.43 def	0.53 fg	105 efg
KOH BL (R2)	7.8 d	0.44 def	0.53 fg	138 c
KOH BL (R3)	7.8 cd	0.44 def	0.46 gh	200 a
KOH/NaOH BL (R1)	7.8 d	0.44 def	0.59 ef	97 efg
KOH/NaOH BL (R2)	7.8 cd	0.47 cdef	0.68 ed	108.ef
KOH/NaOH BL (R3)	7.9 bc	0.41 ef	1.00 c	165 b
NaOH BL (R1)	7.8 d	0.44 def	0.76 d	89 g
NaOH BL (R2)	7.9 b	0.48 cde	1.19 b	97 fg
NaOH BL (R3)	8.1 a	0.53 cd	2.25 a	115 de
---------- Contrast ---------	---------- p values ----------			
KCl vs. KOH BL	< 0.0001	< 0.0001	0.011	NS
KOH BL vs. KOH/NaOH BL	0.03	NS	< 0.0001	< 0.0001
KOH BL vs. NaOH BL	< 0.0001	NS	< 0.0001	< 0.0001
KOH/NaOH BL vs. NaOH BL	< 0.0001	NS	< 0.0001	< 0.0001
Linear KCl rates	< 0.001	< 0.0001	NS	< 0.0001
Linear KOH BL rates	0.001	NS	NS	< 0.0001
Linear KOH/NaOH BL rates	0.009	NS	< 0.0001	< 0.0001
Linear NaOH BL rates	< 0.001	NS	< 0.0001	0.0052

§: BL: black liquor; R1, 2, 3: application rates; KCl (R1, R2 and R3): 62.5, 125 and 250 mg K kg^{-1} soil; three BL (R1, R2 and R3): 5, 10 and 20 mL kg^{-1} soil, corresponding to 11 to 44 mg N kg^{-1} soil.

[£] Means with the same letter are not significant differences ($p \leq 0.05$).

Chapter 4

Soil Microbial Responses to Potassium-Based Black Liquor From Straw Pulping

Abstract

Sodium-based black liquor from fiber pulping for papermaking creates challenging waste disposal issues. By substituting potassium hydroxide for sodium hydroxide in the pulping process, the resulting black liquors may be land applied as an environmentally beneficial disposal alternative. Incubation studies examined the effect of potassium hydroxide based black liquor on soil pH, electrical conductivity (EC), and microbial biomass, CO_2 evolution and soil enzyme activities in a sandy soil. Amended soils with black liquor at rates up to 67.2 mL kg^{-1} (corresponding to 1,200 kg K ha^{-1}) soil were incubated at 24 °C for 60 days. Increasing application rates increased soil pH, indicating that black liquor has potential as a mild liming material. Soil EC increased with black liquor application rates, but only up to 1.04 dS m^{-1}, suggesting that black liquor application at these rates would not cause a salinity problem. The CO_2 evolution rate peaked at 2 days of incubation, and then gradually declined with decreasing differences among the amended soils until the end of incubation. Metabolic quotient significantly increased with increasing application rates of black liquor. Microbial biomass, microbial respiration, dehydrogenase, β-glucosidase and arylsulfatase activities generally increased with increasing application rates throughout the incubation duration. In contrast, increased soil pH by addition of KOH alone resulted in decreased soil respiration and soil enzymatic activities, indicating that black liquor stimulation of soil

microbial activity is attributable to organic compounds and inorganic nutrients contained in black liquor rather than its high pH.

Key wordS: Soil, microbial biomass, soil respiration, black liquor, and enzyme activity.

1. Introduction

Black liquor is an environmental concern for the papermaking industry due to its high levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solid, and lignin and their derivatives (Juwarkar and Subrahmenyam, 1987; Hanmer, 1988). The discharge of black liquor into surface waters is not only a serious aesthetic problem, but decreased penetration of solar radiation reduces algal and aquatic plant productivity (Ali and Sreekrishnan, 2001). Presently, strict environmental protection regulations and higher public awareness have limited the discharge of untreated black liquor (Ali and Sreekrishnan, 2001). As the pulp and paper industry begins to adapt crop straw into its feedstock stream to supplement wood-based fiber, the development of straw pulping processes should account for these environmental concerns.

A current common disposal approach is through a chemical recovery process where black liquor is concentrated, burned for producing energy, and recycled for recovering inorganic chemicals (Grover et al., 1999). However, this disposal approach is not cost effective nor environmentally friendly (Girovich, 1996). Typically, NaOH is used in straw pulping, producing Na-based black liquor. Land application of this liquor raises concern for deterioration of soil structure (Balba, 1995). Reducing Na content of

black liquor by substituting NaOH with KOH may render black liquor that can be used as a K fertilizer and soil amendment, while offering a cost-effective and environmentally acceptable disposal alternative. However, other effects on soil quality parameters are less well defined, but the alkalinity and soluble organic matter in the black liquor could be beneficial.

The objectives of our study were to: (i) determine the temporal impacts of KOH based black liquor on soil biological and chemical indicators of soil quality, and (ii) distinguish the causal factors of these impacts by the black liquor between pH modifications vs. other factors.

2. Materials and Methods

Three soil incubation experiments were conducted to address the objectives stated above. The soil (fine-silty, mixed, mesic Xeric Torripsamments) was collected from the Washington State University experiment station in Othello, WA. Chemical characteristics were as follows: pH 7.5; electrical conductivity (EC) 0.26 dS m^{-1}; organic C 6.4 g kg^{-1}; total N 0.70 g kg^{-1}; $NaHCO_3$ extractable K 125 mg kg^{-1}; cation exchange capacity (CEC) 15 cmol kg^{-1}; and water holding capacity 14.7 mL 100^{-1} g.

The black liquor was obtained by pulping wheat straw with KOH according to the Universal Pulping (M. Jackson, Tolovana, Park, OR, personal communication, 2000). Briefly, wheat straw was mixed with KOH, concentrated HNO_3, and alum at a ratio of 10:1, 100:1, and 1000:1 on a weight basis, respectively, and water was added to keep the ratio of water to straw of 10:1. The mixture was cooked at ambient pressure and 90 ˚C for 1 hour. After cooling, black liquor was separated from the pulped straw by draining, and tested for the residual alkalinity based on acid titration neutralization. The separated

liquor was reused as part of the cooking liquor for the next cook after fortification with KOH to the target level (ratio of straw: KOH 10: 1). Black liquor was recycled eight times. General characteristics of final black liquor are shown in Table 1.

Application rates were based on the K contents in black liquor. The rates ranged from the recommended K rate in Washington for field corn at the lowest rate (200 kg K ha^{-1}), to the highest rate (1,200 kg K ha^{-1}).

2.1. Experiment 1

Air-dried soil was passed through a 2-mm sieve and amended with black liquor as a K source at rates of: 0 (non-amended control), 200 (recommended K rate), 400, 800, and 1200 kg K ha^{-1}. Black liquor application rate for each treatment was calculated based on its K concentration (1.49 g K 100^{-1} mL). The corresponding volume of black liquor for treatments was 0, 11.2, 22.4, 44.8, and 67.2 mL kg^{-1} soil, respectively assuming a soil bulk density of 1.19 g cm^{-3} and a soil depth of 10 cm (1.19 x 10^6 kg soil ha^{-1}). Black liquor was thoroughly mixed with 25 g air-dried soil for each treatment. There were four replicates. Additional water was added to keep the soil moisture content at 60% of the field water holding capacity.

Samples were placed individually in sealed 1 L Mason jars, each containing two vials, one with 5 mL 1 M NaOH to trap evolved CO_2, and one with 10 mL CO_2-free water to maintain soil moisture during the incubation period. The soils were incubated in a growth chamber at 24 °C for 60 days using a randomized complete block design. The vials containing NaOH were removed periodically at days, 2, 5, 10, 20, 40, and 60 for the determination of CO_2 evolved.

2.2. Experiment 2

The same rates of black liquor used in experiment 1 were applied to 500 g air-dried soil with 4 replicates per treatment. The treated soils were incubated under the same conditions as described in experiment 1, and destructively sampled at days, 2, 5, 10, 20, 40, and 60 for the measurements of pH, EC, microbial biomass C, and soil enzyme activity.

2.3. Experiment 3

Experiment 2 demonstrated that soil microbial activity and soil pH increased with increasing black liquor application rates, raising a question of whether pH was a controlling factor of microbial activity. To separate the chemical and pH modifying effects of black liquor, KOH was added alone to soil. Addition of black liquor at a rate of 11.2 to 67.2 mL kg^{-1} soil in experiment 1 resulted in soil pH increases by 0.1 to 0.5 units over 60 days of incubation. A preliminary soil incubation found that the addition of 0.4 M KOH at rates of 10, 30, and 50 mL kg^{-1} increased soil pH to 7.7, 7.9 and 8.0, respectively, mimicking the soil pH modifications found in experiment 2.

Twenty five g of air-dried soil samples were amended with 0.40 M KOH at a rate of 0, 10, 30 and 50 mL kg^{-1} soil with 4 replicates for the determination of CO_2 evolution over 60 days of incubation under the same conditions as described in experiment 1. The same rates of KOH were applied to 200 g air-dried soils with 4 replicates under the same incubation conditions as described in experiment 2. The treated soils were destructively sampled at days of 5, 20, 40 and 60 for the determination of soil enzyme activity.

2.4. Black liquor and soil sample analysis

The pH and electrical conductivity (EC) of black liquor were determined with a pH meter model (211/digital pH meter, Orion Research Inc., Boston Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH), respectively (Jansen, 1993). Black liquor was oven-dried at 85 °C for 24 hours for determination of solid content. Polysaccharides and lignin in black liquor were determined by the method as described by Sun and Tomkinson (2001). Black liquor was digested using HNO_3-H_2O_2 (Jones and Case, 1990), and the digestion solution was analyzed by an inductively coupled agron plasma spectrometer 61 model (Thermo Jarrell Ash, Franklin, MA) for K, Ca, Mg, Si, Mn, Cu, Zn, and Cr concentrations. A 0.5-mL sample of black liquor was used for the determination of total C and N with a Leco analyzer (LECO, CNS2000, St. Joseph, MI).

A soil-saturated paste was equilibrated for one hour for the determination of pH and electrical conductivity (EC) with a pH meter model (211/digital pH meter, Orion Research Inc., Boston Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH). The CO_2 absorbed in NaOH was measured by titration (Anderson, 1982). The cumulative CO_2 amounts were expressed as μg CO_2-C g^{-1} dry soil, and CO_2 evolution rate as μg CO_2-C g^{-1} dry soil day^{-1}. The microbial biomass C was determined by the fumigation - incubation method (Horwath and Paul, 1994). Microbial biomass C was calculated based on the equation:

Biomass C (μg/g dried soil) = (Fc – UFc)/Kc, where, Fc = CO_2 flush from the fumigated sample, UFc = CO_2 produced by the unfumigated control, and Kc, coefficient = 0.41 (Anderson and Domsch, 1978).

Dehydrogenase, β-glucosidase and arylsulfatase activities were measured by the method as described as Tabatabai (1994).

2.5. Statistical analysis

Analyses of variance (ANOVA) were conducted on each parameter. There was interaction between treatments and sampling times for all measured parameters except for soil pH and electrical conductivity. Therefore, separate analyses were conducted for microbial parameters at each sampling time. Protected LSD test was performed when treatments were significant at $p \leq 0.05$ (SAS Institute, 2002) to compare the means of all different treatments within the same day and the same parameter.

3. Results and Discussion

3.1. Soil pH and electrical conductivity (EC)

Addition of alkaline black liquor (pH 10) at rates of 11.2 to 67.2 mL kg^{-1} resulted in rapid increases in soil pH by 0.1 to 0.5 pH units within 2 days and then stabilized throughout the remaining 58 days of incubation (data not shown). This result was in agreement with other studies that reported increased soil pH in soil with irrigation of alkaline paper mill effluent (Kannan and Oblisami, 1990a), and in soil amended with alkaline mill sludge (Beyer et al., 1997). Increase in soil pH with addition of black liquor indicates that black liquor has potential as a liquid liming material. Liquid liming material is advantageous over solid one because of rapid effect in raising soil pH and ease in mechanical land application, but its transportation and application costs are relatively high (Mahler, 1994).

Addition of black liquor at these rates increased soil electrical conductivity (EC) over the untreated control by 0.06 to 0.26 dS m^{-1} over 60 days of incubation (data not

shown). The high salt concentration of the black liquor (EC 27.5 dS m^{-1}) was due to the KOH used in pulping and soluble salts released from the wheat straw. Electrical conductivity is an indicator of salt concentrations in soil solution. Salinity problems with land application of organic wastes with high EC such as paper mill effluent have been an important concern. However, the low soil EC levels of 0.67 to 1.04 dS m^{-1}(data not shown) corresponding to application rates of 11.2 to 67.2 mL kg^{-1} soil black liquor would not cause a soil salinity problem in this soil. The maximum application rate caused an increase in EC of only 0.26 dS m^{-1}, which would not cause salinity problems in most soils with initial EC of $\leq$ 3 dS m^{-1}.

3.2. CO_2 evolution

Cumulative CO_2 evolution differed among the 5 treatments over the incubation period (Fig. 1a), ranging from 316 μg CO_2-C g^{-1} soil in the unamended control to 831 μg CO_2-C g^{-1} soil in the treated soil receiving 67.2 mL kg^{-1} soil. There was also a lineary increase with increasing rates of black liquor ($r^2 = 0.99$; $p < 0.01$).

CO_2 evolution rates were most rapid during the first 2 days of incubation, and treatment differences were most apparent during this initial time period (Fig. 1b). The evolution rates for all treatments drastically decreased to a low and constant level by 10 days after application where treatment differences diminished. For example, at 2 days of incubation, CO_2 evolution rate was 180 μg CO_2-C g^{-1} soil day^{-1} in the highest rate of black liquor (67.2 mL kg^{-1} soil), more than 4 times higher than that in the unamended control (40 μg CO_2-C g^{-1} soil day^{-1}). In contrast, at 60 days of incubation CO_2 rates were 3.9 and 2.6 μg CO_2-C g^{-1} soil day^{-1} in the highest application rate and unamended control, respectively. Similarly, Bardgettt et al. (1995) demonstrated that the application

of silage effluent to soils resulted in a temporary increase in microbial respiration, as measured by CO_2 evolution.

CO_2 evolution rate is an index for organic matter turnover (Debosz et al., 2002) and an indicator of the effect of organic waste amendments on soil microbial activity (Anderson, 1982). In the present study, application rates were correlated with the CO_2 evolution rate. This increase in CO_2 evolution may have been related to the supply of the easily decomposed polysaccharides (1.23 g 100^{-1} mL) (Table 1) contained in black liquor. In addition to the 138 to 827 μg soluble polysaccharides g^{-1} soil (data not shown), application of black liquor also provided other nutrients such as N, K, P, Ca, Mg, and Zn for utilization by soil microorganisms. The black liquor itself may contain microorganisms that survive the pulping process or proliferate between processing and application. The decline in CO_2 evolution in the later incubation period was probably caused by depletion of polysaccharides and or the accumulation of toxic metabolites during the incubation period (Saviozzi et al., 1993; Wong and Wong, 1986). In later stage of incubation, CO_2 evolved increased with increasing rates, suggesting that more nutrients with higher application rates may have stimulated soil microorganisms, greater utilization of recalcitrant substrates such as lignin (0.65 g 100 mL^{-1}) (Table 1) contained in black liquor.

The CO_2-C evolved from the control soil was subtracted from black liquor treatments to obtain CO_2-C evolved from black liquor rates. A similar percentage (18.3% at day 2; 39.1% at day 60) of black liquor C was evolved as CO_2-C from all application rates during the incubation (Fig. 1c). This suggests that black liquor at 11.2 to 67.2 mL kg^{-1} soil had no detrimental effect on soil microbial activity in terms of CO_2 evolution,

with a similar proportion of total added C evolved as CO_2 regardless of application rates within this range.

3.3. Microbial biomass C

Soil microbial biomass C increased with increasing application rates at 2, 5, 10 and 60 days of incubation (Table 2). In contrast, no significant differences in microbial biomass C were observed among application rates at 20 and 40 days of incubation. These results indicated that the black liquor had no detrimental effect on microbial biomass C. Studies have shown that soils amended with silage effluent and urban wastewater (Bardgett et al., 1995; Meli et al., 2002) increased soil microbial C, partly due to the addition of readily available nutrients into soil, and partly due to the input of microorganisms into the soil from the effluent. In our study, this temporal increase in microbial biomass was probably attributed to the addition of easily decomposable substrate such as soluble polysaccharides (Table 1).

The increased microbial biomass may cause temporal biological immobilization of black liquor-derived P. This immobilization and subsequent slow release of nutrients by the microbial biomass may reduce the risk of nutrient leaching (Bardegett et al., 1995).

3.4. Metabolic quotient

The metabolic quotient (specific respiration, qCO_2) is the ratio of microbial respiration CO_2 – C rate to microbial biomass C. An increase in metabolic quotient (qCO_2), as an indicator of reduced microbial efficiency (Anderson, 1994; Wardle and Ghani, 1995), can be explained as a response by soil microorganisms to disturbance to an ecosystem or environmental stress (Anderson and Domsch, 1990). In contrast, higher

qCO_2 was considered to reflect increased input of easily decomposable substrates in limed soils (Persson et al., 1989; Bääth and Arnebrant, 1994) or in fire treated soils (Fritze et al., 1994). Increased substrate availability, could favor the growth of zymogenous microorganisms, or cause other changes in soil microbial community structure (Bääth et al., 1995).

In our study, except at 60 days of incubation, the metabolic quotient increased with increasing application rates throughout the incubation period (Fig. 2). This finding is in contrast to that of other studies, which showed that application of easily available substrates to soil, such as cattle manure slurry (Kandeler and Eder, 1993) or inorganic fertilizers (Insam et al., 1991) caused a decrease in the specific metabolic quotient.

The effect of black liquor on microbial activity was complicated by the fact that it provided a source of substrate C and other nutrients, in addition to raising soil pH. In our study, a higher qCO_2 may not mean that soil microorganisms were stressed. The increase in soil pH induced by the amendments could result in a number of changes that affected the composition of the microbial community. There were no differences in the proportion of black liquor derived C evolved as CO_2 among application rates (Fig. 2c). Microbial biomass C and soil respiration rate both also increased with increasing application rates, which indicated there may be no stress on soil microorganisms (Badalucco et al., 1992). Increases in specific respiration may also reflect shifts in microbial community structure in the amended soils (Bardgett et al., 1995). Bacteria have a higher metabolic activity than fungi (Anderson and Domsch, 1975). High salinity and heavy metal contents of sludge can inhibit microbial activity (Tester and Parr, 1983; Wong and Lai, 1996). Black liquor obtained by wheat straw pulping with KOH had low

or undetectable levels of heavy metals such as Zn, and Cr (data not shown). Application of black liquor at a rate of 11.2 to 67.2 mL kg^{-1} soil resulted in low levels of electrical conductivity within a safe range. Therefore, the increasing qCO_2 values with increasing application rates in our study may be partly related to the increased soil pH in the amended soils. Soil pH affects available of nutrients and controls the composition and diversity of the microbial community (Dick et al., 2000). Sims et al. (1995) found that many of the detrimental effects to microorganisms were attributed to high pH levels in fly-ash amended soils.

3.5. Enzyme activities

Black liquor application increased dehydrogenase activity over the 60 days of incubation period (Fig. 3a). Dehydrogenases activity was the highest at the start of our incubation and decreased over time (Fig. 3a). Dehydrogenase activity was considered to be an index of total viable microorganisms in the soil (Taylor et al., 2002). Further, β-glucosidase activity was relatively stable throughout the incubation, and generally increased from 10 through 40 days of incubation (Fig. 3b). β-glucosidase, an important extracellular enzyme, is one of the three enzymes responsible for decomposition of cellulose, the main components of plant polysaccharides, and has potential as a biological indicator of soil quality (Turner al., 2002). Similar to dehydrogenase and β-glucosidase activities, arylsulfatase activity was significantly influenced by application rates of black liquor. For example, its activity increased with increasing application rates at 10, 20, 40 and 60 days of incubation (Fig. 3c), but not at 2 and 5 days of incubation. Arylsulfatase is an enzyme that catalyzes the hydrolysis of an arylsulfate anion by cleavage of the O-S

bound and is considered partly responsible for S cycling in soils and also an indicator of fungi activity in soil (Oshrain and Wiebe, 1979)..

The addition of nutrients and organic matter with application of organic wastes, stimulated microbial activities and subsequent enzyme synthesis (Sastre et al., 1996). Kannan and Oblisami (1990b) found that irrigation of paper mill waste effluent for 15 years resulted in increases in dehydrogenase, amylase and phosphatase activities, in addition to increase in soil pH. They attributed the increases in enzyme activities to addition of organic matter and nutrients. Increased microbial activities in treated soils in our study may be related to the addition of polysaccharides and nutrients, and indicated that application of black liquor had beneficial effects on soil microbial activities.

3.6. Effects of increased soil pH on soil microbial activities

Generally, increased soil pH decreased or did not affect soil respiration in terms of cumulative soil CO_2 evolution, CO_2 evolution rate, dehydrogenase, β-glucosidase and arylsulfatase activities over the 60 days of incubation (data now shown). Increased soil pH may have decreased the proliferation of microbial species already present in soil that were relatively active, or altered the diversity of the soil microbial community, thus reducing soil microbial respiration, or decreasing soil enzymatic activities. This result differed with other reports of increases in soil respiration and soil enzyme activities with pH increases (Neale et al., 1997; Zimmermann and Frey, 2002), but these studies were conducted on strongly acid soils that were adjusted to near neutral pH.

4. Conclusions

Addition of KOH based black liquor caused rapid increases in soil pH, suggesting that it may be used as a mild liming material. Black liquor significantly increased soil electrical conductivity levels, but within a safe range, indicating that KOH based black liquor at a rate of 11.2 to 67.2 mL kg^{-1} soil would not result in a salinity problem. Addition of black liquor in our study also caused significant increases in the microbial biomass and microbial activities, suggesting that addition of black liquor had beneficial effect on soil microorganisms. There was a general trend that increased soil pH resulted in decreasing soil microbial activities in terms of CO_2 evolution, and soil enzyme activities, suggesting that beneficial effects on soil microbial activities were attributable to organic constituents and nutrients contained in black liquor rather than its high pH.

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Table 1. General properties of black liquor (n = 3)

Properties	Contents	
	Concentrations (W/V)	(W/W dry solid)
pH	9.95	
Electrical Conductivity (EC)	27.4 (dS m^{-1})	
Solid content	57.9 (g L^{-1})	
Total organic C	21.2 (g L^{-1})	366 (g kg^{-1})
Polysaccharides	12.3 (g L^{-1})	212 (g kg^{-1})
Lignin	6.5 (g L^{-1})	112 (g kg^{-1})
K	14.9 (g L^{-1})	257 (g kg^{-1})
Total N	6.6 (g L^{-1})	114 (g kg^{-1})
P	62 (mg L^{-1})	1071 (mg kg^{-1})
Ca	64 (mg L^{-1})	1105 (mg kg^{-1})
Mg	22 (mg L^{-1})	380 (mg kg^{-1})
Si	116 (mg L^{-1})	2003 (mg kg^{-1})
Mn	0.28 (mg L^{-1})	4.8 (mg kg^{-1})
Cu	0.23 (mg L^{-1})	4.0 (mg kg^{-1})

Table 2. Soil microbial biomass C as influenced by black liquor (BL) application rates

BL rates (mL kg^{-1} soil)	Incubation days					
	2	5	10	20	40	60
	----------µg microbial biomass C g^{-1} dry soil----------					
0	205 c	255 c	252 d	311	264	291 c
11.2	298 b	294 b	260 d	321	280	301 bc
22.4	331 ab	324 a	306 b	291	285	293 c
44.8	307 ab	337 a	287 c	304	288	327 ab
67.2	315 a	323 a	321 a	354	297	338 a
$L.S.D._{.0.05}$	32.2	25.9	15.1	NS	NS	30.2

Different letters means significant differences among treatments at the same incubation day at $p \leq 0.05$; and NS: no significant differences.

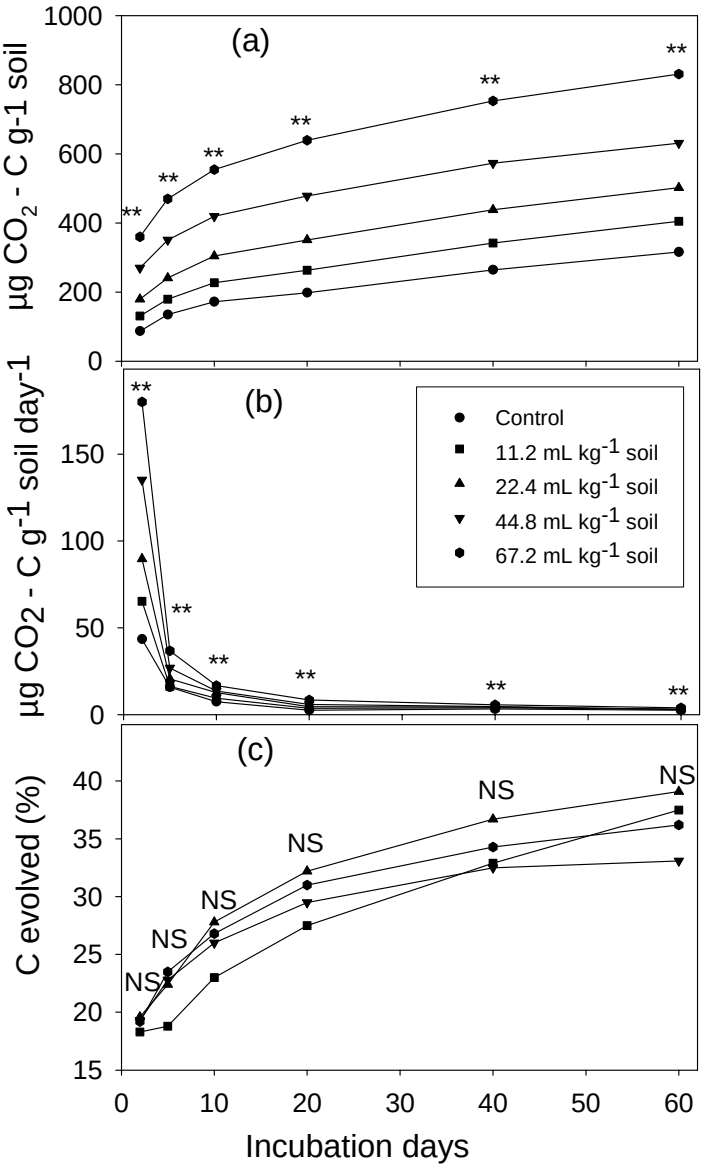

Fig. 1. (a) Cumulative CO_2 evolution, (b) CO_2 evolution rate, and (c) apparent percentage of black liquor derived C evolved as influenced by black liquor application rates. NS: no significant differences among treatments at each sampling date, and **: significant differences at $p \leq 0.01$.

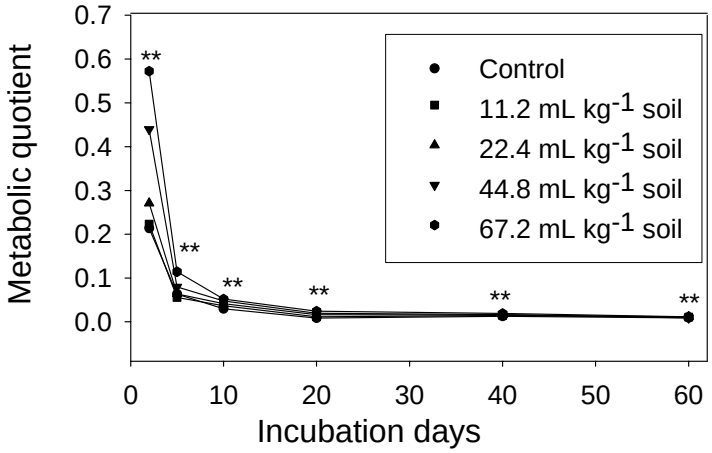

Fig. 2. Metabolic quotient (respired C rate : microbial biomass C) as influenced by black liquor application rates. **: significant differences among treatments at each samping date at $p \leq 0.01$.

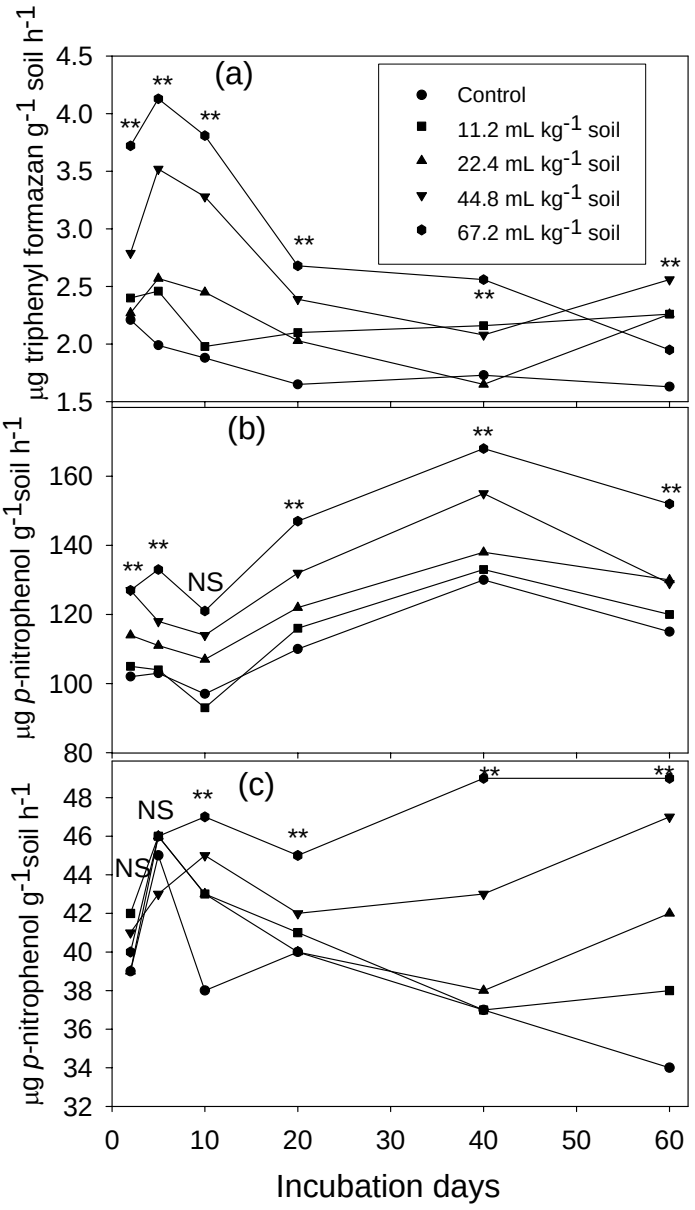

Fig. 3. (a) Dehydrogenase, (b) ß-glucosidase, and (c) arylsulfatase activities as influenced by black liquor application rates. NS: no significant differences among treatments at each sampling date, and **: significant differences at $p \leq 0.01$.

Chapter 5

Soil Aggregation Responses to Black Liquor and Fine Fiber from Bluegrass Straw Pulping

Abstract

Black liquor produced by the pulp and paper industry is an environmental problem when disposed as an industrial waste due to its high chemical oxygen demand (COD) and biological oxygen demand (BOD) levels. The major organic components, polysaccharides, lignin, phenols and lipids of black liquor are potentially important in soil aggregation. Fine fiber, another waste byproduct that is generated by the filtering process of black liquor may share similar characteristics with black liquor. Incubation studies were conducted to examine: (i) soil aggregate formation of black liquor (BL) and fine fiber (FF), (ii) the relative importance of fungi and bacteria in soil aggregate formation through selective inhibition of fungi and bacteria, and (iii) whether soil dehydrogenase and β-glucosidase activities can be used as indicators of microbial activity of BL or FF amended soils treated with biocides. Soil respiration rates peaked 2 days following the addition of BL or FF, but maximum wet stable macro-aggregation occurred after 20 days of incubation. Fungicide treated BL or FF did not result in differences in wet stable macro-aggregates compared to the non-amended control. Decreased wet stable macro-aggregates in fungicide treated BL or FF were observed compared with BL or FF alone, and bactericide treated BL or FF increased wet stable macro-aggregates compared to non-amended control or respective BL or FF, suggesting that the fungal activity was responsible for the increases in wet stable macro-aggregates. Dehydrogenase and β-

glucosidase activities did not correlate with the soil respiration in the presence of biocides (fungicide, bactericide or both) treated BL or FF, suggesting that dehydrogenase and β-glucosidase activities could not be used as indicators of soil microbial activity under these conditions. Additions of BL and FF at a rate of 1.5 g C kg^{-1} increased soil respiration, dehydrogenase and β-glucosidase activities, and also improved the wet stability of macroaggregates, suggesting that black liquor or fine fiber could be a beneficial soil amendment.

Key words: soil aggregation; soil structure; fungi hyphae; black liquor, pulping; soil respiration

1. Introduction

It is well documented that soil aggregate formation is influenced by several factors such as the type and amount of organic material, clay contents and iron and aluminum oxides (Lynch and Bragg, 1985). Three main groups of binding agents are involved in aggregation including (i) transient agents which include easily degraded organic materials by microorganisms (e.g., polysaccharides), (ii) temporary agents such as roots and fungal hyphae, and (iii) permanent binding agents which consist of degraded humic material associated with amorphous iron, aluminium and aluninosilicates (Lynch and Bragg, 1985; Oades, 1984; Tisdall and Oades, 1982).

Soil aggregation is affected by the amounts and quality of organic wastes added (Lynch and Bragg, 1985). Aggregate formation has been observed to increase with increasing amounts of organic residues added under laboratory (Roldan et al., 1994) and

field (Diaz et al., 1994) conditions. However, in other studies no such effects (Gagnon et al., 2001) or negative effects (Roldan et al., 1996) were reported.

Polysaccharides of plant and microbial origin are one of important pools of labile C for soil microorganisms and act as transient binding agents for soil aggregation (Tisdall and Oades, 1982; Murayama, 1984; Hu et al., 1995; Kay, 1998;). Polysaccharides bind soil particles (Kay, 1998; Martens, 2000) and also can form a gel-like substance acting as a glue to bind particles into aggregates (Haynes and Beare, 1997). Phenolic molecules form cationic bridges, and phenols and polyphenols are precursors of humic substances, which increase soil aggregation (Martens, 2000). Lignin is a precursor of humic substances, enhancing soil aggregation (Palm and Rowland, 1997; Magill and Aber, 1998). Mucilage and other compounds produced from degradation of lignin by fungi improve soil aggregation (Caesar-TonThat, 2002). Lipids improve soil aggregate stability due to their hydrophobic nature (Pare et al., 1999).

Black liquor (BL), a byproduct produced in the chemical pulping of wood or non-wood fibers such as bluegrass or wheat straw processing, contains organic substances such as polysaccharides, dissolved alkali-lignin, phenols and lipid products (Fukuzumi et al., 1980; Ali and Sreekrishnan, 2001; Puértolas et al., 2001, Lara et al., 2003; Navia et al., 2003) and inorganic nutrients. Therefore, land use of BL may improve soil aggregation.

Fine fiber (FF), another waste byproduct generated during the filtering process of alkaline crop straw pulping BL, contains more hemicelluloses with short fibers than wood fiber pulping (Rouse et al., 2000). Filtering pulping black liquor (BL) has a

positive influence on the ash content and the pulp and paper properties (Rousu et al., 2002).

The objectives of this study were to examine: (i) the effect of BL and FF from KOH based pulping bluegrass straw on macro-aggregate formation, (ii) the relative importance of fungi and bacteria in macro-aggregate formation, and (iii) whether soil dehydrogenase and β-glucosidase activities can be used as indicators of microbial activity of BL or FF-amended soils treated with biocides.

2. Materials and methods

2.1. Soil, black liquor and fine fiber

Soil (fine-silty, mixed, mesic Xeric Torripsamments) was collected at a depth of 0 to 15 cm from the Washington State University experiment station in Othello, WA. The soil had pH 7.5; electrical conductivity (EC) 0.26 ds m^{-1}; total C 6.4 g kg^{-1}; total N 0.69 g kg^{-1}; Olsen K 125 mg kg^{-1}; cation exchange capacity (CEC) 15 cmol kg^{-1} ; and water holding capacity 147 mL kg^{-1} soil.

BL was obtained by pulping bluegrass straw with KOH according to the modified Universal Pulping process (M. Jackson, Consultant, Tolovana Park, OR, personal communication, 2000). Briefly, bluegrass straw was mixed with KOH and water at a ratio of 10:1:100 on a weight basis. The mixture was cooked at ambient pressure and 90 ˚C for 1 hour. Eight cooks were made in which the pulping BL was separated from the treated bluegrass straw by draining after each cook. FF was obtained by filtering the separated black liquor with a 53 μm stainless steel sieve to obtain fine fiber, and the filtered BL was tested for the residual KOH based on acid titration neutralization (M. Jackson, Consultant, Tolovana Park, OR, personal communication, 2000). The separated

liquor was reused as part of the cooking liquor for the next cook after fortification with KOH to the target level (the ratio of straw : KOH 10: 1). BL was recycled eight times, and FF obtained from each cook was collected for this study. Selected characteristics of the BL and FF are shown in Table 1.

2.2. Experimental setup

Three soil incubation studies were conducted to address the objectives stated above. A randomized complete block design was employed with four replications for the three studies. Both BL and FF were applied at a rate of 1.5 g C kg^{-1} soil. A fungicide, captan, N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide and a bactericide, oxytetracycline were applied at rates of 3.0 and 1.5 g kg^{-1} soil as described by Bossuyt et al. (2001). Studies 1 and 2 consisted of nine treatments: (1) non-amended control (control), (2) black liquor (BL), (3) BL + bactericide (BL + B), (4) BL + fungicide (BL + F), (5) BL + bactericide + fungicide (BL + B + F), (6) FF, (7) FF + bactericide (FF + B), (8) FF + fungicide (FF + F), and (9) FF + fungicide + bactericide (FF + B + F).

2.2.1. Incubation study 1

This incubation was conducted to study soil respiration. Twenty-five grams of air-dried soil samples were treated with amendments, and then placed into 1.0 L Mason jars. Treated soils were incubated at 70% of field water capacity in a dark environment for 30 days. A small vial containing 5 mL of water was included to keep soil moisture. Another small glass vial containing 5 mL of 1.0 mol L^{-1} NaOH was used to trap CO_2 evolution. Trapped CO_2 was titrated with 0.5 M HCl at days 2, 5, 10, 15, 20, and 30 for determination of soil respiration.

2.2.2. Incubation study 2

This study was conducted to assess the effect of amendments on soil enzyme activities and wet stable macro-aggregates (WSM). The treated samples, 250 g air-dried soils were incubated at 70% of field water capacity under a dark environment for 30 days. Soil samples were destructively taken at days 0, 2, 5, 10, 15, 20 and 30 for the determination of wet stable macro-aggregates, dehydrogenase and β-glucosidase activities.

2.2.3. Incubation study 3

This study was conducted to investigate the relative importance of fungi and bacteria in soil macro-aggregation. Incubation studies showed that BL or FF had similar effects on soil macro-aggregation and soil respiration. Therefore, only BL was selected for this study, which consisted of 5 treatments: (1) non-amended control (control), (2) BL, (3) BL + bactericide (BL + B), (4) BL + fungicide (BL + F), and (5) BL + bactericide + fungicide (BL + B + F). The BL application rates, fungicide and bactericide in this study were the same as those used in the incubation studies 1 and 2.

Twenty five grams of treated soil sample was kept at 70% of field holding capacity, and was put into a 60 x 15 mm Petri dish, and then all treated Petri dishes were incubated in a large zip plastic bag with moisturized paper towels. Zip plastic bag was opened every 3 days for 10 minutes to maintain aerobic conditions. Each Petri dish was viewed randomly 4 times for digital image of fungi hyphae on the surface under a binocular dissecting scope (LEICA MZ 6) at 20 x magnification at 1, 2, 5, 10, 15, 20 and 30 days.

2.3. *Black liquor, fine fiber and soil analysis*

The pH and electrical conductivity (EC) of BL and FF were measured with a pH meter (211/digital pH meter, Orion Research Inc., Boston, Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH), respectively (Jansen, 1993). Five mL of black liquor and 5 g of fine fiber were oven-dried at 85 °C for 24 hours for determination of solid content. Polysaccharides and lignin of BL and FF were determined as described by Sun and Tomkinson (2001). A 10 mL sample of black liquor was neutralized with 9.68 M H_3PO_4 to a pH of 7.0, 30 mL of 95% ethanol was added to precipitate polysaccharides, and then filtered to get polysaccharides. Ethanol was evaporated with heat. After cooling, the residual filtrate was adjusted to pH 2 using 9.68 M H_3PO_4 to precipitate lignin. Two mL subsamples of BL and 1.0 g of fine fiber subsamples were digested with concentrated HNO_3 with H_2O_2 (Jones and Case, 1990). The digestion solution was analyzed for K, Ca, Mg, Si, Mn, Cu, Zn, and Cr concentrations by inductively coupled agron plasma spectrometer 61 (Thermo Jarrell Ash, Franklin, MA). A 0.5 mL sample of black liquor or a 0.5 g of fine fiber was used for the determination of total C and N by Leco analyzer (LECO, CNS2000, St. Joseph, MI).

The CO_2 absorbed in NaOH was measured by titration (Anderson, 1982). The cumulative CO_2 amounts were expressed as μg CO_2-C g^{-1} dry soil, and rates of CO_2 evolution as μg CO_2-C g^{-1} dry soil day^{-1}. Dehydrogenase and β-glucosidase activities were measured by the method as described by Tabatabai (1994).

A soil-saturated paste was equilibrated for one hour for the determination of pH with a pH meter (211/digital pH meter, Orion Research Inc., Boston Mass) and soil electrical conductivity (EC) with an EC meter (YSI Model 35, Yellow Springs

Instrument, Co., Inc., Yellow Springs, OH) (Jansen, 1993). Water stable macro-aggregates were measured on soil samples at 70% of field holding capacity by the method of Cambardella and Elliot (1993). Briefly, a 50 g of air-dried sub-sample was submersed into 250μm sieve with 3 cm depth of water in a tube for 5 minutes, and then gently manually moving the sieve up and down for 50 times during a period of 2 minutes. The remained aggregates on the sieve were dried in an oven at 105 ˚C. The water stable macro-aggregates were calculated by the following equation:

Water stable macro-aggregates (%) = 100 x [soils remaining on the 250 μm sieve (g)/Initial soils used (g)].

2.4. Measurement of length of fungal hyphae

The image of each treated Petri dish was printed, and fungal hyphae were delineated on plastic films using fine point permanent marker. The length of fungal hyphae was calculated by the method of measurement of root length in an image of area of 2 mm x 1.5 mm (3 mm^2) as described by Pan et al. (1991).

2.5.Statistical analysis

Analyses of variance (ANOVA) were conducted on each parameter. There was interaction between treatments and sampling times for all measured parameters except for soil pH and electrical conductivity (EC). Therefore, separate analyses were carried out for microbial parameters at each sampling time. Protected LSD test was performed when treatments were significant at $p \leq 0.05$ (SAS Institute, 2002) to compare the means of the same parameter in all different treatments within the same day.

3. Results and discussion

3.1. Soil wet stable macro-aggregates

BL or FF that was not treated with biocides (fungicide or bactericide or both) had similar effects in increasing wet stable macro-aggregates (e.g., from 20% at 2 days of incubation to 40% at 30 days of incubation) (Fig. 1). When treated soil samples were immediately measured for the determination of WSM, which was considered to be at 0 day of incubation, we found that slightly significant increases in WSM were observed in soils amended with BL or FF or when both biocides (bactericide or fungicide) were added with the BL or FF compared to non-amended control. It has been recognized that lipids are important binding agents due to their hydrophobic nature (Palm and Rowland, 1997; Magill and Aber, 1998; Pare et al., 1999). Increased soil aggregate stability has been reported following the addition of waste organic materials containing hydrophobic substances (Ternan et al., 1996). The major organic components lipids and phenols contained in BL or FF may chemically promote aggregation. Floating soil particles were observed in the treatments receiving BL or FF alone or combined with biocides while determining wet stable macro-aggregates. The increased wet stable macro-aggregates were also observed in soils treated with both biocides (bactericide and fungicide) compared to BL or FF, which we cannot explain based on this experiment.

Initial increases in WSM following additions of biocides disappeared after 5 days of incubation. For example, compared to the non-amended control, no significant increases in the WSM contents were observed in treated soils with the BL or FF combined with fungicide (Fig. 1), and significant reduction in WSM was found after 5 days till 30 days of incubation in the fungicide treated soils compared to the respective

BL or FF alone, indicating fungi was more responsible for the increases in WSM than bacteria, which was in contrast to the other studies (Tisdall Oases, 1982; Lynch and Bragg, 1985). Bactericide treated BL or FF, which is intended to inhibit the growth of bacteria significantly increased WSM compared to non-amended control or respective BL or FF alone, suggesting that fungi was important aggregate binding agents. These results were in agreement with those of soil treated with sludge (Metzger et al., 1987), and of soil treated with wheat straw (Bossuyt et al., 2001).

Both fungicide and bactericide treated BL or FF resulted in significant increases in WSM compared to the non-amended control or the fungicide treated BL or FF (Fig. 1). However, compared to the respective bactericide treated BL or FF, BL with both biocides only caused a significant decline in WSM. FF with both biocides had the similar effects on WSM, especially in a subsequent incubation period. These results may be explained by the facts that both biocides may be effective before 5 days of incubation, and the killed bacteria and fungi in the treated soils may provide more C sources for soil microorganisms, stimulating fungal growth, thus leading to an increased WSM.

Easily decomposed organic C has rapid effects on aggregate binding but the effects are short-lived (Kay, 1998). The aggregate response to slowly decomposing organic C is delayed but longer-lived (Martens, 2000). However, in our study, the wet stable macro-aggregates increased with incubation time, which may be related to the composition of black liquor and fine fiber. The easily labile polysaccharides contained in black liquor and fine fiber contributed to their rapid effects on aggregation, and the recalcitrant lignin contained may have led to its longer-lived binding effects.

A comparison between the treatments by analysis of variance showed that the order of soil wet stable macro-aggregates was: BL treated with both biocides ≈ FF treated with both biocides > BL treated with bactericide ≈ FF treated with bactericide > BL ≈ FF > BL treated with fungicide ≈ FF treated with fungicide ≈ non-amended control.

3.2. The length of fungal hyphae

Amendments had significant impacts on the length of fungal hyphae on the surface of treated Petri dishes (Table 2). No visible fungal hyphae were observed for non-amended control, fungicide, and both fungicide and bactericide treated BL at 1 and 2 days of incubation. In contrast, BL and bactericide treated BL resulted in significant increases in the length of fungal hyphae at these incubation times. This result provides a further evidence that fungal haphae is important aggregate binding agents. After 2 days throughout 30 days of incubation, amended soils had no consistent effects on the length of fungal hyphae on the Petri dishes, which we cannot explain based on this experiment.

Significant correlations between fungal growth and initial increase in aggregate stability have been reported in several studies (Molope et al., 1987; Bossuyt et al., 2001). Improved macro-aggregation is related to the increased fungal hyphal length and the concomitant increased extracellular polysaccharides following addition of fresh residue (Lynch and Bragg, 1985; Beare et al., 1993). Fungal hyphae improves macro-aggregate formation by binding micro-aggregates (Tisdall and Oades, 1982; Oades, 1984; Metzger et al., 1987; Tisdall, 1991). The binding effects of soil fungal hyphae were found by both biochemical analyses (Molope et al., 1987) and direct observation of scanning electron microscopy (Golchin et al., 1994). Molope et al. (1987) found that aggregate stability increases with increasing growth of fungal hyphae.

3.3. Soil respiration

Similar to the WSM, amended treatments had significant effects on cumulative CO_2 over incubation period (Fig. 2). BL or FF alone resulted in significant cumulative CO_2 evolution compared to the non-amended control (Fig. 2), which could be related to labile organic C contained in black liquor or fine fiber, providing energy sources for soil microorganisms. This suggests that additions of BL and FF at a rate of 1.5 g C kg^{-1} soil had beneficial effects on soil microorganisms. Compared to the respective BL or FF amended soils, additions of fungicide or both fungicide and bactericide resulted in significant reductions, and when applied with bactericide, significant increases in cumulative CO_2 evolution were observed over incubation period, suggesting fungi may be more effective than bacteria in utilization of C sources. The order of cumulative CO_2 evolution by comparing the means of the treatments by analysis of variance was: BL ≈ BL treated with bactericide > FF ≈ FF treated with bactericide > BL treated with both biocides ≈ FF treated with both biocides > nnon-amended control.

All treatments had also significant effects on CO_2 evolution rate (Fig. 2). Soils receiving BL, FF, or both amendments with bactericide or non-amended control had the maximum CO_2 evolution rate at 2 days of incubation, and soils receiving BL, or FF with fungicide, or with both fungicide and bactericide had the maximum CO_2 evolution rate at 5 days of incubation (Fig. 2). The largest differences in CO_2 evolution rates among treatments occurred at 2 days of incubation. These differences tended to be smaller with further incubation. These results offer another evidence that fungi are more efficient in utilization of C sources, and biocides were the most effective before 5 days of incubation.

A comparison between Fig. 1 and 2 indicates that maximum aggregation occurred some time later than maximum CO_2 evolution rate, which was in agreement with other reports (Metzger et al., 1987; Bossuyt et al., 2001). Soil respiration has been considered to be an important index for evaluation of the amount of the overall microbial activity in soils (Metzger et al., 1987). In this study, there was no significant correlation between WSM and cumulative CO_2 evolution or CO_2 evolution rate ($p > 0.05$), and the peak WSM did not coincide with the maximum CO_2 rate, suggesting that microbial activity may not directly affect soil aggregation, but the products produced by soil microorganisms promote soil aggregation.

The apparent percentage of BL or FF derived C evolved was estimated by subtracting CO_2-C evolved from non-amended control. The largest C evolution occurred in the initial phases of incubation (5 days) among treatments, reflecting that biocides had the strongest effect before 5 days of incubation (Fig. 2).

3.4. *Soil enzyme activities*

Compared to non-amended control, addition of BL or FF significantly increased dehydrogenase and β-glucosidase activities over incubation (Fig.3), which may be related to the organic C substances contained in BL or FF, stimulating microbial activities and subsequent enzyme synthesis (Sastre et al., 1996). This further indicates that BL or FF have potential as a beneficial soil amendment.

It is widely reported that soil dehydrogenase activity is considered to be an index of total viable microorganisms in the soils. β-glucosidase is one of the three enzymes responsible for decomposition of cellulose (Sparling, 1997; Turner al., 2002), and has been widely used to investigate the effects of organic amendments on soil microbial

activity (Kannen and Oblisami., 1990; Quemada and Menacho, 2001; Meli et al., 2002). Dehydrogenase and β-glucosidase activities are sensitive indicators in assessing the effect of soil treatments (Sparling, 1997) because they are involved in organic matter decomposition, and nutrient recycles, and has potential as a biological indicator of soil quality (Dick, 1997; Yakovchenko et al., 1996). Measurement of soil respiration has been recognized as a better method for reflecting overall microbial activity than the plate count method for determining the size of microbial populations (Alexander, 1977).

The addition of biocides (fungicide or bactericide or both) to BL or FF resulted in significant reductions in dehydrogenase and β-glucosidase activities through the incubation period compared to the non-amended control or respective BL or FF (Fig. 3), especially for fungicide or both fungicide and bactericide treated BL or FF. When compared to CO_2 evolution rate (Fig. 2), we found that the soil microbial activities in amended soils with biocides did not correlate with the changes of soil respiration (CO_2 evolution rate) in these treatments. Similar finding was reported by Chander and Brookes (1991), who observed a decreased dehydrogenase activity in soils contaminated with Cu-contaminated sludges but no significant differences in soil CO_2 evolution rate compared to uncontaminated control or sludges without Cu contamination. They ascribed the decreased enzyme activity to a reaction between triphenyl formazan and Cu, which interfered with the enzyme assay. Barnah and Mishra (1986) also observed higher levels of dehydrogenase activity at low doses of pesticides and vice versa. Thus, addition of fungicide or bactericide may have interfered with the color formation for dehydrogenase and β-glucosidase activity assay, which limited its use as an index for soil microbial activity.

4. Conclusions

Black liquor and fine fiber increased soil respiration, dehydrogenase and β-glucosidase activities and soil wet stable macro-aggregates, suggesting that they had potential as beneficial soil amendments. Increased macroaggregate formation occurred when BL or FF was treated with bactericide, suggesting that bacteria were not responsible for macro-aggregation. No macro-aggregate formation and a significant suppression of microbial activity were observed in the treatments receiving fungicide over incubation period compared to non-amended control, suggesting the fungi had a significant positive effect on macro-aggregation. The microscopic images offered further evidence that fungal hyphae produced were important aggregate binding agents. Dehydrogenase and β-glucosidase activies in biocides (fungicide and bactericide or both) treated BL or FF did not justify the soil respiration, suggesting that they may not be used to reflect soil microbial activities. Addition of biocides (fungicide, bactericide or both) may interfere with color formation for the dehydrogenase and β-glucosidase assay.

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Table 1. Selected characteristics of black liquor and fine fiber (n=3, on a weight basis)

Properties	Black liquor	Fine fiber
pH	10	10
Electrical conductivity (EC)	39 (dS m^{-1})	39 (dS m^{-1})
Solid content	95 (g L^{-1})	122 (g kg^{-1})
Total organic C	34 (g L^{-1})	4.56 (g 100 g^{-1})
Total N	1.1 (g L^{-1})	1.5 (g kg^{-1})
Polysaccharides	23 (g L^{-1})	49 (g kg^{-1})
Lignin	6.3 (g L^{-1})	11 (g kg^{-1})
Total K	21 (g L^{-1})	22 (g kg^{-1})
Total P	265 (mg L^{-1})	543 (mg kg^{-1})
Total Ca	63 (mg L^{-1})	654 (mg kg^{-1})
Total Mg	21 (mg L^{-1})	306 (mg kg^{-1})
Total Na	2110 (mg L^{-1})	2708 (mg kg^{-1})
Total Si	108 (mg L^{-1})	368 (mg kg^{-1})
Total Fe	4.8 (mg L^{-1})	168 (mg kg^{-1})
Total Mn	4.0 (mg L^{-1})	28 (mg kg^{-1})
Total Cu	0.7 (mg L^{-1})	1.3 (mg kg^{-1})
Total Al	2.6 (mg L^{-1})	117 (mg kg^{-1})
Total Cr	Undectable	Undectable
Total V	Undectable	Undectable

Table 2. The length of fungal hyphae as influenced by amendments over incubation days

Treatment	Incubation days 1	2	5	10	15	20	30
	Fungal hyphae length (μm mm^{-2})						
Control	0.0 c	0.0 c	45.3 b	476 b	381 b	537	695 a
BL	633 b	770 b	0.0 c	698 b	525 b	361	263 bc
BL + F	0.0 c	0.0 c	0.0 c	777 b	982 a	373	22 c
BL + B	1043 a	1261 a	605 a	639 b	477 b	680	186 c
BL + F + B	0.0 c	283 c	680 a	1357 a	1076 a	673	513 a
$L.S.D._{.0.05}$	127	288	117	407	302	NS	256
p values	<0.0001	<0.0001	<0.0001	<0.0007	<0.0001	0.074	<0.0001

Lowcase letters: significant differences among treatments ($p < 0.05$), NS: insignificant differences among treatments. BL: black liquor, BL + F: black liquor + fungicide, BL + B: black liquor + bactericide, and BL + F + B: black liquor + fungicide + bactericide.

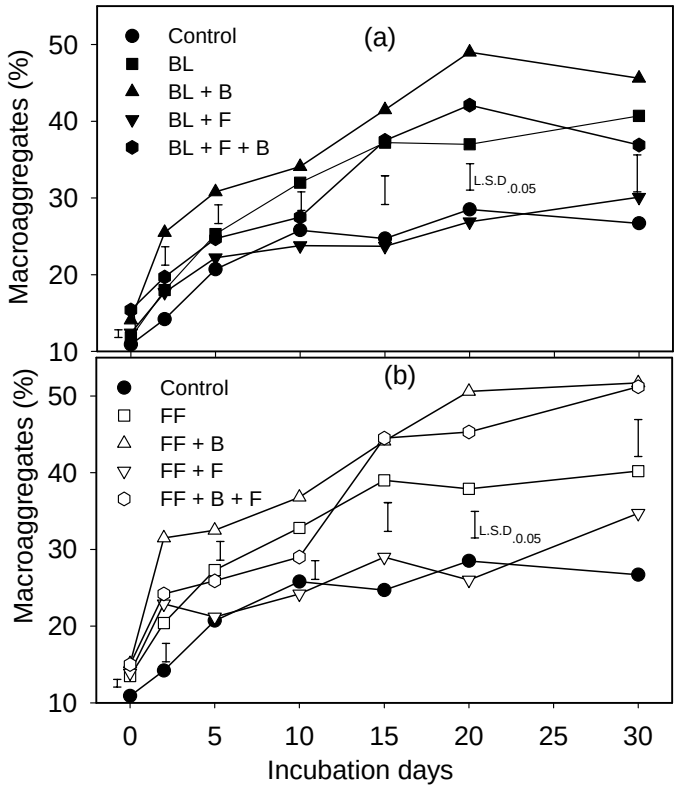

Fig. 1. (a) and (b) Wet stable macroaggregates as influenced by black liquor and fine fiber treated with biocides. I: LS.D.0.05 bars, significant differences among treatments at each incubation date at $p \leq 0.05$, BL (FF): black liquor (fine fiber), BL (FF) + B: black liquor (fine fiber) + bactericide, BL (FF) + F: black liquor (fine fiber) + fungicide, and BL (FF) + B + F: black liquor (fine fiber) + bactericide + fungicide.

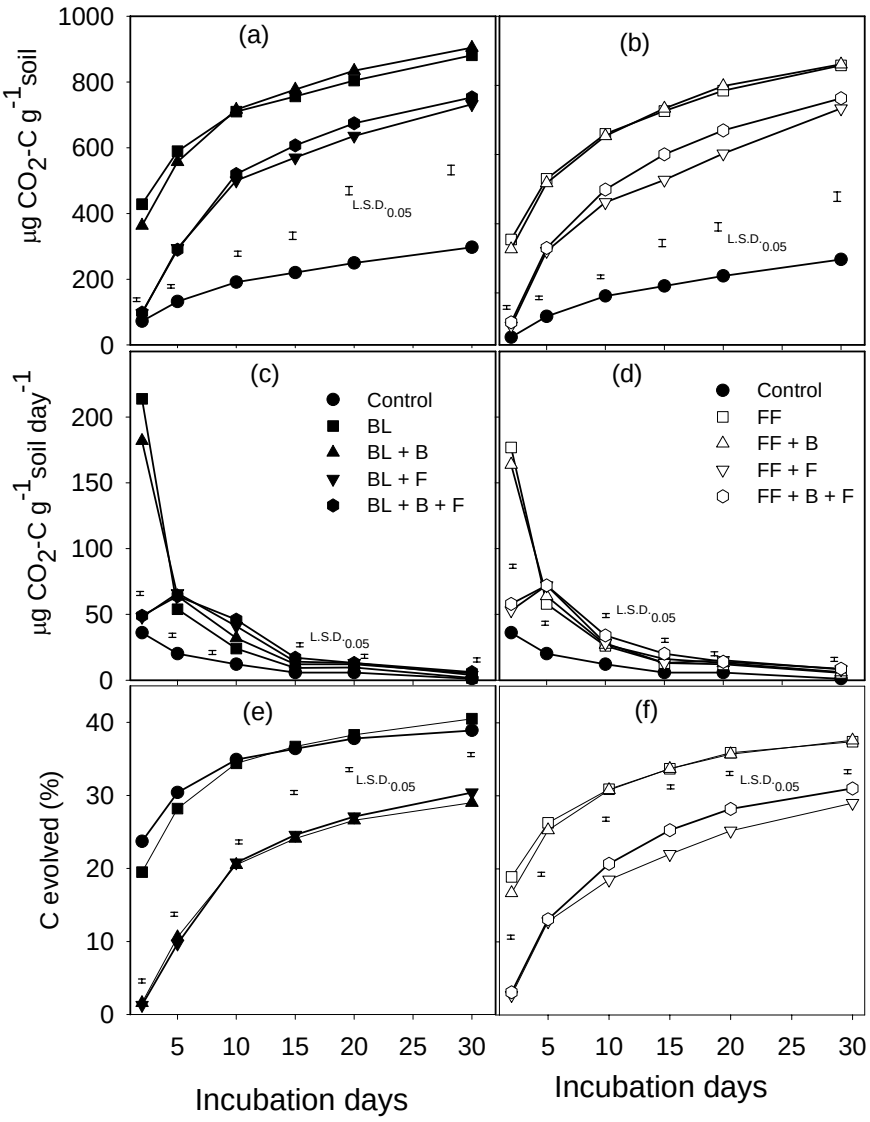

Fig. 2. (a), (b) Cumulative CO_2, (c), (d) CO_2 evolution rate, and (e), (f) Apparent perecntage of BL or FF derived C evlolved as influenced by BL or FF treated with biocides. I: L.S.D.$_{0.05}$ bars, significant differences among treatments at each incubation date at p 0.05, BL (FF): black liquor (fine fiber), BL (FF) + B: black liquor (fine fiber) + bactericide, BL (FF) + F: black liquor (fine fiber) + fungicide, and BL + B + F: black liquor (fine fiber) + bactericide + fungicide.

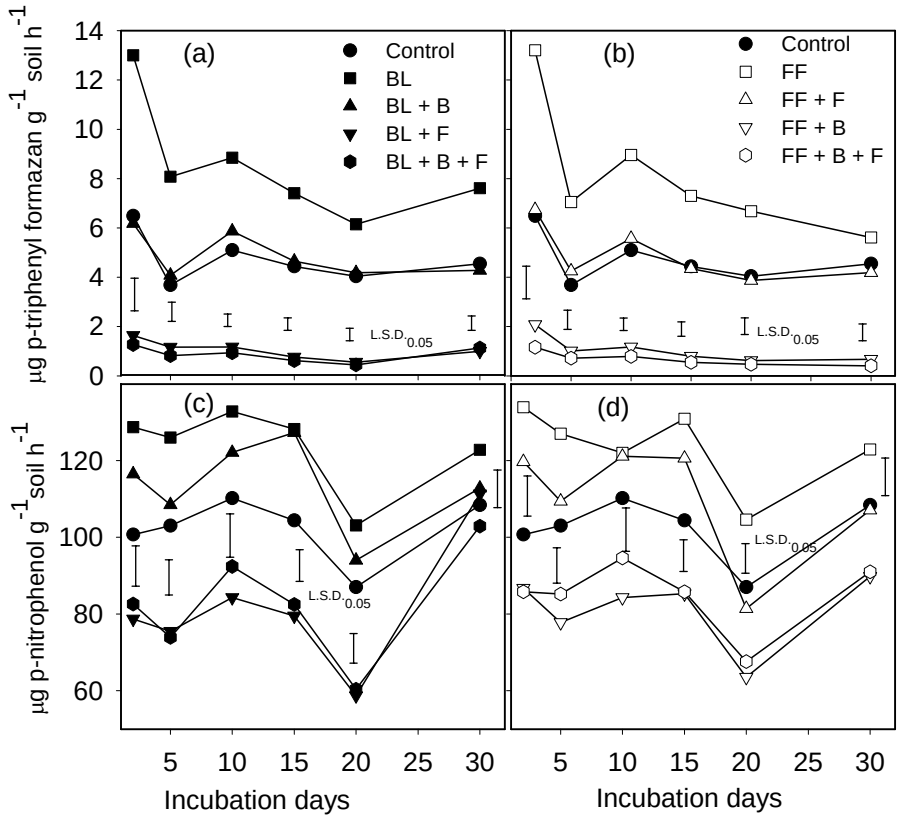

Fig. 3. (a) and (b) dehydrogenase, and (c) and (d) β -glucosidase activites as influenced by black liquor or fine fiber treated with biocides.
I: $LS.D._{0.05}$ bars, significant differences among treatments at each incubation date at $p \leq 0.05$, BL (FF): black liquor (fine fiber), BL (FF) + B: black liquor (fine fiber) + bactericide, BL (FF) + F: black liquor (fine fiber) + fungicide, and BL (FF) + B + F: black liquor (fine fiber) + bactericide + fungicide.

Chapter 6

Soil Microbial Activity, Aggregation and Nutrient Responses to Straw Pulping Black Liquor in Corn

Abstract

Crop straw represents an abundant, inexpensive and renewable fiber source for liquor that has potential as a beneficial soil amendment. A two-year field trial was conducted to evaluate use of wheat straw black liquor as a soil amendment for: (i) improving soil aggregation, microbial biomass and microbial activities, and (ii) increasing soil K availability for crop production. A complete randomized block experiment was established with 7 treatments and four replicates with corn grown at two locations in central Washington. Black liquor applied at rates to deliver 168 and 336 kg K ha^{-1} increased soil pH by 0.2 to 1.1 units to a depth of 5 cm and soil electrical conductivity by 0.37 dS m^{-1} at both locations, suggesting that black liquor application has potential as a mild liming material without salinity concerns. The KOH-based black liquor also increased soil Olsen K by 199 to 368 mg K kg^{-1} soil and by 44 to 200 mg K kg^{-1} soil at a depth 5 cm at two locations. No significant differences in soil Olsen K were observed between KOH-based black liquor and comparable K rates applied as KCl at both locations. Corn had no growth or yield response to K applications of either K sources. The apparent lack of response to K application may be related to high initial available soil K levels at both sites. The KOH-based black liquor increased soil dehydrogenase, ß-glucosidase, and arylsulfatase activities, microbial biomass C, and soil

wet macroaggregates compared to non-amended control and comparable KCl rates, suggesting that KOH based black liquor had beneficial effects on soil quality.

Key words: Soil microbial activity, Soil enzymes, soil aggregation, wet stable aggregates, corn.

1. Introduction

Increasing demand for pulp and paper products is leading to a growing shortage of wood materials and a gradual deforestation in many countries (Oinonen and Koskivirta, 1999). Use of non-wood fibers may help solve the fiber shortage problems and serve as an alternative wood fiber while lessening deforestation. Non-wood crop straws represent a very abundant, inexpensive, and renewable source for papermaking. Wheat straw is one of the most promising agricultural residues for papermaking with good quality compared to the other non-wood fibers such as olive tree fallings, sunflower stalks, vine shoots and cotton stalks (Alcaide et al., 1993).

Non-wood fibers are typically pulped with NaOH under pressure (Mimms et al., 1989). This pulping process generates a large quantity of highly polluting black liquor (Hanmer, 1988, Juwarker and Subrahmanyam, 1987). Black liquor, an environmental concern and disposal problem for the pulp and paper industry, accounts for 15% of the total waste effluents, but contributes nearly 95% of the total pollution load of pulp and paper mill effluent (Rangan, 1987).

Generally, the approach to disposal is through combustion process where the pulping black liquor is concentrated, burned for producing energy, and recycled for recovering inorganic chemicals (Grover et al., 1999). However, combustion not only

requires high capital and operational costs that are not economically viable (Girovich, 1996). On the other hand, high Si content in black liquor from straw pulping makes this recovery process costly, which also makes installation unprofitable for small non-wood mills (Rousu et al., 2002). Therefore, the pulp and paper industry has a growing interest in finding disposal alternatives.

Black liquor is rich in organic matter and beneficial inorganic nutrients, and rarely considered hazardous with respect to lower heavy metals (Cox et al., 1997). Studies have shown that land use of organic wastes with low levels of heavy metals help maintain soil fertility by improving physical, chemical and biological properties, and thus improving crop yields (Johansson et al., 1999; Debosz et al., 2002). However, black liquor generated in NaOH pulping process may enhance soil dispersion due to the Na additions (Brady, 1990; Paul, 1995). Pulping of crop straw with KOH would address this problem. Recycling residual KOH in black liquor on land could provide K and a high pH liming material. However, benefits to soil quality and crop growth must be demonstrated before black liquor can be considered for land application. Soil amendments must not be toxic to plants or interfere with soil microbiological metabolism (Abrosimova, 1988).

Soil quality is determined by the physical, chemical and biological components of the soil (Johansson et al., 1999). Soil enzymes, considered to be involved in soil structure (Roldan et al., 1994), may be a useful indicator of changes in soil quality (Dick, 1992; Visser and Parkinson, 1992) and as bioindicator of external impacts (Dick and Tabatabai, 1993) because they are involved in organic matter decomposition, and nutrient recycling (Wright and Reddy, 2001). Although soil C and N are indicators of soil quality (Anderson and Domsch, 1989), soil microbial biomass C is more sensitive to changes in

soil quality. Soil structure is also an important indicator of soil quality (Low, 1973; Allison, 1973; Dick, 1997).

The objectives of this study were to determine whether: (i) KOH based black liquor improved soil aggregation, microbial biomass and activities under field conditions; and (ii) KOH based black liquor could be an effective K source in corn production.

2. Materials and methods

2.1. Black liquor

Black liquor was produced from pulping wheat straw with KOH according to a modified Universal Pulping (M. Jackson, M. Jackson, consultant, Tolovana Park, OR, personal communication, 2000) at University of Washington, Seattle, Washington. Briefly, wheat straw was mixed with solid KOH and water with a ratio of 10:1:100 on a weight basis. The mixture was cooked at ambient pressure and 90 ˚C for 1 hour. After cooling, black liquor was separated from the pulped straw by draining, and tested for the residual alkalinity based on acid titration neutralization. The separated liquor was reused as part of the cooking liquor for the next cook after fortification with KOH to the target level (ratio of straw: KOH 10: 1). Black liquor was recycled 3 to 4 times. Selected characteristics of black liquor are shown in Table 1.

2.2. Site description and experimental design

A two-year field trial was performed at two sites in central Washington. The first site was located at a grower's irrigated field, Paterson, Washington under sweet corn (*Zea mays L., spp. mays* supersweet jubilie) from November 2002 to September 2003. The second site was located at the Irrigated Agriculture Research and Extension Center (IAREC) at Washington State University, Prosser, Washington under field corn (*Zea*

mays L., Croplan Genetics 396RR PP) from November 2003 to November 2004. The respective soil types for both sites were coarse-silty, mixed, superactive, mesic Calcidic Haploxerolls, and silty, mixed, mesic Xeric Torripsamments. Soils had the following characteristics for the Paterson and Prosser sites, respectively: pH, 5.9 and 6.9; organic C, 5.2 and 5.2 g kg^{-1}; total N, 0.55 and 0.56 g kg^{-1}; Olsen P, 29 and 13 mg kg^{-1}; and Olsen K, 246 and 102 mg kg^{-1}.

The field trial was a randomized complete block design with four replications for both sites. Plot size was 4.6 by 10.6 m. There were seven treatments for both sites, consisting of two K sources, KOH-based black liquor and KCl; two rates, 168 and 336 kg K ha^{-1}; two application times, winter and spring applications for KOH based pulping black liquor; a non-amended control. Fluid KCl was used for the Paterson site, and solid KCl used for the Prosser site. The treatment combinations are shown in Table 2.

The K concentrations of pulping black liquor were variable (Table 1) due to the differences in recycling black liquor times and the proportion of KOH to straw used in pulping. Before application, black liquor was filtered to pass through a 0.5-mm screen to avoid clogging spray nozzles. The black liquor and fluid KCl were applied to the soil surface with a nozzle spray applicator. Solid KCl was broadcasted on the soil surface.

The treated plots were plowed with a moldboard plow to a depth of 15 to 20 cm, 1 to 2 days before corn seeding. Sweet corn and field corn were sown with a corn planter in rows at a depth of 3 to 4 cm at a density of 91,358 and 69,136 seeds ha^{-1} on April 16, 2003, and April 30, 2004, respectively. A total of 224 kg N ha^{-1} was applied to the sweet corn, with 134 kg N ha^{-1} applied preplant, and 90 kg N ha^{-1} applied four 4 times after planting with a sprinkler (22.5 kg N ha^{-1} application each). A total of 224 kg N $ha^{-1}$and

25 kg P ha^{-1} was applied to field corn prior to seeding. The herbicide "alachlor": 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide, was applied to sweet corn, and "roundup": glyphosate, applied to field corn at approximately 15 cm of corn height.

2.3. *Soil and plant sampling*

Soil samples were taken from treated plots at two depths (0 to 5 cm and 5 to 30 cm) at the first site, Paterson on April 14, 2003, and at the second site, Prosser on April 22, 2004 after spring application before corn seeds were sown. Soil samples at 0 to 5 cm depth were analyzed for soil pH, electrical conductivity (EC), available K, soil microbial biomass C, enzyme activities (dehydrogenase, ß-glucosidase and arylsulfatase) and soil wet stable aggregates. Soil samples at the lower depth were analyzed for soil pH, electrical conductivity (EC), and available K.

Composite samples of 12 ear leaves were taken randomly from non-harvest rows at silking at Paterson site on August 5, 2003, and at Prosser site on August 5, 2004. The samples were oven dried at 60 º C and ground to pass through a 2-mm sieve for leaf K analyses. On September 15, 2004 at silking, a subsample of 6 stalks were taken randomly from a 6.1-m section of 2 rows, weighed and chopped with a chipper machine. Chipped subamples were weighed, and oven dried at 60 º C for 6 days for determination of moisture content, and then ground to pass through a 2-mm sieve for the determination of stalk K concentration.

Fresh ear yield of sweet corn was determined by handpicking all the ears from 4 rows 1.5 m in length from each plot on August 22, 2003. The number of plants and ears was counted and the total ear weight with spurs and without spurs was weighed. Field

corn yield was determined by handpicking all the ears from 2 rows 6.1 m in length from each plot on November 15, 2004.

2.4. Black liquor, soil and plant sample analysis

The pH and electrical conductivity (EC) of black liquor were measured with a pH meter model (211/digital pH meter, Orinon Research Inc., Boston Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH), respectively (Jansen, 1993). Black liquor was oven-dried at 85 °C for 24 hours for determination of solid content. Polysaccharides and lignin in black liquor were determined as described by Sun and Tomkinson (2001). A 10 mL aliquot of black liquor was neutralized with 9.68 M H_3PO_4 to a pH of 7.0, 30 mL of 95% ethanol was added to precipitate polysaccharides, and then filtered to get polysaccharides. Ethanol was evaporated with heat. After cool, the residual filtrate was adjusted to pH of 2 using 9.68 M H_3PO_4 to precipitate lignin. Black liquor was digested using a HNO_3-H_2O_2 (Jones and Case, 1990), and the digestion solution was analyzed by an inductively coupled agron plasma spectrometer 61 (Thermo Jarrell Ash, Franklin, MA) for K, Ca, Mg, Si, Mn, Cu, Zn, and Cr concentrations. A 0.5-mL sample of black liquor was used for the determination of total C and N by a Leco analyzer (LECO, CNS2000, St. Joseph, MI).

A soil-saturated paste was equilibrated for one hour for the determination of pH with a pH meter (211/digital pH meter, Orinon Research Inc., Boston Mass) and electrical conductivity (EC) by an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Spriings, OH) (Jansen, 1993). Available soil K was extracted by 0.5 M $NaHCO_3$ at a pH of 8.5 according to Schoenan and Karamanos (2000).

Microbial biomass C was determined by the fumigation - incubation method (Horwath and Paul, 1994). Following the removal of chloroform, 25 g of fumigated soils, and non-fumigated soils at optimum soil moisture content (60% of water-holding capacity) were then incubated in a 0.5 L Mason jars at 24 °C in the dark for 10 days. The CO_2 flush from fumigated and unfumigated soils were trapped by titration (Anderson, 1982). Microbial biomass C was calculated based on the equation:

Biomass C (μg/g dried soil) = (Fc – UFc)/Kc, where: Fc = CO_2 flush from the fumigated sample, UFc = CO_2 produced by the unfumigated control, and Kc, coefficient = 0.41 (Anderson and Domsch, 1978).

Dehydrogenase, β-glucosidase and arylsulfatase activities were measured according to the methods of Tabatabai (1994). Soil wet stable aggregates were determined by the method as described by Cambardella and Elliott (1993). A 200 g of air-dried sample was gently passed through a 2 mm sieve. Three 50 g oven dried equivalent subsamples were submersed on a 250 μm sieve in water in a shallow pan for 5 minutes, and then hand moved gently up and down the sieve 3 cm vertically 50 times over a period of 2 minutes. Soils remaining on the 250 μm were transferred to a forced air oven at 105 °C. The oven dried macroaggregates were weighed and soil wet stable macroaggregates were calculated based on the equation:

Wet stable macroaggregates (%) = 100 x [weight of soil >250 μm (g)/total soil weight (g)].

The ground subsamples of 0.5 g ear leaves and field corn stalks were digested using HNO_3-H_2O_2 (Jones and Case, 1990), and digestion solution was analyzed by

inductively coupled agron plasma spectrometer 61 (Thermo Jarrell Ash, Franklin, MA) for K concentrations.

2.5. Statistical analysis

Analysis of variance and contrasts were performed with SAS (SAS Institute, 2002) for a randomized incomplete block design. Interaction between the study years and treatments were not considered due to difference in treatments at both sites, e.g., different application timing for KCl. Therefore, the data were separately analyzed for each site. Multiple comparisons were made with a protected significance least difference. Treatment effects were considered to be significant at $p \leq 0.05$. Contrasts were used to compare groups of treatments to one treatment or anther groups of treatments.

3. Results and discussion

3.1. Soil pH

Treatments had significant effects on soil pH at a depth of 0 to 5 cm at both sites (Table 3). Soil pH increased by 0.3 to 0.9 units at Paterson and 0.2 to 1.4 units at Prosser following application of KOH based black liquor at a rate of 168 and 336 kg K ha^{-1} compared to the non-amended control. These results reflect the alkaline nature of KOH based black liquor with pH values from 7.9 to 8.9 (Table 1). These results are in agreement with our previous incubation study (chapter 4 of this dissertation), offering additional evidence that KOH based black liquor has potential as a mild liming material. Alkaline wastes such as compost and fly ash (Cox et al., 2001), and paper mill effluent (Kannan and Oblisami, 1990) have been widely reported to increase soil pH.

The KCl applied at 168 and 316 kg K ha^{-1} decreased the soil pH at the surface depth compared to the non-amended control or black liquor at both sites. It is known that

neutral salts such as KCl decrease soil pH because high K^+ concentrations can exchange with exchangeable Al^{3+} or H^+ in soil clays, leading to increased H^+ or Al^{3+} concentrations in the soil solution.

Soil amendments had no significant impacts on soil pH of soils at the deeper depth depth of 5 to 30 cm (Table 4) at both sites, suggesting that major proportion of soil amendments applied stayed in the 0 to 5 cm depth.

3.2. Soil electrical conductivity (EC)

Treatments had significant effects on soil electrical conductivity (EC) at the surface depth at both sites (Table 3). Increases in soil electrical conductivity (EC) were observed in soils with KOH-based black liquor applied only in spring compared to non-amended control at both sites (Table 3). For example, soil EC increased by 0.1 to 0.2 units at Paterson, and by 0.32 to 0.47 units at Prosser. High salt concentrations of the black liquor (EC of 18 to 30 dS m^{-1}) were due to the KOH used in pulping and soluble salts released from the wheat straw. Salinity problems with land application of organic wastes of paper mill effluent with high EC have been a concern. However, the low soil EC of 0.8 to 1.0 dS m^{-1} at Paterson, and 0.3 to 0.8 dS m^{-1} at Prosser from KOH-based black liquor were below EC of 4 dS m^{-1} for saline soils (McBride. 1994), suggesting that land use of black liquor as a K source would not cause salinity concern at these application rates.

Applied KCl at 168 and 316 kg K ha^{-1} resulted in the highest soil EC in soils at the 0 to 5 cm depth among the amendments at both sites. Contrasts showed that there were significant differences in EC between KOH based black liquor and KCl. Similar to soil pH, soil amendments did not result in significant differences in electrical

conductivity among treated soils at a depth of 5 to 30 cm (Table 3), providing further evidence that major proportion of soil amendments applied remained at a the 0 to 5 cm depth.

3.3. Available soil K

Both K sources applied at 168 and 336 kg K ha^{-1} significantly increased available soil K at the surface in depth at both sites (Table 4). For example, available soil K was increased by 199 to 368 mg kg^{-1} at Paterson, and by 42 to 200 mg kg^{-1} at Prosser. Contrasts showed no differences in available soil K between K sources, or black liquor applied in fall vs. spring, suggesting that black liquor was an effective potassium source.

Both K sources applied at these rates did not cause significant difference in Olsen K of soils at a depth of 5 to 30 cm, suggesting little movement below the 5 cm depth.

3.4. Soil microbial activity

Treatments had significant effects on soil enzyme activities at both sites (Table 5). KOH based black liquor generally increased soil dehydrogenase, β-glucosidase, and arylsulfatase activities compared to the non-amended control. Soil enzyme activities generally increased with increasing rates of KOH based black liquor, especially for soils receiving black liquor in spring, which may be related to the fact that black liquor applied in spring was still in the initial stage of decomposition. The more easily decomposable organic components were likely available for microorganisms, allowing for the synthesis of soil enzymes. Black liquor applied in fall was likely in later stage of decomposition at sampling date with more resistant components such as lignin (Berg et al., 1984; Melillo et al., 1989).

Soil dehydrogenase, β-glucosidase or arylsulfatase activities were either not affected or decreased in soils applied with KCl compared to non-amended control (Table 5). KOH-based black liquor generally increased soil dehydrogenase, β-glucosidase and arylsulfatase activities compared to KCl rates, illustrating that K addition was not the determining factor of the microbial stimulation by K-based black liquor. Kannan and Oblisami (1990) found that irrigation of paper mill waste effluent for 15 years increased in dehydrogenase, amylase and phosphatase activity. They attributed the increases in enzyme activities to addition of organic matter and nutrients.

Dehydrogenase activity is considered an index of total viable microorganisms in the soil (Taylor et al., 2002) and a good indicator of soil microbial activity in semiarid areas (García-Gil et al., 2000). Many researchers have reported increases in soil dehydrogenase activity following application of organic wastes (García-Gil et al., 2000; Bardgett et al., 1995; Meli et al, 2002). The β-glucosidase activity is considered an important component of soil quality because it is involved in soil organic matter cycling (Müller and Wegener, 1988; Turner al., 2002). Arylsulfatase is an enzyme that catalyzes the hydrolysis of an arylsulfate anion by cleavage of the O-S bound (Oshrain and Wiebe, 1979). It is considered to be partly responsible for S cycling in soils and an indicator of fungi activity in soil. Generally significant positive correlations between enzyme activities, total organic C and dissolved C were found (Tabatabai, 1994; Madejón et al., 2001). Therefore, it was likely that lignin and polysaccharides in the KOH-based black liquor stimulated microbial activity.

3.5. Microbial biomass C

KOH-based black liquor applied in spring, but not fall resulted in significant differences in microbial biomass C compared to the non-amended control at both sites (Table 6). Silage effluent and urban wastewater increased soil microbial C (Bardgett et al., 1995; Meli et al, 2002), partly due to the addition of readily available nutrients into soil, and partly due to the input of microorganisms into the soil from the effluent. In our study, application of black liquor at a rate of 168 to 336 kg K ha^{-1} provided 195 to 578 kg C ha^{-1} and 36 to 226 kg polysaccharides ha^{-1}, which likely stimulated the growth of indigenous soil microorganisms, thus leading to the increase in microbial biomass (García-Gil et al., 2000).

KOH based black liquor increased soil microbial biomass compared to the KCl treatments, which could be explained by the fact that soil microorganisms obtain their energy from decomposition of organic matter for the growth requirements, and KCl did not provide any C source for soil microbial growth.

3.6. Soil wet stable macro-aggregates

Increased wet stable macro-aggregates were observed in soils with KOH-based black liquor applied at 336 kg K ha^{-1} in spring at Paterson, and in soils with KOH-based black liquor applied at 168 and 336 kg K ha^{-1} in fall at both Paterson and Prosser sites (Table 6) compared to the non-amendment control and comparable KCl rates. More stable macroaggregates were observed in soils with KOH based black liquor applied in spring than in fall; and KCl applied at 168 and 336 kg K ha^{-1} did not cause differences in soil stable macroaggregates compared to the non-amended control.

Wet stable macroaggregation is an important indicator of soil quality because it is an indicator for soil to resist to dispersion and erosion, and a general indicator of favorable soil structure for rooting and water infiltration (Karlen and Stott, 1994). Good soil structure promotes favorable water relations, root environment, and reduces susceptibility to erosion (Bossuyt et al., 2001). Application of paper mill sludge improved soil structural stability (Chantigny et al., 1999). Ferguson (1956) observed that aggregate stability increased due to the application of black liquor. Application of lignin increases soil aggregate stability and reduces soil erosion (Abrosimova, 1988). Mahoney (1998) also showed that increased water-stable aggregation due to addition of ammoxidized lignin. In our study, increased wet stable macroaggregates may be partly attributable to added substrate C, which stimulated microbial activities, thus leading to more binding agents such as polysaccharides and fungi hyphae produced by microorganisms; the added lignin from application of black liquor may also partly be responsible for the increased wet stable macroaggregates.

3.7. Leaf K, corn stalk biomass, K uptake and the yields of corn

There were no effects of treatments on ear leaf K concentrations in either site-year, field corn stalk biomass, stalk K concentrations (Table 7), sweet corn fresh yields (with spurs or without spurs), and field corn dry grain yields (Table 8). Contrasts also indicated no significant differences in these parameters between amendments vs. non-amendment control, black liquor vs. KCl, or black liquor applied in fall vs. spring (Tables 8 and 9), suggesting that corn had no response to K applications at both sites. This may be related to high initial available soil potassium levels (246 and 102 mg K kg^{-1}) for both

sites. It is known that corn would have no responses to potassium applications in a soil with available Olsen K greater than 100 mg K kg^{-1} soil (Hawkes et al., 1985).

Treatments had significant effects on field corn stalk K uptake (Table 7). There was a general trend that K applied at 336 kg ha^{-1} increased K uptake over non-amended control or K applied at 168 kg ha^{-1}, and KOH based black liquor applied at 168 and 336 kg ha^{-1} increased corn stalk K uptake compared to the non-amended control (Table 7), suggesting that KOH based black liquor or KCl applied at these rates improved field corn K availability. Contrasts showed no differences in field corn stalk K uptake between amendments vs. the non-amendment control, black liquor vs. KCl, or black liquor applied in fall vs. spring (Tables 7 and 8), providing another evidence that KOH based black liquor had similar effect with KCl on corn production as a K source.

4. Conclusions

Land application of KOH based black liquor at a rate of 168 and 336 kg K ha^{-1} increased soil pH by 0.2 to 1.1 units compared to non-amended control, indicating that black liquor may have potential as a mild liming material. No significant differences in Olsen K were observed between KCl and KOH based black liquor. KOH based black liquor applied at 168 and 336 kg K ha^{-1} increased soil dehydrogenase, β-glucosidase and arylsufatase activities, microbial biomass C, and wet stable macrooaggregates compared to the non-amended control or respective KCl rates, suggesting black liquor could be an effective K source and beneficial soil amendment. The lack of corn growth or yield response to K sources was related to high initial available soil K levels at both sites.

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Table 1. Selected characteristics of black liquors of each application timing at both sites (on a wet basis, n = 3)

Properties	Paterson		Prosser	
	---------- Application timing ----------			
	12/19/2002	3/14/2003	12/5/2003	4/15/2004
pH	8.8	8.9	7.9	8.5
EC (dS m-1)	27	21	18	30
Solid content (g L^{-1})	68	46	40	70
Total organic C (g L^{-1})	26	21	15	24
Total N (g L^{-1})	0.8	0.6	0.2	0.5
Polysaccharides (g L^{-1})	8.2	8.3	2.3	4.4
Lignin (g L^{-1})	15	4.7	6.8	4.1
Total K (g L^{-1})	15	11	8.9	18
Total P (mg L^{-1})	18	13	13	58
Total Ca (mg L^{-1})	43	40	30	36
Total Mg (mg L^{-1})	20	18	7.0	59
Total Na (mg L^{-1})	76	80	115	186
Total Si (mg L^{-1})	70	74	45	62
Total Fe (mg L^{-1})	1.4	3.9	2.8	2.6
Total Mn (mg L^{-1})	0.62	0.53	2.7	2.4
Total Cu (mg L^{-1})	0.25	0.2	0.27	0.50
Total Al (mg L^{-1})	0.45	0.38	3.7	1.5
Total Cr (mg L^{-1})	Undectable	Undectable	Undectable	Undectable
Total V (mg L^{-1})	Undectable	Undectable	Undectable	Undectable

Table 2. Treatments and application timing for KCl and black liquor for both sites

Treatment ID	Treatment	K rate (kg ha^{-1})	Paterson	Prosser
			----------Application timing ----------	
1	Control	0	-	-
2	KCl (R_1)	168	12/19/02	4/15/04
3	KCl (R_2)	336	12/19/02	4/15/04
4	BL fall (R_1)	168	12/19/02	12/5/03
5	BL fall (R_2)	336	12/19/02	12/5/03
6	BL spring (R_1)	168	3/14/03	4/15/04
7	BL spring (R_2)	336	3/14/03	4/15/04

R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring.

Table 3. Soil pH and electrical conductivity (EC) as influenced by soil amendments in both sites

Treatment	Paterson		Prosser		Paterson		Prosser	
	0 – 5 cm	5 – 30 cm	0 – 5 cm	5 – 30 cm	0 – 5 cm	5 – 30 cm	0 – 5 cm	5 – 30 cm
	---------- Soil pH ----------				---------- Soil EC (dS m^{-1}) ----------			
Control	6.4 d	6.2 ab	6.0 b	6.5	0.8 d	0.8	0. 30 d	0.35
KCl (R_1)	5.8 e	5.9 b	6.1 b	6.6	1.2 b	0 9	1.58 b	0.35
KCl (R_2)	5.6 f	6.2 a	6.0 b	6.5	1.5 a	0.8	2.91 a	0.36
BL fall (R_1)	6.7 c	6.3 a	6.2 b	6.3	0.8 d	0.7	0.27 d	0.27
BL fall (R_2)	6.8 bc	6.2 a	6.5 b	6.6	0.9 cd	0.8	0.37 d	0.30
BL spring (R_1)	6.9 b	6.1 ab	7.0a	6.4	0.9 cd	0.8	0.62 c	0.29
BL spring (R_2)	7.3 a	6.2 ab	7.4 a	6.3	1.0 bc	0.8	0.77 c	0.29
L.S.D.$_{0.05}$	0.18	0.35	0.54	NS	0.24	NS	0.22	NS
---- Contrast ---	---------- p values ----------							
Amendments vs. control	NS	NS	0.62	NS	<0.0051	NS	<0.0001	NS
BL vs. KCl	< 0.0001	NS	<0.0002	NS	<0.0001	NS	<0.0001	0.02
BL fall vs. BL spring	<0.0001	NS	<0.0001	NS	NS	NS	<0.0001	NS

§ values in a column followed by the same letter are not significantly different from one another. NS: insignificant differences among treatments or between treatment group.
R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring.
₤: sampled at 2 weeks of black liquor spring application.

Table 4. Available soil potassium (Olsen K) as influenced by soil amendments in both sites

Treatment	Paterson		Prosser	
	0 – 5 cm	5 – 30 cm	0 - 5 cm	5 – 30 cm
	---------- mg K kg^{-1} soil ----------			
Control	334 c	297	114 c	102
KCl (R_1)	560 b	279	168 b	100
KCl (R_2)	661 a	291	321 a	106
BL fall (R_1)	533 b	282	156 b	102
BL fall (R_2)	645 a	329	335 a	96
BL spring (R_1)	570 b	282	169 b	98
BL spring (R_2)	702 a	285	314 a	96
$L.S.D._{0.05}$	75	NS	22.3	NS
------ Contrast -------	---------- p values ----------			
Amendments vs. control	< 0.0001	NS	< 0.0001	NS
BL vs. KCl	NS	NS	NS	NS
BL fall vs. BL spring	NS	NS	NS	NS

§ values in a column followed by the same letter are not significantly different from one another. NS: insignificant differences among treatments or between treatment group.
R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring.
£: sampled at 2 weeks of black liquor spring application.

Table 5. Dehydrogenase, ß-glucosidase and arylsulfatase activities as influenced by soil amendments at both sites

Treatment	Paterson			Prosser		
	Dehydro-genase	ß-glucosidase	Arylsul-fatase	Dehydro-genase	ß-glucosidase	Arylsul-fatase
	µg TPF g^{-1} h^{-1}	---- µg PNP g^{-1} h^{-1} -----		µg TPF g^{-1} h^{-1}	---- µg PNP g^{-1} h^{-1} -----	
Control	1.23 cd	41 cd	11.6 d	0.89 c	54 bcd	11 c
KCl (R_1)	1.05 d	42 bc	12.3 d	0.77 c	50 cd	9.5 c
KCl (R_2)	0.84 e	40 c	14.3 c	0.86 c	48 d	9.9 c
BL fall (R_1)	1.44 bc	47 a	14.5 cd	0.91 c	57 bc	11.1 c
BL fall (R_2)	1.61 b	47 a	16.2 c	1.03 c	60 b	13.6 b
BL spring (R_1)	1.64 b	46 ab	19.6 b	3.47 b	73 a	14.5 b
BL spring (R_2)	2.42 a	47 a	24.4 a	4.89 a	77 a	17.1 a
$L.S.D._{0.05}$	0.27	5.5	3.0	0.42	7.0	2.2
----- Contrast ------	---------- p values ----------					
Amendments vs. control	NS	0.05	< 0.009	< 0.0001	< 0.0094	NS
BL vs. KCl	< 0.0001	< 0.0016	< 0.0001	< 0.0001	< 0.0001	< 0.0001
BL fall vs. BL spring	< 0.0001	NS	< 0.004	< 0.0001	< 0.0001	< 0.0003

§ values in a column followed by the same letter are not significantly different from one another. NS: insignificant differences among treatments or between treatment group.
R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring.
† TPF: triphenyl formazan, and PNP: p-nitrophenol.
£: sampled at 2 weeks of black liquor spring application at soil 0 to 5 cm depth.

Table 6. Soil microbial biomass C and wet stable macroaggregates as influenced by soil amendments

Treatment	Paterson		Prosser	
	Microbial biomass C (μg CO_2-C g^{-1})	Wet stable macroaggregates (%)	Microbial biomass C (μg CO_2-C g^{-1})	Wet stable macroaggregates (%)
Control	134 c	11.6 d	211 cd	25.4 b
KCl (R_1)	135 c	12.3 d	191 d	24.8 b
KCl (R_2)	124 c	14.3 cd	215 cd	24.6 b
BL fall (R_1)	142 c	14.5 cd	212 cd	25.3 b
BL fall (R_2)	142 c	16.2 c	219 c	27.7 b
BL spring (R_1)	197 b	19.6 b	249 b	33.7 a
BL spring (R_2)	235 a	24.4 a	292 a	38.7 a
L.S.D.$_{0.05}$	25	3.0	27	5.1
------- Contrast -------	---------- p values ----------			
Amendments vs. control	< 0.006	< 0.0001	NS	NS
BL vs. KCl	< 0.0001	< 0.0001	< 0.0001	< 0.0004
BL fall vs. BL spring	< 0.0001	< 0.001	< 0.0001	< 0.0001

§ values in a column followed by the same letter are not significantly different from one another. NS: insignificant differences among treatments or between treatment group.
R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring.
£: sampled at 2 weeks of black liquor spring application at soil 0 to 5 cm depth.

Table 7. K concentrations of ear leaves and field corn biomass as influenced by soil amendments

Treatment	Paterson	Prosser			
	Ear leaf K ($g\ K\ kg^{-1}$)	Ear leaf K ($g\ K\ kg^{-1}$)	Stalk K ($g\ K\ kg^{-1}$)	Stalk biomass ($kg\ ha^{-1}$)	K uptake ($kg\ K\ ha^{-1}$)
Control	28.2	20.6	10.0	10.97	109.96 bc
KCl (R_1)	27.2	20.6	10.8	9.79	106.54 c
KCl (R_2)	27.1	21.5	12.1	11.37	137.53 a
BL fall (R_1)	28.3	21.0	10.5	10.52	109.55 c
BL fall (R_2)	27.5	21.2	12.0	11.24	134.92 ab
BL spring (R_1)	28.9	21.5	11.6	10.83	137.60 a
BL spring (R_2)	26.6	21.7	11.4	10.97	122.55 abc
$L.S.D._{0.05}$	NS	NS	NS	NS	25.2
------ Contrast --------	---------- p values ----------				
Amendments vs. control	NS	NS	0.028	NS	NS
BL vs. KCl	NS	NS	NS	NS	NS
BL fall vs. BL spring	NS	NS	NS	NS	NS

§ values in a column followed by the same letter are not significantly different from one another. NS: insignificant differences among treatments or between treatment group.
R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring.

Table 8. Corn yields as influenced by soil amendments

Treatment	Paterson		Prosser	
	Fresh ear yield without spurs	Fresh ear yield with spurs	Ear yield	Dry grain
	---------- ton ha^{-1} ----------			
Control	22.20	26.35	11.86	9.36
KCl (R_1)	24.18	29.06	11.11	8.85
KCl (R_2)	21.64	25.86	11.27	8.97
BL fall (R_1)	23.57	28.50	11.67	9.35
BL fall (R_2)	22.42	26.77	12.32	9.68
BL spring (R_1)	23.79	27.72	11.03	8.54
BL spring (R_2)	24.33	29.14	11.96	9.78
$L.S.D._{.0.05}$	NS	NS	NS	NS
------ Contrasts ------	---------- p-values ----------			
Amendments vs. control	NS	NS	NS	NS
BL vs. KCl	NS	NS	NS	NS
BL fall vs. BL spring	NS	NS	NS	NS

§ values in a column followed by the same letter are not significantly different from one another. NS: insignificant differences among treatments or between treatment group.
R1 and R2: 168 and 316 kg K/ha, BL fall, spring: black liquor applied in fall and spring

APPENDIX

Table A-1. Characteristics of recycled black liquor[£]

Recycles	Solid	Polysaccharides	Lignins	K	pH	EC (dS/m)
	---------- g L^{-1} ----------					
1 x	25	5.2	3.4	4.9	10	12
2 x	38	8.1	4.7	8.2	9.5	19
3 x	39	8.3	4.8	8.5	11	19
4 x	57	9.7	5.2	10	10	22
5 x	55	10	5.2	13	10	24
6 x	60	11	5.4	14	10	26
7 x	57	11	6.6	14	10	26
8 x	58	12	6.5	15	10	27

£: Black liquor from wheat straw pulping by a modified universal pulping process (M. Jackson, Consultant, Tolovana Park, OR, personal communication, 2000). The pulping conditions were as follows: Wheat straw was mixed with solid KOH and water at a ratio of 10:1:100 (on a weight basis), and cooked under a temperature of 90 –95 °C and ambient pressure for 1 hour. Black liquor was separated to recycling residual KOH for the following cook.

Table A-2. Aggregate size distributions as influenced by lignin derived C rates

Rates (lignin derived C g kg^{-1} soil)	Incubation (weeks)	>2 mm	2-1 mm	1-0.5 mm	0.5-0.25 mm	0.25-0.15 mm	0.15-0.053 mm	<0.053 mm
		%						
Control	2	1.68 ns	4.68 ns	4.38 ns	8.45 ns	25.3 ns	46.8 ns	6.98 ns
1.67		1.79 ns	4.51 ns	4.45 ns	9.09 ns	26.3 ns	45.9 ns	6.13 ns
3.34		1.70 ns	4.87 ns	4.47 ns	9.28 ns	25.3 ns	46.1 ns	6.62 ns
L.S.D $_{0.05}$		0.67	1.42	0.14	1.75	0.59	6.81	2.14
Control	4	1.59 ns	4.87 ns	4.46 ns	8.39 ab	25.1 ns	47.9 ns	6.69 ns
1.67		1.37 ns	4.10 ns	4.22 ns	7.66 b	25.0 ns	49.1 ns	7.47 ns
3.34		1.38 ns	4.80 ns	4.52 ns	8.94 a	23.8 ns	47.7 ns	6.86 ns
L.S.D $_{0.05}$		0.32	1.06	0.44	1.16	2.47	2.45	1.41
Control	6	1.12 c	4.14 ns	4.29 ns	8.71 ab	25.6 ns	49.0 ns	6.48 ns
1.67		1.98 b	4.29 ns	4.15 ns	8.32 b	23.8 ns	50.3 ns	6.78 ns
3.34		2.74 a	4.30 ns	4.32 ns	9.65 a	26.6 ns	46.6ns	5.55 ns
L.S.D $_{0.05}$		0.50	0.62	0.60	1.15	3.85	3.97	1.57
Control	8	1.45 c	4.57 ns	4.12 ns	9.07 ns	27.4 a	47.3 ns	6.40 ns
1.67		2.43 b	5.15 ns	4.46 ns	9.28 ns	24.2.b	47.2 ns	6.69 ns
3.34		3.34 a	5.33 ns	4.19 ns	10.1 ns	25.5 b	45.2 ns	6.16 ns
L.S.D $_{0.05}$		0.30	0.79	0.58	2.05	1.89	4.00	0.70

£: NS: no significant differences among treatments. Different letters: significant differences among treatment ($p \leq 0.05$).

Table A-3. Wet aggregate stability (%) as affected by lignin C derived C rates

Rates (lignin derived C g kg^{-1} soil)	Incubation (weeks)	>2 mm		2-1 mm		1-0.5 mm		0.5-0.25 mm	
		macro	micro	macro	micro	macro	micro	macro	micro
Control	2	54.0 ns	38.1 ns	67.0 ns	26.2 ns	68.3 ns	26.1 ns	75.6 ns	19.3 ns
1.67		50.7 ns	40.5 ns	64.8 ns	28.6 ns	67.3 ns	25.7 ns	75.8 ns	18.1 ns
3.34		53.5 ns	39.0 ns	63.2 ns	29.1 ns	63.1 ns	28.9 ns	74.7 ns	19.5 ns
L.S.D $_{0.05}$		12.7	9.97	4.37	3.82	7.86	7.86	6.34	5.42
Control	4	55.2 ns	35.7 ns	63.0 ns	29.3 ns	63.6 ns	28.9 ns	74.2 ns	19.5 ns
1.67		49.9 ns	38.3 ns	62.6 ns	30.7 ns	61.7 ns	30.6 ns	73.7 ns	19.0 ns
3.34		54.4 ns	35.5 ns	62.8 ns	29.5 ns	64.3 ns	28.9 ns	74.6 ns	18.6 ns
L.S.D $_{0.05}$		12.81	18.59	4.23	5.59	3.42	5.53	1.37	3.69
Control	6	58.2 ns	34.2 ns	60.1 ab	31.5 ns	65.0 ns	28.3 ns	73.3 ab	20.4 ab
1.67		48.3 ns	43.5 ns	64.8 a	28.2 ns	68.0 ns	24.9 ns	76.3 a	17.6 b
3.34		51.5 ns	40.4 ns	58.8 b	32.7 ns	64.3 ns	27.1 ns	70.7 b	22.1 a
L.S.D $_{0.05}$		10.19	9.75	4.69	4.87	1.18	8.07	5.20	4.28
Control	8	57.7 a	35.6 b	60.9 ns	31.3 ns	61.6 ns	30.2 ns	72.8 ns	20.7 ab
1.67		49.8 b	38.4 ab	61.0 ns	30.5 ns	60.7 ns	32.1 ns	72.9 ns	18.6 b
3.34		50.2 b	43.7 a	64.1 ns	29.0 ns	61.8 ns	30.5 ns	71.3 ns	22.8 a
L.S.D $_{0.05}$		4.32	7.38	6.30	5.94	5.27	4.78	4.14	3.33

£: NS: no significant differences among treatments. Different letters: significant differences among treatment ($p \leq 0.05$).

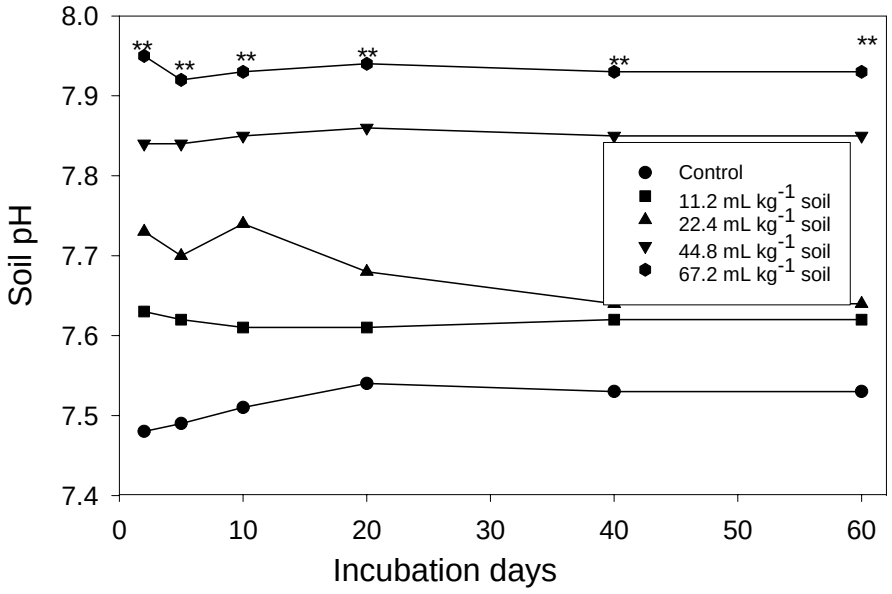


Fig. A-1. Soil pH as influenced by black liquor application rates. **: significant differences among treatments at each sampling date at $p \leq 0.01$.

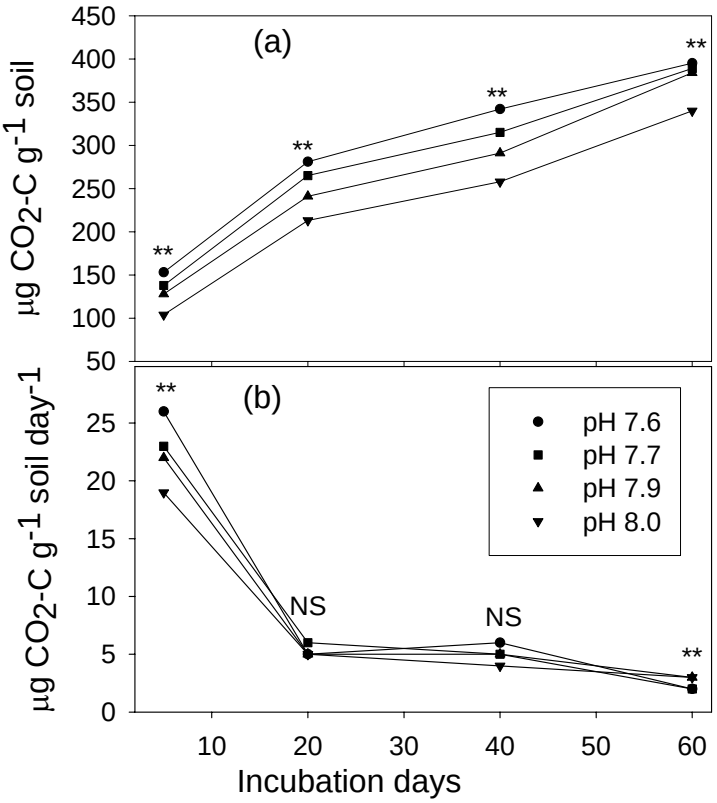


Fig. A-2. (a) Cumulative evolved CO_2-C, and (b) CO_2-C evolution rate as influenced by soil pH. NS: no significant differences among treatments at each sampling date, and **: significant differences at $p \leq 0.01$.

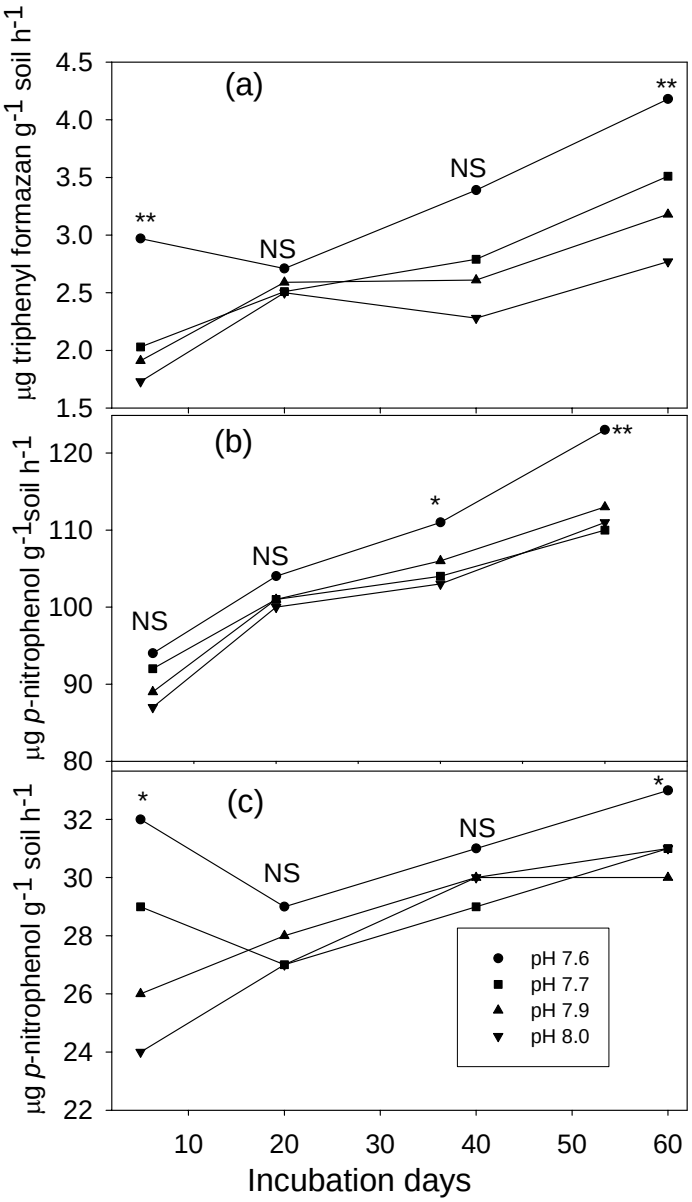
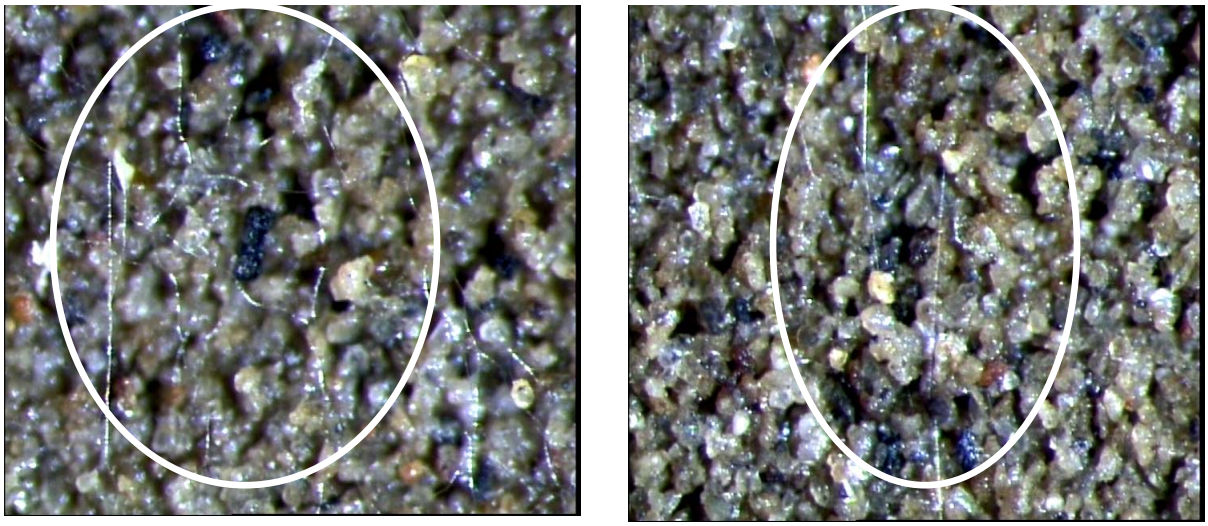


Fig. A-3. (a) Dehydrogenase, (b) ß-glucosidase, and (c) arylsulfatase activities as influenced by soil pH. NS: no significant differences among treatments at each sampling date, * and **: sigificant differences at $p \leq 0.05$, and ≤ 0.01, respectively.

Fungal hyphae microscope (200 x) images in soils treated with black liquor (1.5 g C kg^{-1} soil)



Day 1 Day 7

Soil treated with black liquor
Incubation condition: 24°C