

Transforming plant carbon into soil carbon: Process-level controls on carbon sequestration

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⁴Department of Forest Sciences, University of British Columbia, 2424 Main Mall, Vancouver, British Columbia, Canada V6T 1Z4; ⁵Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario, Canada K1A 0C6; and ⁶Lethbridge Research Centre, Agriculture and Agri-Food Canada, 5403 1st Avenue South, Lethbridge, Alberta, Canada T1J 4B1.

Received 6 May 2013, accepted 15 October 2013. Published on the web 27 October 2013.

Whalen, J. K., Gul, S., Poirier, V., Yanni, S. F., Simpson, M. J., Clemente, J. S., Feng, X., Grayston, S. J., Barker, J., Ishimaru, K., Gregorich, E. G., Angers, D. A., Rochette, P. and Janzen, H. H. 2014. **Transforming plant carbon into soil carbon: Process-level controls on carbon sequestration.** *Can. J. Plant Sci.* **94**: xxx–xxx. Plants figure prominently in efforts to promote C sequestration in agricultural soils, and to mitigate greenhouse gas (GHG) emissions. The objective of the project was to measure the transformations of plant carbon in soil through controlled laboratory experiments, to further understand (1) root-associated CO₂ and N₂O production during a plant's life cycle, (2) decomposition of plant residues leading to CO₂ production, and (3) stabilization and retention of undecomposed plant residues and microbial by-products in the resistant soil C fraction. Experimental plant materials included transgenic near isolines of *Zea mays* L. and cell wall mutants of *Arabidopsis thaliana*, selected for their diverse residue chemistry. Phenology, morphology and above-ground biomass affected soil respiration and N₂O production in root-associated soils. Mineralization of C and N from incubated plant–soil mixtures was complemented with stable isotope tracing (¹³C, ¹⁵N) and ¹³C-phospholipid fatty acid analysis. Advanced chemical techniques such as nuclear magnetic resonance spectroscopy and physical separation (particle size and density separation) were used to track the transformations of plant C into stable soil C compounds. Conceptual models were proposed to explain how the plant residue chemistry × soil physico-chemical interaction affects C sequestration. Incorporating single gene mutations affecting lignin biosynthesis into agricultural and bioenergy crops has the potential to alter short- and long-term C cycling in agroecosystems.

Key words: Biodegradation, lignin, maize, mineralization, soil microbial community, soil organic matter

Whalen, J. K., Gul, S., Poirier, V., Yanni, S. F., Simpson, M. J., Clemente, J. S., Feng, X., Grayston, S. J., Barker, J., Ishimaru, K., Gregorich, E. G., Angers, D. A., Rochette, P. et Janzen, H. H. 2014. **Convertir le carbone végétal en carbone minéral : contrôle des processus de séquestration du carbone.** *Can. J. Plant Sci.* **94**: xxx–xxx. Les plantes figurent en bonne place dans les efforts déployés pour séquestrer plus de C dans les sols agricoles et réduire les émissions de gaz à effet de serre (GES). Ce projet devait mesurer la transformation du carbone végétal dans le sol grâce à des expériences contrôlées en laboratoire, et permettre ainsi de mieux comprendre (1) la production de CO₂ et de N₂O par les racines pendant la vie de la plante, (2) la décomposition des résidus végétaux débouchant sur le dégagement de CO₂ et (3) la stabilisation et la rétention des débris végétaux non décomposés ainsi que des sous-produits microbiens dans la fraction de C résistante du sol. Les matières végétales employées pour l'expérience comprenaient des quasi isolignées transgéniques de *Zea mays* L. et des plants d'*Arabidopsis thaliana* présentant une mutation au niveau de la paroi cellulaire, sélectionnés en raison de la composition chimique différente. La phénologie, la morphologie et la biomasse aérienne affectent la respiration du sol et la production de N₂O dans la zone racinaire du sol. La minéralisation du C et du N par incubation des mélanges de débris végétaux et de sol a été complétée par le traçage des isotopes stables (¹³C, ¹⁵N) et l'analyse des acides gras à phospholipides ¹³C. Pour suivre la transformation du C végétal en composés du C stables dans le sol, les auteurs ont recouru à des techniques d'analyse chimique évoluées comme la spectroscopie par résonance magnétique nucléaire et la séparation physique (séparation des particules selon leur granulométrie et leur masse volumique). Ils proposent des modèles

Abbreviations: GC-MS, gas chromatography–mass spectroscopy; GCN, Green Crop Network; GHG, greenhouse gas; GM, genetically modified; NMR, nuclear magnetic resonance; PLFA, phospholipid fatty acid; SOC, soil organic carbon; UV, ultra-violet

conceptuels pour expliquer comment la chimie des résidus végétaux et les propriétés physicochimiques du sol interagissent et affectent la séquestration du C. Incorporer des mutations génétiques simples modifiant la biosynthèse de la lignine aux cultures vivrières et à celles employées pour produire de la bioénergie pourrait modifier à court et à long terme le cycle du C dans les systèmes agronomiques.

Mots clés: Biodégradation, lignine, maïs, minéralisation, microflore du sol, matière organique du sol

Soil organic C content is about twofold greater than the CO₂-C found in the Earth's atmosphere and about three times the C contained in entire world's vegetation (Cotrufo et al. 2011). Consequently, small changes (positive or negative) in soil organic C stocks have a large impact on the global C cycle. Soils under agricultural production (including cropland, managed grasslands, agroforestry and bioenergy crops) account for nearly 50% of the Earth's land surface. Historically, these soils were depleted of C when forests and grasslands were cleared for agricultural use, so they represent a sink where additional C could be stored. Lal (2004) estimated that agricultural and degraded soils have the capacity to capture about 20–60 Pg of C.

Carbon sequestration in soil refers to the process whereby atmospheric CO₂-C is transferred to soil C pools, namely organic C and secondary carbonates, with long residence times. Plants figure prominently in efforts to promote C sequestration in agricultural soils and to mitigate greenhouse gas (GHG) emissions. Agricultural practices that increase the input of photosynthesized C to soil and/or slow the return of stored C to CO₂ via respiration will cause a net gain in C sequestration. Smith et al. (2007) proposed to mitigate GHG from agriculture with agronomic practices such as using improved crop varieties, extending crop rotations to include more perennial crops that allocate relatively more C to root biomass than annual crops, avoiding or reducing fallow periods, planting temporary vegetative covers, trees and careful management of crop residues. How these practices contribute to soil organic C and GHG mitigation is described in Table 1.

In global C cycle models, it is relatively simple to increase soil C by adding more plant residue C to an agricultural soil or by planting more trees. However, the transformation of plant C to soil C is more complex and there are subtleties that are not yet fully understood. We know that the residence time in soil of plant cellular components varies from a few days (e.g., water-soluble sugars and amino acids) to many years (e.g., lignin, suberin and cutin). Can plant chemistry be manipulated to alter the rate at which plant C is transformed into soil C or returned to the atmosphere as CO₂? Can soil physico-chemical properties and soil biological activity moderate the decomposition of plant root exudates and residues with altered chemistry, or facilitate the transformation of plant residues into microbial by-products that are stabilized for long periods of time in association with soil organo-minerals *sensu* Kleber et al. (2011)? These questions were the foundation upon which project 2d “Transforming plant carbon into soil carbon: process-level controls on carbon sequestration” was built.

This article reviews the progress towards answering these questions by researchers and students who were supported by the Green Crop Network over a 5-yr period. The specific project objectives and relevance of the work to GHG management are described in conceptual models that show how modified plants affect C sequestration, CO₂ and other GHG emissions from soils. Experimental materials and analytical approaches are described, and key research findings are presented. We conclude with some ideas for future research.

Table 1. Selected mitigation strategies using plants to reduce CO₂ from the agricultural sector, and their expected impact on soil C sequestration in aerated mineral soils (adapted from IPCC 2007)

Mitigation strategy	Interpretation
Improved agronomic practices (e.g., reduce fallow period, crop rotations with perennial crops)	Greater photosynthesis possible with high-yielding crops, increasing yields generates higher inputs of residue C, which can increase soil C storage
Cover (catch) crops	Temporary vegetative cover, increases photosynthesis and captures nutrients left by main crops to reduce N ₂ O emissions, additional input of residue C for soil C storage
Agroforestry	Trees have a longer lifespan than most agricultural crops and have a greater standing stock of carbon. Tree residues contain a greater proportion of recalcitrant C compounds than annual agricultural crops, which can increase soil C storage
Residue management	Avoiding residue burning and reducing tillage intensity leaves more intact residue on the soil surface, which can slow decomposition of the residue C and enhance its eventual transformation into soil organic C
Soil erosion controls	Soil erosion removes topsoil that is rich in soil organic C and redistributes it across the landscape. Reducing bare soil area and implanting runoff controls to prevent water erosion is important to maintain soil structural integrity and soil organic C stocks

OBJECTIVES AND RELEVANCE TO GHG MANAGEMENT

Conceptual Model of Plant C Transformation to Soil C

The transformation of plant C to soil C is fundamentally a question of decomposition, a microbially mediated process, and of stabilization, a process mediated by the properties of the soil mineral matrix. As shown in Fig. 1, the decomposition process involves the biochemical breakdown of complex organic substrates from a living plant or dead plant residues into simple monomers and eventually CO_2 . Root exudates include carbohydrates (simple sugars and polysaccharides), amino compounds, organic acids, nucleotides, flavones, enzymes and growth factors. When degraded by extracellular enzymes to simple sugars and amino acids, they can be absorbed and metabolized by soil microorganisms, which release CO_2 and mineral N (NH_4^+ , NO_3^-) as end products of aerobic mineralization and reduced substrates such as nitrous oxide (N_2O) and methane (CH_4) under anaerobic soil conditions. Decomposition of dead plant residues containing a mixture of labile (e.g., water-soluble sugars, low molecular weight peptides, cellulose) and recalcitrant (e.g., lignin, suberin, cutin) compounds proceeds in the same manner. In both pathways, there is a resistant fraction that does not undergo the full biodegradation due to interactions with the soil matrix (e.g., occlusion within macro- and micro-aggregates, adsorption onto

mineral surfaces forming organo-mineral complexes). We expect part of recalcitrant compounds to contribute to the resistant fraction due to the relative difficulty in degrading such material. This view is supported by Marschner et al. (2008), who reported the mean residence time of lignin from grassland, bioenergy and agricultural crop residues to range from 13 to 22 years in soil. It was also outlined by Heim and Schmidt (2007) that lignin is partially preserved by association with fine particles in the soil. Plants with contrasting patterns of root exudation during their lifespan, and with different concentrations of recalcitrant compounds (e.g., lignin) in their residues, are expected to affect CO_2 and other GHG emissions from soil.

The resistant fraction from incompletely decomposed plant molecules is augmented by microbial by-products, such as extracellular polysaccharides, proteins (including surface-active glycoproteins), chitin and hydrophobic long-chain fatty acids ($n\text{-C}_{21:0}$ to $n\text{-C}_{34:0}$) synthesized by microbial cells (Kögel-Knabner 2002; Whalen and Sampedro 2010). The resistant fraction may persist in soil for decades or longer, depending on the strength of its association with physical fractions or organo-mineral surfaces. Marschner et al. (2008) reported that bioproducts synthesized by soil microorganisms had the following mean residence times: polysaccharides, 44–161 yr; protein and chitin, 48–284 yr; unspecified compounds, 30–3880 yr. Our conceptual model is consistent with

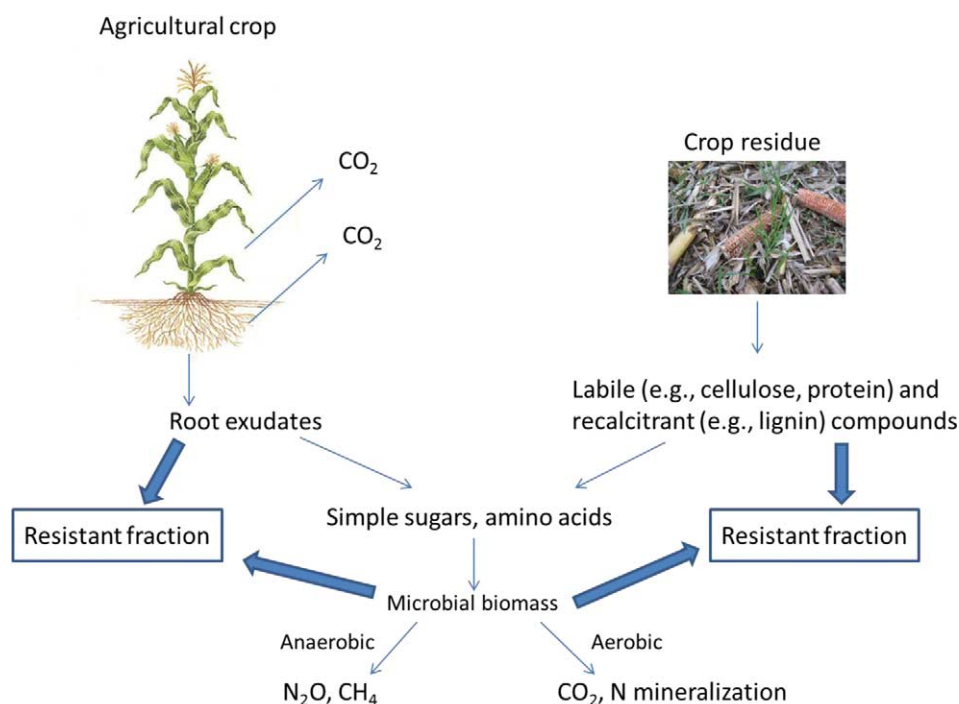


Fig. 1. Conceptual model showing the decomposition of plant C products (root exudates, crop residues) through biochemical breakdown to simpler monomers and gases (CO_2 , N_2O and CH_4). The soil C pool is maintained by the resistant fraction, which contains undecomposed plant C that is protected from further biodegradation by soil physico-chemical factors. Microbial synthesis of polysaccharides, proteins, chitin and long-chain fatty acids also contribute to the resistant fraction.

emerging views of soil organic C dynamics reviewed by Schmidt et al. (2011), who postulate that soil physico-chemical and biological factors control the decomposition process and the persistence of organic C in soil.

Although much of the “old” soil organic C probably originated from microbial synthesis, we still do not fully understand the rates and reactions involved in the decomposition and stabilization processes. The quantity and chemical composition of plant C inputs is expected to be important in fueling the decomposition and subsequent stabilization processes due to the reliance of heterotrophic microorganisms on C substrates for energy and growth (Kögel-Knabner 2002). Soil temperature, moisture and organic matter content, texture, nitrogen availability and other factors also affect the metabolic activity and community structure of soil microorganisms, which can have large effects on decomposition rates (Ågren et al. 2001; Fang et al. 2007; Stewart et al. 2008). Texture, and the clay content in particular, also controls physical interactions between plant residue and soil such as occlusion of residue C within aggregates and sorption of residue C on organo-mineral surfaces sensu Kleber et al. (2007). Consequently, we expected that plant residues inputs (quantity and chemistry) and soil physico-chemical factors would interact to affect CO₂ and other GHG emissions from soil.

Experimental Materials

The experimental materials for this project were genetically modified plants [near-isolines of corn (*Z. mays*) and single gene knock-out or over-expression mutants of *A. thaliana*]. These plants were selected because they were genetically similar, yet had diverse chemical composition with respect to the lignin concentration and chemistry. Since lignin is the second-most abundant plant molecule after cellulose and is hypothesized to be more recalcitrant to biodegradation, it served to modulate plant residue decomposition in short- to medium-term studies in the laboratory and the field.

Our first studies were conducted with near-isolines of Bt (*Bacillus thuringiensis*) and non-Bt corn due to the fact that Bt hybrids could produce as much as 47% greater silage yield and above-ground biomass than non-Bt hybrids in the field (Yanni et al. 2010), suggesting greater C inputs for heterotrophic microorganisms. There were also reports of differences in the chemical composition, primarily lignin, of Bt and non-Bt hybrids (e.g., Saxena and Stotzky 2001), although testing by our group did not find differences in lignin concentration in tissues from nine pairs of near-isolines grown under field conditions for 2 yr (Yanni et al. 2011a). There were, however, differences in lignin concentration between tissues, with roots > stems > leaves (Yanni et al. 2011b), which permitted us to compare decomposition from tissues with distinctive chemical composition in genetically similar plants (near isolines of corn).

Through the GCN, we obtained down-regulated and over-expression lines of *A. thaliana* cell wall mutants

having different lignin concentration in stem tissues than their wild ecotypes (Li 2009; Bhargava et al. 2010). The Cinnamoyl-CoA Reductase 1 (*CCR1*) coding gene is involved in lignin biosynthesis in stems and its down-regulation reduced lignin concentration in stems by up to 50% (Goujon et al. 2003). Gul et al. (2012a, b) reported lower C:N ratio, lignin:N ratio and lignin content in stem residue from a *CCR1* knockout mutant, which resulted in significantly more C mineralization in soil amended with that stem residue, compared with residue from the wild ecotype. In contrast, down-regulation of the production of Anthocyanin 1 (*PAP1*, also known as *MYB75*) coding gene increased lignin deposition in inflorescence stems and higher guaiacyl (G) to syringyl (S) ratio of lignin monomers in *A. thaliana* under controlled environmental conditions (Bhargava et al. 2010). Similarly, down-regulation of the Knotted Arabidopsis Thaliana 7 (*KNAT7*) coding gene caused a thickening in the interfascicular fiber cell walls and greater lignin concentration in stems than the wild ecotype (Zhong et al. 2008; Li 2009). The expression of these genes is tissue specific, with little or no change in lignin detected in roots, as confirmed by Gul et al. (2012a).

Analytical Approaches

We relied upon short-term, controlled laboratory studies to evaluate the impact of plants on CO₂ and N₂O emissions during their lifespan and during the initial stages of residue decomposition in soil [51 to 63 d laboratory incubations (Gul et al. 2012a, b; Poirier et al. 2013)]. Longer laboratory incubations (252 d) and in-field decomposition trials (1 yr in duration) were also used to evaluate residue decomposition in response to the initial lignin concentration in plant residue and an exogenous lignin source [laboratory study (Yanni et al. 2011b; Clemente et al. 2013)] and response to herbivory on plant stems [field study (Yanni et al. 2011c)].

Key analytical tools used by our group to evaluate the effect of plant residues inputs (quantity and chemistry) and soil physico-chemical factors on decomposition were gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) spectroscopy, stable isotopes (¹³C and ¹⁵N) as tracers of the C and N transformations in the plant-soil system, and stable isotope probing (SIP) of phospholipid fatty acids (PLFA) to assess microbial community responses. The copper oxidation method, followed by GC-MS analysis, was used to quantify lignin monomers in residues and extracted from soil organic matter (Otto and Simpson 2006). Among the NMR techniques employed were solid-state ¹³C CP-MAS NMR, which distinguishes a variety of C functional groups (carboxyl, carbonyl, phenolic, aromatic, anomeric and O-alkyl). The O-alkyl group was considered as a degradation parameter to report the extent of residue biodegradation occurring at various points in time during an incubation study. Another technique was solution-state ¹H NMR, which differentiates

between CH_2 found in proteins and lipids of microbial origin (Clemente et al. 2012). Stable isotopes tracing provided insight into the fate and mass balance (loss and retention) of ^{13}C and ^{15}N from recent plant inputs in the whole soil and soil fractions determined by particle-size (Poirier et al. 2013) and density separation techniques (e.g., particulate organic matter, macro- and micro-aggregates, and organo-mineral complexes). The ^{13}C SIP-PLFA method permitted identification of active decomposers and permits the researcher to track the succession of microbial colonization on labeled substrates during time-series experiments.

GENERAL FINDINGS AND RELATIONSHIP TO THE OTHER PUBLISHED WORKS

As described in Fig. 1, root exudation by plants during their lifespan constitutes a C input that supports microbial growth, respiration and contributes to the production of the resistant C fraction. The growth pattern (phenology), resource allocation (morphology) and biomass accumulation of plant above-ground components is expected to feedback on root-associated processes like water uptake, nutrient cycling and GHG production. Gul and Whalen (2013a) examined the CO_2 -C and N_2O -N emissions from root-associated soil of *A. thaliana* lines (wild ecotypes and down regulated mutants of *MYB75*, *KNAT7* and *CCR1* having altered lignin concentration in secondary cell walls) at various plant developmental

stages. The major finding was that morphology and biomass exerted a strong influence on soil respiration, and that the *CCR1* mutant line, which had a prolonged vegetative growth phase, reduced fertility and biomass, lowered evapotranspiration and left more mineral N in root-associated soils, leading to higher N_2O emission from soil (Fig. 2). While some of these feedbacks in the plant-soil system have been predicted or measured previously (Nord and Lynch 2009), the link between plant-induced changes in the soil environment and GHG emissions was a new contribution from this work.

Decomposition leading to CO_2 production from residues of genetically modified plants (Bt corn, cell wall mutants of *A. thaliana*) was affected by the concentration of lignin and other recalcitrant substances in plant residues (Yanni et al. 2011b; Gul et al. 2012a, b). There are a number of ways that lignin may affect decomposition of plant residues (Table 2). The lignin concentration and lignin chemistry have an impact on the biochemical reactions that are necessary to unravel the three-dimensional structure and cleave monomers from complex lignin and ligno-cellulosic macromolecules (Yanni et al. 2011b). The size, physical integrity and exposure of residues to ultra-violet (UV) radiation also affects their decomposition (Feng et al. 2011; Yanni et al. 2011c). Finally, physical interaction of residues with soil minerals or sorption to organo-minerals is expected to limit microbial colonization and therefore

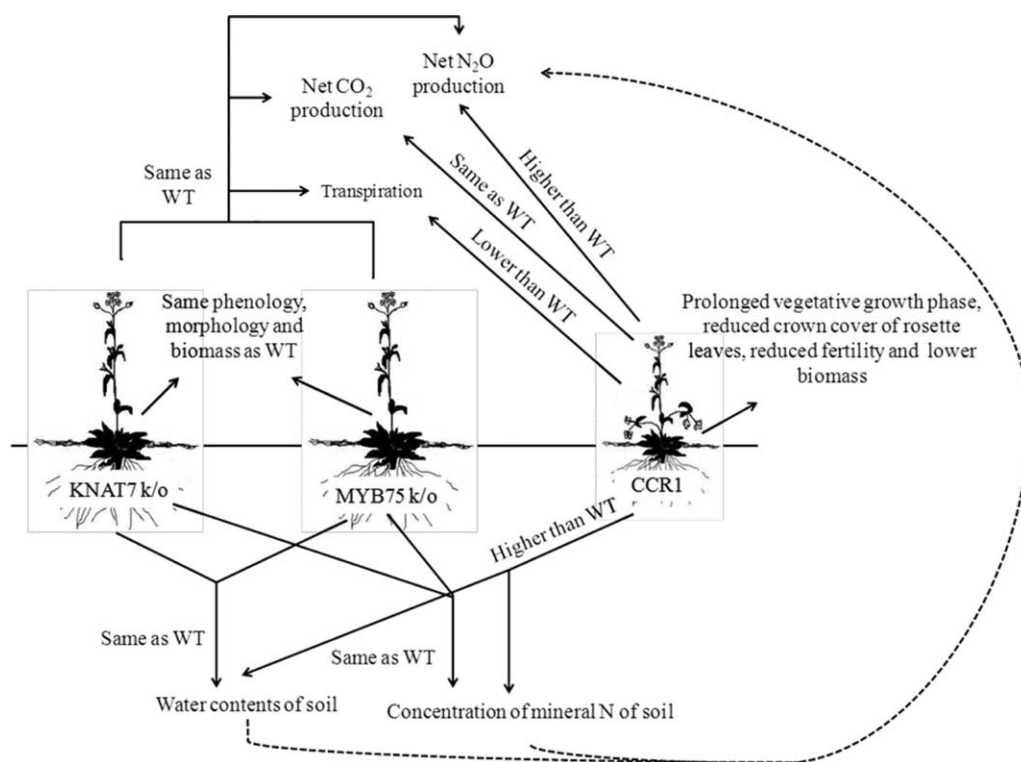


Fig. 2. Influence of *A. thaliana* knockout mutants of *KNAT7*, *MYB75* and *CCR1* on CO_2 and N_2O emissions from soil during their growth and development. WT = wild ecotype. Model is based on findings from Gul and Whalen (2012).

Table 2. Plant lignin chemistry, plant residue characteristics and physico-chemical interactions between residue and soil affecting residue decomposition

Plant lignin chemistry	Expected outcome
Increasing lignin content	Slower decomposition of lignin and ligno-celluloses in affected plant tissue(s)
Changing lignin chemistry: greater guaiacyl: syringyl (G:S) ratio	Slower decomposition of lignin monomers
Residue characteristics	Expected outcome
Larger residue size	Less surface area for microbial colonization, slows decomposition
Residues free of insect damage	Residues remain intact and physically limit access to decomposer organisms, slows decomposition
Residues left on soil surface	Exposure to UV radiation accelerates the breakdown and decomposition of residues
Physico-chemical interactions (residues and soil)	Expected outcome
Incorporation into macro- and micro-aggregates	Reduces decomposition through occlusion (short- to medium-term)
Complexation with mineral surfaces	Reduces decomposition through adsorption (long-term)

slow decomposition (Table 2). This concept is also presented graphically in Fig. 3, which illustrates a switch from physical controls (soil texture) to chemical controls

(recalcitrant compounds) on decomposition with increasing residue lignin concentration. When lignin inputs are sufficiently high, the recalcitrance of this material to decomposition can limit C mineralization in the initial stages of decomposition and in C-rich soil horizon (von Lützow et al. 2008).

We were also interested in the contribution of plant molecules and microbial synthesis to the resistant C fraction during biodegradation processes (Fig. 1). Clemente et al. (2013) examined the transformation of plant C to soil C by solid-state ¹³C and solution-state ¹H NMR spectroscopy. A key finding was that humic substances extracted with 0.1 M NaOH from leaf-amended soils had higher concentrations of aliphatic components, likely due to higher concentrations of aliphatic compounds in leaf tissues, which suggests that compounds derived from leaves are potential contributors to the stable pool of soil organic C. The contribution of microbial-derived organic matter was greatest in alkaline humic extracts from root-amended soils, indicating that root amendments may promote the formation of the resistant C fraction, consistent with the conceptual model illustrated in Fig. 1. Changes in soil organic matter composition during a 252-d incubation study was controlled by the chemical composition of the original plant tissue, demonstrating the important link between plant chemistry and soil organic C dynamics.

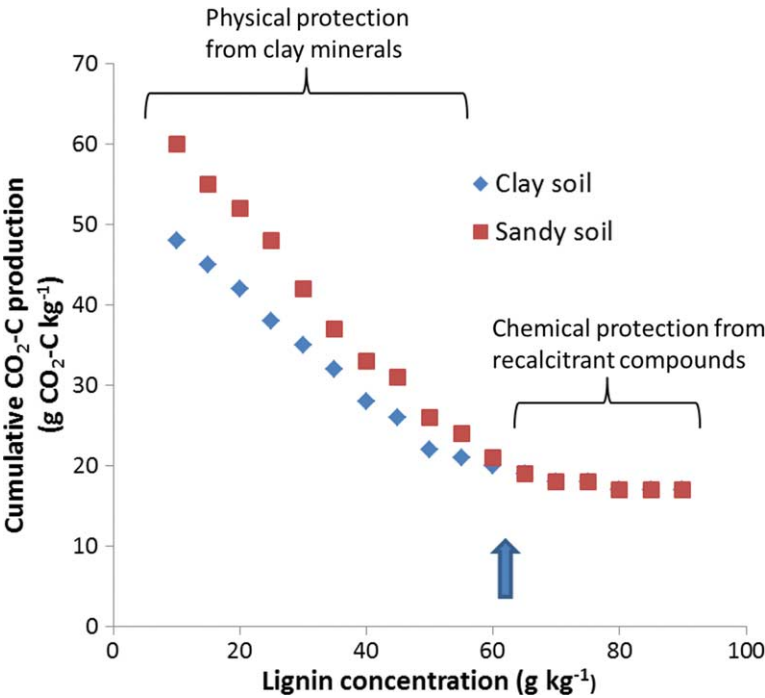


Fig. 3. Hypothetical model showing how the plant residue chemistry × soil texture interaction affects decomposition of plant residue (presented as C mineralization = cumulative CO₂ production during an incubation study, for example). In the initial stages of decomposition, C mineralization is limited by physical interactions with the soil matrix (e.g., with clay minerals) and by the chemical composition of resistant compounds (e.g., lignin) in the plant residue. The arrow indicates the point of convergence of the decomposition curves on two soil types, where decomposition is controlled less by physical interactions and more by residue chemistry.

Another important component of the study was the physical interaction between plant residue carbon and soil particles. Our results indicate that initial soil organic matter content influenced the partitioning of residue C between the silt + clay (<50 μm) particle-size fraction and the whole soil. Poirier et al. (2013) showed that when low amount of residues were added to the soil, the greater microbial activity in a C-rich topsoil resulted in more residue-C retained by silt+clay particles in this soil than in a C-poor subsoil. In the latter, however, high residue input levels resulted in greater residue-C retention by silt and clay particles than in the C-rich topsoil, likely due to the greater C saturation in the C-rich topsoil. Our results also show that clay minerals can indeed provide protection of soil organic matter components from biodegradation. Lignin residues associated with clay minerals are the most oxidized components in soil organic matter. Thus, clay minerals may assist with the long-term protection of stable forms of plant-derived carbon. We also found that other organic matter components, such as long-chain fatty acids, lipoproteins and peptidoglycans from microbial metabolism, can physically protect lignin from chemical attack (and presumably biodegradation). We also examined organic matter in three native prairie soils from Alberta to identify which components are attenuated by clay minerals in soil (Clemente et al. 2011). Lignin associated with minerals was more oxidized than in

the other soil density and size-fractions. Clay-size fractions also had increased amounts of cutin-derived compounds (likely from leaf waxes) and microbially derived peptides. We further tested the role of minerals in preserving lignin and other soil organic matter compounds using soil clay-size fractions and lignin-clay complexes (Clemente and Simpson 2013). Lignin is protected by both organic matter and clay minerals from chemical oxidation. These results collectively show that lignin interactions with minerals as well as other soil organic matter components are responsible for the long-term stability of the resistant C fraction observed in the environment.

CONCLUSIONS REGARDING RELEVANCE OF FINDINGS TO SHORT- AND LONG-TERM GHG MANAGEMENT

The overall goal of this GCN-supported project was to gain a better understanding of C cycling in the plant-soil system to ensure that soil C stocks are maintained or increased with time. There is a sense of urgency to this research, given that agricultural practices have historically depleted soil C stocks and soil degradation continues in some regions of the world due to food, fiber and fuel demands of the growing population. Even in regions where ample food is produced, crop residues are now being viewed as a source of renewable energy and their removal from agroecosystems could result in a loss of

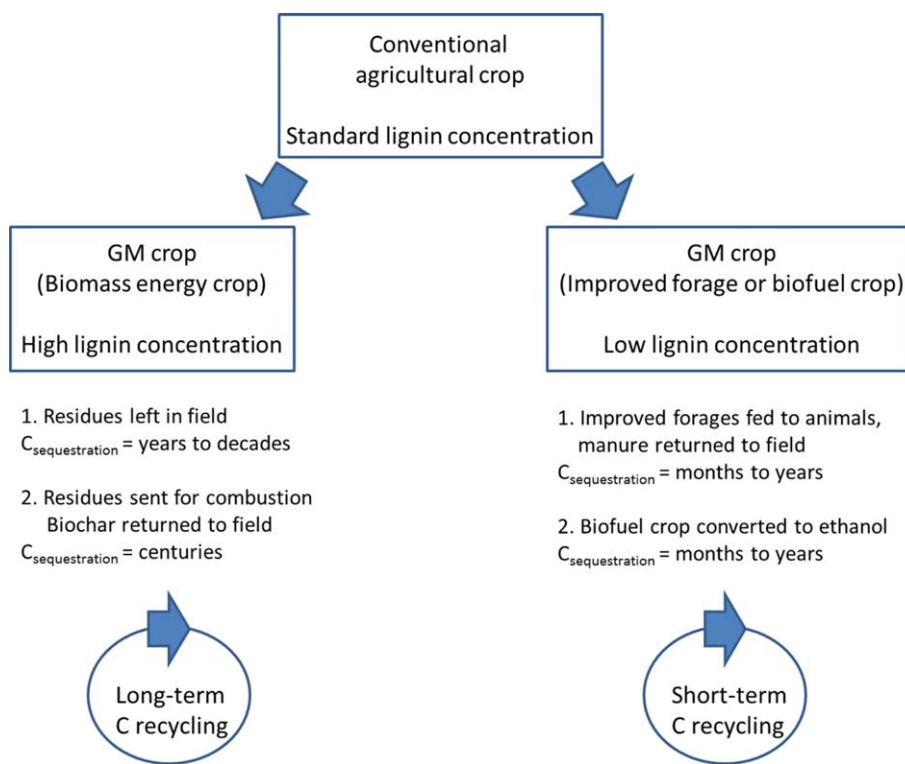


Fig. 4. Carbon sequestration ($C_{\text{sequestration}}$) potential of genetically modified (GM) crops with altered lignin concentration, expressed as the relative residence time of C in the soil-plant system before it returns to the atmosphere as $\text{CO}_2\text{-C}$.

soil organic C. Further discussion of the environmental impacts were covered by Whitman et al. (2011), who performed an agroecosystem-level life cycle assessment of corn stover removal for cellulosic bioethanol production, based on data from Quebec, Canada. Global warming continues steadily and is expected to induce a positive feedback on the C cycle, so that higher temperatures and greater CO₂ concentration in the atmosphere not only promote plant growth but also accelerate decomposition of recently added plant C. Whether the stabilization process is sufficiently robust to offset the C lost from soil respiration in this future climate scenario remains to be determined.

This GCN project has clearly shown the potential of using plant residues with greater lignin concentration and altered chemistry to slow the biodegradation of plant residues (less CO₂ produced) and enhance C storage associated with the clay mineral fraction of soil. Crop modification with single gene mutants, such as those used in this project, could be seen as an option to meet C sequestration objectives. Figure 4 outlines the C sequestration potential of biomass energy crops that were modified to increase their lignin concentration, considering that the residues of such crops could have a longer residence time in the soil, or that biochar, a by-product of pyrolysis, could be returned to the agroecosystem to further enhance the soil C sequestration potential. These modifications have the potential to promote long-term C recycling.

Gul and Whalen (2013b) discussed how down-regulation of genes controlling lignin biosynthesis would allow plant breeders to lower lignin concentration or lignin chemistry (e.g., lower the guaiacyl:syringyl ratio) in above-ground biomass. This could result in forage crops with greater digestibility, improve short rotation woody crops for the wood-pulping industry and create second generation biofuel crops with low ligno-cellulosic content. However, unharvested residues from such crops are expected to decompose quickly, potentially increasing CO₂ and N₂O emissions from soil in the short-term. Thus, improved forages and biofuel crops with lower lignin concentration have a role for providing necessary forages and energy, but would recycle C more quickly than conventional crops in the short-term. Further research is warranted to explore the potential and possible adverse effects (if any) of such crop modifications on soil C sequestration at the field scale.

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