

# Sustaining soil carbon in bioenergy cropping systems of northern temperate regions

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## Abstract

Soil organic carbon (SOC) has an essential role in controlling ecosystem functions associated with soil physical, chemical and biological properties. Maintaining the SOC pool size in agroecosystems is important to sustain food security, protect soil biodiversity and buffer environmental impacts. The SOC pool is dynamic, with losses occurring due to CO<sub>2</sub> mineralization and gains from microbially mediated humification of organic substrates into stable C compounds. Bioenergy production from lignocellulosic feedstock implies that greater amounts of plant residues will be removed from agroecosystems and could deplete the SOC pool, based on empirical models and experimental results from long-term field trials. In northern temperate regions, several management practices are suggested to conserve the SOC pool, such as the application of biochar, judicious use of organic and inorganic fertilizers, crop rotations that include high biomass producing non-bioenergy crops or intercropping systems that combine perennial bioenergy crops with other crops (annuals or trees). Moreover, new technologies such as genetically modified (GM) bioenergy crops are recommended to enhance bioenergy production per unit energy input. Those modifications include GM crops with higher resource-use efficiency (i.e., for water, nutrients and light), GM crops with cellulase/ligninase enzyme systems for biofuel production and GM crops with higher calorific values that release more energy during combustion.

**Keywords:** Genetically modified bioenergy crops, Crop rotation, Biochar, Tree-based intercropping, Soil organic carbon, Humification.

**Review Methodology:** The data bases ISI Web of Science and Science Direct was searched for articles for this review.

## Introduction

Global energy demand (424 Exajoules/year) is increasing by an estimated 2.2% per year [1]. Approximately 1.86 million barrels per day of bioenergy were produced in 2012 [1], supplying approximately 10% of the world's energy demand, mainly for liquid fuels and household use, e.g. for cooking [2]. In order to mitigate the climate change effect associated with burning of fossil fuel, alternative measurements such as the use of renewable resources for energy production is inevitable. The largest source of renewable energy is the bioenergy and the

world supply of bioenergy is predicted to increase from 50 EJ today to 160 EJ in 2050, of which 100 EJ will be available for generation of power and heat [2]. The Energy Independence and Security Act of 2007 of United States mandates the production of renewable fuels as 36 billion US gallons by 2022, of which 16 billion US gallons ought to be produced from lignocellulosic feedstock. As first-generation bioenergy technology (i.e. using grains and sugarcane and vegetable oils for bioenergy production) compete with food, lignocellulosic biomass or second generation feedstock (i.e. non-food components of plants, from agriculture, forestry and industrial sources) are

preferred for bioenergy production, which also have the potential to provide benefits such as making use of abandoned lands and consuming waste residues [3].

Although second generation bioenergy production is considered a better social and environmental option than first-generation bioenergy production, the major constraint at this point is the lack of availability of low-cost feedstocks. Until large tracts of dedicated bioenergy crops such as perennial grasses and trees are planted, bioenergy production facilities will rely on crop residues, specifically the non-food components (i.e. stems and leaves) as feedstock. However, the removal of non-food residues from agricultural fields reduces the amount of residue C that is returned to the soil, which can cause a drastic reduction in the soil organic carbon (SOC) pool size and associated ecosystem services [4, 5]. For example, Blanco-Canqui and Lal [6] reported that removal of 50% of the corn stover residues from a long-term (>8 years) no-tillage corn agroecosystem in Ohio during a 2.5-year period reduced the SOC pool by 1.63 Mg/ha in an erosion-prone soil.

Conservation of the SOC pool is important because it controls soil physical, chemical and biological properties that contribute to soil health and ecological functions. Among the soil properties related to the SOC content are soil structure (aggregation), porosity, pH, water and nutrient retention, retention and detoxification of pollutants, carbon sequestration and emission of greenhouse gases (i.e. carbon dioxide, nitrous oxide and methane), and population size and diversity of soil organisms [e.g. 7–9]. Globally, the SOC is as a reservoir for C sequestration, due to the fact that soils (to 1 m depth) store three times more C than is contained in terrestrial vegetation or the atmosphere [10]. Worldwide, soils contain about 1550 Pg C in the upper 1 m layer, excluding soil C reserves in permafrost soils and peatlands [see 11–13]. Due to the large size of the SOC pool, small changes in this reservoir may have a large positive or negative impact on the concentration of greenhouse gases in atmosphere [14].

It is clear that SOC reserves need to be protected globally, and there is a pressing need to do so in regions with high bioenergy demand like northern temperate regions of North America and Europe. In these northern climates, energy is required for heating and transportation by every segment of the population and targets for renewable energy use need to be met now and in the foreseeable future [15–17]. Sorda *et al.* [15] presented an exhaustive overview of biofuel policies across the world that encourage expansion of the global biofuel industry. Assuming rapid growth and demand for bioenergy, which could be deleterious to SOC pools if we rely upon non-food crop residues as feedstock for bioenergy generation, we discuss (1) the factors controlling SOC dynamics, (2) describe agricultural residues and dedicated bioenergy crops and (3) present management options to sustain or improve SOC amount in bioenergy cropping system of

northern temperate regions. We will eventually need to shift our bioenergy feedstock from non-food crop residues to dedicated bioenergy crops, which do not grow very fast in this climate. The last section of the review will discuss new technologies and production techniques to boost the yield of bioenergy crops in northern temperate regions.

### Soil Organic Carbon: Nature and Quantity in Cold-Temperate Regions

The SOC pool includes dead organic matter (OM) such as fresh-fallen leaves, twigs, microbial byproducts and animal tissues in varying states of decomposition [e.g. 18, 19]. Due to their close association with decomposing organic matter and short generation time, living microorganisms are also considered to be part of the SOC pool [20]. Chemical compounds commonly found in SOC include acid-soluble polyphenolics, lignin monomers, lignin dimers, *n*-alkanes, alkanolic acids, fatty acids, bacterial hexosamines, proteins, glucosamine and saccharides [10, 11, 20]. Various benzene compounds, alkyl-benzenes, poly-aromatic compounds, toluene, phenanthrene, mono-, di-, tri- and tetra-methylphenanthrene and alkyl-aromatic compounds were also reported in soils of Bainsville, Canada by Schnitzer and Monreal [20]. The functional groups found in SOC, based on isotope NMR spectroscopy, are alkyl-C and *O*-alkyl groups, aldehydes, ketones and alkenes [e.g. 11], while the elemental composition of the C–C skeleton of SOC is generally covalently bound to H, O, N and S [20].

Climate and ecosystem type also influence SOC composition. For instance, using pyrolysis – GC/MS technique, Vancampenhout *et al.* [21] observed that the NaOH-extracted SOC from tropical forest, temperate forest, tundra, taiga and steppe ecosystems possessed different chemistry. The SOC from cold climates had more long-chain alkanes and levosugars as compared to N-compounds, whereas tropical SOC had larger quantities of N-compounds relative to lignins and recalcitrant fractions (i.e. aromatic and aliphatic compounds) were not detected. The ratio of  $\sum \text{levosugar}:\sum \text{N}$  for temperate coniferous and temperate broad-leaved forests ranged from 0.36 to 3.48, with a ratio of 7.64 in taiga and from 0.12 to 0.53 in tropical rain forests. The ratio of  $\sum \text{lignins}:\sum \text{N}$  for temperate coniferous and temperate broad-leaved forests ranged from 0.65 to 4.21, for taiga it was 5.25, and for tropical rain forests the ratio was 1.03–2.08.

Within a particular ecosystem, the amount of SOC depends on land use and history of vegetation type (Tables 1 and 2, Figures 1 and 2). Qi *et al.* [26] reported that the change in vegetation from *Stipa baicalensis* steppe to arable land (from 1953–2000) in Inner Mongolia, China caused a 28.2% reduction in SOC amount in the top 0–10 cm depth, 26.8% in the 10–20 cm depth and 20.5%

**Table 1** Influence of conversion of grassland into forests and shrubland on SOC pool size, residence time, bulk density, total soil respiration, heterotrophic respiration and contribution of root respiration (adopted from Wang *et al.* [22])

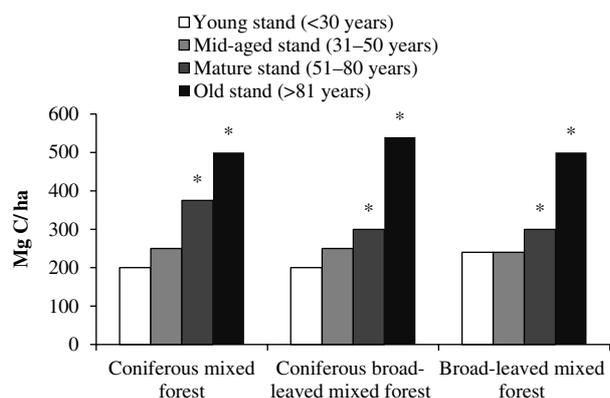
Vegetation type	SOC (Mg/ha)	SOC residence time (yr)	Bulk density	Soil respiration (Mg C/ha/yr)	Heterotrophic respiration (Mg C/ha/yr)	Contribution of root respiration (g/kg)
Grassland	14.767	3.4	0.92	4.504	4.290	47
~45 years old deciduous broad-leaved forest	28.307*	6.3	0.69*	5.489*	4.505	179
~15 years old evergreen coniferous forest	20.227	5.3	0.98	4.648*	3.800	183
~15 years old deciduous coniferous forest	29.939*	7.7	1.06*	5.137*	3.902	240
Shrubland	18.415	5.0	0.71*	4.795*	3.668	235

Values with \* within a column are significantly different ( $P < 0.05$ ) from the grassland ecosystem.

**Table 2** Amount of SOC (g/kg) in soils of various land use (data summarized from Sun *et al.* [23] and Qi *et al.* [24])

Region	Soil depth (cm)	Soil type	Type of land use			Reference
			Forest	Grassland	Arable land	
China (Heilongjiang) Canada (Alberta)	0–20	Kastanozem (heplic) for Chinese soil and Albic Luvisol for Canadian soil	67.1 ± 5.3*	30.3 ± 0.99	41.2 ± 1.58	[23]
			22.6 ± 3.25	24.8 ± 1.37	24.5 ± 1.69	
Region Inner Mongolia China	0–10 10–20	Chernozem (loam)	Stipa baicalensis steppe			[24]
			29.5 ± 2.7	18.10 ± 1.0	18.5 ± 0.8	

Values followed by \* are significantly different ( $P \leq 0.05$ ) other values of a given region [23].



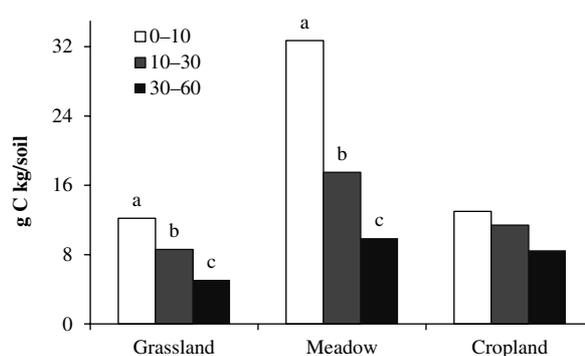
**Figure 1** SOC amount in various forest stand types of different ages (adapted from Wei *et al.* [25]). The bars with \* are different at  $P \leq 0.05$  within a given stand type, between age classes.

in the 20–30 cm depth (Table 2). Sun *et al.* [23] also found that forest had a significantly ( $P < 0.05$ ) greater amount of SOC than grassland and shrubland (Table 2). Li *et al.* [19] reported that the SOC stocks in top 30 cm depth of cold-temperate forest ecosystems in Japan were 12–20 kg/m<sup>2</sup> and were positively correlated with stand density, aboveground biomass and mean tree height ( $P < 0.01–0.05$ ). Likewise, in a study with three temperate forest types: coniferous mixed forest, coniferous broad-leaved mixed forest and broad-leaved mixed forest in north-eastern China, Wei *et al.* [25] reported that forest stands that were older than 51 years had significantly ( $P < 0.05$ ) more organic carbon than younger forest stands (Figure 1).

Sandy soils typically have lower SOC content than clayey soils, due to greater physical protection of SOC in association with clay minerals [27]. The SOC concentration generally declines with depth, as shown in Figure 2, due to the fact that the C input to the SOC pool comes largely from dead organic residues deposited on the soil surface or within the topsoil layer, where fine root biomass is concentrated and turns over on an annual or semi-annual basis. Soil erosion redistributes SOC across the landscape and may contribute up to 0.5–0.085 Mg SOC/ha in various land-use types in cold-temperate climate in Coshocton, Ohio [28], but erosion constitutes a relatively minor input to the SOC pool in most temperate ecosystems [28, 29]. Lal [30] reported that the global amount of total C displaced by erosion may be 4.0–6.0 Pg/year.

### Factors Affecting Soil Organic Carbon Pool

Dead organic residue undergoes two major processes: (1) it is metabolized as a C substrate by microorganisms and is lost from the system as carbon dioxide (CO<sub>2</sub>) and (2) it is transformed into humus, which is recalcitrant to mineralization. The transformation to humus may be



**Figure 2** SOC amount of various land uses, at three depths (0–10, 10–30 and 30–60 cm). Bars with \* are different at  $P \leq 0.05$  between depths within a given land use (modified from Wang *et al.* [22]).

achieved through biotic processes (e.g. microbial byproducts that are stabilized in organo-mineral complexes and become physically or chemically resistant to further decomposition *sensu* [10]) or abiotic processes, e.g. quinone reduction, adduction of amino acids to phenols, reducing sugars etc. as documented by Scott *et al.* [31] and Fukushima *et al.* [32].

### Decomposition Leading to CO<sub>2</sub> Loss from the SOC Pool

The amount of CO<sub>2</sub> released from decomposition of SOC pool depends on biotic factors, litter type, environmental conditions, soil texture and stand type.

#### Biotic controls

Soil microbial diversity influences decomposition as different microorganisms have different preferences and efficiency for mineralizing a given organic substance [e.g. 33–37]. There is empirical evidence for a positive relationship of microbial diversity with catabolic and functional diversity of microorganisms [38–43]. Soil microbial responses depend upon the amount of plant and litter biomass, plant and litter diversity, and the level of SOC in the soil environment [38–43]. For example, Liu *et al.* [40, 41] observed highly significant positive correlations (on average  $P < 0.0001$ ) of biomass, catabolic diversity, catabolic activity and catabolic evenness of microorganisms with the aboveground plant biomass, root biomass and nitrogen:phosphorus ratio in soils of temperate grassland and temperate steppe ecosystem in Inner Mongolia, China. In another study, Thoms *et al.* [42] found a positive relation of microbial diversity and total microbial phospholipid fatty acid (as an indicator of microbial biomass) with leaf litter diversity in temperate deciduous forest in Thuringia, Central-Germany. Zhang *et al.* [43] also found that the natural level of SOC was positively

**Table 3** Amount of organic C, soil microbial biomass, total bacterial PLFAs, total actinomycetes PLFAs and total fungal PLFAs in 0–50 cm depth soils of hilltop (HT) and foot slope (FS) areas from litter protected (+L) and litter removed (–L) research plots at ecological Experimental station, Chinese Academy of Sciences, Yingtan, Jiangxi Province, China (adapted from Zhang *et al.* [43])

	Organic C (g/kg)		Soil microbial biomass (mg/kg)		Total bacterial PLFAs (nmol/g)		Total actinomycete PLFAs (nmol/g)		Total fungal PLFAs (nmol/g)		Total PLFAs (nmol/g)	
	+L	–L	+L	–L	+L	–L	+L	–L	+L	–L	+L	–L
HT	11.8	13.0	115.1	193.5	58.8	83.1	8.2	9.8	4.4	4.5	73.5	100.3
FS	33.2 <sup>*a</sup>	13.0	522.8 <sup>*</sup>	472.2	228.6 <sup>*a</sup>	89.2	30.7 <sup>*a</sup>	10.5	13.6 <sup>*a</sup>	7.6	281.5 <sup>*a</sup>	111.0

Values with \* are significantly different between HT and FS treatments (within column differences). Values with lowercase letters indicate significant differences between LP and LR treatments ( $P < 0.05$ ).

related to microbial biomass carbon and total bacterial, actinomycetes and fungal PLFAs (Table 3).

The C use efficiency of microorganisms controls how much organic C from substrates is converted into CO<sub>2</sub>, where a higher C use efficiency means more C retained in microbial cells. Moreover, the degree of influence of microbial community structure and diversity on CO<sub>2</sub> emission from decomposing C substrates is also related to the level of SOC in the soil environment [e.g. 22, 33, 44]. For instance, Maire *et al.* [33] found a positive relationship between the amount of SOC and microbial biomass, microbial (richness of fungal and bacterial PLFA) and faunal species richness, whereas the CO<sub>2</sub>-ATP ratio was lower with greater SOC concentration. Despite the greater level of SOC in a 15-year-old forest stand, Wang *et al.* [44] found less heterotrophic respiration (3.90 Mg/ha/year) in the forest soil than in soil under shrubland (4.29 Mg/ha/year) in a temperate region of Inner Mongolia, China (Table 1). Similarly, Gong *et al.* [45] found that old forest stands had greater amounts of SOC but less microbial soil respiration than young forest stands, despite the greater microbial biomass carbon in old than young forest stands in northern China. Birkhofer *et al.* [46] found that the agricultural land in a cold-temperate region with long-term organic wheat farming system had significantly higher SOC concentration (15.5 g/kg organic C,  $P < 0.05$ ) than the conventional wheat farming system, which received inorganic fertilizer (13.5 g/kg organic C). The long-term organic wheat system also had ~50% higher MBC, ~60% higher microbial biomass nitrogen, ~27% more total PLFA, 40% higher MBC:SOC ratio, and 43–50% larger populations of nematodes, spiders and earthworms ( $P \leq 0.05$ ) than the conventional system. Moreover, during a 1 week of incubation of soil at optimum laboratory conditions, they found 42% less microbial respiration in the soil from the organic wheat system than the inorganic wheat system ( $P < 0.001$ ), suggesting higher C use efficiency of microorganisms in the soils with more SOC.

Soil fauna contribute to SOC mineralization directly, by consuming and metabolizing decomposing organic matter, and releasing CO<sub>2</sub> as a byproduct of their metabolism. Indirectly, soil fauna facilitate SOC decomposition by their symbiotic gut microflora (bacteria and protists) that degrade cellulose and hemicellulose efficiently [e.g. 47]. Moreover, soil macrofauna are responsible for translocation of organic residues, which enhances microbial activities in soil [e.g. 48], making habitat for other soil fauna and microorganisms to invade their nests, burrows, etc. [49–52], which enhances microbial activities in the soil as new substrates are incorporated. In addition, the soil restructuring of soil macrofauna (including aggregate formation via their casts and pore creation with their burrows) provides habitats for microorganisms and soil micro- and meso-fauna to access those substrates. In a 5-week microcosm-based laboratory study under elevated CO<sub>2</sub> with four leaf litter types from trees in eleven

different combinations/treatments, with and without the millipede species *Glomeris marginata*, Rouifed *et al.* [53] reported that mass loss of litter in the presence of *G. marginata* was ~70–370 g/kg higher than the litter without the presence of *Glomeris* (significant for five out of eleven litter type combinations). In a microcosm study with alder litter mixed with clay spoil, exposed in a forest field of cold-temperate climate for 1 year, Frouz [54] reported four times greater CO<sub>2</sub> production from microcosms that were accessible to macrofauna than the control (microcosms not accessible to macrofauna).

#### Abiotic controls

**Soil physicochemical characteristics.** Soil temperature and soil moisture are positively related to SOC decomposition [e.g. 24, 55–59]. Warming accelerates the proteolytic enzyme activity of soils at moderate soil moisture contents [60], indicating that the impact of soil temperature on SOC mineralization is moisture dependent. Based on radioactive <sup>14</sup>C analysis, Frank *et al.* [61] reported that the turnover time of SOC of northern boreal forests, tundra and deserts was 1277–2151 years, whereas upland and bottomland tallgrass prairie (moister and comparatively warmer than tundra and northern boreal forest) had SOC turnover times of 158 and 260 years, respectively, and the shortest SOC turnover time was 60 years for tropical forests (warmer and moister than the other biomes). This example illustrates how soil temperature and moisture exert a fundamental control on decomposition and turnover of the SOC pool, which is expected to be affected at a global scale due to climate change. Soil parent material and texture are the major determinants of soil physical properties such as structure, hydrology, gas diffusion and heat-transfer. These characteristics also depend on the SOC content, which has a role in water retention and promotes aggregation, which in return increase aeration [e.g. 62] and may also participate in regulation of soil temperature directly and indirectly by affecting plant growth and the plant-associated microbial community.

Soil chemical characteristics such as pH levels influence the activity of soil biota responsible for SOC transformations. Soil pH was identified as an important controller of decomposition in a review by Walse *et al.* [63]. Bacterial growth rates are more sensitive to low pH levels than fungi growth rates due to the effect pH exerts on the activity of extracellular enzymes and the metabolic functions of cell (see also section 'Biotic controls'). Bacteria are known to decompose easily decomposable material (e.g., cellulose is hydrolysed with cellulases that have an optimal pH range of 5–8 [64–69], with no reduction in

their activity at pH 10, as reported by Dilek and Ozlem, [70]. Fungi contribute more to the decomposition of recalcitrant matter such as lignin with extracellular laccase enzymes [optimum pH of 3–6 e.g. 71–75]. Soil pH also influences the adherence of OM to mineral surfaces and subsequently its decomposition rate. As pH increases, hydroxylated surfaces become increasingly negatively charged and thus more repulsive to negatively charged OM [76, 77], causing organic molecules to remain in soil solution where they are more susceptible to hydrolysis by extracellular enzymes. When residue inputs exceed decomposition rates, resulting in net SOC accumulation through organo-mineral complexes, pH buffering and higher cation exchange capacity are expected [78–80].

**Chemistry of organic residue.** The chemical nature of organic residue is an important determinant of its influence on SOC dynamics. Readily available organic C has a positive priming effect on both residue and SOC decomposition [81–83]. Lignin, C:N ratio, lignin:N ratio are important determinants of the mineralization of plant residue and residual SOC [36, 38, 77]. Lignin provides protection to plant residues against decomposition and subsequent N mineralization [9,36]. Moreover, as the level of SOC in the soil environment and soil aggregation are positively related, as fragments of undecomposed plant residues and complex polymers (i.e., lignin and cellulose) are protected physically from biodegradation within soil aggregates. This phenomenon is well explained in Gul and Whalen [36], Blanco-Canqui and Lal, [84] and Gul *et al.* [9].

#### Humification

Humification is the biological process of conversion of organic matter into non-tissue colloidal heterogeneous substance known as humus. It is the outcome of the alteration of biologically derived organic substances into chemically complex organic substances that resist further biochemical degradation [78]. Humus is a recalcitrant fraction of SOC and its turnover time ranges from decades to several millennia [10, 78, 85–88]. Generally, humus contains plant- and animal-derived organic substances, microbial byproducts (e.g. extracellular enzymes and lysed cell contents) and other substances, which may be plant- and animal-derived organic substances that were covalently linked with microbial byproducts by enzymatic activities, i.e., newly biosynthesized compounds [see 20].

Plant residues have diverse chemical composition and therefore have different rate of decay in soil. Due to their higher concentration of lignin, stems and roots decompose 1.5 and 2.8 times slower than leaf residue [meta analysis by Langley and Hungate 89]. In a field litter bag

study, Katterer *et al.* [90] reported that the humification coefficient of root-derived C (including rhizodeposits) was 2.3 times higher than the same amount of above ground residue C in the long-term (50 years) agricultural land in Sweden cultivated with nine different grass and leguminous annual crops. Studying humification of oak and beech litter buried in field in temperate deciduous forest in Japan, Ono *et al.* [91] observed that mass loss for O-alkyl group (hemicellulose and cellulose) was 38 and 44% for oak and beech litter respectively, for aromatic carbons (lignin and acid-soluble polyphenolics) it was 17–6%, for aliphatic carbons it was 20–10% while carbonyl carbons (C=O containing organic substances such as certain flavonoids, carbonyl-nicotinamide, etc.) were very stable with only 2% mass loss during a 3-year period. The chemistry of lignin determines its residence time in soil. For example, lignin with higher guaiacyl:syringyl (G:S) or guaiacyl:*p*-hydroxycinnamyl (G:H) lignin monomer ratio degrades slower than the lignin with lower G:S or G:H ratio [92–97].

Soil animals possess diverse organic substances in their body tissues that have variable biochemical resistance to decomposition in soil [e.g. 98]. Many soil arthropods produce chitins, such as egg shells of soil fauna (e.g. nematodes) that have chitinous layers, which are resistant to decomposition compared to other animal-derived organic substances (e.g. earthworm mucus) [99]. Earthworms secrete mucus to lubricate internal and external body surfaces and facilitate their movement through soil. In a microcosm-based incubation study with *Octolasion lacteum*, incubated in soil obtained from a beechwood forest on limestone from Northern Germany, Scheu [100] found that the daily C loss from mucus secretion from the body surface and in freshly-deposited casts was 0.2 and 0.5% of total animal C, respectively. Chen *et al.* [101] reported that in 24 h time period, earthworms produce 5.6 mg mucus/g fresh weight biomass. Earthworm mucus contributes to humification of plant residue. For example, Bityutskii *et al.* [99] observed that addition of earthworm mucus in fresh leaf tissue of *Elytrigia repens* mixed with quartz sand caused 2.25 mg C humification/g plant tissue in 30 days of incubation while the control soil (plant-sand mixture without mucus solution) had 1.57 mg C humus/g plant tissue during the same period ( $P < 0.05$ ).

Microbial-derived organic substances constitute the majority of stable SOC fraction, and the remainder is undecomposed plant- and animal-derived organic substances [86, 102–106]. Simpson *et al.* [107] reported that microbially derived organic matter was ~45% of humin fraction, >50% of the extractable SOC fraction and accounted for >80% of soil nitrogen in the 0–15 and 15–40 cm depth of brunisols from pine forest, native prairie grasses, and mixed aspen-dominated forest, based on NMR spectroscopy.

Microbial-derived extracellular secretions contain various types of lipids, polysaccharides, melanin, enzymes and

polyketides [e.g. 20, 98]. The longer residence time of microbial-derived organic substances than plant or animal residues in soil is due to (1) their strong bonding with mineral complexes [e.g. 20, 108–111] and due to the fact that (2) during the process of decomposition, microbial-derived organic substances depolymerize and repolymerize with other decomposing organic substances (e.g. plant derived polyphenolics) to produce stable biomolecules [e.g. 20, 58, 102]. Metadata analysis based on  $^{13}\text{C}$  tracer studies of SOC in surface horizons of 20 long-term field experiments in temperate climates revealed that the residence time of soil polysaccharides is approximately 5–120 years, microbial-derived proteins may persist ~30–65 years, phospholipid fatty acids (PLFA) of gram positive and gram negative bacteria and bacterial hexosamines may last ~5–75 years in soil [10]. Likewise, based on pyrolysis-field ionization mass spectrometry (Py-FIMS) analysis of soil, the putative polyketides as aromatic, alkylaromatic, phenolics and lipid pyrolytic products in humus are reported to have the residence time >1000 years and represent the majority of SOM in clay fractions [see 20]. The long residence time of microbial-derived polyketides is attributed to their high adsorption to inorganic colloids [see 20].

The size of SOC pool and the fraction of recalcitrant organic substances are positively related [112]. Based on a study of SOC turnover times in soil horizons of two temperate agricultural lands and two temperate acid forests, Lutzow *et al.* [112] concluded that the selective preservation of recalcitrant organic substances from active C pool (e.g. fresh root exudates, fresh plant residues, faunal and microbial residues and faunal feces) is positively related to the SOC concentration, which is consistent with other reports [113–116]; as well, a positive relationship between SOC, microbial biomass and humification is expected [114, 117]. In an 8-year field experiment with conventional versus organic farming system in cold-temperate climate, Marinari *et al.* [114] reported that the soil from 5–20 cm depth of organic farming system had 41% higher MBC, 36% higher humification rate and 32% higher degree of humification as compared to conventional farming. A review of the ecological significance of humus in soil ecosystem services is provided in Gul *et al.* [9].

### Bioenergy Crops and their Influence on SOC Dynamics

In this review, we focus on second generation bioenergy crops and their influence on SOC dynamics as the residues of these crops are removed from field to be utilized for bioenergy production. Several studies reviewed potential lignocellulosic biomass sources for bioenergy, considering the environmental impacts associated with their cultivation, processing and use [e.g., 118]. These crops include cereals, perennial grasses and trees.

### Annual Crops

The residues of annual crops such as corn, wheat, oat, barley and rice are being used as a source of bioenergy [e.g. 119–124]. Lal [5] reported the estimated annual lignocellulosic residue production from cereal crops for the world as 2800 million Mg/year with 367 million Mg/year in the US alone. The average annual crop stalk production at Inner Mongolia Autonomous Region, China is estimated to be increasing by 16.3% with corn stalk accounts for 60% of corn production [125]. England has the potential to produce 5.27 Mt of cereal straw from arable farms, 62% of which is currently used for bioenergy and is equivalent to ~1% petrol consumption demand per annum [122].

Removing non-food residues from cereal crops will generally have a negative effect on SOC levels in northern/cold-temperate climates [6, 115, 126–128]. Blanco-Canqui and Lal [6] reported that the removal of 75% corn stover caused 26% reduction in SOC amount in upper 0–2 cm soil depth in silt loam soil in Coshocton Ohio over 2½ years whereas in clay loam soil in Hoytville Ohio the effect was non-significant. Using same field sites, they [127] reported that ≥25% corn stover removal from field for 4 years reduced macroaggregates (>4.75 mm) by ~40%. Using the AMG simulation model with data from eight long-term agricultural fields in a cold-temperate climate, Saffih-Hdadi and Mary [115] predicted that straw removal would reduce SOC levels by 2.5 to 10.9% SOC n after 50 years of those fields. Laird and Chang [128] reported that removal of ~90% of residues from continuous maize (12 years) and continuous soybean (7 years) fields near Rosemont Minnesota reduced the organic C by 12% total N by 12.6%, cation exchange capacity by 7.3%, total respiration by 12.3%, N mineralization potential by 27.7% and macroaggregation by 13% in the 0–15 cm depth. Likewise the study based on residue removal influence on SOC contents in 47 fields (grown with annual crops) within Midwestern USA and Canada, revealed a decline in SOC content by 0.5 to 32% in the top 20 cm depth in 29 fields, depending on the degree of residue removal, tillage practice, duration and fertilizer application [129]. While these results almost universally indicate a reduction in SOC content following the harvest of non-food residues from cereal and grain-based cropping systems, further study is warranted to evaluate the influence of crop residue removal on SOC dynamics (i.e. humification, microbial biomass/abundance, amount of dissolve organic carbon and particulate organic carbon).

### Perennial Grasses

Perennial crops extensively investigated for bioenergy production in northern temperate climates include switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus*

*sp.*), reed canarygrass (*Phalaris arundinacea*), alfalfa (*Medicago sativa*) and giant reed (*Arundo donax* L.) [130–134]. Other species under consideration for bioenergy production include bermudagrass (*Cynodon dactylon*), napiergrass (*Pennisetum purpureum*), eastern gamagrass (*Tripsacum dactyloides*), and prairie cordgrass (*Spartina pectinata*) [131]. However, due to competition with food crops and pastures for domestic animals, limited land is available for the cultivation of dedicated bioenergy crops [121]. In the United States, out of 182 million ha, only 30 million ha of land can be available for alternative use [135]. In Europe, England, Ireland, Finland, Sweden, Denmark and Italy are the major producers of dedicated bioenergy crops using 19 000, 2905, 18 700, 13 865, 4285 ha of land respectively [136]. The native high-diversity prairies can provide higher useable energy per hectare as compared to soybean and corn [137, 138]. Tilman *et al.* [137] found a linear increase (84–238%) in bioenergy production per ha plant harvest with increasing number of native prairie plant species (2–16 species) than in monocultures. Over 20 years of study in Midwestern US states, Gelfand *et al.* [138] reported that the successional herbaceous vegetation (probably perennial prairie vegetation), once established on marginal lands and fertilized properly, has the capacity to produce  $63 \pm 5$  GJ of energy/ha as compared to  $41 \pm 1$  GJ from no-till corn–soybean–wheat rotation.

The energy output from perennial grasses is expected to be much higher than that from annual crops. Data from commercial farms in an agricultural region of western Germany showed that net energy production by rapeseed, maize and miscanthus was 66 GJ/ha, 91 GJ/ha and 254 GJ/ha/year respectively, using the German Association for Technology and Structures in Agriculture (KTBL) online calculator to model diesel fuel consumption of agricultural machinery Felten and Emmerling [139]. Moreover, the energy output:input ratio for miscanthus was ~10 times higher than rapeseed and ~8.5 times greater than maize. Angelini *et al.* [140] reported ~74% higher energy yield of *A. donax* than *Miscanthus* during 9 years of cultivation in temperate climate of central Italy.

Perennial grasses have considerable potential to sustain or increase SOC reserves. Perennial grasses have higher root:shoot ratio and more root distribution than annual crops [e.g. 141, 142]. Zan *et al.* [141] reported 5 times higher root biomass, ~20% greater total biomass, and ~4–6 times greater root:shoot ratio of switchgrass as compared to corn in farms in southwestern Quebec. Root exudation and fine root turnover contribute to the pool of labile C substrates that can be readily metabolized by soil microorganisms and either used for metabolic processes or transformed into SOC via humification. Compared to arable and bioenergy annual crops, greater SOC sequestration is expected for these perennial crops due to long root life, greater root biomass and rhizodeposition [e.g., 133, 135, 143–148].

Gelfand *et al.* [138] reported GHG mitigation potential of well-established successional vegetation in Midwestern US as  $-851 \pm 46$  g of  $\text{CO}_2/\text{m}^2/\text{year}$  as compared to  $-397 \pm 46$  g of  $\text{CO}_2/\text{m}^2/\text{year}$  for no-till corn–soybean–wheat rotation. Likewise, in 10-year-old bioenergy cropping systems, with corn, switchgrass and prairie in Southern Wisconsin USA, Liang *et al.* [149] reported  $\sim 2$  times lower microbial biomass (based on total PLFA concentration) in soil under corn production as compared to the prairie, while soil under switchgrass had intermediate soil microbial biomass that was not significantly different from that of corn and prairie soils.

Perennial grasses also prevent SOC loss through erosion by providing year-round soil cover, which also favours protection of the physically-bound SOC in aggregates, since the soil is not tilled [134, 148, 150]. Other benefits of perennial grasses include lower N leaching, due to the fact that perennial grasses typically require less N fertilizer [see 151, 152] than annual crops like corn (up to 200 kg N/ha/year according to Iowa State University Agronomy Extension calculator using the central Illinois dataset (<http://extension.agron.iastate.edu/soilfertility/nrate.aspx>)). For instance, Smith *et al.* [153] reported 83–89% lower N leaching from the soil planted with switchgrass and prairie as compared to corn–corn–soybean plots during second, third and fourth years of growth ( $P < 0.05$ ) while for miscanthus the N leaching was 55 and 98% lower than annual crops during that period. The fields grown with annual cereals received approximately three times more fertilizer than the plots grown with switchgrass. The lower leaching of N from soils grown with perennial crops can also have a positive role in the biomass production of bioenergy crops [e.g. 154].

Table 3 summarizes the SOC sequestration potential and greenhouse gas emissions from select bioenergy crops, which varied due to soil type, initial soil conditions and climate [e.g., 155]. Perennial grasses favour higher microbial biomass, which could be important for converting plant residue C into humus [e.g. 118, 147, 156]. Haney *et al.* [118] reported that 10-year-old monocultures of switchgrass, sideoats grama, coastal bermudagrass and buffalograss had 54, 70, 64 and 76% greater SOC contents, respectively and 35, 59, 42 and 62% higher MBC concentration, respectively, than a long-term corn field ( $P < 0.05$ ). They also observed significantly higher C and N mineralization in soils under perennial grasses than corn, which is interpreted to mean that cultivation of perennial grasses favours the accumulation of a pool of labile SOC that is physically protected from decomposition until the soil is sieved in preparation for C and N mineralization in the laboratory. The effect of perennial grasses on SOC pools, including microbial biomass and soil fauna, is most noticeable when they are compared to soils that are tilled for annual crop production. In contrast, Liang *et al.* [149] reported no influence on soil microbial biomass of a 10-year-old stand of switchgrass

when compared to a mixed prairie in southern Wisconsin USA. No difference in the abundance and number of earthworm species was observed when comparing a 14-year-old miscanthus stand to uncultivated fallow and grassland soils in Trier Germany [139].

### Woody Crops

Short-rotation woody species, forest residues, and non-renewable forest biomass, such as 'disturbance' wood are considered to have a high potential to meet bioenergy production needs in Canada [16]. Fast-growing woody species investigated for bioenergy production include *Populus* sp. and hybrids, *Salix* sp. and hybrids, and *Pinus* sp. [157, 158]. In their evaluation of nutrient use efficiency and SOC sequestration potential of woody crops, Sochacki *et al.* [159] concluded that tree harvesting strategies and nutrient management could be employed (i.e. harvesting strategies that allow for the reapplication of woody biomass wastes after processing, incorporation of legumes) to improve SOC sequestration in the bioenergy production system [159]. To reduce environmental impacts associated with lignocellulosic biomass cultivation, these potential bioenergy feedstock sources would ideally exhibit high nutrient- and water-use efficiency and requires low external inputs (fertilization, irrigation) [e.g., 160].

Woody crops grown for bioenergy have a positive influence on SOC content and soil aggregation as compared to annual crops [e.g. 161–163]. This is due to the absence of soil disturbance (no tillage) when trees are grown and the greater root biomass of trees than annual crops. Greater fungal:bacterial (F:B) ratio is reported for bioenergy poplar and aspen as compared to annual crops in cold-temperate climate [e.g. 162, 164]. Yannikos *et al.* [164] reported  $\sim 1.5$ – $2.5$  times greater F:B ratio in soils under aspen and 7 and 14 years of short rotation of coppice (SRC) system of hybrid poplar as compared to alfalfa fields in Orthic Gray Luvisols in Saskatchewan, Canada. They also reported higher contents of phenols, lignin monomers and pentose and hexose carbohydrates in the 0–10 cm of soil depth from coppice and aspen fields than alfalfa fields. Rytter [165] estimated that the cultivation of bioenergy polar and willow in abandoned arable lands in Sweden has the potential to sequester 0.4–0.5 Mg C/ha/year and can accumulate 9–10.3 Mg C/ha in soil over first 20–22 years. However, experimental measurements do not necessarily support these predictions. Pacaldo *et al.* [166] found no difference in SOC contents in soil under 0, 5, 12, 14- and 19-year-old stands of shrub willow biomass crops with almost the same planting density, evaluated in the 0–15, 15–30 and 30–45 cm soil depths. Dowell *et al.* [167] reported reduction in SOC contents by 46% in the upper 0–12.5 cm depth after 5 years of bioenergy poplar growth on a site that was previously a permanent pasture. Compared to pastureland, bioenergy

willow and poplar plantations in northern Michigan had about ~15 and 20 times higher losses of N (as  $\text{NO}_3^-$ ) and lower root respiration during the first 2–3 years of the plantation [168]. There are a few reasons why SOC gains may not be observed when agricultural land is converted for woody crop production: (1) land preparation, including plough-down of pastures and land levelling, can reduce the SOC content, (2) in the first year after establishment, the C inputs from tree litterfall and fine root turnover may be less than the annual C inputs of the previous vegetation (especially if it was permanently vegetated with grasses), (3) SOC accumulation is affected by soil texture, and there is a finite SOC saturation capacity depending on the mineral surface area where SOC can be adsorbed [169, 170], (4) measurement of SOC content in the top 15 to 45 cm may not capture C inputs from tree roots that extend deeper in the soil profile, and (5) SOC pool size (considering SOC content and soil bulk density) is a more accurate way to evaluate the SOC accrual in soil.

### Genetically Modified Bioenergy Crops

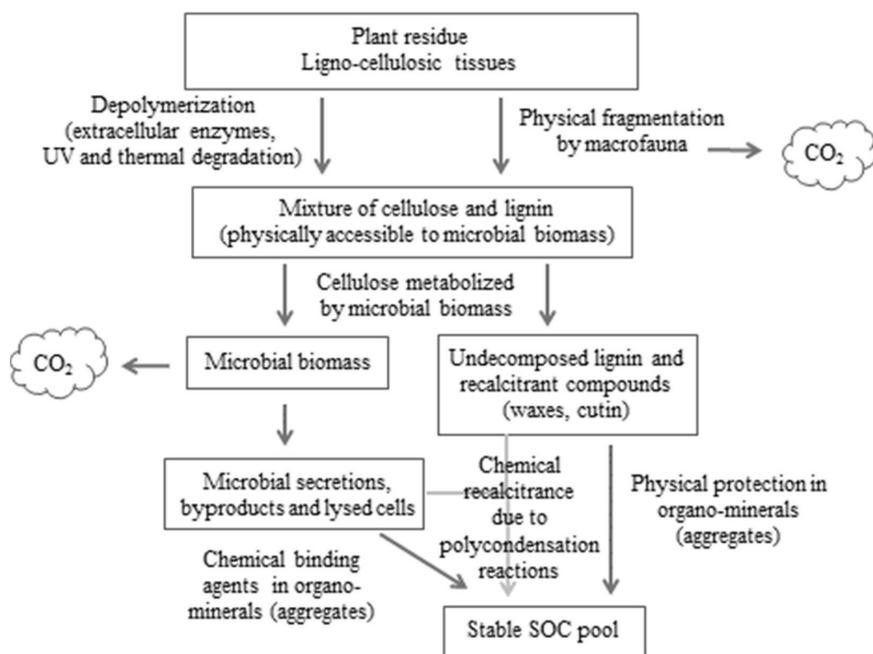
Optimal traits for bioenergy production are well studied for crops such as maize [171] and poplar [172], and there are a range of traits, determined by major genes or quantitative trait loci, that are researched using existing genetic variations [173] or genetic modification (GM) technologies [174]. Karp and Shield [175] assessed GM traits for bioenergy crops, and identified three main challenges in the context of sustainable production systems: (1) changing the 'thermal time sensitivity to extend the growing season'; (2) increasing aboveground biomass without depleting belowground biomass; and (3) increasing aboveground biomass without limiting water availability. The DOE Great Lakes Bioenergy Science Center (GLBSC), led by the University of Wisconsin-Madison in partnership with the Michigan State University, conduct research to produce GM crops with high energy output and high-level expression of target proteins. A major focus is the development of synthetic enzyme mixtures to evaluate the best enzymes and optimal enzymatic ratios for efficient digestion of cellulose [176–178]. The core set of enzymes under investigation includes endoglucanase,  $\beta$ -glucosidase, cellobiohydrolase,  $\beta$ -glucosidase and endoxylanase [178]. This initiative is an example of the research efforts underway worldwide to improve the conversion efficiency and energy output from bioenergy crops.

Genetic modifications aim to achieve high production of bioenergy per unit weight of crop residue by increasing their conversion efficiency (i.e., the digestibility of the lignocellulosic feedstock). These modifications involve: (1) crossing over (2) reducing the concentration of lignin, (3) reducing the G units in lignin, (4) reducing the degree of polymerization of lignin, (5) introducing the amide and

ester interunit linkages in lignin polymers and (6) cellulase/ligninase enzyme biosynthesis in plants [e.g. 123]. A detailed account of these advancements for bioenergy crops was provided by Hirasawa *et al.* [123]. In short, many strains of miscanthus are developed by crossing over the miscanthus species in European Miscanthus Improvement Project [123]. Genetic modifications regarding reducing the concentration of lignin in bioenergy crops involve the down regulation of Cinnamoyl CoA Reductase 1 (CCR1), Cinnamyl alcohol dehydrogenase (CAD), Coniferaldehyde 5-hydroxylase (CALD5H), Caffeoyl CoA 3-O-methyl transferase (CCOMT), Caffeic acid O-methyl transferase (COMT), *Populus tremuloides* 4-coumarate:coenzyme A ligase (Pt4CL1), MYB61, MYB2, MYB308 etc. [62, 123]. To decrease the polymerization of lignin, a monolignol 4-O-methyl transferase is synthesized by the substitutions of amino acid residue on the active site of isoeugenol 4-O-methyl transferase enzyme [179]. This mutated enzyme methylates monolignol precursors and therefore prevents laccases and peroxidases from catalyzing their further synthesis into lignin polymers. Another advance in bioenergy crops is the introduction of easily hydrolysable amide and ester interunit linkages into lignin polymers, which can be achieved by upregulation of hydroxycinnamic acid amides [123].

Although these genetic modifications would eliminate the need for extensive pre-treatment of the lignocellulosic feedstock, they must be compatible with the phenotypes and cultivars selected for field cultivation. Many of these mutations reduce the growth and biomass accumulation in herbaceous plants [see 62] while for trees, higher growth rates or no influence of these mutations is reported. For instance, an antisense inhibition of Pt4CL1 gene in *Populus tremuloides* caused enhanced leaf, stem and root biomass, with 45% reduction in lignin and 15% increase in cellulose content in the stem [180]. Likewise, Leple *et al.* [181] found no difference in the growth of transgenic poplar tree with CCR1 down regulation, which reduces lignin deposition in the secondary cell wall. Therefore, woody crops for bioenergy are likely the best candidate for genetic modification.

Another novel advancement in improving the energy output from bioenergy crops is the modification of various cellulase/ligninase enzymes in crop biomass. Such modifications aimed to depolymerize the crop biomass and ensure its fast hydrolysis to produce ethanol [e.g. 182]. For example, Ziegler *et al.* [183] introduced the catalytic domain of thermostable ( $T_{\text{opt}} = 81^\circ\text{C}$ ) endo-1,4- $\beta$ -D-glucanase from *Acidothermus cellulolyticus* bacteria in *Arabidopsis thaliana* and tobacco (*Nicotiana* spp.) plants, where it was expressed in leaves and in the apoplast of BY-2 suspension cells, respectively. The authors also reported that *A. thaliana* transformants accumulated as much as 26% of total protein in leaves, meaning that protein could be isolated more efficiently by separating leaves from the feedstock. This enzyme had low activity at ambient temperatures and optimum activity at  $81^\circ\text{C}$ ,



**Figure 3** A general mechanism of plant residue transformation into stable SOC by microbial biomass.

which is lower than the usual temperature required for lignocellulosic feedstock predigestion. It implies that such modifications can reduce the cost of biomass hydrolysis by reducing the demand of ligninase and cellulase enzymes from microbial source, moreover; less energy will be required to digest/hydrolyze biomass into ethanol. The introduction of thermostable endo-1,4- $\beta$ -D-glucanase in bioenergy crops holds promise to greater yield of bioenergy with lower cost and energy consumption.

From the perspective of feedstock conversion, there are many advantages to reducing the lignin content in bioenergy crops. However, lignin provides physical protection to cellular components of plants, slowing their degradation in soil [38, 77], and also contributes to the SOC as a complex polymer that can be bound to soil surfaces and physically protected within aggregates, as depicted in Figure 3. Unharvested residues with reduced/modified lignin content are subject to faster rates of decomposition [see 9, 36] and associated degradation of soil quality [e.g. 9, 184].

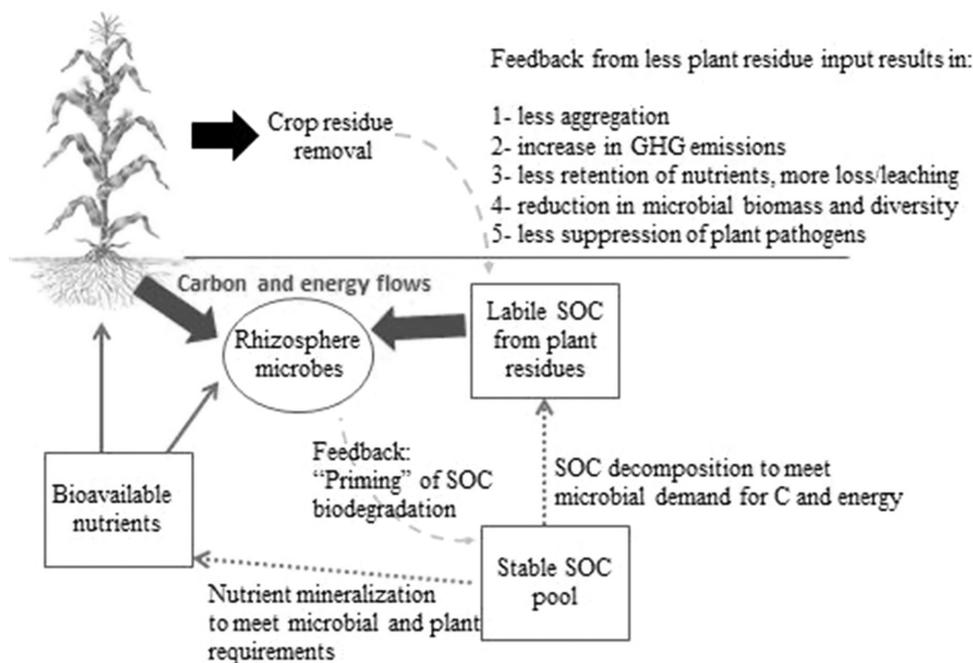
### Management Practices to Sustain Soil Organic Carbon in Bioenergy Cropping System

Bioenergy cropping systems are predicated on the assumption that most, if not all, of the aboveground biomass will be removed from the system and converted to bioenergy. The high level of residue removal is expected to impact soil quality, as nutrient removal by grass-based and woody crop species will nearly always exceed naturally-occurring nutrient inputs due to the limited  $N_2$ -fixing

ability of these crops. As depicted in Figure 4, this would induce a negative feedback whereby soil microorganisms will need to decompose the SOC reserves to sustain their growth and metabolism. If microbial diversity and population levels decline as a consequence of low residue C inputs, other effects such as reduced resistance to plant pathogens could occur. Management practices that sustain or increase SOC content in bioenergy cropping systems need to be adopted to avoid such negative impacts.

### Degree and Frequency of Residue Removal

The influence of residue removal on the SOC pool of annual crops in cold-temperate climates depends on the degree and duration of harvest, tillage system and application of inorganic fertilizer [129]. Muth *et al.* [185] estimated that the sustainable crop residue removal of 26.5 million Mg/year in Iowa, USA could increase to 40 million Mg/year if no till systems were adopted. Likewise, a study conducted in fields in Chazy NY, Moebius-Clune *et al.* [186] reported that >90% maize residue harvest under no-till system had no significant effect on total organic matter in the 0.5–6.6 cm soil depth, but aggregate stability was reduced by 16%. Removing most of the maize residue reduced SOC content ~26% in plow tillage than a no till system ( $P < 0.05$ ). Similarly, Blanco-Canqui and Lal [6, 127] found no influence on SOC content in the 0–15 cm depth after 2.5 years in a no-till system with 25% corn stover removal, but macroaggregation declined by 40% after 4 years of these practices.



**Figure 4** Influence of crop residue removal on the stable SOC pool, considering the priming effect of rhizosphere microbial communities on decomposition and nutrient mineralization required to meet their C, energy and nutrient demands. This diagram is considered to depict the feedbacks in a cropping system with grass-based or woody plants that are under minimal disturbance (e.g. no tillage) and have low nutrient inputs (e.g. no external fertilizer inputs, no legumes).

These studies and the findings of Campbell and Coxworth [187] suggest that in regions with northern temperate climate, under no till system, moderate residue harvest of annual crops ( $\leq 25\%$ ) may not significantly influence SOC amount. Lemke *et al.* [188] predicted a 13% non-significant reduction in the SOC content after 50% crop residue removal from agricultural fields in Saskatchewan, western Canada under fallow–wheat–wheat rotation and no-till system since 1990, based on estimates from the Introductory Carbon Balance Model (ICBM) and Campbell model. They also found that the application of N fertilizer to those fields where wheat straw was removed continuously for 50 years increased the SOC pool significantly, by 3 Mg/ha.

The influence of residue removal on SOC contents in perennial cropping systems may not be as severe as for annual crops due to the fact that perennial crops have an extensive root system and associated higher rhizodeposition. However, the complete aboveground biomass removal of perennial grasses in winter can reduce SOC contents [e.g. 148]. For woody crops such as poplar and willow in cold-temperate climates, frequency of harvest ought to be considered to sustain SOC contents. More frequent harvesting can result in more SOC depletion in the 0–15 cm soil depth [e.g. 167, 189 and references therein]. Shibu *et al.* [189] proposed a 5-year interval between harvests of SRC of poplar and willow bioenergy crops in soils of Scotland, which have SOC contents ranging from 110–360 Mg/ha.

#### **Habilitation of Marginal and Abandoned Lands**

Considerable land is required to grow the lignocellulosic feedstock to meet the demand of renewable energy. For instance, in order to meet the target set by European Union of increasing the renewable energy from 9% in 2010 to 20% in 2020 of total energy consumptions [190], an estimated 17–21 million ha of additional land needs to be converted to bioenergy crop production [191–193]. Likewise the US legislation has a mandate for 80 gigalitres of ethanol production per annum from lignocellulosic feed stock [194] to meet the  $\sim 25\%$  of liquid transportation fuel need by 2050 [195]. The challenge is – where should these bioenergy crops be grown while maintaining SOC contents?

Marginal and abandoned lands are the areas vulnerable to erosion or have low productivity, therefore not suited for food crops [138]. Cultivating lignocellulosic feedstocks on these lands can increase the SOC content of soil. Therefore, putting marginal and abandoned lands back into production will offset the expected SOC depletion in agricultural lands where non-food crop residues are removed for second generation bioenergy production, moreover will not compete with food production [e.g. 138]. Perennial bioenergy crops such as switchgrass and the native successional herbaceous perennial vegetation are adapted to grow in lands with high soil erodibility, reduced fertility and water limitation [138, 156, 196–200]. Gelfand *et al.* [138] reported that native successional herbaceous species established on marginal lands (more

than 11 million ha land in Midwestern US) would have the capacity to fulfil ~25% of 2022 biofuel production mandate of US EISA 2007. They predicted same results if switchgrass were grown on marginal lands in this region. These estimates highlight the potential of marginal lands to support perennial bioenergy crops for both energy generation and GHG mitigation objectives, along with providing other ecosystem services [138].

### Organic and Inorganic Amendments

Organic fertilizers such as compost and animal manure have considerable amount of C (~15%; [201]). Their amendment to soil in cold-temperate climate is reported to have a positive influence on SOC content including microbial biomass [e.g. 202–206]. The application of these fertilizers in bioenergy cropping systems can play a substantial role in sustaining SOC contents. For instance, Thelen *et al.* [120] reported that in corn–soybean–corn rotation system in East Lansing Michigan with >95% corn residue removal, the application of composted dairy manure and cattle feedlot manure at a rate of 22 Mg/ha mitigated the net global warming potential (GWP; equivalent of CO<sub>2</sub>) by –784 and –934 g/m<sup>2</sup>/year, respectively while the field without organic fertilizer had GWP of 52 g/m<sup>2</sup>/year, suggesting a very high C sequestration by organic amendments. Likewise, Fronning *et al.* [207] reported 25 and 41% increase in SOC contents when manure and compost amendments were applied for 3 years to corn–soybean–corn rotation system with >95% corn residue removal, whereas control fields had 3% SOC reduction during the same period.

The effect of organic fertilizer on SOC contents in short-rotation coppice (SRC) system in northern temperate climate is also reported to be positive [208]. For instance, in response to the application of biosolid compost to soil under willow coppice system at the rate of 150 and 200 kg N/ha, Quaye and Volk [208] found 25–38% increase in organic matter in the top 15 cm of soil depth during first and second year of fertilizer application. Johnson [210] reported the optimum N fertilizer in SRC willow as 40, 60 and 100 kg N/ha after first, second and third year of each 3-year cycle. However, the author did not document the influence of N fertilizer on SOC contents was not documented. The influence of N fertilizer on SOC contents in SRC willow depends on its initial status. For instance, Shibu *et al.* [189] reported that in Scotland soils, the application of N fertilizer to poplar and willow had a positive influence on SOC contents in soils that had an initial SOC content less than 180 Mg/ha in upper 0–10 cm depth, but soils with greater initial SOC levels in the upper 0–10 cm depth did not gain more SOC after the second and tenth harvest cycles. Jung and Lal [209] reported linear increase of SOC contents in upper 30 cm depth of soils under switchgrass, in response to 50, 100 and 200 kg N/ha inorganic fertilizer

at two sites at the Ohio Agriculture Research and Development Centre, for an overall gain of 17–21.5% in SOC content.

### Biochar

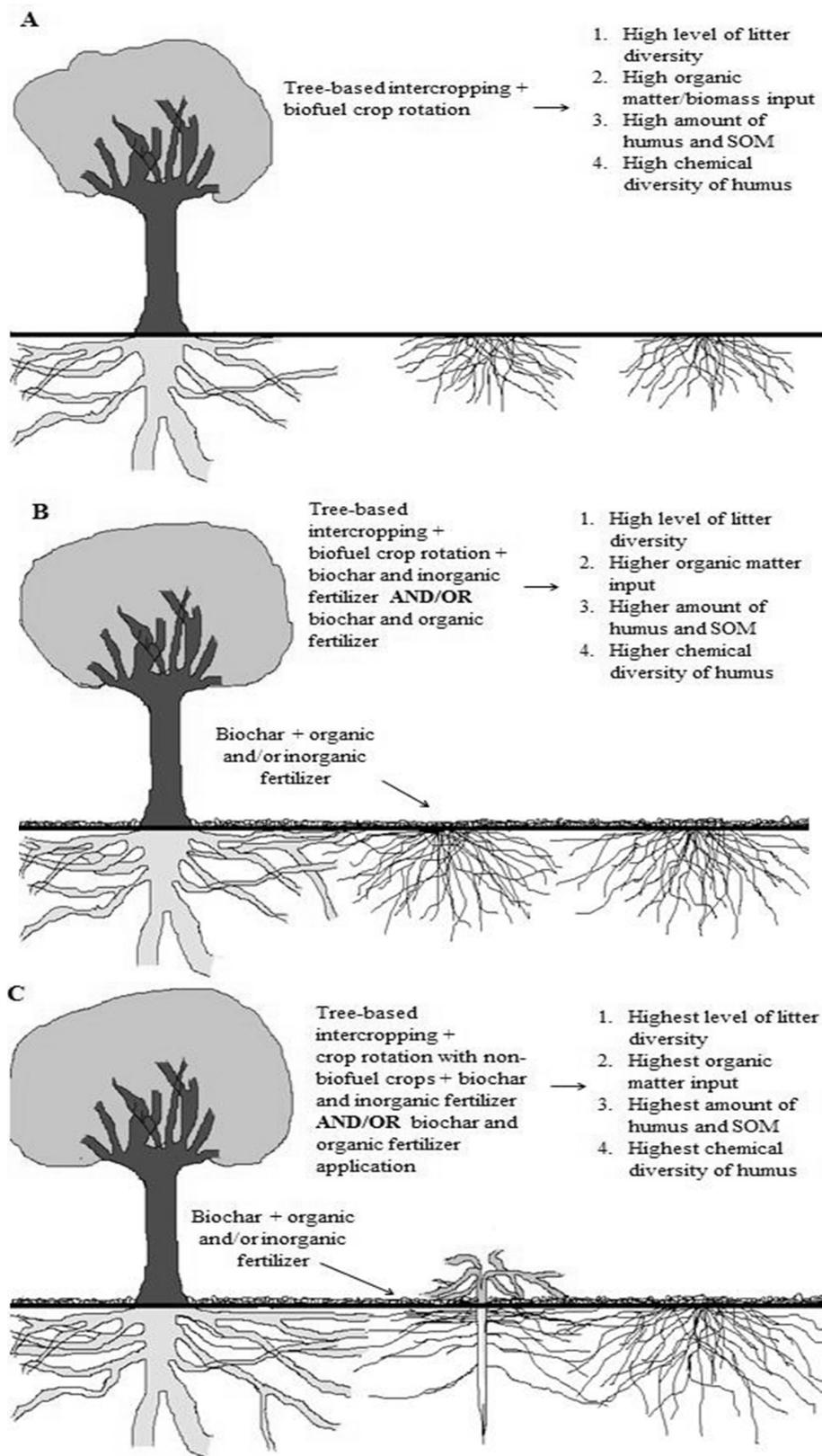
Biochar, a coproduct of biofuel production (e.g. fast pyrolysis biofuels) that contains 45–91% C [211, 212], is known to improve crop yield by enhancing physical, chemical and biological properties of soil [e.g. 139, 201, 213, 214] and contributes directly to the SOC content [121]. Biochar is considered a good strategy to increase SOC contents of bioenergy cropping systems [211]. Kauffman *et al.* [215] stated that biochar-induced yield improvement of second generation annual bioenergy crops can range from 1 to 8% gains in the US Midwest, and can sequester C via an indirect land use change credit of between 1.65 and 14.79 t CO<sub>2</sub> equivalent/ha/year over the next 30 years.

The addition of biochar to the field along with organic and/or inorganic fertilizers or composted biochar could be a good management practice to enhance organic matter as microbial (increased MBC) and non-microbial source (added biochar, organic fertilizer and increased input of crop underground residue e.g. 216, 217). Omil *et al.* [221] reported that the addition of biochar + P fertilizer to two soil types (i.e. sandy loam and clay loam) of two *Pinus radiata* plantations enhanced ~60 and ~53% MBC in clay loam soil after second and third year of biochar + P application while in sandy loam soil during third year of biochar + P application caused ~33% increase in MBC.

As the literature provides limited information at present, further study is warranted on how biochar influences SOC contents, SOC pool dynamics and soil physical properties (e.g. aggregation) in bioenergy cropping systems of northern temperate regions. Biochar may have a positive influence on the growth of dedicated perennial bioenergy crops situated on marginal and abandoned lands, but this still needs to be determined. Moreover, there is a need for research that evaluates the influence of biochar with or without other organic amendments on sustenance of SOC contents in GM bioenergy cropping systems in northern temperate climate.

### Biodiversity Management

Soil biodiversity is important in terms of maintaining soil quality and sequestering SOC through the process of humification [9]. Moreover, aboveground biodiversity is positively related to belowground biodiversity [9]. Biodiversity in bioenergy cropping systems can be achieved by crop rotation and growing polycultures (i.e. intercropping with trees or other crops) rather than monocultures [150, 198]. Perennial crops are considered to have a positive influence on soil biodiversity via eliminating



**Figure 5** Influence of management practices on increasing SOC pool size in bioenergy cropping system; (a) tree-base intercropping and crop rotation of bioenergy dicot and monocot crops without fertilizer application (b) tree-based intercropping crop rotation of bioenergy dicot and monocot crops with biochar + inorganic or organic fertilizer application (c) tree-based intercropping and mix cropping or crop rotation of non-bioenergy dicot and monocot crops with biochar + inorganic or organic fertilizer application after bioenergy cropping.

**Table 4** Expected reduction in greenhouse gas (GHG) emissions and gains from soil carbon (C) sequestration when bioenergy crops are grown in cold-temperate regions

Bioenergy crop	C3/C4	GHG emission reduction (kg CO <sub>2</sub> /ha/yr)	Soil C sequestration (kg C/ha/yr)	Reference
Alfalfa	C3	0.2–5.8	164–2500	[216, 218]
Switchgrass	C4	0.2	800–1200	[216–218]
Miscanthus	C4	0.2	682	[218]
Prairie cordgrass	C4	0.2–5.8	164–245	[218]
Reed canarygrass	C3	0.2–5.8	164–245	[218]
Low-input high diversity (LIHD) prairie	C4	nd	1200	[137]
Willow	C3	0.2–1.5	436–900	[216, 218]
Poplar	C3	nd	1100	[160]

kg, kilogram; ha, hectare; yr, year; nd, not determined.

tillage, providing habitat to animals, for degrading organic pollutants and having tighter nutrient recycling capacity, which reduces nutrient loss to the environment [150, 198]. Given that SOC content is positively related with soil biodiversity [e.g. 9], the adoption of polycultures for bioenergy cropping systems, as illustrated in Figure 5, could be beneficial for maintaining SOC levels in cold-temperate climate. Tilman *et al.* [137] reported that over 10 years of experiment in Minnesota US, the net C sequestration for the agriculture plots grown with mixed native prairie vegetation grown on marginal lands was  $2.7 \pm 0.29$  Mg/ha/year, while it was non-significant for monoculture systems. The positive influence of mixed prairie perennial vegetation on greater GHG mitigation potential reported by Gelfand *et al.* [138] is mentioned in section 'Perennial grasses'.

### Crop Rotations and Intercropping

As crop rotation positively influences SOC content in cold-temperate climate [218], by sustaining/increasing below ground biodiversity [9], it could be a management practice to sustain the SOC content in bioenergy cropping systems. However, this practice can be possible if the demand of a farmer for grain and residue production is not compromised. Moreover, as leguminous crops have a positive influence on N contents in soil and in microbial biomass [219], if their residues are not harvested, they can play a positive role in rebuilding soil humus that was depleted by last year residue removal [207] (Table 4).

Similarly, intercropping of cover crops (e.g. fescue, *Festuca eliata*) can also play a positive role on sustaining the SOC content [123] as they prevent leaching of nutrients and soil erosion. Tolbert *et al.* [145] reported a 6% lower SOC contents in agricultural plots grown with sweetgum without fescue cover crops in cold-temperate climate regions in US. Likewise, a positive influence of tree intercropping with annual crops on tree biomass production is also documented. For instance, Rivest *et al.* [220] reported ~40% increase in leafless biomass of

hybrid poplars at third year of growth when intercropped with cereal and legume crops in Southern Quebec as compared to the poplars grown as monocultures. A comprehensive description about the positive influence of agroforestry on sustainable agriculture is provided by Nair [212]. We expect greater belowground biomass of plants in intercropping system, which in return can result in greater SOC contents as compared to monocultures. However, for the better yield of feedstock from annual and woody bioenergy crops, the distance between cover crops and bioenergy crops need to be taken into consideration to overcome possible competition between them [e.g. 213, 214].

### Conclusions and Future Directions

Bioenergy cropping systems are expected to produce aboveground biomass that is mostly removed for bioenergy generation, which reduces the C inputs to soil and over time will reduce the SOC pool size. Given the importance of SOC for ecosystem functions, it is essential to minimize or reverse this tendency. Management practices that conserve SOC vary with the type of bioenergy crop produced. The SOC content in soil under annual bioenergy crops may be maintained by controlling the amount of residue removed, adopting no-tillage, applying organic fertilizers and biochar, and including cover crops in the rotation to minimize SOC loss through soil erosion during the non-growing season period. Intercropping systems, including tree-based intercropping, may be an option in some areas. For perennial grasses, abandoned and marginal lands are preferred to avoid displacing food crops and offer a new opportunity for SOC sequestration that can be enhanced with appropriate cultivar selection and amendments of biochar and organic fertilizers. Likewise for woody crops, the possible management practice to sustain SOC amount is to control the harvest frequency and implement these cropping systems on marginal agricultural lands that have potential to sequester SOC. While biochar and organic fertilizers could be beneficial to boost SOC content in woody crop

production, research is needed to evaluate the amount and type of these organic substances to be applied to fields. The introduction of GM bioenergy crops such as cellulase/ligninase enzyme production holds promise to reduce the cost and energy of feedstock conversion, required to achieve higher efficiency bioenergy production. However, the influence of such GM bioenergy crops on SOC dynamics during their life cycle and after harvest, their influence on microbial community structure, and the decomposition rate of non-harvested GM crop residues under field conditions needs to be evaluated.

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