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Chapter 14

LIGNIN CONTROLS ON SOIL ECOSYSTEM SERVICES: IMPLICATIONS FOR BIOTECHNOLOGICAL ADVANCES IN BIOFUEL CROPS

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ABSTRACT

Lignin is a complex phenolic polymer, mainly derived from the three monolignols: *p*-coumaryl, coniferyl, and sinapyl alcohols. As an important component of secondary cell walls in vascular plants, lignin is the second most abundant plant derived organic substance after cellulose. Relative to most other plant derived organic substances (i.e., structural and non-structural carbohydrates), lignin is recalcitrant to mineralization by soil microorganisms. The recalcitrance of lignin is due to the fact that only few microorganisms (i.e., white rot fungi and few bacterial species) can completely degrade polyphenols, and catabolism is often required to fully break down plant lignin. Consequently, lignin directly and/or indirectly influences soil microbial community structure, which in turn controls soil quality through the provision of several key ecosystem services: 1) reducing the emissions of greenhouse gases from soil, 2) retaining soluble nutrients, 3) promoting soil aggregate formation and stabilization, which reduces soil erosion, and 4) bioremediation and detoxification of natural and man-made organic pollutants. As lignin is a heterogeneous polymer composed of phenylpropanoid units, the vanillyl:syringyl ratio of lignin is considered an indicator of its effect on ecosystem services. The influence of global warming on accelerating lignin degradation and the consequence of reduced lignin concentration and soil organic matter levels in the soil ecosystem are discussed. Biotechnology permits manipulation of lignin concentration and/or lignin chemistry in plants in order to produce crops designed for the production of advanced biofuels and other bioproducts, but greater attention needs to be paid to the

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feedbacks on soil quality and ecosystem services. Genetic manipulations to increase the content and/or change the chemistry of lignin in the non-harvested parts of bioenergy crops, such as the roots, are recommended to preserve soil ecosystem services.

Keywords: lignin, ecosystem functioning, ecosystem services, humus, soil aggregation, carbon dioxide

INTRODUCTION

Lignins are three-dimensional cross-linked phenolic macromolecules found in the secondary cell walls of vascular plants [1,2]. They are heterogeneous molecules made up of aromatic rings with side chains. The building blocks of lignins are phenylpropanoid units called *p*-hydroxyphenyl, guaiacyl, and syringyl units, which are linked together in a complex network to form lignin [3].

Lignin is relatively recalcitrant to biodegradation in soil as compared to other plant derived organic substances [4-6]. Moreover, few microorganisms can degrade lignin and only white and brown rot fungi, also referred to as wood decay fungi, can completely decompose lignin into CO_2 and water [7-12]. The recalcitrance of lignin has recently come into question with the advancement of analytical techniques indicating that mean residence time (MRT) of lignin in soil is shorter than previously thought; it can be as short as 1-5 years [13,14]. Although it initially exhibits slow degradation rates, lignin can decompose faster than bulk soil organic matter (SOM) in the long term. Still, the decomposition of lignin is related to the environmental and soil conditions and to the soil management practices, which can favor lignin preservation in soil. Under such circumstances, the chemical characteristics of lignin such as its complex structure, its aromaticity and side chains, and its resistance to hydrolytic enzyme, lend it this 'recalcitrance' character. Because of the complex structure of lignin, its degradation generates by-products that form secondary complexes or bind to other organic molecules in the soil, further slowing the decomposition of organic matter and aiding in the formation of humus and humus-like matter. These characteristics allow lignin to play an important role in controlling various ecosystem functions. For instance, lignin contributes to the formation of humus [15-18] directly as a transient part of the non-decomposed SOM [see 4, 19] and indirectly during its transformation into other more resistant aromatic forms (i.e., phenanthrene, benzene and naphthalene) that are concomitantly bound to microbial-derived organic substances [19], by promoting fungal growth, and by improving soil aggregation and soil structure. Good soil structure is important for soil ecosystem services such as water and nutrient retention within the soil profile, cation exchange capacity and other buffering functions [20] and protection of soil enzymes that operate abiotically or within microbial biomass [21-24], which are the engine of nutrient cycling [22,25] and detoxification of organic substances [26]. The degradation of lignins in soil is influenced by lignin concentration and its composition such as V:S ratio [13,27], elemental composition of plant residues such as the C:N ratio, and lignin: N ratio [5,6,28], photooxidation, soil moisture and temperature, and availability of nutrients.

Lignin plays an important role in environmental safety. It helps reduce emissions of greenhouse gases from soil by serving as a physical barrier to the decomposition of organic substances in plant residues (e.g., protection of cellulose in ligno-cellulosic complexes) while

lignin-degrading fungal enzymes (e.g., laccase, Mn-peroxidase) play a role in the detoxification of xenobiotics such as chlorinated compounds (i.e., DDT, dichlorodiphenyl-trichloroethane), azoic dye, and aromatic hydrocarbons such as toluene, benzene and xylenes [26]. Moreover, lignin-degrading fungal enzymes can also participate in disease suppression due to their ability to degrade fungal melanins in soil environment [29].

Biotechnological advances in the field of biofuel and bioproducts (e.g., pulp and paper industry) aim to reduce the concentration of lignin and/or V:S ratio of lignin units in plants. Such alterations in plant residues can accelerate the depletion of SOM, leading to an increase in greenhouse gas emissions from soils. Blanco-Canqui and Lal [30] highlighted the negative influence of residue removal from agricultural fields for production of biofuel. Hence, the decrease in SOM due to residue removal can be accelerated if crops have lower lignin content and/or have lignin with lower V:S ratio.

Being an important controller of soil quality and a player in environmental safety, lignin provides provisional, regulative, and supporting ecosystem services to mankind. This chapter discusses 1) factors influencing degradation of lignin and its feedback on SOM dynamics, 2) the soil ecosystem functioning and environmental safety services provided by lignin, 3) trends in biotechnological manipulation of lignin biosynthesis pathways in plants grown for biofuels and bioproducts, and 4) points out future directions for soil management practices to reduce the risk of SOM depletion due to climate change or growth of genetically modified plants with low lignin content and altered lignins.

LIGNINS IN PLANTS

Chemical Structure and Occurrence

Lignins are phenolic polymers found in secondary cell walls of vascular plants [1,2]. Lignin is often described as a random three-dimensional polymer made up of phenylpropanoid units, monolignols, with various linkages. These monolignols are hydroxycinnamyl alcohols that have varying degrees of methoxylation. The monolignols are the coniferyl alcohol (4-hydroxy-3-methoxycinnamyl), the sinapyl alcohol (3,5-dimethoxycinnamyl), and the *p*-coumaryl alcohol (4-hydroxycinnamyl) [1,2]. Monolignols are synthesized in the plants through the phenylpropanoid pathway which begins with the conversion of phenylalanine (produced by plants through the shikimate pathway) into cinnamic acid in the presence of PAL (phenylalanine ammonia-lyase) [31]. Linkages between monolignols are mainly C-C or C-O bonds such as the *β*-O-4 (arylglycerol-beta-aryl ether), *β*-5 (phenylcoumaran) and the 5-5 aryl-aryl (biphenyl) linkages as shown in Figure (1) taken from Talbot et al. [32]. Monolignols are incorporated into the lignin polymer and the resulting units are called guaiacyl (G or V units), syringyl (S units), and *p*-hydroxyphenyl (H units) when they originate from coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol, respectively [1]. Gymnosperm lignin is composed mainly of G units and minor amounts of H units, whereas angiosperm lignin is made up of both G and S units [2]. The polymerization of the lignin structure is still poorly understood; it is initiated by an enzymatic reaction that produces free radicals on the monolignols, which couple together through the above

mentioned bonds to form lignin dimers that couple further to form the complex lignin structure in an apparently random manner.

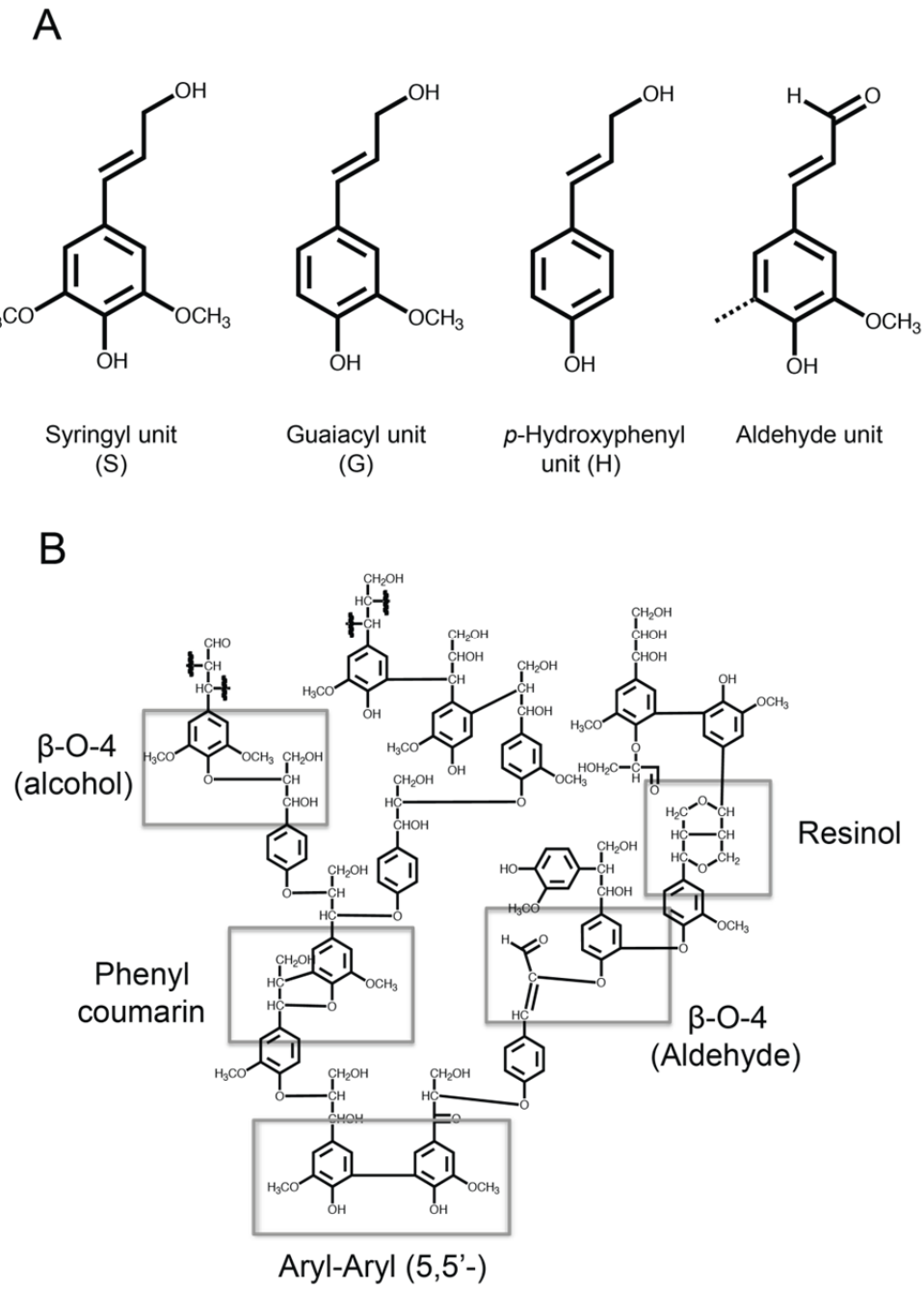


Figure 1. Phenylpropanoid pathway. Structure of monolignols (A) and types of linkages between monolignols (B) from Talbot et al. (2012).

Lignin is the second most abundant terrestrial biopolymer after cellulose, constituting about 30% of the organic carbon in the biosphere [2]. The deposition of lignin occurs during the secondary thickening of cell walls especially in fiber cells and conducting tissue (xylem and phloem) [33]. Secondary cell walls consist of the following three layers: S1 the outer layer, S2 the middle layer and S3 the inner layer [2,33]. The formation of lignin proceeds by the deposition of carbohydrates [2,33] in the region of the middle lamella and the primary wall at the cell corners during S1 formation. Lignification proceeds in the secondary cell wall after completion of the polysaccharide matrix of the S2 and S1 layers [2]. Lignin in the cell walls is associated with hemicellulose-protein matrices in which cellulose microfibrils are also embedded [32]; this is one mechanism by which the presence of lignin physically protects the more labile cellular polysaccharide components against degradation. This connection with other cell wall structural polysaccharides i.e., cellulose and hemicelluloses provides strength and rigidity to cell walls [11,32,34,35]. Amounts and types of lignin vary with the types of plants and with the age of the tissue. Also, the concentration of lignin varies in plant tissues of individual species and generally has a greater concentration in roots compared to stems and leaves [36,37]. The chemical composition of lignin (V:S ratio) can also vary in various plant organs of the same species [36,37]. For instance, Heim et al. [36] observed that roots of maize plants had ~50% greater concentration of the *p*-hydroxyphenyl lignin monomer than shoots. Likewise, Abiven et al. [37] observed H:V and S:V ratio in various organs of maize and wheat followed the order of leaves < stems < roots.

Quantifying the amounts and chemical compositions of lignin is most commonly done through the gravimetric [38] and the CuO oxidation [39] methods. The former method is based on washing plant samples in various detergents and finally in 72% concentrated sulfuric acid to remove non-structural and structural cellular components, leaving un-dissolved lignin (acid insoluble lignin) from the sample. However, depending on plant species and plant organ, this acid insoluble fraction contains traces of insoluble lipids, condensed tannins and acid-soluble phenolics along with lignin for which reason this fraction is termed acid insoluble residue or acid unhydrolyzable fraction (AUF) [40-43]. In this chapter the terms lignin or AUF are used interchangeably. Chemical characterization of lignin is achieved by treating samples with alkaline CuO and extracting up to 11 simple lignin-derived phenols with ethyl ether, followed by analysis by gas chromatography.

Persistence in the Environment

Lignin plays an important role in the persistence of plant residues in the environment through its roles of physical and chemical protection of plant material, which slows the biodegradation of the residues in soil.

Mechanical strength to plants

Lignin imparts mechanical strength to plant components, leaves, stems, and roots [44-51]; toughness of leaves in lowland tropical forests was found to positively correlate with fiber content [52] and determined susceptibility of plants to damage from insect feeding. As well, Li et al. [50] found a positive correlation between concentration of lignin and mechanical strength in herbaceous Peony inflorescence stems. This mechanical strength affects the decomposition rates of plant residues because it decreases incidence of stem and

leaf breakage and susceptibility to herbivory; less stem breakage and less pest injury leads to better success in plant reproduction and greater biomass production [51] and reduces the release of nutrients and leaching from plant litter [53] as well as bacterial and fungal infection of plant material before it enters the soil as litter. Pérez-Harguindeguy [53] reported that leaf litter chemistry (C:N ratio) and leaf tensile strength were strongly correlated with decomposition across a wide range of functional types and habitat.

Protection to plants against pests and diseases

Lignin provides a physical barrier against initial attack and penetration by saprophytic microorganisms and it also plays more physical and chemical roles in the defense against pest and disease damage. Cell wall thickening and rigidity that comes from lignin deposition is a constitutive defense mechanism against injury and damage. It prevents stylet penetration of pests to the vascular tissues [54] and therefore increases the resistance of plants to pest attack [55,56] and reduces infection from disease agents [57,58], as some insects are viral carriers and serve as a conduit for viral infection in plants. Schrotenboer et al. [58] reported a positive relationship between plant tissue digestibility (low lignin/AUF concentration) and infection of plants with aphid-vectored cereal and barley yellow dwarf viruses (B/CYDVs) in switchgrass (Panicum virgatum) that was selected for biofuel production. Maher et al. [57] found that transgenic tobacco plants with suppressed levels of PAL and low levels of chlorogenic acid developed about 60% more lesions after 5 days of infection by the fungal pathogen *Cercospora nicotianae*, compared to the wild type.

There is some evidence that lignin controls and prevents crop diseases. Bellaloui et al. [60] reported that soybean genotypes moderately resistant to charcoal rot disease had significantly higher lignin concentration (10%-63%, $P<0.05$) in seed coats along with higher concentration of water soluble polyphenolics, total boron, isoflavones and sugars compared to the susceptible soybean genotype.

Lignin is a chemical defense against pests because it reduces nutrient availability to pests and is directly toxic to larvae, e.g., larvae of spruce bark beetle *Dendroctonus micans*as as reported by Wainhouse et al. [44]. Induced lignification of tissue and the presence of different phenolic compounds at the site of injury prevent diffusion of fungal enzymes, bind to and protect polysaccharides and glycoproteins from fungal enzyme activity, and form a barrier against diffusion of enzymes and toxins further into the plant tissue [61]. The presence of free radicals and lignin precursors at the injury site can also inactivate microbial enzymes and toxins. A more comprehensive listing of chemical defenses attributed to lignin and factors that induced lignification in various plants is available in Vance et al. [61] and references therein.

Resistance against biodegradation

Lignin is difficult to degrade compared to other plant derived organic substances such as structural and non-structural carbohydrates and water soluble polyphenolics [4,62]. The resistance of lignin to degradation is due to its complex structure and the heterogeneity of the lignin macromolecules; while hydrolytic enzymes can attack the outer structure of the lignin molecule, extracellular lignolitic oxidative enzymes are required to de-polymerize and mineralize it and these are produced only by a few soil organisms. Microorganisms that can degrade lignin are mainly the basidiomycetous white rot fungi, which have shown the fastest degradation rates [11,63], followed by some soft rot fungi (typically species of ascomycetes)

and brown rot fungi [7,8,11,61,64]. White rot fungi can degrade lignin completely into CO_2 and water [9,65]. Some bacteria such as those belonging to actinomycetes, α proteobacteria and γ proteobacteria degrade lignin but at a much slower rate than fungi [7,8,61,66-68]. Huang et al. [68] reported that out of 140 strains of bacteria from rainforest soils of Peru, *Bacillus pumilus* and *Bacillus atrophaeus* possessed high laccase activity and were able to degrade the lignin dimer guaiacylglycerol-*β*-guaiacyl ether.

There is evidence that the chemical composition of lignin affects its decomposability; syringyl and *p*-hydroxyphenyl units are preferentially degraded over guaiacyl [13,27,32,69-72] and the V:S and G:H ratios are indicators of the ease of degradation of plant residues. Lignin monomers are linked together by various covalent bonds [13,32], which result in the formation of different lignin structures. These bonds vary in their strength and affect the degradability of lignin; guaiacyl lignin forms aryl-aryl linkages which are more condensed and stronger that the *β*-O-4 linkages favorably formed by syringyl lignin [13,27,32]. Moreover, fungi seem to preferentially attack the *β*-O-4 linkages over aryl-aryl linkages [39,73]. Lignin/AUF and its chemistry therefore plays a role in controlling the biodegradation of plant residues and this phenomenon was well explained in our previous article [74]. Lignin being relatively resistant to biodegradation and providing physical protection to plant residues allows undecomposed plant residues to persist in soil and thus contributes to the formation of SOM.

ROLE OF LIGNINS IN ECOSYSTEM SERVICES

Ecosystem services are defined as the benefits provided by managed or natural ecosystems to mankind [75]. Based on the Millennium Ecosystem Assessment [75], ecosystem services are classified into four groups; provisional, regulative, supporting and cultural services. The soil ecosystem services provided by lignin are presented in Figure (2). As an important soil quality controller, lignin provides supporting and regulating services to mankind; this is done through influencing the formation of humus, soil aggregation, nutrient cycling, maintaining or increasing the number and diversity of soil organisms, and disease control in plants. Lignin also provides environmental safety through reduction of greenhouse gas emissions and detoxification of organic pollutants and increasing biological resilience in the soil and thus provides regulative and provisional services to humans. Lignin plays a direct and an indirect role in this regard, as documented in the following sections.

Provisioning and Supportive Ecosystem Services: Lignin as a Soil Quality Controller

Direct and indirect relation of lignin to humus and SOM

Humus is a non-living, non-tissue, colloidal heterogeneous component of SOM. It is a complex mixture of plant and microbial biopolymers and their degradation products [76-81] with variable chemical composition [79-81].

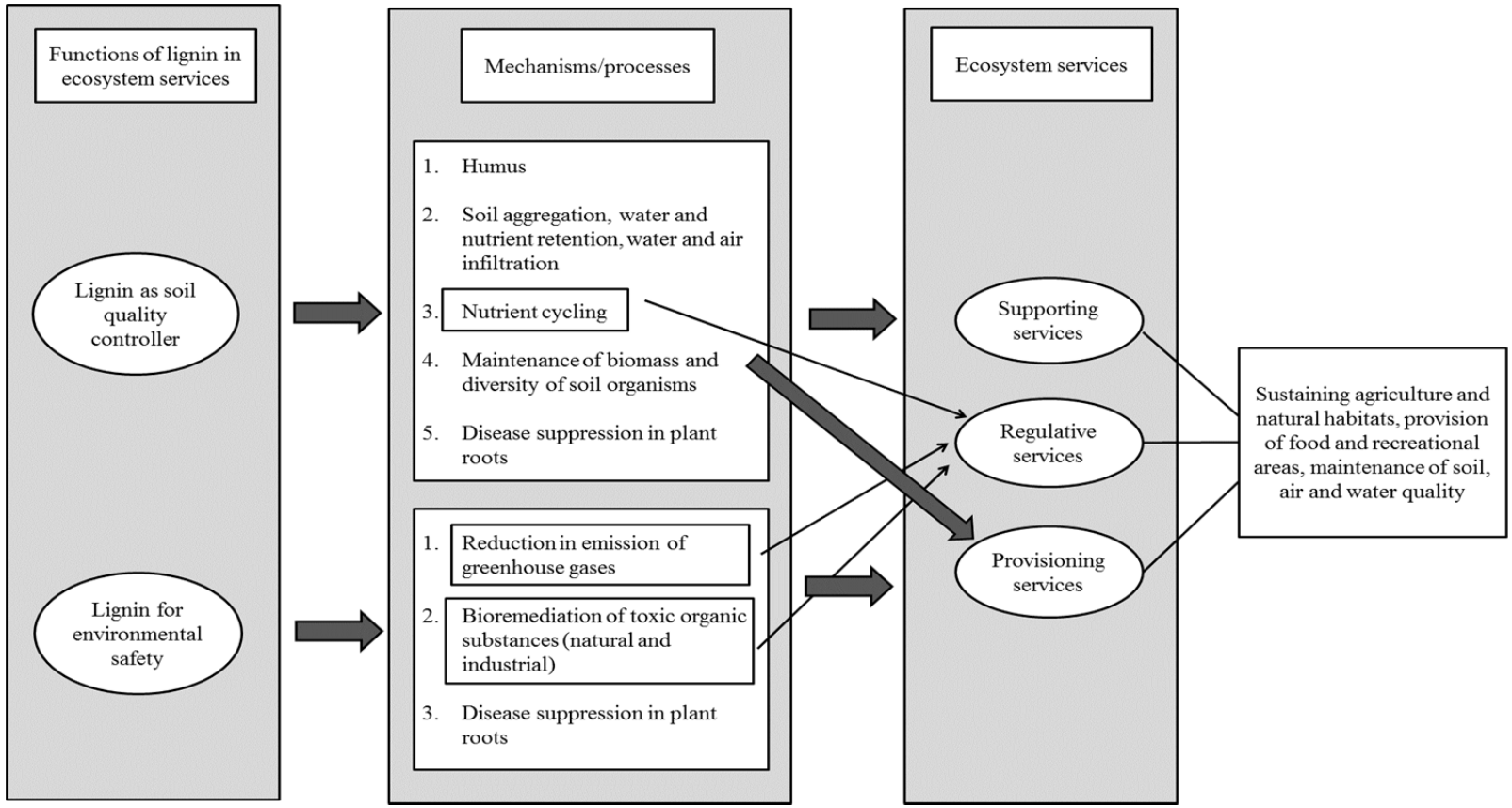


Figure 2. Role of lignin in controlling the various ecosystem services i.e., supporting, regulative and provisioning.

A true definition of the shape, form, and size of humic substances remains unavailable; humic substances were thought to be large complex molecules that represent the largest pool of recalcitrant fraction of organic matter in terrestrial ecosystems [79]. However, recent research has shown that humus/SOM also includes compounds that have a smaller, simpler molecular structure [14,82,83] that are not necessarily inherently recalcitrant. Decomposability of humus/SOM depends on a number of factors including the chemical form of the organic materials in 'humus', the surrounding soil environment including biotic and abiotic factors, the physical properties of the soil, and the soil management in cultivated soils. Traditionally, humified substances and SOM were thought to resist degradation because their chemical structure rendered them recalcitrant to enzymatic hydrolysis. Howeve,r new insight and advances in SOM analysis suggest that this understanding might be incomplete and that physical association with mineral particles and physical protection of humus/SOM give it its 'recalcitrant' nature, meaning that disturbance of this physical association will lead to the decomposition of apparently recalcitrant material. Under the right set of conditions, humus/SOM can be degraded at a relatively rapid rate.

Humus and SOM contribute greatly to the productivity and health of soil [84,85]. They provide bulking (aggregation), water-holding [77], cation exchange [20,76,86], buffering and chelating [20] capabilities to soil [18], which support the building and maintenance of soil structure and buffering capacity. Humus/SOM also plays a role in disease suppression for plants [84,87,88] and in protection of soil enzymes from pyrolysis, thermal denaturation, and damage from high pH [21-24]. Humic substances have small pores that prevent the passage of the high molecular weight substances such as proteases [89]. Through their protection of soil enzymes, humic substances indirectly contribute to nutrient cycling [90,91], formation of soil aggregates [82,92] and detoxification of xenobiotics [26,93,94].

Lignin contributes to humus formation. Directly, undecomposed lignin is counted as part of the soil humus while indirectly, lignin is a substrate for microbial growth and its decomposition releases degradation products that are chemically recalcitrant and form part of the humus constituents. Moreover, the incorporation of lignin into microbial biomass and subsequent turnover via microbial byproducts (cellular debris, extracellular secretions) also contribute indirectly to humus formation. Humus formation is not completely understood and could occur through different processes and pathways, however it is initiated via the decomposition of plant and animal residue by the action of soil microorganisms, with fungi being more important for the final stages of residue decomposition [18,95-98]. There are two main models that describe the formation of humus: (1) degradation models, which postulate the selective preservation of slowly decomposable/recalcitrant microbial and plant derived macromolecules, and (2) condensation models, which demonstrate the degradation of macromolecules into low molecular weight substances and their subsequent abiotic condensation into humus polymers [92]. The slow turnover rates of primary resources that are rich in slow-decomposing substances such as lignins and lipids would contribute more to humus formation than fast-decomposing carbohydrates of plant origin [15,16,19,80,83,99]. Schnitzer and Monreal [19] reported that lignin accounted for 20-40% of the peak height intensity measured by pyrolysis-field ionization mass spectrometry (Py-FIMS) in various components of humus (i.e., humic acid, humin, fulvic acid) in Bainsville soil. In general, the residence time of lignin in SOM is less than 50 years [4,83] and the degradation products of lignin participate more in the formation of the recalcitrant fraction (i.e., humic acid) of SOM [80]. For instance, recent studies based on ^{13}C NMR spectroscopy reveal that the aromatic

lignin degradation products such as phenanthrene, benzene and naphthalene are the major building blocks of humic acid while alkanes from microbially-originated-decarboxylated fatty acids participate largely in the formation of alkyl aromatic structures with lignin degradation products [81].

Although lignin persists longer in soil than other plant-derived carbohydrates and tannins [80], plant-derived alkanoic acids and *n*-alkanes have even longer residence times than lignin in soil [83]. How much lignin persists in SOM depends on its chemistry (i.e., V:S ratio), soil texture [13], depth of soil [100], climate [13] and C:N ratio of plant residues [6,28]. Therefore, the fraction of lignin of a given plant residue that participate in the formation of humic acid and the fraction of lignin that persists as lignin in humus is expected to depend on the lignin concentration, chemistry and soil physico-chemical properties.

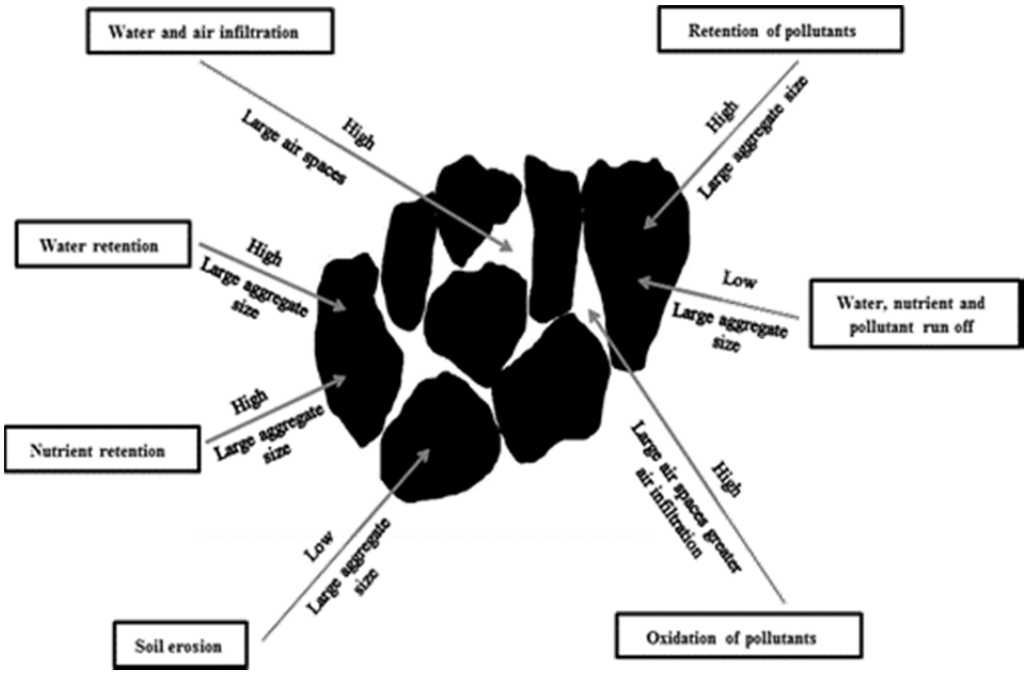
Direct and indirect role of lignins in soil aggregation

Soil aggregation is an important indicator of soil quality and supports a number of soil ecosystem services. Aggregation promotes aeration, water retention, water and air infiltration, it prevents soil erosion, plays a role in detoxifying pollutants, and protects organic matter from decomposition or leaching [see also 30]. The influence of soil aggregation on soil ecosystem services is conceptualized in Figure 3. Soil aggregation is the result of binding of plant and animal residues (or animal wastes) and biota (microbes) with mineral particles [101,102]. Physical binding of these materials occurs through the mesh of roots, fungal and mycorrhizal hyphae, algae and bacterial cells [101], whereas chemical binding is through polysaccharides and organic mucilage derived from exudation of roots, fungal hyphae, bacteria and decomposing plant and animal materials [30,101-105]. Humified and highly decomposed materials, which are more stable, aid in the formation of microaggregates [101]. Lignin promotes macroaggregation in soil [101,105,106] and it increases aggregate stability [106]. According to Xiao et al. [106], application of 1.67-3.34 g C kg^{-1} soil in the form of sulfuric acid-precipitated lignin from rice straw pulping resulted in more than two fold increase (~59%, $P<0.05$) in macroaggregate formation over the control soil during an 8 week incubation. Moreover, the lignin-amended soil had 43% wet microaggregate stability of macroaggregates, compared to 36% wet microaggregate stability of macroaggregates in the control soil.

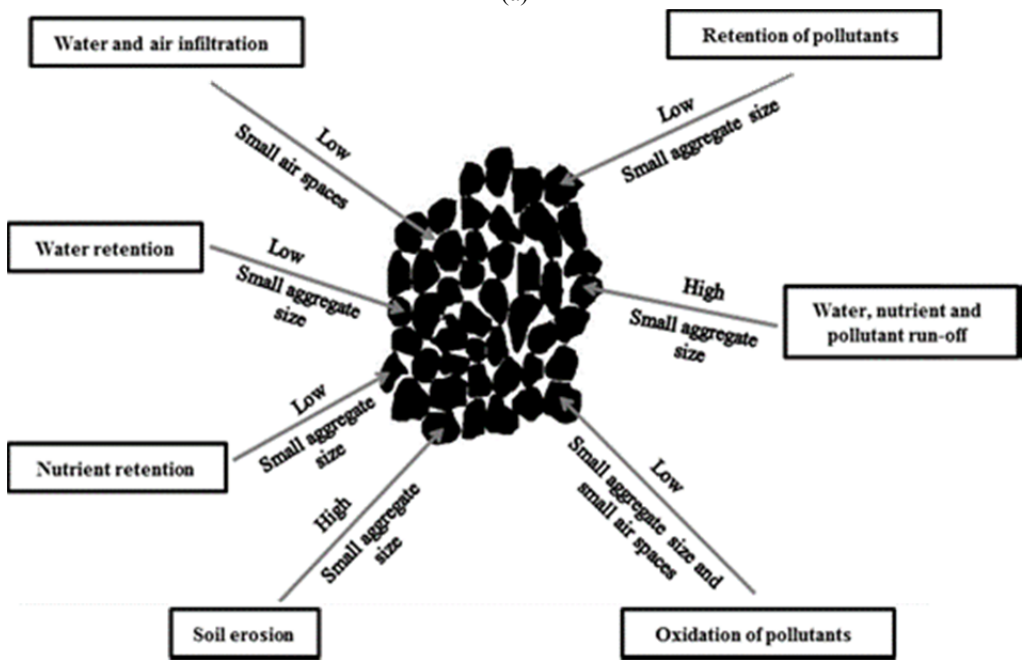
The direct effect of lignin on soil aggregation is achieved by its binding to organo-mineral substances in soil. For instance, lignin has hydrophilic regions such as hydroxyls (-OH), carboxylic acids (COOH) and small alkyl chains i.e., methane groups ($-CH_3$) (Figure 1). These functional groups and the non-polar regions of lignin enable it to bind with organic matter and mineral particles through polar, covalent, and hydrogen bonding as well as Van der Waals forces. These chemical interactions of lignin with organic matter and mineral particles help make organo-mineral complexes in soil.

Lignin also has an indirect effect on soil aggregation through alteration in the community composition of soil microorganisms. For instance, lignin promotes the growth of brown and white rot fungi, which prefer lignin as substrates. Fungi are important for the formation of macroaggregates [105,106]. Fungi hyphae are often found at the core of macroaggregates, and the concomitant mucilage secretions strengthen and stabilize macroaggregates [105,107-109]. Indirectly, fungal extracellular secretions aid in the decomposition of plant residues by releasing substrates for bacterial growth [110,111]. Both fungi and bacteria secrete

extracellular compounds such as polysaccharides that promote the formation and stability of aggregates [108,98].



(a)



(b)

Figure 3. Diagrammatic presentation of influence of soil with large aggregates and the soil with small aggregates on various soil processes and their mechanisms.

Lignin control over nutrient cycling in soil

Nutrient immobilization prevents the leaching of soluble nutrients and gaseous loss of nutrients such as nitrogen, which can be lost as N_2O, N_2, NOx and NH_3 from soil. Lignin helps prevent leaching of nutrients through immobilization into microbial biomass or by promoting soil aggregation.

As described above, lignin has –OH, COOH, -OCH_3 functional groups. These groups along with hydrophobic sites (e.g., resinol, C-H groups) make lignin a good adsorption/desorption surface for cations and organic molecules [112]. Lignin also stimulates nutrient retention in soil by providing a carbon (C) source to microorganisms. Actively-growing microorganisms absorb and retain nutrients from the soil solution; if nutrients in ionic forms (e.g., NH_4^+) are transformed into organic compounds (e.g., amino acids and proteins) within microbial cells, those nutrients will remain associated with cellular compartments in organic forms upon the death of the microorganisms.

Humus has a role in retention of nutrients as described above. As for soil aggregation, it helps prevent leaching of nutrients from soils [113,114,115] through its prevention of water loss [30]. This prevention is either physical or chemical through the binding of nutrients to mineral and organic matter [114]. McDowell et al. [115] reported that reducing the aggregate size increased leaching of organic and inorganic phosphorous (Figure 4a). Likewise, Six et al. [116] observed that the amount of total N increased with the increase in aggregate size (Figure 4b). In a review of 14 field studies about the influence of long term (>10 years) fertilizer and organic manure amendment on soil properties, Edmeades [113] found that the soil with greater aggregate stability had higher enrichment of nutrients in top (Mg, Ca, K) and sub soils (N). As plant residues with higher concentration of lignin promote formation and stabilization of aggregates, lignin can protect nutrients from leaching.

Organic substances that have relatively higher lignin concentrations release nutrients at a slower rate because of nutrient immobilization and slower decomposition [117-120]. Thomas and Asakawa [117] showed that there is a negative correlation between the loss of N from litter and the initial concentration of lignin and C:N ratio in litter, as well as the ratios of lignin:N and lignin+polyphenols: N. Table (1) summarizes data from Thomas and Asakawa [117] and Osono and Takeda [118], showing that the plant residues with higher AUF or lignin and lower initial N concentration caused immobilization of mineral N from the soil solution. Since lignin is a physical barrier to the degradation of plant residues, the rate of mineralization of organic N from plant residues with higher lignin and low N contents is slower. In this case, microbes acquire the mineral N they need for cellular components and metabolic functions from the soil solution, not plant residues. This phenomenon is illustrated in Figure (5). Likewise, in a 10 year field study in a semi-arid shortgrass steppe ecosystem, Burke et al. [120] reported that industrial lignin caused immobilization of inorganic N in soil for a longer period of time than sawdust plus sugar treatment.

Microbes are important sinks for soil nutrients [11,122]. They incorporate nutrients in their cellular components and the nutrients return back to the soil when microbial cells die and their cellular contents are lysed and mineralized by the next generation of microorganisms. Environmental factors that cause the death and lyses of microbial cells can cause nutrient loss from soil in leachates [123]. Some microorganisms may have greater resistance to certain disturbances such as drying and re-wetting cycles in soil than others [123,124]. For instance, in a study with two soil types, Gordon et al. [123] found that the soil with higher fungal:bacterial (F:B) ratio (0.02) were significantly more resistant to drying and re-wetting

cycles that affect N leaching than the soil with lower F:B ratio (0.01). Ouyang and Li [125] stated that fungi are more resistant to drying/rewetting cycles than bacteria and this was corroborated by Huang et al. [126], who found that the community structure of fungi was less sensitive to drying/rewetting cycle than bacteria while fungal abundances were more dynamic over time after rewetting than bacterial abundances.

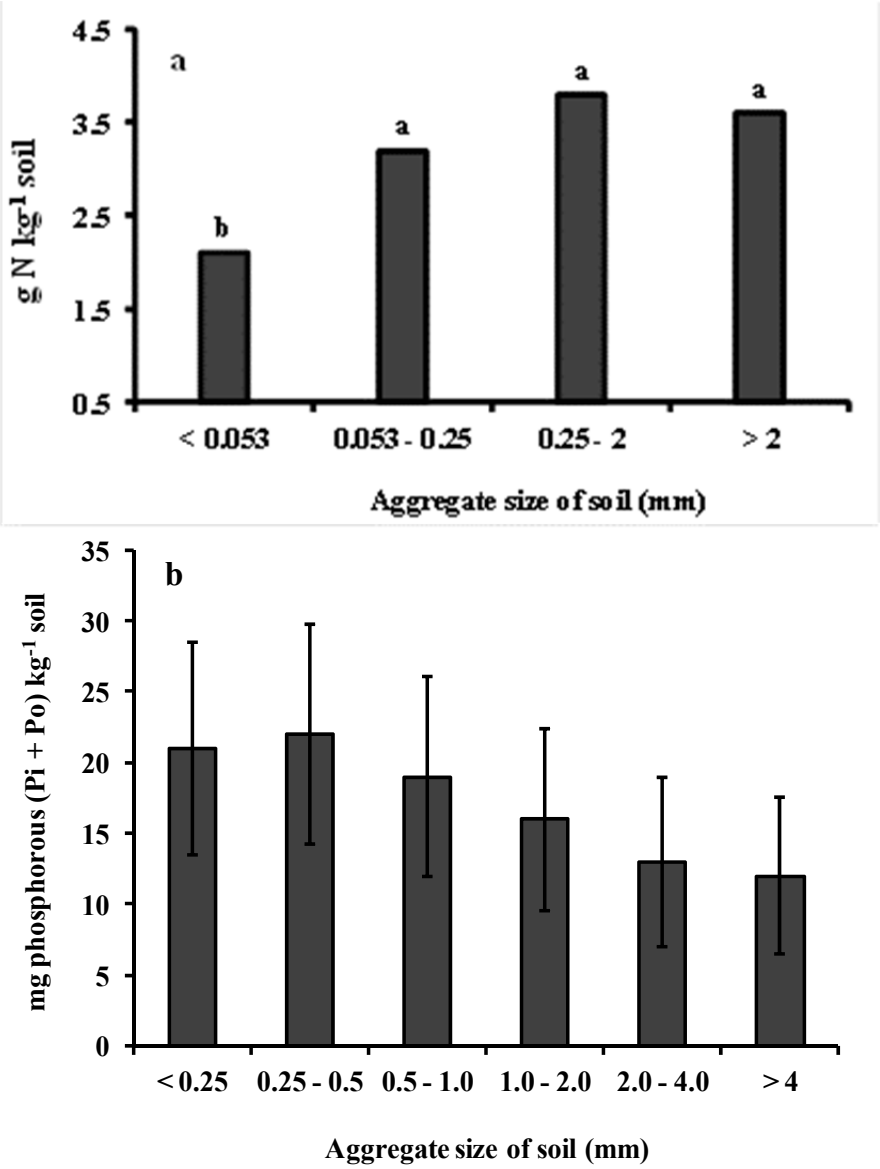


Figure 4. (a) Concentration of total nitrogen in various aggregate size fractions (< 0.053 - > 2mm) values with different letters are significantly different at $P < 0.05$ (Six et al., 1998), (b) concentration of inorganic (Pi) + organic phosphorus (Po) in leachate of soils with variable aggregate sizes (< 0.25 - > 4) during 32 weeks growth period of ryegrass (McDowell et al., 2006), the aggregate size and concentration of P in leachate interaction was significant ($P < 0.01$).

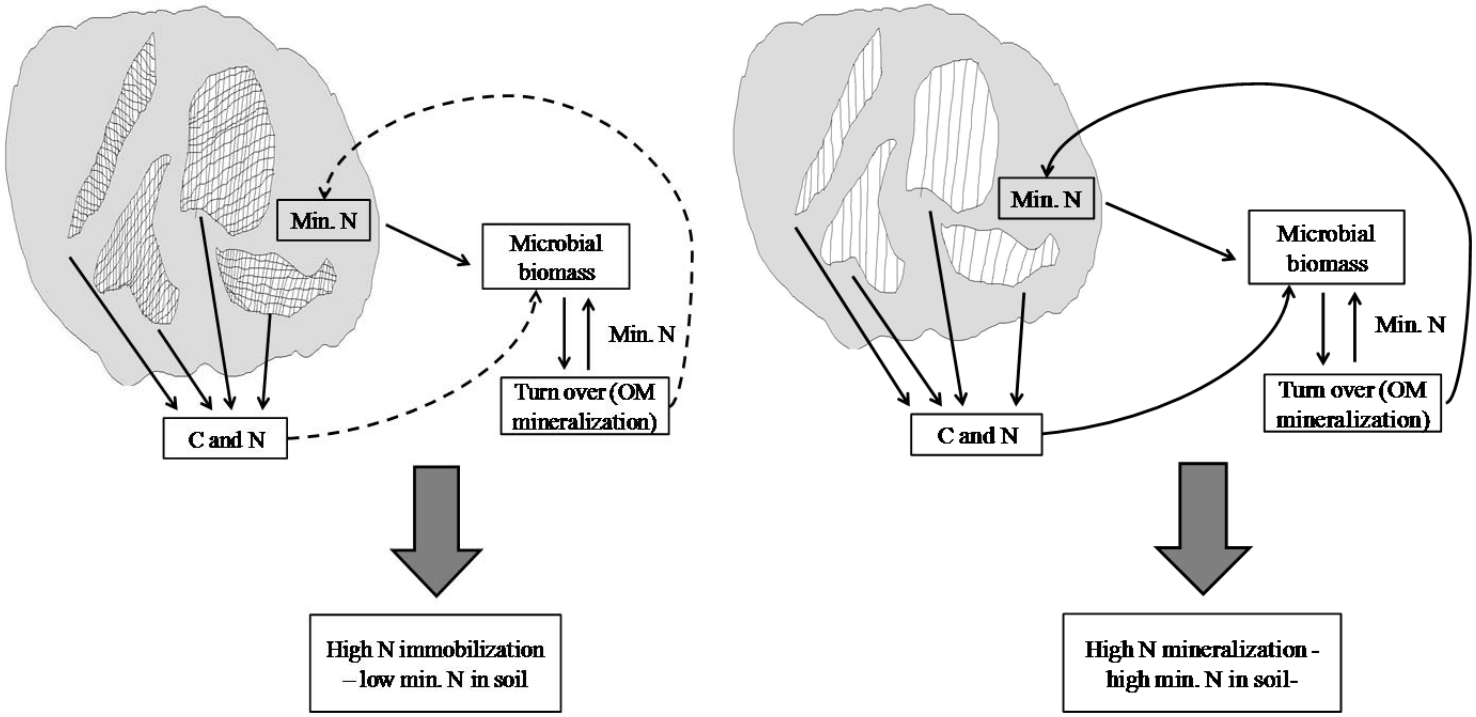


Figure 5. Mechanism of N immobilization and N mineralization in soil amended with plant residue of high lignin and low N contents (a) and soil amended with plant residues of low lignin and high N content (b). Gray background show mineral N environment, plant residue debris with low lignin and high N contents, plant residue debris with high lignin and low N contents.

Table 1. AUF, AUF/N ratio and total N and P concentration in leaf litter of five species before and after 3 years of burial in soil in litter bags (Osono and Takeda, 2004), AUF, AUF/N ratio and total N and P concentration in litter of four species before and after 3 months of burial in soil in litter bags (Thomas and Asakawa, 1993)

Plant species	AUF	AUF/N ratio	Initial concentration	Final concentration	Initial concentration	Final concentration	Reference
			N		P		
Pterostyrax hispida	300	10.3	78	17	3.70	0.50	Osono and Takeda (2004)
Aesculus turbinate	501	46.5	30	35	1.25	1.50	
Pterocarya rhoifolia	462	27.5	42	35	2.00	1.25	
Acer refinerue	464	80.0	15	21	0.75	1.25	
Acer mono	323	39.9	20	25	1.00	1.20	
Arachis Pintoi	28.8	13.4	2.08	1.95	0.08	0.10	Thomas and Asakawa (1993)
Stylosanthus capitata	16.6	8.60	1.92	1.65	0.07	0.07	
Desmodium Ovalifolium	38.5	27.3	1.46	1.67	0.07	0.06	
Andropogon gayanus	10.2	24.7	0.41	0.83	0.03	0.06	

Lignin may positively influence the retention of nutrients in soil by directly promoting fungal growth. Moreover, microorganisms within aggregates are more protected against drying/rewetting cycle, than the ones on surfaces [125]. Therefore, lignin can also contributes to nutrient retention by positively influencing aggregation via fungal growth. The interaction between lignin, fungal abundance, aggregation and microbial abundance inside of aggregates for nutrient retention merits further study.

Lignin: Direct and indirect influence on the number and diversity of soil organisms

Soil microorganisms regulate soil ecosystem functioning through nutrient cycling, immobilization and slow release of nutrients from organic matter into soil, soil aggregation and detoxification of pollutant in soil. Through their interactions with plants, soil microorganisms can prevent plant diseases (i.e., rhizosphere microorganisms), [127,128] and enhance the nutrient availability to plants from symbiotic nitrogen fixing bacteria and symbiotic fungi i.e., endomycorrhizae and ectomycorhhizae [128]. The role of soil invertebrates in ecosystem functioning and how lignin influence soil invertebrates is conceptualized in Figure 6. The mounds, channels, burrows and nests of invertebrate soil animals provide suitable micro-sites/habitats for smaller invertebrates to invade [129,130], which help increase community richness and population densities of soil invertebrates and microorganisms. For example, ants generally favor ammonifying bacteria, and some ants culture fungi in their nests like leaf cutter ants [130]. Likewise certain groups of termites (i.e., higher termites belonging to Termitidae and Macrotermitinae) culture basediomycete fungi in their nests [131] while earthworms favor the growth of actinomycetes in their guts [130,132]. It follows that species richness of soil fauna is related positively to species richness of the soil microbial community.

Diversity of microorganisms and soil fauna is important for efficient nutrient cycling in soil [129,133,134]. Whalen et al. [135] reported that in humid temperate climates, the N mineralization by bacteria and fungi alone can account for 10% of the total soil N per annum; while soil micro-fauna, which predate on bacteria and fungi, also release mineral N as ammonium, which is estimated as 32 to 38% of total N mineralization per year. Soil meso- and macro-fauna also take part in nitrification and mineralization of N by making soil habitat favorable for microbial activity and by accelerating the decomposition of organic substrates [135].

Diversity of microorganisms and soil fauna is also important in making soil more suppressive to plant diseases [127,136]. For instance, in a study with continuously cropped soybean with long-term (10 years) application of herbicide, Wang et al. [136] found that the diversity of soil organism *Pseudomonas spp* decreased by 50% (results based on amplified ribosomal DNA restriction analysis (ARDRA) biomarkers), which also reduced the resistance of soil against soil-borne pathogens *Fusarium graminearum, Fusarium oxysporum*, and *Rhizoctonia solani*.

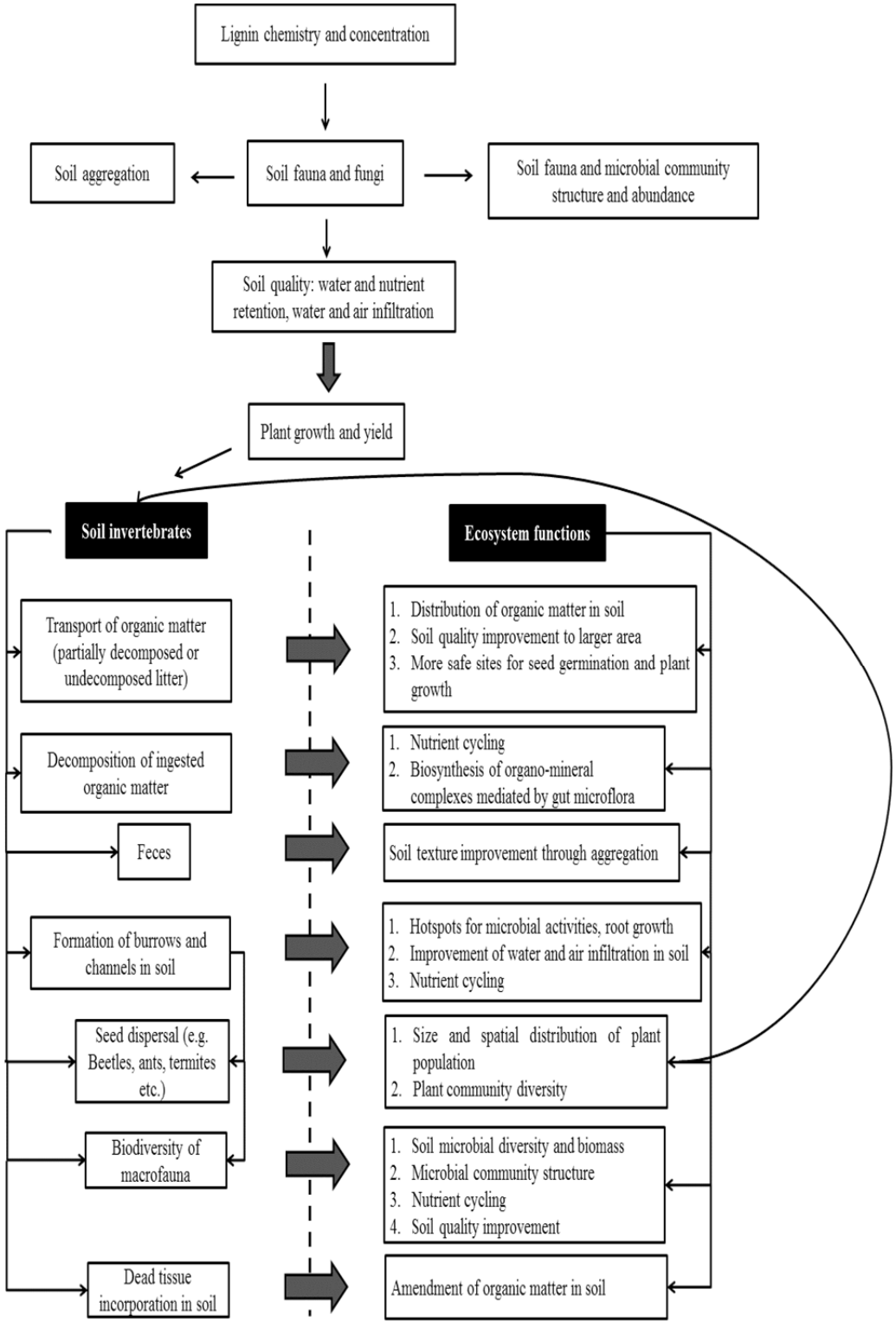


Figure 6. Hypothetical model illustrating the relationship between lignin, soil quality, plant yield and invertebrates, and the influence of soil invertebrates on soil ecosystem functions.

Table 2. AUF (g kg^{-1}), AUF:N ratio, total fauna density of soil and liter and species richness in various agroforestry systems (Moco et al., 2010)

	Agroforestry system		
Study observation parameter	**CRF**	**CE**	**NF**
AUF	368	264	348
AUF:N ratio	24	14	23
Total fauna density of soil and liter	1829 ± 307	1205 ± 227	1051 ± 248
Species richness of soil and liter fauna	11 ± 1	10 ± 1	10 ± 1

CRF is cacao system renewed under natural forest, CE is cacao system under *Erythrina* sp. and NF is natural forest near agroforestry system.

The control of lignin on soil fauna and microbial populations is conceptualized in Figure 6. There are many published reports indicating a positive influence of lignin on the abundance and diversity of soil fauna [137,138,139,140]. In a study in the humid tropics in a field with various litter types, Tian et al. [137] observed that the plots with slower decomposing litter (higher lignin:N ratio in litter) had a higher abundance of termites and millipedes, no change in the abundance of ants compared to control, while under such litter type there was a reduction in the abundance of earthworms likely linked to the low palatability of the mulch type. Adejuyigbe et al. [138] found a positive relation between concentration of AUF and density of microarthropods in both rainy (R^2 = 0.71) and dry (R^2 = 0.86) seasons in the degraded soil at Ibadan in southwestern Nigeria. Likewise, Moco et al. [139] observed that the litter type with greater concentration of AUF resulted in greater abundance and richness of soil fauna in cacao agroforestry systems in south of Bahia Brazil (Table 2). As well, Merciris et al. [141] found a positive relation between the palatability of litter (low concentration of AUF and high N content) with the abundance and species richness of soil fauna in two stands of a tropical semi-evergreen secondary forest with different plant community composition, however their results can be attributed to the species richness of plants at the study site rather than the palatability of liter. Diversity of soil macrofauna was also reported to relate to litter quality; Fujii and Takeda [142] reported that collembola community composition and abundance in root liter were greater than those in leaf litter in a litterbag field study in a coniferous forest. This and the findings of Tian et al. [137] suggest that litter type with greater concentration of lignin may influence soil faunal community composition but the abundance of soil organisms may increase or not be changed by litter type.

Lignin also influences soil microbial community structure. Fungal [143,144] and bacterial [110,111] niches develop during residue decomposition that contain lignin. The bacterial niche development is caused by the release/provision of degradable organic compounds from the degrading lignin, from fungal extracellular secretions, and from the direct bacteria feeding on fungal mycelia or development of a symbiotic relationship with the fungal mycelia [110,111]. Therefore, lignin degrading fungi favor colonization of a large number of bacterial species that range from mutualistic exudate-consuming bacteria such as certain species of *Bacillus*, *Burkholderia* and *Pseudomonas* genera [111] to endosymbiotic bacterial species belong to *Burkholderia*, *pandoraea* and *Ralstonia* genera [145] and mycophagous bacteria belonging to Collimonas [110,111] and Trichoderma [146,147] genera. Fungi also cause the colonization/growth of actinomycetes as this group of microorganisms feed on fungal cell walls [148].

Regulative Ecosystem Services: Lignins for Environmental Safety

Mitigating the greenhouse gas emissions

Globally, soils contain twice the amount of carbon (in the form of CO_2) as that in the Earth's atmosphere and three times of the carbon contained in the world's vegetation [149]. Small changes in this reservoir of carbon can have a large impact on greenhouse gas emissions and concentrations [149]. This carbon binds with nutrients which depending on soil conditions (i.e., anaerobic conditions), can produce other greenhouse gases besides CO_2 such as nitrous oxide (N_2O) and methane (CH_4). Of these greenhouse gases the CO_2, N_2O and CH_4 contribute ~60%, 6% and 20% to global warming, respectively [150]. Moreover, the global warming potential of N_2O is 298 times greater than CO_2 over a 100 year time frame due to its greater retention in the atmosphere; the extensive use of inorganic nitrogen fertilizer in agricultural fields is the major source of N_2O emissions from soils [150].

Mitigation occurs when CO_2 outputs from the soil ecosystem are less than CO_2 inputs from plant litter. That is, if we consider the annual C balance in a particular soil and there is more C entering from plant residues than leaving (from respiration/decomposition), the soil stores more C overall just like a carbon sink.

A more detailed description of how lignin/AUF controls the emission of CO_2 and N_2O is provided by Gul and Whalen [74]. Briefly, the production of CO_2 occurs under aerobic conditions while the production of N_2O occurs both under aerobic and anaerobic conditions. Lignin/AUF as discussed before, are not only slowly decomposable organic substances compared to other organic compounds, but they also provide physical protection to other organic compounds against biodegradation. Therefore, they play an important and direct role in slowing down the emissions of CO_2 from soil [6,28,151-155]. Yanni et al. [6] provided evidence that including indulin lignin by 0.5 % with stem residues of corn in soil, significantly reduced the emission of CO_2 by ~9%.

Dissolved organic carbon (DOC), a byproduct of crop residue and SOM decomposition, is a readily available source of carbon for microorganisms. Empirical evidence suggests a positive relationship between DOC and the emission of CO_2 from soil ($r = 0.823$, $P < 0.05$) [156]. On the other hand, lignin concentration is negatively related to the amount of DOC in soil. For instance, Moore et al. [154] found a negative relation between the concentration of lignin in plant litter and the production of DOC and a positive relation between DOC production and cellulose after 395 days of a laboratory incubation; furthermore, they found a significant negative relation between DOC production and CO_2:DOC quotient ($R^2 = 0.403$, $P < 0.001$). Overall, soil that contains more lignin can be expected to have lower decomposition of SOM and therefore less CO_2 emitted to the atmosphere.

The influence of lignin/AUF on reduction of N_2O and underlying mechanisms is documented in Gul and Whalen [74]. We are not aware of studies that demonstrates the control of lignin on CH_4 emission from soil. Due to the complexity of reactions and paucity of studies that can make a direct correlation between residue lignin concentration and N_2O and CH_4 emissions from soil, the topic will not be discussed. This topic is worthy of further study to better understand the mechanisms that control the emissions of these potent greenhouse gases to enable proper management and control their emissions from soil.

Bioremediation of toxic chemicals: An indirect influence of lignins

White-rot fungi produce a group of peroxidases that degrade lignin. Besides the degradation of lignin this lignolytic system oxidizes a large variety of environmental pollutants, both naturally-occurring and man-made [26,157-161]. Paszczynski and Crawford [26] in their literature review, provided a detailed report on the role of lignin-degrading peroxidases produced by a white-rot fungi *Phanerochaete chrysosporium* in detoxifying a large variety of xenobiotics (see also a literature reviews by Gianfred and Rao, [157], Baldrian [158], Couto and Herrera [162] Haritash and Kaushik [163], Arora and Sharme [164], Canas and Camarero, [159] and Gao et al. [165]). Examples of environmental pollutants which the lignolytic enzymes can degrade include chlorinated compounds such as DDT, simple and polycyclic aromatic hydrocarbons (PAHs) such as toluene, benzene, xylenes (BTEX), ethyl-benzene, pyrene, certain polymeric dyes from textile, leather, and paper industries such as poly R-478, Ramazol brilliant blue R, azo dyes (3,5-dimethyl-4-hydroxy-zobenzene-4-sulfonic acid), nitrosubstituted compounds such as nitro-arenes and certain cyanides [26]. Levin et al. [161] reported that a white-rot fungi *Criolus antarcticus* via solid-state fermentation on grape stalks produced laccase activities (as 33.0 U/g dry solid) and Mn-peroxidase (as 1.6 U/g dry solid) that were able to decolorize 93, 86, 82 and 58% of indigo carmine, malachite green, azarc B and xylidine dyes, respectively from textile processing effluents in 5 hours. Lignin participates in the bioremediation of xenobiotics by promoting fungi, which can metabolically degrade xenobiotics.

Disease suppression to soil

Melanins are thought to cause pathogenesis in plants by shielding plant pathogens from recognition by plant defense systems [29] and are required for the penetration of pathogenic fungi in host plants [166,167]. Ligninases (lignin degrading enzymes) produced by white rot fungi can completely degrade melanins [29]. In another study, Montanari and Innocenti [168] reported a suppressive effect of lignosulphonates, the by-products of the pulping process, against sclerotia viability of *Sclerotinia sclerotiorum*. A 1.5% v/v amendment of lignosulphonates in peat + coconut fiber potting mix caused 50% reduction in the viability of sclerotia by enhancing the activity of the indigenous mycoparasitic fungi i.e., *Fusarium oxysporum* and *Trichoderma* spp. However, there are certain studies which report a disease-inducing role of lignin degrading enzymes. For instance, Michielse et al. [169] reported that 3-carboxy-cis, cis-muconate lactonizing enzyme, from the β-ketoadipate pathway for further degradation of degraded lignin products, induced pathogenicity of *Fusarium oxysporum* in tomato. Because their study was solely laboratory based, we cannot conclude from their finding that this effect could be achieved in the natural soil environment. Concentration of humus and the density and diversity of microorganisms may substantially contribute in the suppression of pathogenicity in plants. For instance, humus may promote microbial diversity, which plays a role in disease suppression to plants through soil. For example, Elo et al. [84] reported a suppressive property of the humus layer of Norway spruce stands due to high proportions of bacterial species, which were antagonistic towards plant pathogens *Botrytis cinerea*, *Fusarium culmorum* and *Rhizoctonia sp*. Likewise, Gur et al. [87] observed enhanced growth of apple trees infected with replant disease in response to composted or earthworm humus.

FACTORS INFLUENCING LIGNIN DYNAMICS IN SOIL

Plant Residue Chemistry

Plant residue chemistry in terms of C:N ratio and the concentration of lignin exerts a significant role in controlling the biodegradation rate of residue in soil [5,7,28,37,39,72,170-172]. During degradation, S, G, and H lignin units (analyzed by CuO degradation) change from their aldehyde to their acid forms [13]. The acid: aldehyde ratio (Ad/Al) of the analyzed lignin monomers are considered biomarkers for the assessment of degradation of lignin in soil [5,6,13]. Gul et al. [155] reported that a 38% lower AUF and 29% lower C:N ratio in the stem residues of *Arabidopsis thaliana* down-regulated for cinnamoyl CoA reductase 1 (*CCR1*) caused 22% increase in Ad/Al ratio of G units and 41% increase in Ad/Al ratio of S units in CuO oxidation products from lignin of residues in soil incubated for 63 days under controlled laboratory conditions. Likewise, Sanaullah et al. [5] found that the young leaves of *Festuca arundinacea*, which had 37% lower C:N ratio and 21% lower concentration of lignin as compared to senesced leaves, saw a ~37% reduction in concentration of lignin as compared to senesced leaves, after 44 weeks of burial in soil. They attributed the higher degradation of lignin in young leaves to lower C:N ratio and lower concentration of lignin.

Inorganic Fertilizers

The influence of inorganic N on the degradation of lignin was described by Gul and Whalen [74]. The published data is contradictory - ome papers reported the negative influence of inorganic N fertilizer on the degradation of lignin [173-179] while others found a positive influence of inorganic N fertilizer on the degradation of lignin [179]. The N that comes from the mineralization of plant residues tends to aid in the degradation of lignin [6,28,32]. For instance, Gul et al. [28] reported that the stem residues of *A. thaliana* down-regulated for CCR1 with significantly reduced C:N ratio and AUF caused 14% increase ($P \leq 0.05$) in the concentration of mineral N of soil and the degradation of soil lignin was also higher for these residue amended soils. Similar results were reported by Yanni et al. [6], who found that corn leaf residues with a lower concentration of AUF and lower C:N ratio than stem and root residues, caused an increase in soil mineral N and showed a greater Ad/Al ratio of lignin phenols.

There is limited data to evaluate the influence of inorganic P fertilizer on degradation of lignin of soil. The available data suggests no effect of P+N fertilizer on the degradation of lignin in soil. For instance, using ^{13}C tracers, Hofmann et al. [180] found no effect of over 36 years N+P fertilizer application in arable soil (Cadriano field experiment, University of Bologna, Italy) on the amount of old SOC and lignin, however, they found indications of enhanced degradation of newly incorporated lignin in soil. In another study, Liu et al. [178] found no influence of N+P fertilizer application on degradation of lignin in Mollisols of northeastern China.

Early experiments on the degradation of lignin by *Phanerochaete chrysosporium* indicated that low soil N concentration was required for the onset of lignin degradation i.e., lignin biodegradation occurs during secondary metabolism [181]. Evidence of this

phenomenon was also found for white rot and other fungi [182,183]. However it cannot be generalized because of contradictory evidence from other lignin degrading fungi. Entry and Backman [184] found that the addition of N without easily degradable C sources increased cellulose degradation but not that of lignin, which was degraded only when both C and N were added, and a similar report was presented by Perie and Gold [185] who found higher mineralization of lignin by *Dichomitus squalens* under high C-low N conditions compared to high C-high N.

Another nutrient that has been shown to affect lignin degradation is manganese (Mn) as it goes into the formation of Mn-peroxidase (Mn-P), which is an important lignin degrading enzyme produced by many fungi [11,181,186] including *P. chrysosporium, D. squalens, C. versicolor, P. radiata, R. lignosus*. Soil Mn concentration regulates the production of laccase and lignin peroxidase in *P. chrysosporium* [187]. High levels of Mn increase the production of Mn-P, which seems to be required for initial depolymerization of lignin, but these levels suppress the production of lignin peroxidase, which is needed for further mineralization of lignin by *P. chrysosporium* [187]. Périé and Gold [185] showed that cultures of *D. squalens* produced Mn-P in the presence but not in the absence of Mn; the expression of laccase was not affected by the presence or absence of Mn in the *D. squalens* culture. As well, Camarero et al. [159] also showed that Mn-P was produced in cultures of several *Pleurotus* species, however the level of Mn-P did not change with the addition of Mn^{2+} but rather this addition stimulated high activity of lignin mineralization, suggesting that the activity of Mn-P was limited by the availability of Mn which oxidizes Mn^{2+} to Mn^{3+}.

Climate Change

Global warming causes a shift in rainfall distribution; arid and semiarid regions are becoming more arid and mesic regions are getting more rainfall [188]. Prolonged exposure of litter to sunlight in arid regions and high humidity for prolonged periods from more rainfall in mesic regions along with increasing temperature may cause an increase in degradation of lignin through photooxidation and also as a result of increased microbial biomass. The following sections will discuss those phenomena.

Photooxidation

Lignin is an effective light-absorbing organic substance, which absorbs light over a wide range of wavelengths [189]. Lignin and aromatic compounds can be chemically altered by UV light and can become more susceptible to photo-degradation [189-194]. The decrease in the concentration of lignin by photoeoxidation in plant residues expedites their mineralization in soil [5,189].

Soil moisture and soil temperature

Soil moisture and soil temperature control microbial activities and associated decomposition of organic matter in soil. Donnelly et al. [195] reported a positive correlation between soil moisture and microbial biomass in a curvilinear relationship ($r^2 = 0.88$, $n = 9$, $P<0.05$). They also found a positive curvilinear relationship between microbial biomass and

degradation of lignin ($r^2 = 0.47$, $n = 9$, $P < 0.05$). Moreover, they found a positive correlation between soil temperature and decomposition of lignin at 40% and 60% soil water contents ($r^2 = 0.92$).

Soil Microbial Community Structure in Relation to Plant Residue Chemistry

Soil microorganisms play a major role in the formation of SOM or humus, which is considered a relatively slow decomposable organic fraction of soil and can contribute to soil C storage [92] under the right environmental and soil conditions. Participation of microorganisms in the formation of SOM is through transformation of organic substances (lignin-rich and other plant residues) into slowly decomposable humus/humus-like forms, and their contribution to the formation of soil aggregates via secretion of extracellular organic compounds (i.e., enzymes, polysaccharides etc.)[83,92]. Cotrufo et al. [92] postulated that plant residues with higher amounts of labile organic compounds contribute more to the formation of SOM than plant residues with higher concentrations of slowly decomposable organic compounds (i.e., lignin). They attributed this phenomena to the carbon-use-efficiency (CUE) of microorganisms which is higher for labile than recalcitrant organic substances. Microbes that scavenge on plant residues with higher concentration of readily degradable organic compounds incorporate higher amounts of plant organic substances into their biomass and therefore, participate more in SOM formation than the microbes that scavenge on lignin-rich plant residues. The conclusion of Cotrufo et al. [92] is contradicted by many published reports demonstrating the higher emission of CO_2 in response to the mineralization of plant residues with lower concentration of lignin/AUF and lower C:N ratio [6,153,28,155,196,197]. Moreover, plant residues with lower concentration of AUF also cause the reduction of soil lignin [5,6,155]. Garcia-Pausas and Paterson [198] found that readily degradable organic substances cause a priming effect on the degradation of residual SOM by promoting the activities of actinomycetes and fungi, which scavenge on the recalcitrant organic substances more effectively than gram-negative bacteria [199,200,201].

Moreover, Cotrufo et al. [92] did not consider the importance of microbial community structure in the formation of soil aggregates. Bacteria and fungi act differently in the formation of aggregates; fungi promote macro aggregation more efficiently than bacteria [108,74]. The stabilization of macro aggregates depends on the activities (i.e., amount of extracellular secretions by microbes) of fungi and bacteria in concert [74] while plant residues provide a primary skeleton to macro aggregate formation [101]. The fast mineralization of plant residues with lower lignin concentration thus can decrease the size of aggregates [30,101], which with an associated lower growth of fungi can reduce macro agregation of soil and in turn reduce the formation of stable SOM.

LIGNIN ALTERATION AND ITS INFLUENCES ON ECOSYSTEM SERVICES

Enhanced Biofuel and High Quality Paper Production

Today, there is a demand for biofuel production to reduce the use of fossil fuel for environmental safety. Production of genetically modified (GM) crops with a reduced concentration of lignin and/or reduced V:S ratio is one of the options to produce more biofuel per unit dry weight of plants [202]. The presence of lignin and lignin with a high V:S ratio [202,203] hinders the process of fermentation of cellular carbohydrates to produce biofuel [204]. Also, the presence of lignin negatively affects paper production. Many advances have been made since the last decade regarding the introduction of various mutations in order to reduce the lignin concentration and/or the V:S ratio of lignin units in bioenergy crops and in *A. thaliana*, a model plant (Table 3). These mutations include the following examples: a) down-regulation of cinnamoil-CoA-reductase 1 (*CCR1*), which is reported to reduce the concentration of lignin by more than 30% (Table 3), b) expression of the maize (*Zea mays*) Corngrass 1 (*Cg 1*) gene which promotes juvenile cell wall identities was reported to cause enhanced biomass production and some increase in lignin concentration in maize and switchgrass (*Panicum virgatum*) [216], c) down-regulation of Caffeoyl CoA-3-O-methyl transferase (*CCOMT*) is reported to cause reduction in the V:S ratio and in the concentration of lignin in *A. thaliana*, tobacco and poplar. A detailed account of various mutations and their influence on the concentration and chemistry of lignins is provided by Li et al. [209], Gul and Whalen [74], and is summarized in Table (3). The influence of various factors on degradation of lignin and its feedback on ecosystem functioning is conceptualized in Figure (7). Plant gene alterations which reduce lignin concentration in plants for biofuel and paper production are reported to enhance the mineralization of GM mutant residues in soil [28,226-229] and are well documented in Gul and Whalen [74]. Moreover, those residues can also enhance the degradation of lignin in soil, which can act as a barrier to the degradation of residual SOM. Those genetic alterations, if coupled with environmental factors that favor the degradation of lignin in soil, can increase the rate of SOM decomposition which in turn affect soil aggregation and all its associated soil-health promoting services. These factors in turn can reduce the biomass yield of crops, enhance soil erosion, increase leaching of nutrients and organic pollutants, and cause poor nutrient cycling [30,101]. Such deteriorations in ecosystem functioning will deteriorate ecosystem services.

High Concentration and High V:S Ratio of Lignins in Plants

If we accept that lignin can play a role in stabilization of SOM under the right soil environmental and management conditions, then it can be said that building up lignin input in the soil is a positive activity for improving soil quality and for environmental safety. Genes have been identified that are responsible for enhancing the concentration of lignin and increasing the V:S ratio of lignin. The down-regulation of KNOTTED ARABIDOPSIS THALIANA 1 (KNAT7) [209,28,155] and PRODUCTION OF ANTHOCYANIN

PIGMENT 1 (PAP1/MYB75) [225] are examples of gene modifications reported to increase the concentration of lignin/AUF and V:S ratio in inflorescence stem tissues of *A. thaliana*.

Another discovery for secondary cell wall biosynthesis was made by Li [209]. They found that down-regulation of OVAT FAMILY PROTEIN 4 (OFP4) increase cell wall thickness in inflorescence stem of *A. thaliana*. Moreover, they also found that KNAT7 down regulation increased the influence of down-regulation of OFP4 and resulted in enhanced secondary cell wall thickness in inflorescence stem of *A. thaliana*. However, they did not quantify the concentration of lignin and the V:S ratio in those mutant lines. Since down regulation of KNAT7 is reported to increase the concentration of AUF and V:S ratio in inflorescence stems of *A. thaliana*, further research is needed to confirm the influence of double down-regulation of OFP4 and KNAT7 on the concentration and chemistry of lignin in stems.

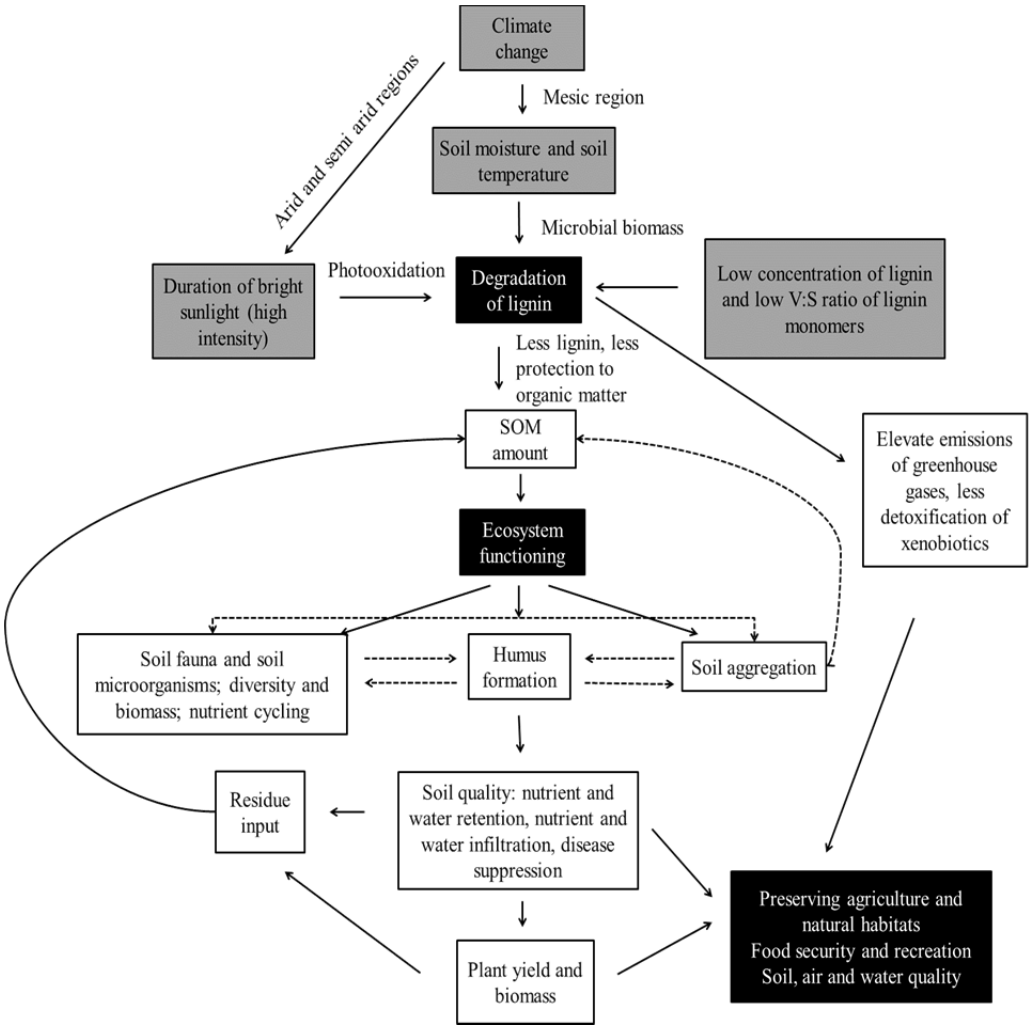


Figure 7. Hypothetical model illustrating the influence of climate change parameters on degradation of lignin, the consequences for the soil organic matter pool, and feedbacks on soil quality, crop production and ecosystem services.

Table 3. Mutation in enzymes/genes responsible for lignin chemistry, abbreviations of the names of those enzymes, concentration of lignin (mg g^{-1} plant tissue dry weight) and V:S ratio in mutated plant species

Mutated enzyme/gene	Abbreviation	Lignin	V:S ratio	Plant species	Reference
4-coumarate, CoA ligase1	*4CL* k/o	Low	nd to High*[2]	*Populus tremuloides* Rice	[205] [206]
Cinnamyl alcohol dehydrogenase	*CAD* k/o	Low	High	*Medicago sativa* *Nicotiana tabacum*	[212] [208]
Cinnamyl alcohol dehydrogenase encoding flexible culm 1 gene	*FC1* k/o	Low	--*[3]	Rice	[47]
Coniferaldehyde 5-hydroxylase	*CAld5H* o/x	nd*[1]	Low	*Populus tremuloides*	[210] [205]
Cinnamyl CoA-reductase 1	*CCR1* k/o	Low	Low high*[2]	*A. thaliana* *Medicago sativa* *Nicotiana tabacum* Poplar Rye grass	[211] [212] [213] [214] [215]
Corngrass 1	*Cg1* expression	Low	--	*Panicum virgatum* …..	[216]
Caffeoyl CoA 3-O-methyl transferase	*CCOMT* k/o	Low	Low	*A. thaliana* Poplar Tobacco	[217] [218] [219]
Caffeic acid O-methyl transferase	*COMT* k/o	nd, low*	High, low*[2]	*A. thaliana* *Nicotiana tabacum* Poplar Rye grass	[220] [219] [221] [222] [215]
Early Arabidopsis aluminum-induced gene 1	*EARLI1* k/o	Low	--	*A. thaliana*	[223]
Shikimate hydroxycinnamoyl transferase	*HCT* k/o	Low	--	*Medicago sativa*	[224]
Knotted Arabidopsis thaliana 7	*KNAT7* k/o	High	High	*A. thaliana*	[137]
Production of anthocyanin pigment 1/ myeloblast 75	*PAP1/MYB75* k/o	High	High, low*[2]	*A. thaliana*	[225] [226]
Double mutations					
4CL k/o + *CAld5H* o/x		Low	Low	*Populus tremuloides*	[205]
CAD k/o, *CCR1* k/o double mutant		Low	--	*A. thaliana*	[227]

*[1] represents not different.

*[2]Article reporting for low, not different and high V:S ratio for a given mutation.

*[3] represents no data.

Gul and Whalen, 2013.

Tilston et al. [230] studied the decomposition of tobacco roots that have been genetically modified for down regulation of cinnamyl alcohol dehydrogenase (CAD) and caffeic acid O-methyltransferase (COMT), which affect lignin formation in stems, and they reported a subtle and short-term effect of lignin modification on decomposition. Such modifications would only increase lignin input to the soil if they are not removed with the harvested plant parts. Therefore they would be beneficial if lignin-rich stems and leaves are returned to the soil after harvest or alternatively if lignin increase is targeted for roots which are not removed from the soil at harvest and which are undisturbed by no-till farming.

Modifying plants for longer and bigger root systems is also suggested as one means of increasing C input deeper into the soil horizon where decomposition is relatively slower than in topsoil however, priming of decomposition by the introduction of labile C into the subsurface soil would need to be considered in this case [231-234]. Lignin-enhanced plant parts can be viewed as a relatively stable C source that is added into the soil and expected to have delayed decomposition when compared to other lignin-poor plant residues. This delay gives a chance for building soil aggregates and forming organo-mineral complexes, which protect C compounds from degradation. Although technical advancement in scientific research will improve the recalcitrance or susceptibility of lignin to decomposition, its delayed decomposition or slow degradation under certain conditions and its degradation by-products that complex with the indigenous SOM and mineral soil particles will still play a positive part in building SOM reserves, and on soil health and soil ecosystem services in general.

CONCLUSION

Lignins are polyphenolic organic substances that provide mechanical support to plants and protect plants from pest and pest-associated diseases. The chemical recalcitrance of lignin makes it relatively difficult to degrade in soil, which contributes directly or indirectly to build SOM and supports a number of essential soil ecosystem services. These services include retention of nutrients and water through formation of humus and soil aggregation, degradation of organic pollutants via lignin-degrading fungi, disease suppression through protection of soil enzymes and melanin degradation by lignin-decomposing fungi, and reduction of greenhouse gas emissions by slowing mineralization of organic matter. The degradation of lignin is affected by its concentration in plant residues and its chemistry in terms of V:S ratio, temperature and moisture of soil, soil microbial composition, and its location (physical protection) in the soil structure.

The expanding biofuel industry provides economic incentives for farmers to harvest and sell plant residues that would normally remain in the field. As well, lignocellulosic residues destined for liquid biofuel production will be more readily transformed through biochemical conversion when they have a lower lignin content or more degradable lignin, thus favoring the selection of cultivars with altered lignin concentration or lignin chemistry. Removal of plant residues for biofuel production and the alteration of lignin in plant tissues can reduce the amount of lignin in soil as well as the SOM. Removal of plant residues reduces lignin and OM inputs to the soil, thus may not replenish soil C pools that are depleted continuously by decomposing microbes, whereas; if the residue that enters the soil has lower lignin

concentration and altered lignin chemistry (i.e., lower V:S ratio), it decomposes more quickly, and in return causes faster CO_2 release back to the atmosphere and therefore, shorter residence time of the plant C in soil; CO_2 mitigation effect of the soil is therefore reduced. Climate change effects such as increase in temperature and sunlight exposure can create favorable environments for SOM decomposition. These changes are predicted to reduce soil quality and therefore affect crop production and the role of soil in environmental safety including an increase of greenhouse gas emissions from soils and reduction of degradation of organic pollutants in soils.

To maintain or increase the quality of soil, there is a need to increase the organic matter content of soil by inputing plant residues with high lignin content or lignins with high V:S ratio. Future research is required to unearth the pathways and genes that can increase the concentration of hard-to-degrade lignin in non-harvested plant tissues, especially in the roots. Assessment of how down-regulating key genes in lignin biosynthesis, such as KNAT7, MYB75 and OFP4, could support the development of C-sequestering crops with potential to boost SOM reserves under field conditions is also needed.

Any negative effects on SOM dynamics from the cultivation of second-generation biofuel crops or from intensive cropping should be offset by alternative measures such as crop rotation, conservation agriculture measures, intercropping and cover crops, and possibly utilizing GM crop varieties with elevated concentrations of lignin, especially in the roots.

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