

Allelopathic and Environmental implications of plant phenolic compounds

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(Received in revised form: February 2, 2012)

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ABSTRACT

Interest in phenolic compounds has greatly developed during the past decade, hence, numerous biological, chemical and agricultural studies have been done. Thus much information has accumulated on the role of phenolic compounds in the environment. These have been investigated as allelopathic agents, plant growth regulators and for chemical defence in plant-pathogen interactions. Ecosystems degradation due to the effects of phenolic compounds has become very significant because it caused problems of natural regeneration failure, reforestation and management with serious ecological consequence. This paper reviews some recent findings, on role of phenolic compounds in plant-plant, plant-soil-microbial and plant-pathogens interactions with special emphasis on ecosystem degradation and regeneration.

Key words: Allelopathy, autotoxicity, forest ecosystems, phenolic compounds, plant-plant interactions, plant-soil-microbial interactions, regeneration failure, soil microbes

1. INTRODUCTION

Phenolics are carbon-based secondary metabolites with a wide range of chemical properties, and ranges from low molecular weight phenolic acids to the high molecular weight condensed tannins. These chemicals are widespread in plants, and play an important role in the soil-plant-environment interaction (50,51,72,73). Phenolics actively released by plants or passively produced during the decomposition process of both above- and below-ground plant residues affect abiotic and biotic processes in the ecosystem, thereby modifying the behavior of not only other plants (72) but also the microbial dynamics as well (6). When released in the soil, phenolics affects the soil nutrients dynamics by forming complexes with proteins and delaying organic matter decomposition and mineralization (12) and by increasing the rhizosphere soil microbial activity and N immobilization (13), resulting in decrease in inorganic N available for plants uptake (19). Additionally, they affect ion transport, protein synthesis, hormone activity and energy metabolism (48,49,85). As allelopathic agents, they inhibit the seed germination and root elongation in higher plants (45), affects photosynthesis (18,45), respiration (39), and water balance (7) resulting in the reduced plant growth and reproduction (98).

Several environmental factors increases the amount of phenolics produced by plants. Generally, intensity, duration and quality of light influences the phenolics production (47). Mineral deficiencies in soil, drought stress, temperature stress, herbicides and other allelochemicals induce greater production of phenolic compounds. It appears that any factor that induces stress can cause an increase in phenolics production and/or release (73). In addition to natural environmental variation, plants cope with a variety of human-induced environmental changes, the rate and magnitude of which have greatly increased during the last decades. Human-induced alterations in the abiotic environment have a significant impact on the production and accumulation of phenolic compounds in plants (91).

Phenolics are highly variable in solubility, and may have a tendency to condense into less soluble forms, or to react with larger molecules (4). Partly hydrolyzed tannins are a common example of soluble phenolics, which either serve as defense against herbivory and are either lignin precursors, or waste products. These chemicals are also recognized as enzyme and general microbial inhibitors as well (36).

In many plant species, root exudates play a direct role as phytotoxins in mediating chemical interference (i.e., allelopathy). In addition, root exudates are critical to the development of associations between some parasitic plants and their hosts. Finally, these may play indirect role in resource competition by altering the soil chemistry, soil processes and microbial populations. Many phenolics produced by dicotyledones have the potential to form complexes with metallic micronutrients and may increase metals availability.

Environmental implications of the phenolic compounds are difficult to quantify and evaluate in field conditions due to numerous confounding factors. Low concentration, little persistence and possibility of chemical alterations by soil microorganisms makes it difficult to determine their *in situ* presence and effects in soils (89). In addition, the interactive nature of phenolic compounds and occurrence of multi stresses under field conditions further complicates the problem. Interest in phenolic compounds have increased in many recent studies as numerous chemical, biological, agricultural and medicinal

aspects have been attributed to them, thus much information has accumulated on them. This paper overviews the recent findings on effects of phenolic compounds in plant-plant, plant-soil-microbial and plant-pathogens interactions.

2. EXPERIMENTAL METHODS

Analytical capabilities for phenols have improved tremendously during the past few decades (62). For example, Reigosa (71) suggested detailed procedures to measure and analyse the inhibitory effects of phenolics on photosynthesis, respiration, transpiration, relative water content and water potential as main physiological functions of plant organs, tissues and cells. Later, Narwal and Roshchina (55), and Narwal *et al.* (56) offered detailed procedures of sampling, analysis and experimental treatments in laboratory, greenhouse or field conditions. Although methods described are focused on phenolics, the methods described are useful for analysis of a variety of chemical compounds including phenols. Finally, Blum (8) new book, describes the underlying criteria and assumption in the development and execution of field and associated laboratory bioassays with details and commentary regarding the materials and methods used.

Olk (62) has reviewed the analytical procedures for soil phenols, from a relatively simple procedure of NaOH extraction of soil, and analysis of phenol species by gas chromatography or HPLC through oxidation with CuO or with tetramethylammonium hydroxide, and detection by gas chromatography. Thus neither approach appears to allow extraction of all soil phenols, even the relatively caustic CuO oxidation, yet both approaches have given useful, reproducible results, and the challenge is to synthesize their results into a fundamental understanding of phenol chemistry. Martens (43) found large amounts of phenols in monocotyledonous plants than in dicotyledonous plants by measuring the soil biochemical compounds including phenols, by using NaOH extractions to distinguish ester-linked phenols (mostly coumaric acid and ferulic acid) from ether-linked phenols (mostly vanillyl and syringyl) in soil and plant samples.

Near infrared reflectance spectroscopy (NIRS) is rapid, cheap and non-destructive technique offering the potential for accurate and repeatable measurements of chemical constituents in organic materials. Therefore, Couteaux *et al.* (14) used NIRS technique to determine the water-soluble and total extractable polyphenols in plant material. The proposed NIRS analysis is based on the use of regression models between the spectral information of a set of samples and their reference values. Authors attempted to fit regression models to total and water-soluble extractable polyphenol content (TEP and WEP) of undecomposed (biomass and necromass) and decomposing plant material and proposed these models as an easy tool for rapid determination of phenolic content in plant material. NIRS method also allows large screening for studies e.g. polyphenols control on decomposition process and phenolics implication in herbivory.

Antioxidant properties of phenolic compounds have been studied by UV-visible and ESR spectrometry, fluorescence, chemiluminescence, chromatography, and electrochemistry. However, Yakovleva *et al.* (93) characterized phenolic acids and flavonoids by cyclic voltammetry and total antioxidant activity in reaction with the ABTS cation radical. Anode peak voltages (E_{ap}) and their pH dependences were determined for the phenolic acids and flavonoids. Authors found correlation between the E_{ap} and Trolox

equivalent antioxidant capacity (TEAC) values for polyphenols, which react with the ABTS cation radical in two steps. Correlation between the half-wave potential ($E_{1/2}$) and TEAC was determined for electrochemically irreversible compounds. They proposed the mechanisms of the reaction of phenolics on the electrode, which involve one- and two-electron oxidation.

To determine the polyphenols from plant extracts, Diaconu *et al.* (16) proposed a biosensor based on the use of redox enzymes and chitosan–MWCNT nanocomposite, with ITO electrode modified with multiwalled carbon nanotubes, and the enzymes laccase and tyrosinase co-entrapped into a chitosan matrix. The resulting biosensor was calibrated at -50 mV (vs. the Ag/AgCl reference electrode) using rosmarinic acid, caffeic acid and gallic acid as the substrates. The new biosensor resulted in a 10.7-fold increase in response sensitivity and a considerable improvement in the detection limit (42 nM for rosmarinic acid). To prevent the fouling of the surface of the biosensor the surfactant Tween 20 was applied. The data recorded in surfactant medium revealed a significant improvement in the operational stability and an enlarged linear concentration range (up to 12 μ M for rosmarinic acid).

To determine the total phenolic content in crude and hydrolyzed plant extracts, Mišana *et al.* (46) developed a rapid resolution HPLC/DAD method. The analysis has been done on a 1.8 μ m, 4.6x50 mm column, to enable a rapid separation of a mixture of 17 compounds, which consisted of hydroxybenzoic acids, hydroxycinnamic acids, flavones, flavonols, flavanone, flavonol-glycoside and anthraquinone, in a single run, within 22 min. Authors stated that a proposed method is precise, accurate and sensitive enough for simultaneous quantitative evaluation of major compounds in crude and hydrolyzed extracts of different plant species. To overcome the inability to quantify all the phenolic compounds present in the samples due to lack of external standards, authors used HPLC to estimate the total phenolic content based on the sum of all integrated peak areas. Considering that there were no significant differences between the results obtained by HPLC and total phenolic content determined spectrophotometrically by Folin–Ciocalteu method, authors proposed this method as a tool for total phenolic content estimation, without complete identification of the individual compounds.

3. PLANT-PLANT INTERACTIONS

Recent studies of plant-plant interaction mediated by chemicals released in the environment showed significant progress by using creative experimental design, sophisticated chemical analyses and careful data interpretation (42). The progress has been made in fundamental understanding of the process as well as its application in agriculture, forestry and aquatic ecosystem management, which should help in solving many environmental issues in the future.

Plant-plant interactions occur due to the action of organic substances released from living plants or decomposing plant residues into the environment. These substances may be oxidized or reduced before influencing associated plants, in a positive or negative manner, either due to direct (e.g., affect root membranes and/or cell process) or indirect (e.g., impact on nodule or mycorrhiza formation, development and/or function) actions of allelopathic agents (8).

3.1. Seed germination and seedlings growth

Allelopathic effects of phenolics inhibits the germination and seedling growth of plants. In disturbed ecosystems, such effects may cause changes in species diversity, a dominance of one species over another. For example, Grombone-Guaratini (25) found inhibitory effects of quercetin, rutin and ferulic acid from aqueous and ethanolic extracts of mature bamboo *Aulonemia aristulata* (Döll) McClure on germination and growth of test plants *Lactuca sativa* L. and *Sesbania virgata* (Cav.) Poir., which explains bamboo-dominance in disturbed areas in Atlantic Forest.

High content of water-soluble phenolic acids in forest soil may inhibit the germination of tree seeds. For example, phenols extracted from the forest soil reduced the seed germination showing phytotoxic effects in *Pinus laricio* Poiret ssp *calabrica*, the predominant and characteristic coniferous species in Aspromonte (Southern Italy). Germination tests were conducted on seeds of *Pinus laricio* Poiret, *Pinus pinaster* Aiton, and *Pinus halepensis* Mill., coniferous species wide spread in the Mediterranean environment (50).

Table 1. Germination (%) of *Pinus* species treated with phenolic acids and phenols mixtures from the litter layer and soil Ah horizon in winter and summer (50)

Treatment	<i>Pinus laricio</i>	<i>Pinus pinaster</i>	<i>Pinus halepensis</i>
Control (H ₂ O)	80(2) ^a	70(3) ^a	75(3) ^a
Vanillic acid (0.1 Mm)	48(2) ^d	35(2) ^d	34(2) ^d
Vanillic acid (1 mM)	40(3) ^e	27(1) ^e	25(3) ^e
Benzoic acid (0.1 mM)	76(3) ^a	68(3) ^a	70(5) ^a
Benzoic acid (1 mM)	70(2) ^b	64(2) ^{ab}	60(1) ^c
Salicylic acid (0.1 mM)	72(2) ^b	62(1) ^b	61(2) ^c
Salicylic acid (1 mM)	65(1) ^c	58(1) ^b	57(1) ^{cd}
Syringic acid (0.1 mM)	72(2) ^b	60(1) ^b	62(2) ^{bc}
Syringic acid (1 mM)	68(3) ^{bc}	55(3) ^c	58(1) ^c
Ferulic acid (0.1 mM)	75(2) ^{ab}	60(2) ^b	65(1) ^b
Ferulic acid (1 mM)	69(4) ^b	53(2) ^c	59(2) ^c
Protocatechuic acid (0.1 mM)	75(3) ^{ab}	65(2) ^{ab}	60(3) ^c
Protocatechuic acid (1 mM)	68(3) ^{bc}	57(3) ^{bc}	55(1) ^d

Numbers in parentheses denote the standard error of the mean, n=5

*Values in the same column, followed by the same letter, are not statistically different at P=0.05

3.2. Photosynthetic pigments

Reduced chlorophyll content in plants treated with phenolics has been frequently reported (94). Treatments with phenolic acids reduced chlorophyll content in leaves. For example, Djurdjević *et al.* (18) studied effects of phenolics from trees on photosynthesis and chlorophyll content of the vernal ephemeroïd *Arum maculatum* L. via the bioavailable rhizosphere soil. They found positive correlation between the Chl a/b ratio with the derivatives of cinnamic and benzoic acids, whereas total chlorophyll content was positively correlated with the derivatives of cinnamic acid, and negatively with the derivatives of benzoic acid. Therefore, authors concluded that chlorophyll biosynthesis in general is related to the phenolics in the rhizosphere soil.

Table 2. Average monthly values for chlorophyll content and growth parameters of *Arum maculatum* (18)

	March	April	May
Chla+b (mg g ⁻¹)	21.6172.05 a	25.2072.60 b	30.7672.28 c
Chl a/b	3.0170.14 a	2.6670.28 b	2.4970.20 b

Chl, chlorophyll content per leaf dry mass; Chl a+b, Chl a/b ratio; n =10; Mean±S.D.; *Different letters mark significant differences at p>0.05

The effects of phenolic acids have been studied on the growth and photosynthetic pigments (chlorophyll a and b, carotenoids) of *Echinochloa crus-galli* L., *Setaria viridis* L., *Chenopodium album* L. and *Galinsoga parviflora* (83). Stupnicka-Rodzynkiewicz et al. (83) found a reduction in plant dry matter and also a reduction of photosynthetic pigments content in *Echinochloa crus-galli* aboveground parts induced by ferulic acid and *p*-coumaric acid. Contrary, in *Chenopodium album* L. a decrease of chlorophyll a and b content was caused by vanillic acid. Similarly, Canakci and Munzuroglu (11) found a loss of chlorophyll a+b in radish cotyledons induced by salicylic acid.

Table 3. Photosynthetic pigments (mg g⁻¹ dry mass) in above ground parts of *Echinochloa crus-galli* and *Chenopodium album* as influenced by different phenolic acids (83)

Phenolics	<i>Echinochloa crus-galli</i>			<i>Chenopodium album</i>		
	Chl a	Chl b	Ccarotenoids	Chla a	Chl b	Carotenoids
Chlorogenic acid	0.677 ^{abc}	0.396 ^{ab}	0.065 ^{ab}	0.396 ^c	0.256 ^d	0.028 ^{ab}
Ferulic acid	0.487 ^a	0.262 ^a	0.046 ^a	0.309 ^{abc}	0.179 ^{abcd}	0.021 ^{ab}
<i>p</i> -Hydroxybenzoic acid	0.906 ^{bc}	0.405 ^{ab}	0.086 ^b	0.323 ^{bc}	0.202 ^{bcd}	0.023 ^{ab}
<i>p</i> -Coumaric acid	0.638 ^{ab}	0.316 ^a	0.061 ^{ab}	0.255 ^{abc}	0.131 ^{abc}	0.031 ^{ab}
Protocatechuic acid	0.494 ^a	0.274 ^a	0.044 ^a	0.244 ^{abc}	0.147 ^{abc}	0.023 ^{ab}
Salicylic acid	0.731 ^{abc}	0.388 ^{ab}	0.061 ^{ab}	0.292 ^{abc}	0.215 ^{cd}	0.022 ^{ab}
Trans-cinnamic acid	0.637 ^{ab}	0.367 ^{ab}	0.043 ^a	0.172 ^{ab}	0.118 ^{ab}	0.014 ^a
Vanillic acid	1.022 ^c	0.557 ^b	0.087 ^b	0.158 ^a	0.097 ^a	0.016 ^{ab}
Mixture of phenolics	0.484 ^a	0.303 ^a	0.040 ^a	0.278 ^{abc}	0.145 ^{abc}	0.036 ^b
Control	0.708 ^{abc}	0.389 ^{ab}	0.075 ^{ab}	0.241 ^{abc}	0.162 ^{abc}	0.019 ^{ab}

*Different letters in the columns indicate differences between treatments (P < 0.05).

However, Sweify and Abdel-Wahid (81) found that application of salicylic acid increased the chlorophyll a and b as well as carotenoids in *Synгонium podophyllum* plants. Likewise, Mady (40) found significant increase in chlorophyll a and b as well as carotenoids in tomato treated with salicylic acid.

3.3. Photosynthesis, respiration and plant-water relations

Any changes in Chl content are expected to bring a change in photosynthesis. The phenolics decreases the Fv/Fm ratio to indicate stress and inhibits the growth, long term photosynthesis and fluorescence. Plant growth and productivity are correlated to both total leaf area and photosynthetic rate per unit of leaf and the factors that depress photosynthesis reduces the plant growth as well. Other variables, such as stomatal conductance and transpiration are also affected (45).

Numerous studies indicate that allelochemicals (including phenolics), may significantly influence the photosynthesis and respiration (18,39,94) and induce reductions

in growth (3,7,39). In higher plants, chemical must be absorbed through roots and transported through xylem to chloroplast, before it works as inhibitor of photosystem II (PSII) electron transport (98). Application of phenolics reduces the photosynthetic efficiency (variable fluorescence/maximum fluorescence Fv/Fm), and the physiological traits and interferes with the efficiency of PSII (18). They may also induce plasma membrane injuries (68). In plant, due to reduction in vegetative parts, phenolics (especially pronounced by derivatives of cinnamic and benzoic acids) have negative influence on yield (18).

Phenolic acids inhibited the transpiration, water use, leaf area and rate of leaf expansion in cucumber (7). The cinnamic acids, ferulic and *p*-coumaric acids were 2-5 times more inhibitory than the benzoic acids, *p*-hydroxybenzoic acid and vanillic acid. Hence, authors chosen the *p*-coumaric acid as the model compound for further study. When *p*-coumaric acid was inhibitory, transpiration, water use, and rates of leaf area expansion of actively growing leaves declined rapidly as the *p*-coumaric acid concentration surrounding the roots decreased. Subsequent resulting changes in the plant water relations and mineral nutrition, secondary and tertiary, effects frequently called as secondary effects (reductions in photosynthesis, carbon allocation to roots, leaf expansion, transpiration, increases in abscisic acid, and closure of stomata).

Likewise, Hussain *et al.* (29) studied the effects of *p*-hydroxybenzoic acid released into soil by root exudates of *Avena fatua* L., leaf leachates and decomposed plant tissues of *Triticum aestivum* L. on photosynthetic efficiency, yield and non-photochemical fluorescence quenching in *Lactuca sativa*. Authors found reduction in quantum efficiency (Fv/Fm) in lettuce seedlings together with effective quantum yield (Φ PSII) of photosystem II induced by *p*-hydroxybenzoic acid. In addition, the photochemical fluorescence quenching (qP) was significantly decreased after the treatment.

3.4. Autotoxicity

Autotoxicity occurs when a plant species releases chemical substances that inhibit or delay germination and growth of the same plant species (69). Inhibition may occurs when plants of a given variety release chemical substances that inhibit or delay germination and growth of other varieties of the same crop species or when plants of a given variety release chemical substances that inhibit or delay the germination and growth of the same variety (92). This mechanism reduces the competition between the members of same species. Inhibition of growth of other plants will increase the availability of nutrients. Although it is an important factor in crop management but the ecological significance of autotoxicity has been overlooked

Earlier research suggested that phenolics are involved in the phytotoxicity caused by sugarcane straw and ferulic, vanillic, and syringic acids were identified as phytotoxins in sugarcane straw (75,76). These compounds increased the root cell leakage, inhibited the dehydrogenase activity and reduced the chlorophyll content in lettuce (75). Leachates from sugarcane straw contained water-soluble phenolics that interfered with seedling growth of beggarticks (*Bidens subalternans* L.) and wild mustard (*Brassica campestris* L.). Leachates added to the biotic soil inhibited the weed root growth more than leachate added to abiotic soil, suggesting that microbial activity is involved in allelopathic activity. Soil characteristics evaluated in soil treated with sugarcane straw leachate suggested that straw phytotoxicity were not related with variations in inorganic nutrients (77).

Because of its importance in agriculture, autotoxicity has been studied in crop species like sugarcane. For example, Viator *et al.* (86) tested autotoxic effects of sugarcane using fresh sugarcane post harvest residues extracts containing benzoic acid, which affected the germination and leaf development. Leaf number was reduced by 0.5 leaves at 2 weeks after treatment at 1.0 and 100% concentrations. At 4 weeks after treatment, 1, 10, and 100% concentrations reduced the leaf number over the control.

Table 4. Comparisons of means for sugarcane growth in a greenhouse as a function of increasing water-soluble extract concentration from sugarcane postharvest residue (86)

Concentration (%)	Leaf number (no. per plant)		Plant height (cm)		Plant dry weight (g)
	2 wk	4 wk	2 wk	4 wk	8 wk
0	5.8ab†	7.1a	27.3a	28.3a	17.3a
0.1	5.8ab	6.7abc	27.7a	28.8a	17.1a
1.0	5.3c	6.3c	26.6a	27.0a	17.5a
10	5.4bc	6.4c	26.4a	28.6a	16.5a
25	5.9a	7.0ab	28.4a	28.8a	18.2a
100	5.3c	6.6bc	23.8a	28.1a	15.9a

Similarly, Alías *et al.* (1) found the autotoxic effects of a species widely represented in the communities of the Iberian Peninsula, *Cistus ladanifer* L. Authors found that aqueous extract from the *C. ladanifer* leaves collected in autumn and winter significantly inhibited its own seed germination and cotyledon emergence, showing the auto-allelopathic potential of *C. ladanifer*. This species has an adaptive strategy for germination to occur preferentially in autumn and winter, coinciding with the rainy period, these results show that the greatest autotoxicity occurs at the time of germination.

Ding *et al.* (17) obtained interesting findings on autotoxic effects of cinnamic acid from root exudates of cucumber, on cucumber and figleaf gourd seedlings. Authors analyzed reactive oxygen species metabolism and plasma membrane H⁺-ATPases activity in roots upon exposure to cinnamic acid. The exposure significantly increased the activities of NADPH oxidase, superoxide dismutase, guaiacol peroxidase and catalase, as well as in O₂⁻² production and H₂O₂ content, in cucumber roots, i.e. the cucumber roots produced significant amount of reactive oxygen species (ROS) immediately after cinnamic acid treatment, consequently increasing membrane peroxidation, decreasing membrane H⁺-ATPase activity and losing root viability. However, such changes were not observed in figleaf gourd roots. Results indicate that there is an interspecies difference in the recognition of allelochemicals, which induced oxidative stress accompanied by root cell death in cucumber, an autotoxic plant, but not in figleaf gourd, a relative of cucumber.

4. PLANT-SOIL AND SOIL-MICROBIAL INTERACTIONS

Plants continuously release phenolics to the soil. Soil solution concentrations of phenolics are product of the inputs of phenolic acids (e.g., leaching of plant materials, microbial activity, root secretions and exudations, and root cell autolysis), losses of phenolic acids (e.g., sorption by soil particles, microbial utilization, uptake by roots), and soil water content (7).

Soil phenols are the second most abundant organic constituent cycled in soil, after cellulose and account for about 40% of the organic C that circulates in the biosphere (62). These chemical compounds have high chemical reactivity and are implicated in numerous soil processes, thereby directly and indirectly influences the soil quality. Phenolics have major ecological functions in ecosystems: C sequestration in soil associated with chemical stabilization of soil N (12,13), nutrients cycling (19,61,99), and interference in ion transport, in protein synthesis, hormone activity and energy metabolism (48, 49, 72). These effects do not exclude each other, but, in general, the specific chemical properties of a given phenolic compound determine its major type of interaction with the N cycle. For example, low molecular weight phenolics, e.g. phenolic acids are easily degraded by microbes and are related to increase in N immobilization, while complex phenolics such as condensed tannins form complexes with proteins, including extracellular enzymes present in the soil, which may slow decomposition or N mineralization (13).

In soil, phenolics can occur in 3- forms: free, reversibly bound, and bound forms. *ortho*-Substituted phenolics, such as salicylic and *o*-coumaric acids, and dihydro-substituted phenolics, such as protocatechuic and caffeic acids, are absorbed by clay minerals by forming chelate complexes with metals. Free phenolic compounds may accumulate in rhizosphere soils, thereby influencing the accumulation and availability of soil nutrients and rates of nutrient cycling, which both ultimately affects the plant growth (38). Decreasing of physiological and biological activity of some soil microorganisms as well as the allelopathic effects of plants in cropping systems are the main reasons of decreasing soil productivity in crop forests. Soil microorganisms are one of basic factors that influence the soil fertility, which along with the plants qualify the direction and character of biochemical processes connected with the matter circulation and energy flow in forest ecosystems. Cultivated trees and their residues as well as soil substances have a selective influence on soil microflora, therefore, each change in microbiocenotical system of cultivated soil negatively influences the biocenotical relation in forests, which cause decrease in yield of cultivated trees. Over abundance of pathogenic and toxin-producing fungi as well as a quantitative reduction of nitrogen fixing bacteria and bacteria involved in nutrients transformation is a symptom of soil sickness (66).

4.1. C and N cycling

Phenolic substances comprise a substantial pool of C substrates in the soil that may increase microbial activity, resulting in short-term immobilization of nitrogen (12). Long-term immobilization of soil-N has been attributed to the formation of tannin–protein complexes in the soil (35) or inhibition of enzyme activity (34,50,74). Some tannins directly inhibit the microbial processes (79). Interactions between the soil microorganisms and tannins appear complex and may relate to the characteristics of the tannins, plant genetics, or vary among the soil microbial groups (53).

The overall effect of phenolics on soil N cycling is a decrease in the availability of inorganic N. By creating an unfavorable environment, phenolic compounds indirectly affect the target species performance and confer a competitive advantage to the phenolic producer. The ecological relevance of phenolic compounds is likely to be most important in ecosystems, where slow-growing, N-poor species rich in phenolics predominate, such as Mediterranean and Boreal ecosystems (13). For example, Castells *et al.* (12) found that, soluble compounds released from the shrubs *Ledum palustre* L. and *Empetrum*

hermaphroditum Hagerup. indirectly affected the performance of white spruce during regeneration by decreasing the soil N availability.

Knowledge on the role of tannins in soil is of ecological and environmental importance because they influence the quantity and quality of soil organic matter (SOM) and nutrient cycling in ecosystems (24). Tannic acid could react quickly with soil proteins linked with soil quality, but tannins might differ from related phenolic compounds in their ability to sorb to soil and effects on soluble soil-N (26). Therefore Halvorson *et al.* (27) continued to search for evidence of tannin sorption on soil, and their effects on soil-N. Authors treated forest and pasture soils with soil tannic acid solutions (mixture of galloyl esters, gallic acid (GA), a phenol, or b-1, 2, 3, 4, 6-penta-O-galloyl-D-glucose (PGG), a hydrolyzable tannin, and measured the soluble-C and N. They found significant amounts of soluble-C, with sorption of nearly 90% of the PGG-C, about 75% of the TA-C but less than 25% of the GA-C in surface soil. Decrease in soluble-C followed a reduction of total phenolic content. PGG and TA reduced the soluble-N from surface soil. Soluble-N in extracts was composed mainly of organic-N. Effects of tannins persisted in surface's soil suggesting the formation of stable complexes with soil. The proportion of initial soil-C and N remaining after all extractions was higher in samples treated with PGG or TA than in control or GA treatment. Authors concluded that PGG readily sorbs to soil and reduces the solubility of soil organic-N unlike GA, therefore these differences are important for surface soil layers where quantities of soil organic matter and biological activity are comparatively large and most easily affected by management.

Earlier studies compared the phenolic compounds production in plants growing in acidity gradient in natural conditions and found higher phenolic concentrations in plants associated with lower pH, which was associated with lower N availability found in acidic soils although some studies have found direct effects of pH (57,37). Lately, Smolander *et al.* (80) studied effects of silver birch (*Betula pendula* Roth) and Norway spruce (*Picea abies* (L.) Karst.) on the soil C and N transformations, and on the organic matter. They found higher soil pH and higher total phenolics in the humus layer under birch. Likewise, the lower C:N ratio, higher organic matter amount, microbial biomass C and N, net N mineralization and net nitrification, and higher concentration of DON were found under birch as well. In spruce soil, however, ferulic and *p*-coumaric acids together with of condensed tannins were more abundant in spruce soil. Smolander *et al.* (81) studied the long-term effects of logging residue removal on soil microbial processes in C and N cycling and on soil organic matter quality, and found that logging residue removal tended to decrease the amount of microbial biomass C and N and the rates of both C mineralization and net N mineralization in the humus layer. In addition they found a decrease of total phenolics in the humus layer as the result of logging residue removal and concluded that it may have a long-term effect on soil microbial processes and organic matter in spruce forests. Later, Smolander *et al.* (82) continued with studies on effects of logging residue removal of in a thinning Norway spruce stand after 5-years on litter decomposition and soil microbial processes in C and N cycling, and the quality of soil organic matter. Authors found that removal of logging residue had no effect on pH or C:N ratio in the humus layer, however, it reduced the concentrations of total water-soluble phenols and condensed tannins in the humus layer of whole-tree harvest (completely removed from forest) than stem-only harvest (left on the site).

In mixed forests, diverse plant litter mixtures frequently decompose differently than expected compared to the average of the component species decomposing alone and it remains unclear why decomposition may respond non-additively to diversity. Therefore Meier and Bowman (44) tested whether litter chemical composition and chemical diversity determine the strength and direction (synergistic versus antagonistic) of non-additive soil carbon (C) and nitrogen (N) cycling responses to litter mixtures. Authors found positive correlation of concentrations of tannins and some low molecular weight phenolics in the mixtures with greater N immobilization. Non-additive N mineralization responses were poorly correlated with N concentration, C:N, lignin:N, and phenolic:N. The results also showed that non-additive N mineralization responses were affected by loss of some species significantly more than other species. Finally, litter mixtures stimulated more microbial biomass N than expected in 45% of cases.

4.2. Soil microbial communities

Despite the availability of new techniques to monitor the microbial activity and diversity in soils, there is still a lack of knowledge on *in situ* dynamics and identities of organisms, which utilize phenolic compounds (78). In soil, phenolics encounter millions of soil microorganisms and microbial communities, including bacteria, nematodes and pathogenic and mycorrhizal fungi (88). Allelopathic interactions are strongly related with microbial activity in the soil, because soil microorganisms are both producers and degraders of allelochemicals and at the same time, they can be affected by allelochemicals (70). From this perspective, soil microorganisms can be considered as allelopathic interaction regulators. This fact, pointed out by Blum (6), is particularly relevant in forest ecosystems.

Soil microorganisms generally utilize phenolic compounds as carbon sources. Schmidt *et al.* (78) hypothesized that the population of phenolic-utilizing microorganisms would be higher in soils with plants producing phenolic compounds than those containing plants that do not produce phenolics. In addition, microorganisms that develop during autumn and winter utilize the phenolic compounds as carbon source, released from the leaf litter in autumn. Therefore, phenolic compounds consumed by microorganisms cannot play any role in plant growth inhibition during spring (31).

The ecological activity of phenolics in the ecosystem depends on their reactivity and water solubility. Phenolic compounds have an activating or inhibiting effect on microbial growth according to their composition and concentration. Inhibitory effects of some of these compounds on microorganism growth is again confirmed (58,59,60). Leaf litter promotes extracellular enzyme activity by induction or reduction in enzyme expression and activity through catabolite suppression. Differences in the quality and quantity of leaf litter phenolics induce different inhibition of some important soil enzymes. Tannins affect the nutrients cycling by inducing toxicity to microbial populations and inhibiting enzyme activities. Tannin inactivation of microorganisms exoenzymes (polygalacturonase, β -glucosidase, cellulase, amylase, urease etc.), is an important part of its inhibitory effect on the decomposition of plant residues (34,41).

Natural variation in the abiotic environment (e.g., soil fertility, moisture, temperature, altitude) may profoundly modify the chemical basis and outcome of interactions between plants and their pathogens. The litter colonization by fungi is regulated both by exogenous or environmental factors and endogenous factors, i.e. litter

chemistry. Ormeño *et al.* (63) studied litter fungal colonization in a Mediterranean ecosystem, by determining ergosterol content (as an indicator of fungal biomass) of *Quercus coccifera* L. leaf litter. Environmental factors have been studied through the fertility of the soil, by comparing plots amended with two rates of compost and plots without amendment. Results indicated that compost had a significant effect on soil fertility but did not increase ergosterol content of leaf litter and soil humidity improved leaf litter colonization by fungi. Endogenous factors have been studied through determining the total phenolic and ergosterol concentrations of seven shrub species leaf litter. They have shown a negative significant correlation between total phenolic compounds and ergosterol concentrations of leaf litter, and a positive significant correlation between total phenolic compound concentrations in green leaves and in leaf litter. Authors concluded that leaf litter colonization by fungi is controlled by soil moisture and plant leaf litter quality.

Recently, climate change models predict both an increase in summer droughts and increased rainfall, depending on region, thus Fenner *et al.* (20) studied the effect of these changes on the microbial population that mediate phenolic degradation in northern peatlands (waterlogged, anaerobic conditions and high levels of phenolic substances). Authors found that both increased drought and increased rainfall events clearly have the potential to alter the bacterial community structure and therefore carbon cycling within peatlands. Drought induced a greater diversity and abundance of phenolic catabolising bacterial species, with concurrent increased total phenol oxidase and *b*-glucosidase activities that are likely to have contributed to the lower phenolic compound abundance and increased CO₂ fluxes. A shrinking carbon storage capacity and increased climate forcing is therefore a serious concern. In contrast, bacterial diversity and abundance is suppressed under increased rainfall conditions, as were total phenol oxidase activities, giving increased phenolic compound abundance and anaerobic trace gas fluxes. Moreover, the effect of drought and increased rainfall in combination may be of even greater concern, due to suppressed decomposition. The studies like this are relevant to the management and restoration of wetlands.

Table 5. The diversity of phenolic compound degrading bacteria using temporal temperature gradient gel electrophoresis under simulated drought and increased rainfall (20)

Transect position	Simulated drought	Drought control	Increased rainfall simulation	Rainfall control
1	10	1	3	6
2	7	5	1	5
3	10	5	1	10
4	10	1	6	6
5	2	5	3	10
Mean	7.8	3.4	2.8	7.4
% Change	129.4 (*)		62.2 (*)	

Number of DNA species possessing the XylE type gene (encoding for Catechol 2,3-dioxygenase), in samples taken from transects through each wetland during treatments. P values are given in parentheses, t-test, * P<0.05.

Different types of soluble phenols (ferulic acid, gallic acid or flavonoids) were found to either stimulate or inhibit the spore germination and hyphal growth of saprotrophic fungi. However, mycorrhizal fungi were even more sensitive to polyphenols.

4.3. Plant mycorrhizal fungi

The colonization and abundance of arbuscular mycorrhizal fungi (AMF) in ecosystems is related to host factors, the fungal community, soil physico-chemical properties, and changes in these variables during soil development are little understood. Piotrowski *et al.* (64) observed a negative correlation between the increases in black cottonwood (*Populus trichocarpa* Torr. and Gray) litter and AMF abundance, and inoculum potential along a riparian chronosequence in northwest Montana. Following this, authors hypothesized that litter inputs negatively affect the native AMF community and may contribute to the shift between AMF and ectomycorrhizas (65). They tested the effects of adding the cottonwood foliage and litter extract on the colonization of AMF of both cottonwood and Sudan grass (*Sorghum sudanese* (Piper) Stapf.) seedlings. Addition of 5% (v/v) dried cottonwood leaves completely inhibited the AMF colonization of *S. sudanese*. AMF colonization of *S. sudanese* was also significantly reduced by litter extract of *P. trichocarpa* foliage, and colonization was negatively correlated with litter extract concentrations. Additions of aqueous litter extract also significantly reduced AMF colonization of cottonwood seedlings. The effect of litter extract on AMF colonization of *S. sudanese* did not appear to be affected by changes in soil pH or plant biomass. Additionally, authors tested pure soluble phenolic compounds common to *Populus* for their effect on AMF colonization by native fungi. All tested compounds significantly reduced the AMF colonization but did not affect the colonization by non-AMF root-colonizing fungi, which suggested that secondary compounds present in cottonwood litter might affect the colonization ability of a native AMF community.

Poor regeneration of black spruce in *Kalmia angustifolia* L.-dominated sites in eastern Canada due to inhibition of primary root growth of black spruce by phenolic acids of *Kalmia* origin, inspired Zeng and Mallik (97) to test if well-adapted conifer ectomycorrhizae can degrade and detoxify the water-soluble phenolic compounds produced by *Kalmia* and use the degraded products as a carbon source to stimulate growth and consequently decrease the inhibition. Authors found stimulation of hyphal growth of *Paxillus involutus* (Fries) Fries, a common ectomycorrhizal fungus of black spruce, by water leachates of *Kalmia* leaf and litter containing ferulic, *o*-coumaric, and *o*-hydroxyphenylacetic acid. No negative effect on fungal growth was found. The *o*-hydroxyphenylacetic (*o*-HPA) acid, which is known to be toxic to black spruce, was found to stimulate the growth of *Laccaria laccata* (Peck) Peck, *Laccaria bicolor* (Maire) P.D. Orton, and *P. involutus*. Pure ferulic, *o*-coumaric, and *o*-HPA acids were degraded by 100, 98, and 79.5%, respectively, in the presence of fungi. Although *o*-HPA acid inhibited the root growth of black spruce but had no effect on seedlings growth of black spruce with ectomycorrhizal fungi. Authors concluded that some ectomycorrhizal fungi, can control species interactions in higher plants by changing the rhizosphere chemistry.

4.4. Plant pathogens

Determination of the role of phenolic compounds on the enzymatic activities of pathogenic bacteria is one of the fundamental interests for the development of new

methods to combat such pathogens. Phenolic metabolites were frequently implicated in chemical defense mechanisms against pathogens in woody plants. The research concerning the role of phenolics in tree–pathogen interactions has entered a new era because it has been shown that some phenolic compounds have important biological function such as toxicity against pathogenic fungi.

Witzell and Martín (91) reviewed the recent findings on the involvement of phenolic metabolites in the resistance of northern forest trees (*Pinus*, *Picea*, *Betula*, *Populus*, and *Salix spp.*) to pathogens. Hjältén *et al.* (28) found negative correlation between the condensed tannins and the frequency of *Melampsora amygdalina* Kleb. rust on clones of *Salix triandra* L., a willow species, with high concentration of condensed tannins. Freiwald *et al.* (23) found a correlation between different phenolic profiles of two aspen species (two hybrid aspen and two European aspen clones) with the resistance of these species to aspen shoot blight (caused by *Venturia tremulae* Aderh.). Cvikrová *et al.* (15) found an increase in total peroxidase activity due to accumulations of lignin, soluble and cell-wall bound phenolics in bark tissues collected at the infection site in Norway spruce infected with the ascomycete *Gremmeniella abietina* (Lagerberg) Morelet, which cause stem canker and shoot dieback. Furthermore, pronounced accumulations of ester- and glycoside-bound benzoic acid derivatives were found in regions of infected branches that were not in contact with the pathogen (5). Evidence for the role of bark-associated phenolics in induced defence has been gained from studies with polyphenolic parenchyma cells and conifer resistance (22).

The use of phenolic compounds (particularly tannic and gallic acids) as antimicrobial as well as enzymatic inhibitors is important for economic and environmental reasons. Zaidi–Yahiaoui *et al.* (96) studied the antimicrobial effects of phenolic compounds and found significant inhibitory effects of phenolic acids (gallic and tannic acids) on the growth of *Pectobacterium chrysanhemi* (Burkholder *et al.* 1953) Brenner *et al.* 1973), and its protease and pectate lyase activities. Likewise, the retardation in enzymatic activity was observed with these two phenolic compounds, but the rate of inhibition varied in both these compound to another. The tannic acid showed the highest antimicrobial potentials and the highest effect on enzymatic activities.

5. ENVIRONMENTAL ASPECTS

The recent emphasis in forestry and agriculture has shifted from a primary goal of maximizing yields over the short term, to a sustainable productivity over long periods. The knowledge of ecological interactions occurring within forestry/agroforestry ecosystems and the sustainable functioning of the system as a whole has become the overall approach. Narwal (54) stated that sustainability could be achieved in an agriculture that is ecologically sound, resource conserving and not environmentally degrading.

5.1. Ecosystems disturbance

During monoculture, homogenous metabolites and plant residues are accumulated in the soil. The release of substances during the decay of dead plant residues plays a role greater than the active secretion of allelochemicals by plants in the phenomenon of soil sickness (66). The phytotoxic potential of decay products depends on plant residue type

and on conditions influencing the activity of microflora, such as temperature, moisture, aeration, inorganic ions and pH (66). The difference between the rate of chemical compounds released into the environment and the rate of their degradation will determine whether they accumulate in the soil to the toxic level. Habitat degradation by allelochemicals, mostly phenolic compounds from understory species has earned great significance in the last decade because this inhibition stunted the growth of planted tree seedlings in forest areas, with serious ecological and economic consequence (12,72).

Type, frequency and intensity of disturbance by interacting with plants' regeneration strategies and allelopathic properties can dictate the direction of succession following the disturbance. For example, forest canopy removal by clearcutting and non-severe fire can stimulate certain ericaceous plants with allelopathic property to dominate the post disturbance landscape and resist tree invasion, transforming forests into ericaceous heath (13,42). Another environmental issue is allelopathic degradation of ecosystems by exotic invasive plants (87). In Series of greenhouse, field and laboratory experiments Callaway and Aschehoug (9) and Bais *et al.* (2) demonstrated that root exudates of *Centaurea diffusa* Lam. and *C. maculosa* Lam., natives of Eurasia and exotics noxious to intermountain prairies of North America, can not only directly affect the root growth of the North American native plants but also their rhizosphere microbes. These exotic plants bring with them novel allelochemicals that adversely affect the germination and growth of native plants, but also their chemicals stimulate the synthesis of allelochemicals by their rhizosphere biota. Therefore, these invasive species with their unique seed regenerating strategy and perennial habit, induce changes in ecosystem-level which results in forming monospecific stands, and changes in the chemistry and biophysical properties of soil (10). Lately, environmental changes are connected to the climate change. Considering that, the microbial communities living in Sphagnum are known as indicators of ecosystem disturbances, Jassey *et al.* (33) tested their relation to climate change. With aid of a microcosm experiment authors tested effects of a temperature gradient (15,20 and 25 °C) on microbial communities including different trophic groups (primary producers, decomposers, and unicellular predators) in *Sphagnum* segments (0-3 cm and 3-6 cm of the capitulum) together with their relationships with abiotic factors (pH, conductivity, temperature, and polyphenols). Their analysis revealed that microbial assemblages differed strongly in Sphagnum upper segments along a temperature gradient in relation to abiotic factors, which recommend them as possible indicators of climate change. In addition, phenolic compounds showed effects on microbial communities as well.

5.2. Natural regeneration of forest ecosystems

In ecosystems, problems of natural regeneration, reforestation and management are often ascribed to the presence of phenolic substances. They at certain concentrations may influence the growth of plants and the activities of soil microorganisms involved in plant-soil interactions. Release of allelochemicals from the leaves or decomposing plant material is often a cause of natural regeneration problem in forests. In forestry, problems of natural regeneration, reforestation, and habitat degradation by phenols has received great attention in the last decades (72). Allelopathy has been implicated as a factor contributing to failure of forest regeneration. In forest ecosystems and agroecosystems, the allelochemicals are added continuously from the perennial trees through leaching, root exudation and litter decay, which might accumulate and exceed threshold levels, thereby

leading to soil sickness (32). Allelopathic effects due to inhibitory effects caused by various phenolic compounds in forest ecosystems delays and reduces the germination, restricts the root development, impairs water balance or nutrients uptake. These lead to reduction in seedling growth and development, impaired photosynthesis and growth stagnation in mature trees.

Allelopathic effects of phenolics have been observed in both natural and managed ecosystems, where they cause a number of ecological and economic problems, such as declines in crop yield due to soil sickness, regeneration failure of natural forests and replanting problems in orchards. For instance, Castells *et al.* (12) examined negative interference of phenolics -containing species commonly associated with white spruce forests in Interior Alaska, on the performance of white spruce (*Picea glauca* (Moench) Voss), which potentially affect its regeneration. They found high concentrations of phenolic compounds in leachates from the shrubs of *L. palustre* and *E. hermaphroditum*. A decrease in net N mineralization was observed in soils amended with *L. palustre* or *E. hermaphroditum* leachates, and this effect was inversely proportional to the phenolic concentrations, dissolved organic matter and leachates C:N ratio. Authors concluded that these shrubs negatively affected the performance of white spruce due to a decrease in soil N availability induced by phenolics. The reported negative effects of these species on boreal ecosystems confirmed the earlier findings on *Picea mariana* and *Picea glauca* at sites dominated by *Ledum sp.*, inhibition of *Pinus sylvestris* regeneration post-fire in sites dominated by *E. hermaphroditum*, and suppression of vascular plant growth in Sphagnum-dominated bogs (30, 95).

Likewise in agricultural crops, the regeneration failure in some tree species has been attributed to the increase of concentration of allelochemicals produced by the dominant trees (21). Politycka and Gmerek (67) have shown that autotoxicity due to addition of residues of several apple cultivars increased the content of phenolic compounds, which negatively affected the root biomass of apple seedlings.

6. FUTURE LINES OF WORK

Phenolic compounds have major ecological functions in primary production and decomposition of organic matter in ecosystems by playing a dominant role in rhizosphere mineral elements and organic matter dynamics through organic matter degradation, humus formation, alteration of microbial activities, mineralization of N and its availability. Additional studies are needed to better understand the role of phenolic compounds in ecosystem functioning.

1. Studies on the effects of phenolics on plant growth in natural and managed ecosystems are needed in the context of ecosystem disturbance because such effects may cause changes in the species diversity and a dominance of one species over another. Such studies need to be done in both forest and agroforestry.
2. Over abundance of pathogenic and toxin-producing fungi, the quantitative reduction in nitrogen fixing bacteria and bacteria involved in nutrients transformation is a symptom of ecosystem disturbance.

3. Despite the availability of new techniques to monitor microbial activity and diversity in soils, there is still a lack of knowledge on *in situ* dynamics and identities of organisms, which utilize phenolic compounds.
4. Determine the role of phenolic compounds in the enzymatic activities of pathogenic bacteria to develop their control.
5. Phenolic metabolites are frequently implicated in chemical defense mechanisms against pathogens in plants. Hence, the role of phenolics in plant-pathogen interactions need to be studied in detail.

ACKNOWLEDGEMENTS

This work was supported by the Ministry for Education and Science, Serbia, Grant No. 173018.

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