

The role of polyphenols in terrestrial ecosystem nutrient cycling

Stephan Hättenschwiler and Peter M. Vitousek

Polyphenols are the most widely distributed class of plant secondary metabolites and several thousand different compounds have been identified. Polyphenols play many different roles in plant biology and human life, including UV protective agents, defensive compounds against herbivores and pathogens, contributors to plant colors, contributors to the taste of food and drink, and pharmaceuticals^{1,2}.

Several papers published in the 1960s showed that polyphenols could inhibit the activity of digestive enzymes and/or precipitate nutritional proteins². Since then, much ecological research has referred to polyphenols as antiherbivore compounds, often neglecting other ecological functions. Polyphenols have also been recognized as regulators of soil processes, where it has been suggested that they inhibit nitrification^{3,4}, as well as decomposition and nutrient recycling^{5,6}, as a by-product of their antiherbivore activity⁷. Alternatively, it has been proposed that plant-produced polyphenols could control the pool and the form of nutrients available for plants and/or microbes⁸⁻¹⁰; this is a suggestion that might require an addition in the functional, and perhaps evolutionary, interpretation of polyphenols. Here, we focus on current experimental and theoretical evidence for interactions between polyphenols and ecosystem nutrient cycling, and suggest possible future research directions.

Chemistry and occurrence of polyphenols

Phenolic compounds are defined chemically by the presence of at least one aromatic ring bearing one (phenols) or more (polyphenols) hydroxyl substituents, including their functional derivatives (e.g. esters and glycosides). (Poly)phenols can be roughly divided into two groups: (1) low molecular weight compounds; and (2) oligomers and polymers of relatively high molecular weight (Box 1). Lignins, although phenolic compounds, are not considered in this review because of their low solubility and distinct functional properties.

Low molecular weight phenolics (LMP) occur universally in higher plants, some of them are common in a variety of plant species and others are species specific. Higher molecular weight proanthocyanidins (PA; also called condensed tannins) are the most abundant polyphenols in woody plants, but are usually absent in herbaceous plants^{11,12}. Hydrolyzable tannins have a more restricted occurrence than PA, being found in only 15 of the 40 orders of dicotyledons^{11,12}. Because of the large variety of analytical methods and problems with choosing the appropriate

Interspecific variation in polyphenol production by plants has been interpreted in terms of defense against herbivores. Several recent lines of evidence suggest that polyphenols also influence the pools and fluxes of inorganic and organic soil nutrients. Such effects could have far-ranging consequences for nutrient competition among and between plants and microbes, and for ecosystem nutrient cycling and retention. The significance of polyphenols for nutrient cycling and plant productivity is still uncertain, but it could provide an alternative or complementary explanation for the variability in polyphenol production by plants.

Stephan Hättenschwiler and Peter Vitousek are at the Dept of Biological Sciences, Stanford University, Stanford, CA 94305, USA
(stephan.haettenschwiler@unibas.ch; vitousek@leland.stanford.edu).

standards^{2,13}, polyphenol concentrations reported in the literature vary immensely and might not be comparable with each other. Nevertheless, the two most frequently used polyphenol measurements (i.e. 'total phenolics' and PA) are accepted reasonably well, and they commonly yield results in the range of about 1% to 25% of total green leaf dry mass. Polyphenol concentrations are determined less frequently in plant parts other than leaves; limited evidence suggests that the concentration of PA can be considerably higher (up to fourfold) in fine roots, although phenolic acid concentrations appear to be lower in fine roots than in leaves of the same plant species¹⁴.

Variation in plant polyphenol production

The general pathways of polyphenol biosynthesis are well described (Box 2), but the regulating and controlling factors determining the quality and quantity of polyphenols in plant tissues remain active and controversial areas of research^{7,15,16}. Much of the controversy arises because regulating factors at several levels, ranging from intrinsic species- and genotype-specific factors to various extrinsic environmental factors, interact with each other; this results in a wide variation of plant polyphenol production among and within species through time and space. Polyphenol production is induced at the level of the phenotype in response to herbivory¹⁷ and/or abiotic factors^{18,19}, which might additionally affect genotype selection in the long term. Both short-term phenotypic variations and genotype selection at a larger timescale have implications for litter quality input and nutrient cycling at the ecosystem level (e.g. Ref. 20). The multitude of intrinsic and extrinsic factors involved in the regulation of plant polyphenol production are comprehensively discussed by Jones and Hartley¹⁶, who also presented an integrative model of regulation at the level of phenylalanine as the common precursor of either protein or phenolic synthesis.

The fate of plant polyphenols

Although most of our information on polyphenols comes from analyses of live tissue, the relative composition and activity of polyphenols can change considerably during plant tissue senescence. Substantial decreases in the number and concentration of LMP, and large increases in the protein-binding capacity of PA, have been observed in leaf litter compared with green leaves¹⁴. Polyphenols enter the soil mainly by two pathways: (1) as leachates from above- and belowground plant parts, and (2) within above- and

belowground plant litter. Little information is available about the relative contributions of these input pathways, especially for belowground fluxes.

For a sugar maple (*Acer saccharum*) stand the amount of soluble polyphenols leached from the canopy and present in throughfall was 23 kg ha⁻¹ yr⁻¹ compared with 196 kg ha⁻¹ yr⁻¹ leached from decomposing leaf litter²¹. Although the amount of leachates in throughfall depends on rainfall patterns and differs among species⁶, it can probably be assumed that larger amounts of polyphenols are released from decomposing litter than are present in throughfall in any natural plant community. In the soil, soluble polyphenols face four different fates (Box 2). They might be degraded and mineralized as a carbon source by heterotrophic microorganisms; they can be transformed into insoluble and recalcitrant humic substances by polymerization and condensation reactions (with the contribution of soil organisms); they might adsorb to clay minerals or form chelates with Al or Fe ions; or they might remain in dissolved form, leached by percolating water, and finally leave the ecosystem as part of dissolved organic carbon (DOC).

The insoluble fraction of PA enters the soil as litter – it might predominate over the soluble polyphenols²². Soluble products are released from the relatively slow microbial decomposition of the insoluble fraction, contributing to the overall pool of soluble polyphenols. Additionally, lignin breakdown, as well as microbial synthesis from nonphenolic compounds, can also contribute to the pool of polyphenols in the soil²³. Some groups of soil fauna are able to digest polyphenols at least in part (e.g. earthworms and termites), but most of the ingested polyphenols are mixed with other litter components, microorganisms and clay minerals during passage through the gut – thus, contributing to either a faster microbial breakdown or a more rapid formation of organo–mineral complexes (Box 2).

Interactions with nutrient cycling

Plant growth and net primary productivity in most little-disturbed terrestrial ecosystems depend on the supply of recycled nutrients; external nutrient inputs generally contribute only a minor proportion to the total requirement. Nutrient mineralization by soil microorganisms is generally viewed as the rate limiting step in the nutrient cycle, and the factors involved in the control of this process include climate, substrate (litter) quality and decomposer organisms²⁴. Polyphenols are known to affect litter quality, at times having a larger effect on decomposition rates than more frequently measured parameters, such as nitrogen or lignin²⁵. However, polyphenols can also interact with nutrient cycling in various ways beyond a simple negative correlation between polyphenol concentration and decomposition rate. These interactions can be considered to fit within two groups of mechanisms – effects on the activity of soil organisms, and physico–chemical effects on the pools and forms of nutrients.

Effects on soil organisms

Phenolic compounds can directly affect the composition and activity of decomposer communities, thus influencing the rates of decomposition and nutrient cycling. The effects of polyphenols on soil microorganisms were reviewed by Kuiters⁶. Different types of soluble polyphenols, such as ferulic acid, gallic acid or flavonoids, have been found to either stimulate or inhibit spore germination and hyphal growth of saprotrophic fungi. Mycorrhizal fungi might be even more sensitive to polyphenols, but again different types of polyphenols can have opposite effects. For

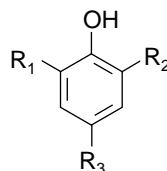
Box 1. Two main groups of polyphenols (a simplified overview)

(1) Low molecular weight compounds [e.g. simple phenols (C₆), phenolic acids (C₆–C₁) and flavonoids (C₆–C₃–C₆)].

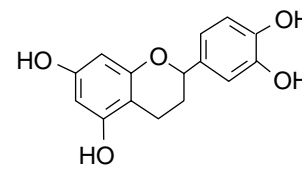
(2) Oligomers and polymers of relatively high molecular weight (e.g. tannins).

(Online: Fig. 1)

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Various phenolic acids.



Flavan-3-ol: a monomeric precursor of condensed tannins.

Tannins are defined as water-soluble phenolic compounds, having a molecular weight between 500 and 3000 daltons. They fall into two major structural groups – proanthocyanidins (PA; also called condensed tannins) and hydrolyzable tannins¹. Proanthocyanidins are nearly insoluble above approximately 7000 daltons (i.e. above 20 flavan-3-ol units). Insoluble polymeric PA can range from 12% to 80% of total PA depending on the type of tissue, plant species and extraction method²². Hydrolyzable tannins are conventionally divided into gallotannins and ellagitannins. Both types are esters of a carbohydrate core (mostly glucose) with gallic acid or hexahydroxydiphenic acids, or their derivatives¹¹.

example, mycelium biomass of an ericoid mycorrhiza was reduced by a mixture of common phenolic acids, but was stimulated by host-derived polyphenols from shoot extracts²⁶ (Fig. 1a). Plant mycorrhizal infection, nutrient uptake and growth can be impaired by specific phenolic compounds released from competitors, as was demonstrated for boreal ecosystems in several studies by Zackrisson and colleagues (e.g. Ref. 27). Polyphenols have also been suggested to inhibit nitrifiers^{3,4}, and (depending on the compound) to either suppress or stimulate the growth and activity of the symbiotic nitrogen (N₂)-fixing bacteria *Frankia* and *Rhizobium*^{6,10} (Fig. 1b). Because most of these effects have been observed under artificial laboratory conditions, their relevance at the ecosystem level remains a matter of speculation. However, Schimel *et al.*¹⁰ recently found that balsam poplar (*Populus balsamifera*) PA – but not thinleaf alder (*Alnus tenuifolia*) PA – inhibited both microbial activity (reduced decomposition and N mineralization) and N₂ fixation by alder (Fig. 1b). LMP from poplar acted as a microbial growth substrate, increasing immobilization and reducing net N availability in soils of later successional stages. From these results, they concluded that plant polyphenols are a control on nutrient dynamics, species interactions and successional dynamics in the Alaskan taiga.

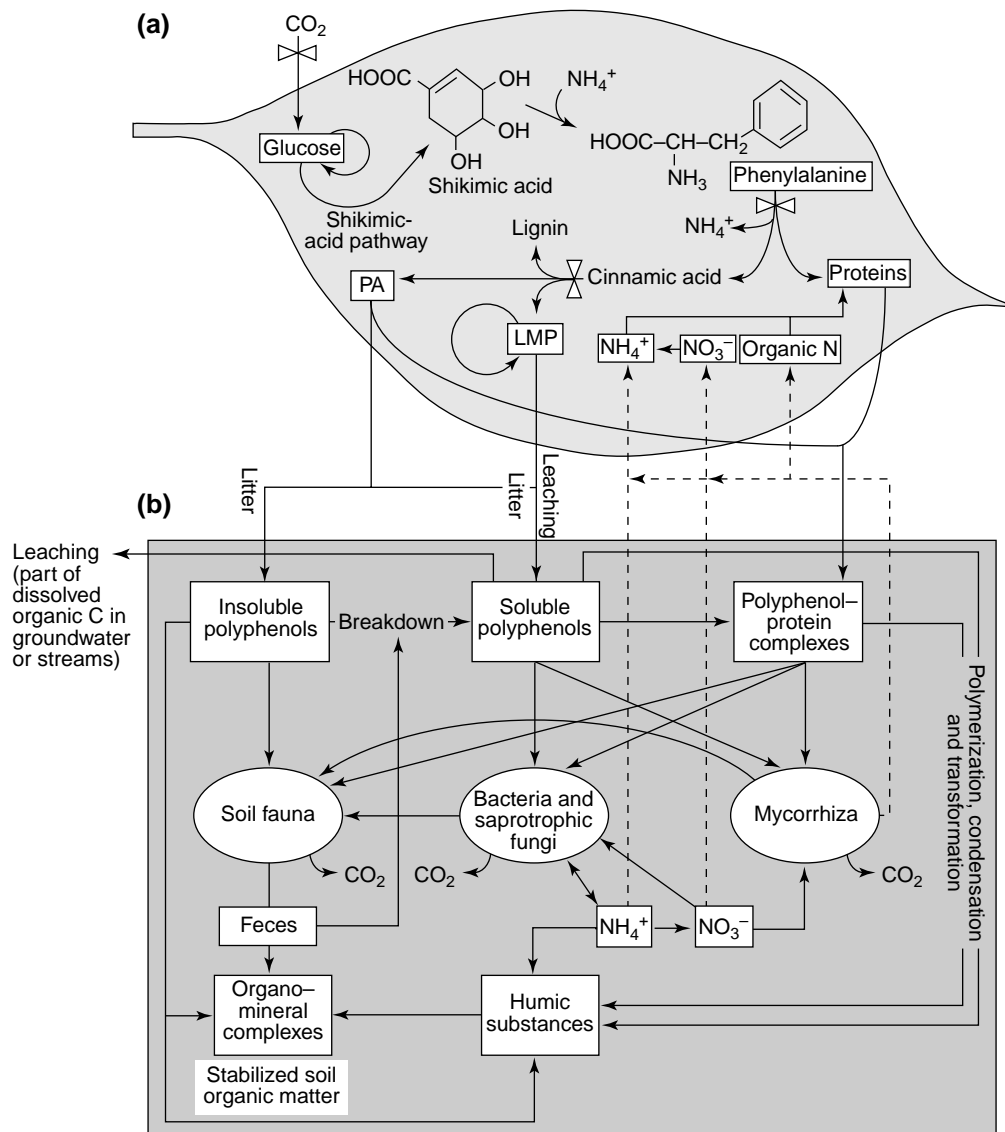
Soil macrofauna can enhance the biodegradation and mineralization of soil organic matter (SOM) by several mechanisms²⁸. High concentrations of polyphenols might restrict the activity and abundance of such fauna²⁹. However, direct effects of polyphenols on soil fauna are difficult to demonstrate because of the covariability of other compounds and the complexity of soil food webs.

Regulation of nitrogen availability

In addition to affecting organisms responsible for decomposition and for specific N transformations, polyphenols can alter N availability by complexing with proteins. Polyphenol–protein complexes (PPC) originate either during senescence of plant tissues, when polyphenols stored in the vacuole come into contact with cytoplasmic proteins, or in the soil, when polyphenols complex with proteins from

Box 2. The biosynthesis and fate of polyphenols

The diagram shows a simplified overview of biosynthesis [(a) represents any living plant tissues], release into the environment and fate of polyphenols in the soil (b). The unbroken lines indicate the biosynthetic pathways of polyphenols, and their fluxes and transformations into and within the soil. The broken lines indicate nitrogen (N) uptake by the plant.



(Online: Fig. 1)

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The aromatic amino acid phenylalanine, synthesized in the shikimic-acid pathway, is the common precursor of proteins and phenolic compounds. Low molecular weight phenolics (LMP) might undergo a high turnover in living plant tissues, whereas high molecular weight proanthocyanidins (PA) are considered to be metabolic end-products with minimal turnover and a tendency to accumulate with the aging of plant tissues^{7,11}. Major control mechanisms (indicated by the regulation symbol \times) occur at the level of the availability of glucose and phenylalanine (mainly quantity of polyphenols¹⁶), and at the level of cinnamic acid (polyphenol quality).

Soil organisms influence not only the uptake and metabolism of phenolic compounds, but also the fragmentation, mixing and translocation of polyphenol-containing litter material (soil fauna), and the production of extracellular enzymes (microorganisms) that drive either the breakdown of insoluble polyphenols or the formation of humic substances from low molecular weight polyphenols (according to the polyphenol theory of humus synthesis²³). The uptake, transformation and/or metabolism of polyphenol-protein complexes by soil organisms might be a major link between polyphenols and nutrient cycling.

use a large portion of N contained in the complexes. Rates of N mineralization decrease substantially with the increasing protein complexing capacity of polyphenols⁸ (Fig. 1c). However, the resistance of PPC to decomposition depends on the specific quality of polyphenols, as was suggested in a classic experiment by Handley³⁰ (Fig. 1d). The same absolute amount of N, provided as either pure protein or one of three different PPC with the same protein but polyphenols extracted from leaves of different plant species, resulted in different biomass accumulation and total N uptake of nonmycorrhizal tree seedlings (Fig. 1d). Seedlings provided with PPC of a plant species growing in relatively nutrient-rich environments [*Circaea lutetiana* (enchanter's nightshade)] attained a sixfold greater biomass than those provided with PPC of a plant species from nutrient-poor environments [*Calluna vulgaris* (Scottish heather)].

Northup *et al.*^{8,9} demonstrated that high levels of polyphenols might not only inhibit N mineralization, but also correlate positively with the release of dissolved organic nitrogen (DON) from pine leaf litter (Fig. 1c). They interpreted these findings as suggesting that plants in strongly N-limited ecosystems might benefit from increasing the DON:mineral N ratio. Positive effects of an increasing DON:mineral N ratio could include a reduction of the overall ecosystem N loss owing to leaching and denitrification of mobile NO_3^- , and a short-circuiting of the microbial mineralization step by allowing increased plant uptake of organic N. Further, they hypothesized that a high polyphenol production by plants on infertile soils might

represent an adaptive attribute to control the fate of N and to influence the plant's competitive advantage for the uptake of limiting N in organic form. This hypothesis is appealing because microbial immobilization of inorganic N is generally greater in infertile N-limited sites and because soil microorganisms are thought to be superior to plants as competitors for inorganic N (e.g. Ref. 31).

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However, there are several uncertainties surrounding this hypothesis. First, DON might actually dominate N losses from unpolluted and, presumably, N-limited forest ecosystems³², and there is no direct evidence that higher polyphenol production increases ecosystem N retention. Second, what is DON? Simple amino acids might be absorbed readily by plants³³, but the extent to which plants are able to use more complex DON is not at all certain. There is evidence that some plants have access to complex organic N via their ericoid or ectomycorrhizal symbionts^{34,35}. However, uptake of this organically bound N by ectomycorrhizae remains controversial³⁶ and their activity depends on other factors, including substrate quality, soil nutrient availability, competition with other microorganisms and host performance. Moreover, there are no data available to support the idea that DON is actually more widely available to the plant-mycorrhiza unit than it is to free-living heterotrophs. The positive correlation between DON release and litter PA concentrations⁸ implies that some of the DON should be in the form of PPC, and it will be crucial to determine if, and how effectively, plant-mycorrhizal associations can access N in either dissolved or insoluble PPC. So far, direct evidence is scarce, but N from PPC was shown to be used by the ericoid mycorrhiza *Hymenoscyphus ericae*²⁶. The mycelia of this species grew even better when supplied with a protein N source plus polyphenol-containing shoot extract of its host plant *C. vulgaris*, than when supplied with the same protein N source alone (Fig. 1a).

The release of a high ratio of DON:mineral N, associated with high litter polyphenol concentration, needs to be confirmed in other studies, and the hypothesis that PPC N is available for plants and enhances their competitive ability for soil N awaits testing. Even if this can be achieved, it remains to be shown that this mechanism acts as a selective force for variable plant polyphenol production, as opposed to an adaptive response to an environment in which other factors have selected for high tissue polyphenol concentrations³⁷. Moreover, because natural selection operates directly at the level of individual fitness, the argument that

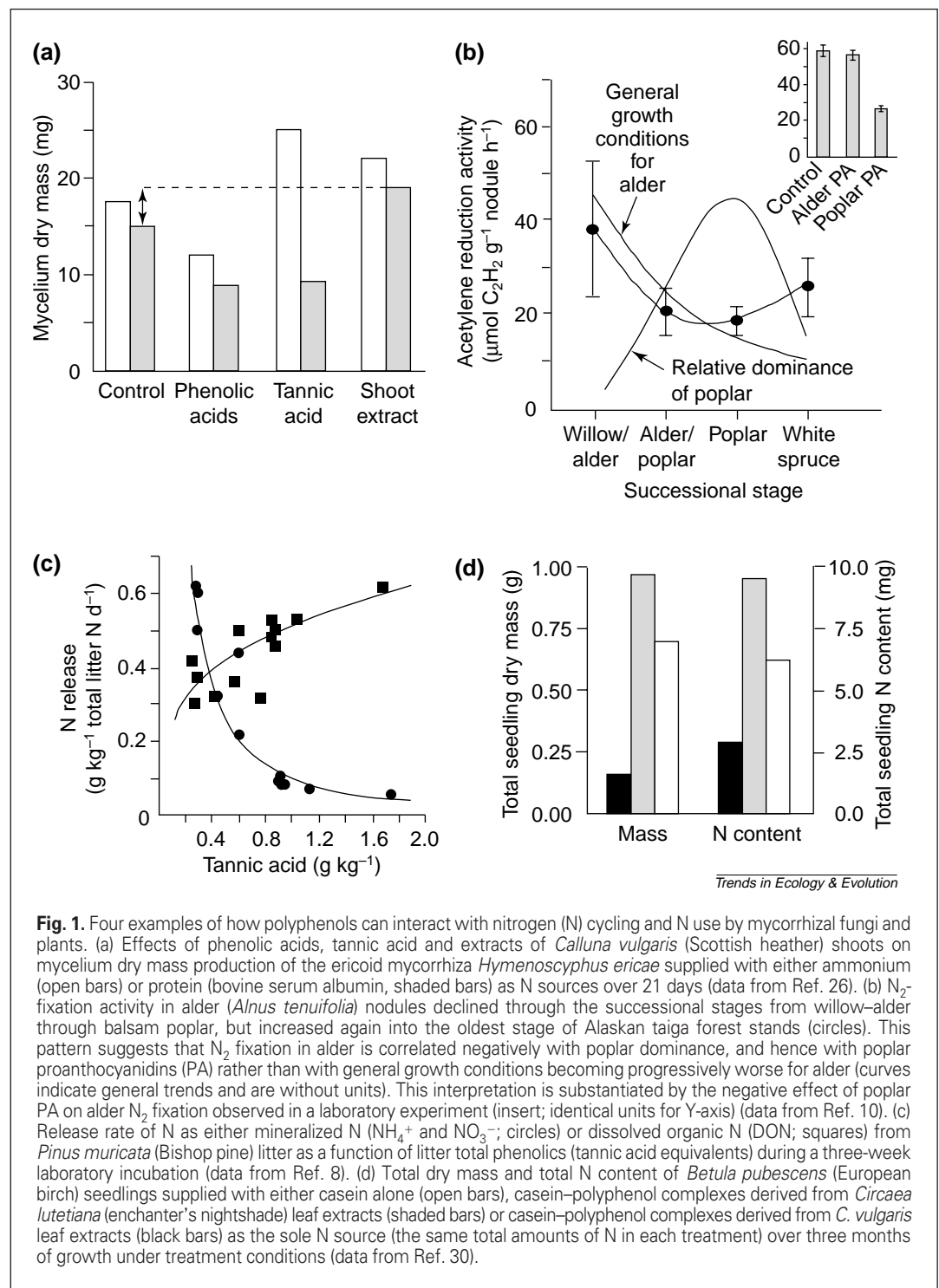


Fig. 1. Four examples of how polyphenols can interact with nitrogen (N) cycling and N use by mycorrhizal fungi and plants. (a) Effects of phenolic acids, tannic acid and extracts of *Calluna vulgaris* (Scottish heather) shoots on mycelium dry mass production of the ericoid mycorrhiza *Hymenoscyphus ericae* supplied with either ammonium (open bars) or protein (bovine serum albumin, shaded bars) as N sources over 21 days (data from Ref. 26). (b) N₂-fixation activity in alder (*Alnus tenuifolia*) nodules declined through the successional stages from willow-alder through balsam poplar, but increased again into the oldest stage of Alaskan taiga forest stands (circles). This pattern suggests that N₂ fixation in alder is correlated negatively with poplar dominance, and hence with poplar proanthocyanidins (PA) rather than with general growth conditions becoming progressively worse for alder (curves indicate general trends and are without units). This interpretation is substantiated by the negative effect of poplar PA on alder N₂ fixation observed in a laboratory experiment (insert; identical units for Y-axis) (data from Ref. 10). (c) Release rate of N as either mineralized N (NH₄⁺ and NO₃⁻; circles) or dissolved organic N (DON; squares) from *Pinus muricata* (Bishop pine) litter as a function of litter total phenolics (tannic acid equivalents) during a three-week laboratory incubation (data from Ref. 8). (d) Total dry mass and total N content of *Betula pubescens* (European birch) seedlings supplied with either casein alone (open bars), casein-polyphenol complexes derived from *Circaea lutetiana* (enchanter's nightshade) leaf extracts (shaded bars) or casein-polyphenol complexes derived from *C. vulgaris* leaf extracts (black bars) as the sole N source (the same total amounts of N in each treatment) over three months of growth under treatment conditions (data from Ref. 30).

selection for polyphenols influences soil processes and nutrient availability is based on at least two premises that are difficult to prove. First, a particular plant (genotype) must acquire most of its nutrients where its polyphenols interact with soil processes, and second, other plants of the same or different species should have limited access to this polyphenol-related nutrient pool.

Interactions with other nutrients

Polyphenols can also influence the fate of other nutrients, although by substantially different mechanisms. Adsorption to clay minerals and/or complexation with sesquioxides protects polyphenols from microbial attack, reduces the toxic effects of aluminum in soils of high acidity and competes effectively with other negatively charged compounds

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for sorption sites⁹. Polyphenol binding to sesquioxides can prevent phosphate sorption and phenolic acids have even been shown to desorb previously bound phosphate³⁸; thus, high polyphenol concentrations might contribute to the maintenance of P availability in highly weathered and acidic soils with high levels of Fe and Al sesquioxides. Several experiments have shown a substantial increase in soil P availability after addition of soluble organics (see Ref. 9), but the contribution of polyphenols *per se* and the importance of indirect effects, such as the stimulation of microbial activity, are not generally known. Polyphenols can also retain exchangeable inorganic cations (Ca, Mg and K) by providing sorption sites in highly leached, acidic soils, and can maintain the availability of metal micronutrients (e.g. Mn, Fe and Cu) by the formation of organic complexes^{39,40}. However, little information on the relative contribution of polyphenols to the overall soil cation exchange capacity is available. The importance of these effects of polyphenols relies heavily on their presence within the soil through time and space. In this respect, root-derived polyphenols might be important, because they are distributed more widely throughout the soil profile and are closer to the 'site of action' than shoot-derived polyphenols.

Although there are several lines of evidence stating that polyphenols produced by plants could affect the bio-availability of P and base cations, the relative importance of plant-derived polyphenols versus phenolic compounds derived from lignin breakdown, or synthesized *de novo* within the soil, is not clear. This differentiation is crucial to determine whether the functioning of plant-derived polyphenols in soil can be an alternative or a complement to the herbivore-defense hypothesis.

Prospects

Analysis of the effects of polyphenols on nutrient cycling is data limited. There are few data available on polyphenol concentrations in senesced plant tissues or fresh litter, and even fewer on the fate of polyphenols from plant tissues to their ultimate mineralization, output from the system as DOC or occlusion in stable organo-mineral complexes. These gaps seriously limit our understanding of how polyphenols interact with nutrient cycling. Although there is evidence for both negative and positive effects of phenolic compounds on the activity of soil organisms, and hence on decomposition and nutrient mineralization, these data were obtained largely from artificial experiments and it remains uncertain how relevant they are to natural conditions. However, the regulation of nutrient mineralization through soil microbial activity is only one way that polyphenols can influence nutrient cycling. Complexation with inorganic sesquioxides, cations and proteins might also allow sustained P and base cation availability in highly weathered soils, and an advantage to the plant-mycorrhiza symbiosis over free-living microorganisms in the competition for N. Well designed experiments to test these hypotheses, combined with systematic measurements of quantitative and qualitative polyphenol inputs (via below- and aboveground leaching, and via litter production in a wide variety of natural ecosystems), should be research priorities.

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The emergence and maintenance of diversity: insights from experimental bacterial populations

Paul B. Rainey, Angus Buckling, Rees Kassen and Michael Travisano

A central problem in evolutionary biology concerns the nature of the forces that maintain variation among individuals within populations^{1–4}. Three principal processes have been proposed: selection, mutation and genetic drift. Although drift undoubtedly has a role in maintaining molecular variation and mutation is essential for the origin of genetic variation, selection is the only evolutionary force capable of maintaining variation in fitness-related traits (Box 1).

Here, we focus attention on recent studies using microbial-based systems, which shed light not only on the forces and mechanisms responsible for the maintenance of ecologically significant variation (i.e. non-neutral), but also on the fundamental issue of how diversity evolves from the monomorphic state. Space restrictions prevent a comprehensive account and we refer the reader to Bell⁵ for greater detail.

Experimental bacterial populations and evolutionary ecology

'Evolutionary change occurs within an ecological context, but the workings of that context can be infinitesimally complex⁶. Therefore, progress requires experimental systems that reduce complexity to the point where mechanistic processes can be observed and rigorously tested. In this

Mechanisms maintaining genetic and phenotypic variation in natural populations are central issues in ecology and evolution. However, the long generation times of most organisms and the complexity of natural environments have made elucidation of ecological and evolutionary mechanisms difficult. Experiments using bacterial populations propagated in controlled environments reduce ecosystem complexity to the point where understanding simple processes in isolation becomes possible. Recent studies reveal the circumstances and mechanisms that promote the emergence of stable polymorphisms.

Paul Rainey and Angus Buckling are at the Dept of Plant Sciences, University of Oxford, South Parks Road, Oxford, UK OX1 3RB (prainey@molbiol.ox.ac.uk; angus.buckling@plants.ox.ac.uk); Rees Kassen is at the Dept of Biology, McGill University, 1205 Docteur Penfield Avenue, Montreal, Quebec, Canada H3A 1B1 (rkassen@bio1.lan.mcgill.ca); and Michael Travisano is at the Dept of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA (mtrav@uh.edu).

respect, bacteria are ideal⁷. They are easily propagated, have short generation times, have large population sizes and are amenable to genetic-level analyses. Environmental factors affecting population growth can be carefully controlled and reproduction by binary fission means that identical populations can be established from a single genotype^{8–13}. From the perspective of evolutionary ecology, large population sizes and rapid generation times ensure the coincidence of ecological and evolutionary timescales. This means that the ecological dynamics of evolutionary change can be observed in real time. Unlike traditional ecological experiments, microbial populations founded from single genotypes allow the evolution (emergence) of diversity, from the milieu of variant genotypes produced by mutation, to be separated from the ecological forces that determine the patterns of diversity that persist in the long term.

The niche-exclusion principle and evolution in asexual populations

Experiments initially performed during the 1930s, with mixed populations of either yeast or *Paramecium* propagated in simple homogeneous environments, led to formulation of the competitive-exclusion principle. This principle