Linkages between plant tannins and the organic nitrogen cycle

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Key words

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Glossary

Non-native invasive species: Some plant species when introduced into novel ranges away from their native range become far more abundant and expand their range compared to when present in their native range. Invasive plants commonly experience biogeographic-evolutionary advantages in terms of mutualistic associations, plant-soil feedbacks, production of allelochemicals and escape from herbivores and pathogens in the novel ranges.

Arbuscular mycorrhizal (AM) fungi: Symbiotic fungi, mainly Glomeromycota, forming an intracellular symbiotic interface in the cortical cells of the roots of a host plant, facilitating the uptake of nutrients and tolerance to abiotic and biotic stresses.

Chitin: Polymer of N-acetylglucosamine comprising the exoskeleton of insects and cell walls of fungi. The second most abundant polysaccharide in nature, after cellulose. Tannin can bind chitin to form tannin-organic N complexes.

Ecto- (EcM) and or ericoid (ErM) mycorrhizal fungi: EcM fungi colonize and encase the fine roots of their host plant and form a Hartig net, a mycelial structure that surrounds root cortical cells. Roots of ErM plants, mostly shrubs, are thin and narrow in diameter and lack root hairs, and ErM fungi penetrate into single-layered epidermal cells of the root.

Hyphosphere: The zone of soil under direct influence of fungal hyphae where the physical-chemical and biological properties different from the bulk or rhizosphere soil due to the influence of hyphal exudates and nutrient uptake. Fungi release hyphal compounds like sugars, amino acids, carboxylates, polysaccharides, proteins, flavonoids, and other secondary metabolites.

Plant-soil feedbacks (PSFs): Plants modify the abiotic and biotic properties of soil, with consequent effects on the establishment and growth of plants, and plant community dynamics.

Saprotrophic fungi: Fungi that grow on dead organic matter and have enzymatic (white rot fungi) or non-enzymatic (brown rot fungi) capacities to decompose soil organic matter (SOM) and participate in N-cycling.

Tannins: High molecular weight plant polyphenols that precipitate proteins. Both condensed and hydrolysable tannins or their mixtures are present in most dicots, but monocots and gymnosperms mainly produce condensed tannins (Box 1).

Tannin-organic N complex: Tannins that enter soil form complexes with soil organic N such as protein and chitin. Such persistent organic N is not directly accessible to plants.

Tanniferous plants: Include boreal and temperate tree species and ericaceous shrubs that produce significant amounts of tannins. The soil environment near these plants is often tannin-rich.

Abstract

Tannins in forest soils bind organic nitrogen into long-lasting complexes, affecting nutrient cycling and ecosystem productivity. Mycorrhizal fungi, especially ectomycorrhizal and ericoid types, can degrade these complexes, releasing nitrogen for plant uptake and influencing community composition. Further, there could be a potential role of arbuscular mycorrhizal fungi in acquiring organic nitrogen from persistent organic nitrogen complexes, albeit largely via interactions with free living bacteria. Understanding how tannins and fungi cooperate or compete to control organic nitrogen availability provides new insights into forest ecology, plant invasions, and biogeochemical cycles. These interactions are crucial in tannin-rich environments like temperate and boreal forests. We propose a conceptual framework to explore the feedback loops between plant chemistry, soil microbes, and ecosystem processes. Such knowledge is vital for predicting how forest communities will respond to climate change, land use, and invasive species, informing sustainable forest management strategies.

Plant tannin: key mediator of the organic nitrogen cycle in temperate and boreal ecosystems

Our understanding of the underlying processes that mediate ecosystem nitrogen (N) cycling has improved tremendously over the last two decades [1-3]. Soil N occurs in a variety of chemical forms, ranging from simple inorganic to complex organic forms, which vary as a function of soil abiotic conditions, plant and soil community composition, litter chemistry, and rates and pathways of decomposition [4-7]. However, most studies to date have focused on inorganic N forms, largely ignoring organic N compounds that typically dominate N pool of temperate and boreal forest soils. However, focusing solely on inorganic N dynamics does not adequately explain ecosystem functioning, especially for those with organic N rich, but inorganic N poor soils [4, 8].

A new paradigm in ecosystem N cycling has emerged that identifies depolymerization of N-containing polymers as a critical point in the N cycle [8, 9], whereby the enzymatic transformation of polymers into monomers (amino acids, amino sugars etc.) makes organic N available to plants and soil microbes. One of the major polymeric persistent forms of organic N in temperate and boreal forests dominated by tanniferous plants are **tannin-organic N complexes** (see Glossary) (including proteins, peptides and **chitin** derived from plant and microbial residues) [10-11]. **Tannins**, which enter the soil from decaying roots, leaves/needles, bark and seeds (nuts) of **tanniferous plants**, are chemically and biosynthetically diverse molecules that are distinguished from other phenolics by their ability to precipitate proteins and chitin to form tannin-organic N complexes that have long residence times in soils [10, 12-15]. Plant litter, from both roots and above-ground plant tissues, provide a significant input of N to soil. Though plants resorb N before their tissues are shed, this resorption reaches only about 50% in leaves [16] and between 18 to 58% for fine roots [17]. Thus, the magnitude of N transfer to forest soil from fine roots ($\leq$ 2 mm) may be equal to, or exceed, that from above-ground litterfall [18]. Moreover, there is large inter-specific variability in foliar nutrient resorption [19]. Thus, significant amounts of organic N derived from decomposing plant litter and roots may interact with tannins in soil to form persistent complexes. Moreover, tannin-organic N complexes are immobile and stay in the immediate vicinity of tannin-producing plants [13], which minimizes N losses [20]. As tannins form complexes with proteins, tannins are also interacting with enzymes, further affecting the rate of soil organic matter (SOM) decomposition [21-23]. However, the underlying mechanisms involved in the cycling of tannin-organic N complexes remain poorly understood.

Trees and shrubs in temperate and boreal ecosystems have association with **ectomycorrhizal (EcM), ericoid mycorrhizal (ErM**) fungi, and **arbuscular mycorrhizal (AM) fungi**. A positive relationship has been observed between community-level biomass of EcM plants and soil carbon stocks [24], and it has been proposed that mycorrhizal type shapes the nutrient economy: EcM plant dominated stands that occur in high-latitude ecosystems, have an organic N nutrient economy, characterised by slow rates of C and N turnover, whereas AM plant dominated stands, which occur in low-latitude ecosystems, have an inorganic nutrient economy with rapid rates of nutrient cycling and a dependency on saprotrophic fungi to mineralize SOM [19, 25]. Some EcM and ErM fungi can oxidize tannins and thus degrade persistent organic N forms into simpler forms that can then be taken up by EcM and ErM host plants. However, our understanding of the potential ecological role of tannin-organic N complexes is still developing. For example, the processes involved in the transformation of tannin-organic N complexes to simpler organic N forms for plant uptake in tannin-rich soil remains unclear. In addition, the potential role of AM fungi in degradation of complex organic matter or, in acquiring organic N from tannin-organic nitrogen complexes, is unknown, although there is evidence to suggest that AM fungi can access organic N, chitin, via interactions with free living bacteria [26]. It also remains unclear whether and how tannins and their interactions with soil organic N affect plant community dynamics by triggering positive **plant-soil feedbacks** (PSFs) to tannin-producing EcM and ErM plants, whereby mycorrhizal fungi associated with tannin-producing plants absorb N from tannin-protein complexes [13]. Similarly, while conceptual models have been proposed to consider plant-microbe linkages and loss and retention of N and depolymerization of organic N [27, 28], these models do not consider the mechanisms regulating N release from tannin-based complexes in ecosystems dominated by tanniferous plants.

Here, we discuss recent advances in our understanding of the interplay between tannins, tannin-organic N complexes, and mycorrhizal fungi (EcM and ErM), in the organic N cycle of temperate and boreal forests. We also discuss the potential role of AM fungi in acquiring organic N from persistent tannin-organic N complexes via interactions with free living bacteria. First, we explore the role of EcM and ErM fungi as mediators of the organic N cycle and positive PSFs for tanniferous plants via the formation of persistent tannin-organic N complexes in soil, and consider how EcM, and ErM fungi mediate plant access to N from these tannin-organic N complexes. We then propose a conceptual framework for linking EcM and ErM fungi to tannin-organic N complexes in temperate and boreal forests, highlighting the interplay between tannins, ecosystem properties, and processes of organic N cycling. Finally, we propose future research priorities to unravel the complex roles of tannin-organic N complexes and EcM and ErM fungi as drivers of community dynamics and the invasion of tanniferous trees in forest ecosystems.

Decomposition of tannin-organic N complexes, and interactions between SOM decomposers

Polyphenol production has been proposed to be an adaptive trait to regulate the retention and uptake of soil organic N in forest ecosystems dominated by tanniferous plants [20]. This proposal was based in part on work demonstrating that seedlings of EcM plants (e.g., *Pinus contorta, Betula pendula*) can use organic forms of N when grown in the presence of EcM fungi [29, 30]. These studies, however, used bovine serum albumin (BSA) for studying the uptake of organic N instead of tannins complexed with plant-derived proteins or fungal-derived chitin. Based on the literature, Northup *et al.* [20] further argued that mycorrhizal fungi, associated with pine (*Pinus muricata*) roots produce extracellular enzymes that release N from tannin-protein complexes and proposed that polyphenols (tannins) released from pine into soil form polyphenol-protein complexes. These authors hypothesized that polyphenol-protein complexes are decomposed by EcM fungi associated with pine roots, resulting in lowering N availability to local competitors, thereby giving pine a competitive advantage. However, there are concerns about their hypothesis because direct evidence supporting the notion that EcM fungi mediated degradation of persistent tannin-organic N complex into simple organic N was not provided [31]. However, several lines of evidence support the idea that both EcM and ErM fungi can access simpler forms of organic N released from persistent tannin-organic N complexes [11, 32].

Free-living saprotrophic, and EcM, and ErM fungi that live in symbiosis with trees may decompose SOM [33, 34], however, they differ in their mode of decomposition and effectiveness in driving this process. Saprotrophic fungi and ErM fungi carry out the white-rot route of SOM degradation including effective degradation of lignin, while EcM mainly use the brown-rot route of degradation with no lignin digestion [35]. Moreover, ErM fungi may have a higher number of plant cell wall-degrading enzyme (PCWDE) coding genes than EcM fungi [34, 36, 37], underscoring their greater capacity to decompose SOM. The view that EcM fungi have a limited ability to decompose SOM [38] is also supported by genome studies showing that many EcM fungi have evolved to lose most of the genes encoding for lignocellulase-degrading enzymes that are present in their saprotrophic ancestors, likely due to increased dependence on host plant sugars [37, 39]. The extent that EcM fungi could use the PCWDEs and microbial cell wall-degrading enzymes (MCWDE) to degrade SOM is poorly understood [37], although it has been hypothesised that due to lower number of PCWDEs, EcM fungi have limited saprotrophic abilities [40]. However, some EcM fungal species (e.g., *Cortinarius*) also use white-rot degradation [34, 41], and the non-enzymatic route of oxidation (i.e., Fenton reaction) may be used during SOM decomposition by both EcM fungi [32, 42] and ErM fungi [43]. In contrast, AM fungi have a limited ability to degrade SOM compared to other fungi [25, 44].

Mycorrhizal fungi can restrict the activities of saprotrophic fungi and other microorganisms through competitive interactions [45]. The ‘Gadgil effect’ refers to this competitive suppression of free-living saprotrophs by EcM fungi, which ultimately slows down the decomposition rates of SOM [46-49]. Since EcM fungi largely depend on carbon allocated by their host in form of sugars, this enables EcM fungi to allocate more resources to explore nutrients (particularly N) in soil, they can suppresses the growth of saprotrophic fungi due to N-limited conditions [50]. However, there is limited evidence to support the Gadgil effect with respect to EcM fungi because this effect depends upon soil moisture and litter quality [46, 48]. Moreover, Gadgil effect is not limited to competition between EcM and saprotrophic fungi [51], as there is also evidence for competition between AM fungi and soil bacteria with consequences for soil N cycling. For example, AM fungi compete with ammonia-oxidizing bacteria for soil N [52-54].

Although saprotrophic fungi have the capabilities to degrade persistent organic N, they are suppressed when competing for water or nutrients with EcM or ErM fungi [45]. However, tannin-organic N complexes are largely present in the underlying organic layer of the soil where EcM fungi dominate [55]. The higher competitiveness of EcM fungi in response to nutrient deficiency helps them to expand to larger area at the expense of saprotrophic fungi [45, 56], thereby making ErM or EcM fungi important in tannin-mediated organic N cycle. ErM and EcM plants may also compete for tannins. For example, *Kalmia angustifolia*, an understory heathland species, has been shown to adversely affect the regeneration of *Picea marina* (black spruce) in boreal forests due to binding abilities of tannins released by *K. angustifolia*, which reduces rates of N release [57]. Due to superior capabilities of *K. angustifolia* tannins over *P. marina* tannins to bind to proteins (BSA), *K. angustifolia* was suggested to have a competitive advantage over *P. marina* in competition for N [57]. These authors also reported that ErM fungi associated to *K. angustifolia* had superior abilities to grow on tannin-protein complex than EcM fungi associated to *P. marina.* EcM, brown rot, and ErM white rot fungi have capabilities to degrade persistent tannin-organic N complexes into simple forms of organic N [58-60].

There is also evidence to support the idea that tannin-rich ErM plants retain N in the soil by forming tannin-organic N complexes that are accessible to ErM fungi and its host, but not to EcM or AM fungi and their hosts [61]. ErM plants occur in approximately two-thirds of the global area of forested land, of which about 90% is dominated by EcM trees [58]. EcM trees in boreal forests typically have ericaceous understory shrubs (e.g., *K. angustifolia, Calluna vulgaris*) that can degrade tannin-organic N complex, releasing simple organic N, which can be used by both trees and shrubs. These findings support the notion that tannins mediate N acquisition by the host of ErM fungi and hinder N acquisition by mycorrhizal roots of neighboring plants. There is also a possibility that some mycorrhizal fungi "steal" nutrients released by exoenzymes of other mycorrhizal fungi, as shown for “cheater” microbes that exploit the catalytic activities of decomposers involved in organic matter turnover [59].

Although AM fungi have a limited ability to degrade tannin-organic N complexes compared to ErM and EcM fungi [25, 58], they could engage in synergistic interactions with rhizosphere bacteria, effective decomposers of tannins, to access N from complexes. This is consistent with mycorrhizal-associated nutrient economy (MANE) framework of Phillips *et al.* [25], which explains the inorganic and organic nutrient economies of AM and EcM fungi-dominated forest stands, respectively. Here, high chemical quality of AM tree litter [62-64] mediates rapid transformation of organic N (protein, chitin or amino acids) into inorganic N (NO_3^-, NH_3^+), enabling AM fungi to utilize inorganic N released from AM tree litter [25]. In contrast, the MANE framework proposes an organic nutrient economy for EcM trees due to their low-quality litter compared to AM trees [25]. The ability of EcM and saprotrophic fungi to transform and utilize organic N under canopies of EcM trees enables them to acquire simpler forms of organic N [65]. Moreover, in mixed AM forests with patches of EcM trees and in mixed conifer-broadleaf temperate forests, AM trees could efficiently use organic N that has been transformed to simpler forms by EcM trees [66, 67].

The weaker ability of AM fungi to directly mobilize organic N compounds could also be compensated by the soil microbes present in the AM fungi **hyphosphere**, which produce enzymes that stimulate the mineralization of SOM [68, 69]. AM fungi hyphae attract distinct microbes into their hyphosphere, a narrow region of soil around the hyphae that have distinct soil properties than bulk or rhizosphere soil due to exudates from the AM fungal hyphae [69], but empirical evidence on the ability of hyphosphere microbial community to decompose tannin-organic N complexes is lacking. Several bacterial (e.g., *Enterobacter aerogenes, E. agglomerans, Cellulomonas, Arthobacter, Bacillus, Lactobacillus, Leuconostoc, Oenococcus, Pseudomonas* or *Staphylococcus*) and fungal (*Aspergillus niger* and *Penicillium*) species are reported to degrade condensed tannins [70, 71]. Also, a synergistic relationship between the AM fungi *Rhizophagus irregularis* and free-living microbial communities was found to help in acquiring organic N from organic matter [72]. Yet, whether synergistic relationships between AM fungi and other microbial communities can help acquiring organic N from tannin-organic N complex remains unknown. Some Acacia species (e.g., *Acacia tortilis, Acacia ehrenbergiana, and Acacia negrii*) associate with AM fungi [73] and AM fungi, for example *Glomus* sp., can acquire organic N and transfer it to its host [74]. Although, there is evidence that AM fungi are negatively affected by EcM fungi due to competition for organic compounds of litter [75], future work is needed to elucidate the mechanisms that explain how EcM and ErM fungi may prevent AM fungi uptake of decomposition byproducts of tannin-organic N complexes (simple forms of organic N).

A framework for organic N cycling in tannin-rich ecosystems

As discussed above, there appear to be linkages between plant chemistry, soil biota and root-associated mycorrhizal fungi that mediate organic N cycling [76]. Here we propose a framework that highlights steps to depict the multilayered interactions between tannins, ecosystem factors and processes in tannin-rich soils dominated by EcM and ErM plants (Figure 1). In our model, mycorrhizal fungi (EcM and ErM) are key players in the integration of tannin-organic N complexes into the N cycle and forest ecology. In the first step, tannins released from litter of roots and above-ground plant parts, form complexes with organic N forms such as proteins, peptides, and chitin (Figure 1, Step 1), slowing down organic N decomposition. Tannins may reduce decomposition of organic N compounds via forming persistent complexes with organic N compounds or directly interact with enzymes inhibiting their activities [55]. The second step relates to the capacity of ErM and EcM fungi, which have enzymatic and Fenton reaction oxidative capacities to oxidize tannins, thus enhancing access of organic N to fungi and plants (Figure 1, Step 2). The next step is the hydrolysis of polymeric organic N forms by proteases, peptidases and glucosaminidases produced both by free-living microbes and mycorrhizal fungi to release simple forms of organic N, such as peptides, amino acids and amino sugars (Figure 1, Step 3). In the following step, a substantial fraction of the N released is immobilized due to development of extensive microbial biomass (Figure 1, Step 4). N immobilization leads to a strong N limitation of plants, resulting in a significant increase in plant investments to below-ground parts (Figure 1, Steps 5 and 6). Plant dependence on mycorrhizal fungi for accessing N from tannin-organic N complexes comes at a cost in term of C supplied to mycorrhizal fungi (Figure 1, Step 7). The resulting plant phenotype may be characterized by a high root mass fraction and extensive C transport to their mycorrhizal fungi. As a result, the N cycle would become constrained with a dominance of organic N over inorganic N, effectively reducing the competitiveness of AM plants and non-mycorrhizal plants of their lack of abilities to degrade persistent organic N-tannin complexes and increasing dominance of EcM and ErM plants over AM plants (Figure 1, Steps 1-6).

The relationships depicted above suggests coevolutionary feedbacks between tannins and ErM and EcM fungi that can degrade complex organic N [13; Figure 2A]. Our framework can also be employed to understand the role of tannins in modifying the organic N cycle in forested habitats invaded by tanniferous invasive species (Figure 3). Some **non-native invasive species** produce tannins, for example *Acacia dealbata* in South Africa, southern Europe, and South America [77], or *A. dealbata* and *A. mearnsii* in the Western Ghats of India.

Invasive plants may alter their surrounding environment, including their rhizosphere microbial communities, to benefit themselves [78, 79]. There is little information available about the mycorrhizal associations that invasive trees such as *Acacias* might have and whether they employ tannin-mediated organic N as a strategy to gain invasion success. However, there is some evidence that tannins produced by the invasive species such as *Polygonum cuspidatum* can suppress rates of soil N mineralization, thereby reducing inorganic N availability to native plants [80]. Further studies are needed to assess the roles of tannins and mycorrhizal fungi in facilitating the success of non-native invasive species via manipulating the organic N cycle (Figure 3) and of tannin-mycorrhizal fungi mediated organic N cycling on the native range of Acacias.

Tannin-mediated interactions have multiscale implications, starting from the molecular scale, including chemical structure (Box 1), through the rhizosphere scale (Figure 1, steps 1-7), regional scale (effect of mycorrhizal fungi on the growth performance of the host i.e., positive

PSFs, Figure 2), and biogeographic (negative effect of soil biota on the host species in native range of the species, and neutral to positive effect on the host species in non-native ranges, Figure 3).

Future research to study the role of tannins in plant community dynamics and plant invasions

Species-specific plant-soil feedback (PSF) mediates the performance of conspecifics and heterospecifics, and plant community dynamics (see Glossary). Evidence suggests that mycorrhizal type regulates plant community structure in temperate forests: EcM trees experience positive PSFs, while AM trees experience negative PSFs [81-84]. Moreover, tannin-mediated formation of persistent organic N complexes, combined with the ability of EcM fungi to degrade tannin-organic N complexes, could help explain why EcM trees experience positive PSFs, while AM trees experience negative PSFs. While we lack empirical evidence that ErM plants experience positive PSFs, it can be speculated that superior abilities of ErM fungi to degrade persistent organic N complex may exert positive PSFs to ErM plants. This idea is supported by ability of tannin-producing ErM species in temperate ecosystems to release tannins in soils to form persistent tannin-organic N complex.

Recent insights gained from PSFs research can be utilized to test whether degradation of tannin-organic N complex by EcM and ErM fungi mediate PSFs in temperate and boreal forests (Figure 2 A, B). For example, studies using soil inocula and amendments of tannins could be designed to test the hypothesis that tannin-organic N complexes transformed by EcM/ErM fungi exert positive PSF to EcM trees. In field experiments, trenching could be employed as a technique to exclude the effects of EcM/ErM fungi [85]. Further, tannin-amended soil can be manipulated by tannin-binding agents like polyvinylpyrrolidone (PVPP) to deactivate tannins, which could help to establish the role of tannin-rich foliar leachate or root exudates of trees in making tannin-organic N complex in soil (Figure 2). Data on growth performance of EcM or AM plants grown in soil amended with or without PVPP could help to measure the direction and strength of PSFs experienced by EcM or AM trees (Figure 2).

Our framework is useful to test the tannin-mediated plant invasion hypothesis, which predicts that multilayered interactions between tannin-mediated organic N and mycorrhizal (EcM and ErM) fungi facilitate invasion of tanniferous plants in novel ranges. Soil inocula (live versus sterile) prepared by using soil associated with the tannin-producing invasive species in its native and non-native ranges or resident species in non-native ranges can be helpful in evaluating tannin-mediated PSFs (Figure 3). The effects of soil biota in the rhizosphere of tanniferous plants, native or non-native ranges or resident non-tanniferous plants in non-native ranges, on the growth performance of a species could help to evaluate biogeographic advantages experienced by species-specific soil biota (Figure 3). Further the manipulation of tannin-amended soil with tannin-binding agents would help to establish the role of tannin-rich foliar leachate or root exudates of trees in making tannin-organic N complex in soil or suppression of N mineralization. Further data are needed on soil concentrations of amino acids, inorganic N and abundance and diversity of mycorrhizal fungi and soil pathogens. This could help establish the linkages between tannin-organic N complex, mycorrhiza type and under-canopy vegetation of tanniferous and native trees in native and introduced ranges (Figure 3).

Future studies could also employ novel approaches, including soil zymography, *in vitro* cultures or isotopic tracing methods to better evaluate mechanistic aspects of tannin-mediated soil organic N cycling. Soil zymography or 2D mapping and visualization of enzymatic activities [86], could be a useful tool to understand the degradation of tannin-organic N complexes by EcM, ErM or saprotrophic fungi [86, 87]. Another technique that could be useful in understanding mechanisms underpinning tannin-mediated organic N cycling is to identify and track microbial degradation of N compounds using ^{15}N isotope [88, 89], for example ^{15}N chitin. There is, however, a need to consider processes that could possibly impact isotopic signatures of a compound [88]. Past studies have largely used N isotope approaches to investigate denitrification, nitrification, comammox or DNRA (dissimilatory nitrate reduction to ammonia), but information on the functional role of mycorrhizal fungi in N cycling can be gained by analyzing natural abundance of N isotope ratios [90]. Moreover, *in vitro* cultures of different types of mycorrhizal or saprotrophic fungi can be used for manipulative studies to test the degradation of tannin-organic N complexes. Another approach is the use of soil mesocosms with purified tannins coupled to "omics" approaches and high-resolution analytical tools to study abundance and functioning of ectomycorrhizal and saprotrophic fungi and mycorrhizal symbiosis [50, 91].

Concluding remarks and future challenges

We hope that our framework will help in understanding the role of tannin-organic N complexes in forest N cycle. Few field-based studies have quantified the fraction of plant N-requirements that are met by organic sources, and tannin-organic N complexes are yet to be integrated into the terrestrial N cycle largely due to uncertainties concerning their formation, stability, and thus their fate have not been elucidated. Also, the measurement of soil tannins, which sorb tightly to soil [92], and tannin-organic N complexes, is challenging, which makes it difficult to assess their formation and loss from soil [93] and to measure their turnover. Understanding of tannins in the N cycle is hampered by the widespread use of unpurified and poorly characterized tannin extracts in experiments because of difficulties in obtaining sufficient purified and well-characterized tannin [22]. The lack of detailed chemical information on the tannins used in most studies impedes the development of mechanistic descriptions of soil tannin-organic N complexes. Future research is needed to overcome these challenges, but also to better understand how the type and amount of tannin in soil regulates organic N cycling, and how their role in plant nutrition is moderated by soil physicochemical conditions. We propose that boreal forests dominated by tanniferous plants could be a promising ecosystem to test how persistent tannin-organic N complexes are degraded into simpler forms of organic N and then used by plants, mycorrhizal fungi or soil microbes. A greater integration of studies on soil organic N with those on plant traits is essential to develop such a general theory that would allow us to predict the feedbacks between plants and soils and thus community dynamics in temperate and boreal forests.

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Declaration of competing interests

Author Torgny Näsholm declares a competing interest as he owns shares in, and works part time for the company Arevo AB that develops, produces, and markets organic fertilizers. Author Torgny Näsholm declares he is the inventor of certain organic nitrogen compositions which are protected by patents. The other authors declare no competing interests.

Author contributions

Inderjit conceived the idea and wrote the first draft of the manuscript. All authors contributed to preparing and finalizing the manuscript.

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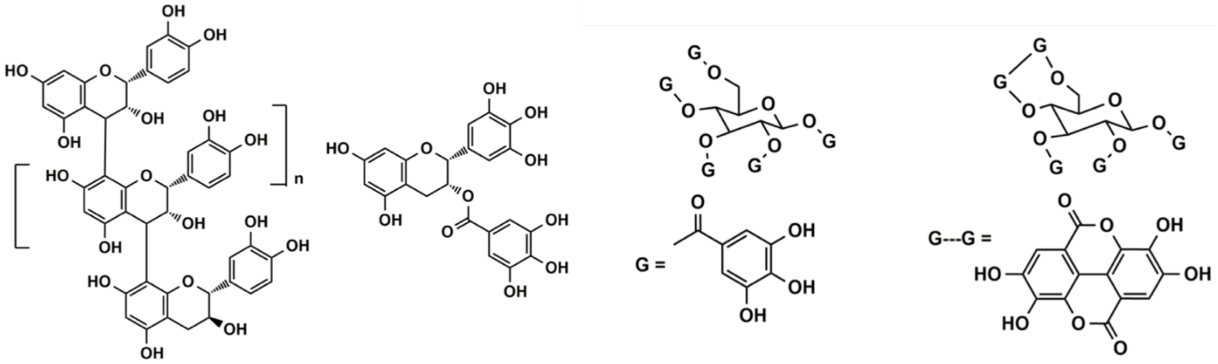
Box 1. Tannin chemistry and reactivity

The high molecular weight plant natural products known as tannins are the most abundant group of plant polyphenols after lignin. Tannins are chemically and biosynthetically diverse molecules that are distinguished from other phenolics by their hallmark ability to precipitate proteins. Both condensed and hydrolysable tannins or mixtures are present in most dicots, but monocots and gymnosperms mainly produced condensed tannins [94]. The condensed tannins, or proanthocyanidins, are products of the flavonoid biosynthetic pathway, and range from oligomers to polymers with molecular weights as high as 20 kD (Figure I, Compound 1). The hydrolysable tannins are based on a parent structure of shikimate-derived gallic acid residues esterified to a core polyol (pentagalloyl glucose; Figure I, Compound 3). The gallotannins are simple esters, while the ellagitannins have undergone further derivatization via oxidative crosslinking between galloyl groups (Figure I, Compound 4). Epigallocatechin gallate, an example of a “catechin tannin” abundant in tea (*Camellia sinensis*), has elements of both major classes of tannins, comprising a trihydroxylated flavonoid gallate ester (Figure I, Compound 2).

The ability of a tannin to precipitate or bind protein cannot be predicted based on the type of tannin, condensed or hydrolysable. For example, it has been claimed that condensed tannins may have higher protein-precipitating capacity than a mixture of hydrolysable tannins and condensed tannins, but the same study showed that mixed tannins have higher abilities to inhibit β-glucosidase and N-acetyl-glucosaminidase activities in gymnosperm-dominated soils [95]. Attempts to develop structure-activity relationships based on plant extracts containing both condensed and hydrolysable tannins suggested that structural features of the tannin are important to protein precipitation, but no specific links to tannin class were revealed [71, 96]. Evidence on comparing a condensed tannin with a hydrolysable tannin showed that the polarity and water solubility of the tannin was a critical determinant of the interaction with protein, rather than the classification of the tannin. Like other macromolecules, tannins have secondary and tertiary structures that dictate their interaction with proteins and other biomolecules. The polarity of tannins can range from virtually water insoluble to freely water soluble, with representatives of both types of tannins across the entire range of partition constants (Kow values 2×10^{-4} to 1.3×10^{6}) [97]. Thus, protein binding depends on the shape and specific chemistry of the tannin, not on its broad structural class or biosynthetic origin.

Tannins are reported to have both negative and positive effects on organisms ranging from microbes to mammals [98]. Similar to chemical activity, bioactivity of tannins cannot be predicted based simply on the broad classification categories of condensed vs. hydrolysable tannins [96]. Condensed and/or hydrolysable tannins have been reported as toxic to some microbes [96], and as deterrents to some herbivorous insects [99]. Nutritional consequences have been linked both to decreased digestibility of tannin-bound protein and to inhibition of key enzymes by tannins [100]. Taken together, all of these studies of tannin function in organisms show that the structural and functional diversity of tannins makes it essential to study individual, defined compounds in order to elucidate specific modes of action.



1 2 3 4

Figure I: Tannin structures. 1, Condensed tannin trimer with one catechin and two epicatechin extenders. 2, Epigallocatechin gallate. 3, Pentagalloyl glucose, a gallotannin. 4, Eugeniin, an ellagitannin.

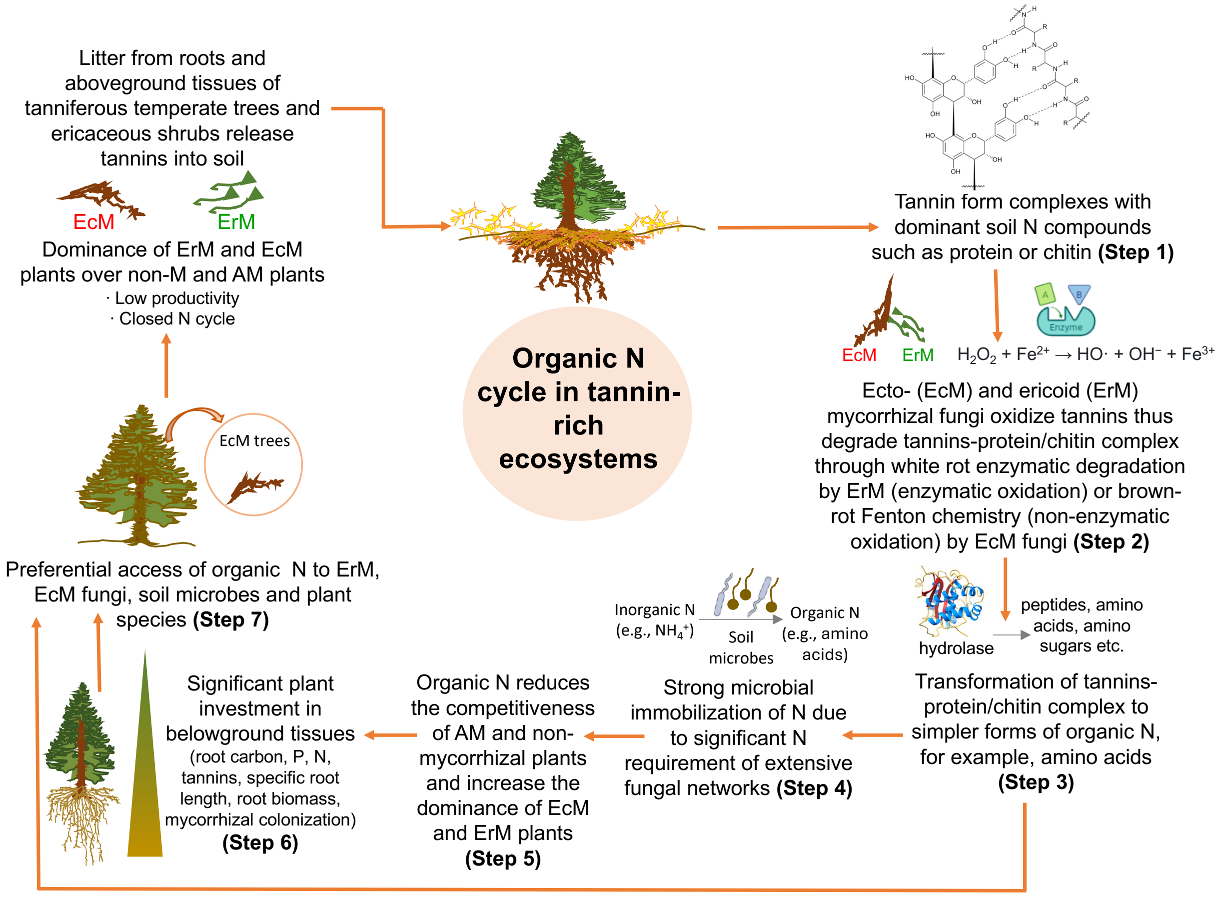


Figure 1. Framework depicting the multiple-layered interactions occurring in tannins-rich ecosystems. Tannins produced by tanniferous plants, including trees and ericaceous shrubs in temperate and boreal forests form complexes with soil organic N, to yield a persistent form of complex organic N that is not directly accessed by plants, mycorrhiza or saprotrophs (Step 1). ErM and EcM fungi oxidize tannins to degrade tannin-protein/chitin complex through white rot enzymatic degradation (enzymatic alteration) or brown-rot Fenton chemistry (non-enzymatic alteration) into simpler forms of organic N (Step 2 and 3). However, some EcM fungi retain white rot capabilities. This results in the immobilization of N due to significant N requirement of extensive fungal networks (Step 4). Dominance of organic N over inorganic N reduces the competitiveness of AM and non-mycorrhizal plants and increase the dominance of EcM and ErM plants (Step 5). Tanniferous plants invest more in the root carbon, P, N, tannins, specific root length, root biomass, mycorrhizal colonization (Step 6). The proposed framework identifies that the mycorrhizal fungal networks (ErM, or EcM fungi) dominate in the temperate and boreal forests due to their higher return on investment of oxidative enzymes compared to bacteria. There is preferential access to complex organic N for plant species that are associated to EcM and ErM fungi because they can degrade tannin-organic N complexes into simpler forms of organic N

(Step 7). The proposed framework identifies that the fungal networks (ErM, EcM or saprotrophs fungi) dominate in the temperate and boreal forests due to their higher return on investment of oxidative enzymes than bacteria.

Figure 2

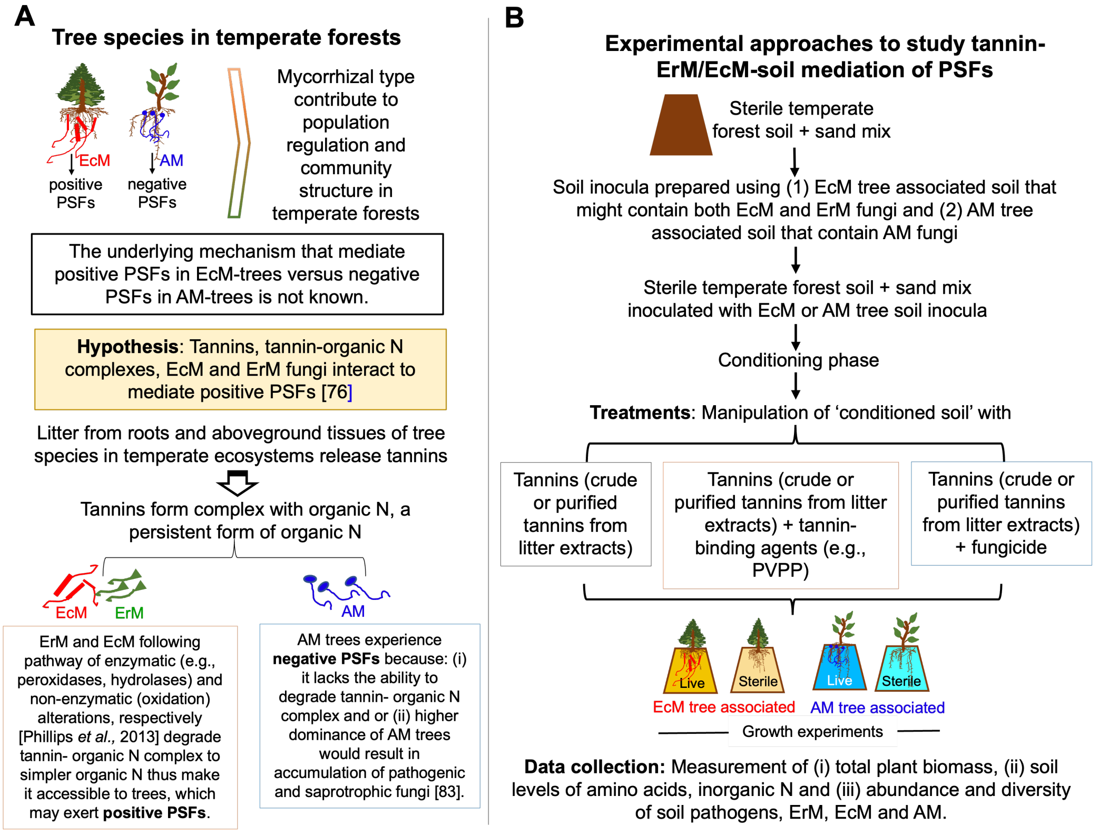


Figure 2. Mycorrhiza mediate plant-soil feedbacks (PSFs) experienced by EcM, ErM or AM trees in temperate forests. A, Tannins released by temperate tree species form complexes with soil organic N (e.g., proteins, chitin), creating persistent form of organic N. The positive PSFs experienced by EcM trees compared to negative PSFs experienced by AM trees could be due to limited ability of AM fungi to degrade tannin-organic N complexes into simple forms of N while ErM and EcM fungi can degrade the complex form of organic N. This aspect, however, needs experimental validation. B, Experimental design to study tannins-mycorrhiza-soil mediation of PSFs in temperate tree species. Conditioning sterile temperate forest soil:sand mix with live or sterile inocula from EcM, ErM or AM trees to test the PSFs. In soil manipulation experiments, tannin-binding agents (like PVPP, polivinylpyrrolidone) could be used to establish the effects of tannins. A comparison of tannins-amended soil in the presence or absence of tannin-binding agents would help to establish the role of tannin-rich foliar leachate or root exudates of trees in making tannin-protein complex in soil. Further, the degradation of the complex by ErM, EcM or AM fungi can be studied by quantifying simpler forms of organic N including amino acids. The measurement of soil pathogenic fungi would help to find the role of AM fungi in increasing the abundance and diversity of soil pathogens that may be linked to negative PSFs experienced by

AM trees. Data on growth performance (e.g., biomass, height) can be collected to measure the direction and strength of PSFs experienced by EcM or AM trees. Further, the degradation of the complex by ErM, EcM or AM fungi can be quantified by measuring simpler forms of organic N such as amino acids. Further, measurement of the abundance and diversity of mycorrhizal fungi and pathogens would help in establishing the linkages between tannin-organic N complex, mycorrhiza type and the direction and strength of PSFs.

Figure 3

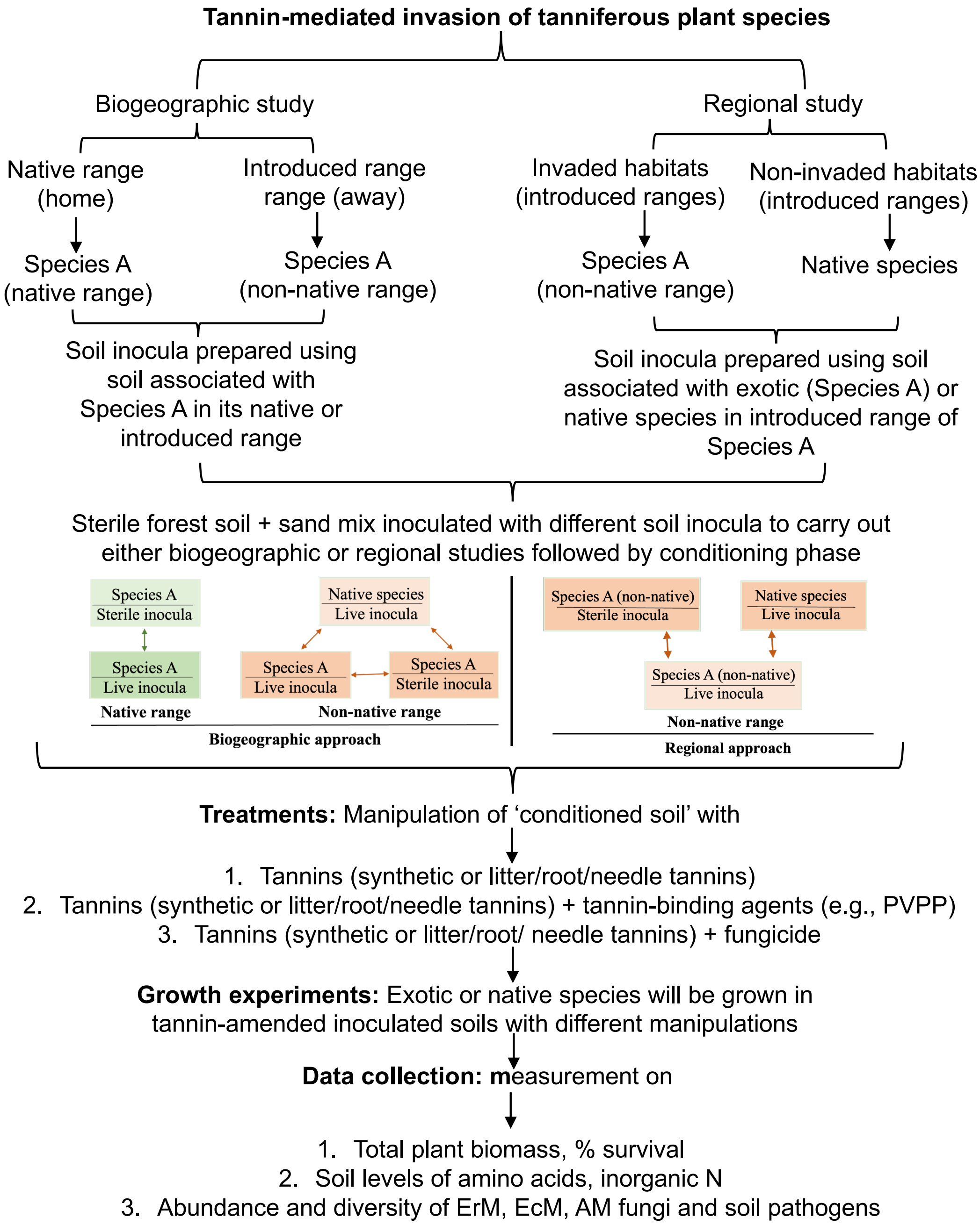


Figure 3. Biogeographic and regional studies to examine tannins-mediated PSFs experienced by alien invasive tanniferous trees in temperate forests. Soil+sand mix can be amended with soil inocula (live versus sterile) prepared by using soil associated with the invasive species or native species (regional approach) and/or by taking soil from its native and non-native ranges and soil associated with native species (biogeographic approach). Further the manipulation of tannins-amended soil with tannins-binding agents or fungicide would help to establish the role of tannin-rich foliar leachate or root exudates of trees in making tannin-organic N complex in soil or suppression of N mineralization. Data on growth performance of non-native and native species,

abundance and diversity of mycorrhizal fungi and soil pathogens and soil levels of amino acids, inorganic N would establish any role of tannin-mediated organic cycle and associate mycorrhizal fungal type in the invasion success of non-native species in non-native ranges.