Nutrient enrichment and plant-diversity loss: belowground multitrophic interactions

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Abstract

Human-induced nutrient enrichment negatively impacts on plant diversity in terrestrial ecosystems. The decline in plant diversity under nutrient enrichment primarily results from species-specific responses and their interactions, which subsequently drive their survival or loss within the community. Here, we synthesize the effects of nutrient enrichment on biotic responses and interactions between plants and their neighbors as well as other organisms. We present a framework of multitrophic interactions and their contributions to structuring plant communities. We focus on mechanisms underlying belowground interactions and highlight both recent progress and gaps in our knowledge of these processes. The proposed framework for belowground interactions would shed light on our mechanistic understanding of plant diversity loss driven by nutrient enrichment.

Challenges in nutrient enrichment and plant diversity research

Biodiversity is crucial for humans as it supports ecosystem services. However, nutrient enrichment resulting from human activities, particularly increased atmospheric nitrogen (N) deposition, is threatening plant diversity in terrestrial natural ecosystems [1]. Loss of plant diversity and changes in plant community composition by atmospheric N deposition are common in many terrestrial ecosystems [2-4]. Understanding the ecological processes and holistically elucidating the mechanisms behind the biodiversity loss driven by nutrient enrichment is essential for developing efficient ecosystem management, and minimizing the negative impacts of the global change.

Numerous studies have investigated the mechanisms underlying N-deposition-induced through manipulative experiments involving N addition in the field [5-8]. The research can be summarized as three major hypotheses: light competition [6, 8-10], N-induced soil legacies [11-13], and secondary stresses [14-18] (Box 1). Our current knowledge on these mechanisms is mainly based on phenomenological work or statistical analyses by investigating the response of plants [3, 10, 19] and microorganisms [13, 17, 20] as well as soil characteristics [2, 11, 12] to N enrichment (Box 2). Few studies specifically focused on the roles of biological processes of multitrophic organisms, particularly belowground interactions, in steering diversity loss. The observed changes in community composition and reductions in plant diversity under nutrient enrichment result from differential responses of plant species and/or functional groups [12, 21, 22] and subsequent interspecific interactions [21, 23, 24]. Therefore, elucidating what happens among plant species and how plants interact with their neighbors and other organisms is essential for a full mechanistic understanding of this ecological phenomenon.

In this article, we highlight multispecies belowground interactions, and explore the pathways by which plants interact with their neighbors, microbes and herbivores, and discuss the consequent effects of these interactions on aboveground communities. Recent advances in belowground interactions among organisms under conditions of nutrient deficiency and enrichment are summarized towards a holistic understanding of how plant communities are structured under nutrient enrichment.

Belowground interactions among organisms

Plant-plant interactions

In nutrient-poor environments, plant species exploit belowground resources in different soil layers through their vertical differentiation in root placement [25]. In addition to the heterogeneity in vertical profiles, nutrients usually display patchy distributions in soil, which further provide diverse niches for plant species coexistence through root proliferation in response to nutrient patches [26] (Figure 2). Root plasticity in response to soil nutrient availability is an important foundation for the coexistence of species in natural ecosystems [27]. Simulation experiments with model plants have demonstrated that low levels of patchy nitrate regulate root branching by stimulating lateral root proliferation, functioning more as a signaling molecule than as a nutrient [28]. However, with eutrophication due to increased nutrient inputs, the spatial distribution of soil nutrients transitions from heterogeneity to homogeneity. This shift intensifies interspecific competition by diminishing niche differentiation, as nutrient patches gradually disappear [29]. The excessive accumulation of inorganic N including nitrate (NO_3^-) and ammonium (NH_4^+), as well as low-molecular-weight organic N (particularly L-glutamate) in the soil, exerts systemic inhibitory effects on root elongation [28, 30]. These morphological changes in roots are modulated by the interplay between signals from the plant's internal N status and the external N supply [28]. Furthermore, the release of toxic metals induced by N enrichment also differentially suppresses root elongation. For example, deep-rooted forb species experience more significant root growth inhibition in response to elevated manganese (Mn^{2+}) concentrations in N-enriched soils compared to shallow-rooted grasses [12]. These varied root responses can lead to trait convergence and increased niche overlap, ultimately posing a threat to species coexistence (Figure 2).

As "belowground highways", root exudates and mycorrhizal networks convey information and allow communication among plants by facilitating or suppressing growth of neighboring plants [31, 32] (Figure 2). For example, under nutrient-deficient conditions, plant species with higher efficiency of phosphorus (P) utilization exude large quantities of carboxylates, protons, and acid phosphatases, which enhance nutrient availability and simultaneously alleviates P deficiency in their neighbors [33]. Another example for root exudates involved in interspecific facilitation is that some cereal plants secrete flavonoids that enhance symbiotic N_2-fixation of adjacent leguminous plants in environments of low soil N availability [34]. In addition to root intermingling and exudates, plants are also capable of interacting with their neighbors by exchanging nutrients via mycorrhizal networks [35]. A recent study utilizing isotope tracing technology in North American prairies revealed that soil fungi serve as a crucial pathway in facilitating N transfer from grass species to leguminous plants [36]. These field and microcosm studies provide clear evidence for the roles of nutrient mobilization and exchange-based facilitation through root exudates and mycorrhizal networks in maintaining plant coexistence in nutrient-poor environments [35, 37].

Nutrient enrichment alters both the quantity and composition of root exudates [38, 39], as well as the structure and function of mycorrhizal fungi [13]. For instance, N addition increases the proportion of oxalate in root exudates of grassland plants, with forb species releasing greater amounts than grasses [39]. These compounds, along with N-rich secondary metabolites in exudates such as alkaloids and benzoxazinoids (BXs), may trigger negative responses in neighboring plants via allelopathic effects [40]. The species-specific and rapid responses of root exudates to nutrient availability [38, 39] enable belowground communications and further shape aboveground community assembly [37] (Figure 2). Additionally, the decrease in the abundance and richness of mycorrhizal fungi by nutrient enrichment [13, 20] may interrupt the nutrient exchange and signal transmission among plant species. This disruption may reduce the resistance of plants to biotic and abiotic stresses [41] (Figure 2), thus driving the gradual loss of species from the plant community.

Plant-microbe interactions

Symbioses between plants and soil microbes have been recognized as an important driver maintaining biodiversity in natural ecosystems [42], as plant species can develop reciprocal plant-microbe symbiotic systems under nutrient-deficient conditions to facilitate coexistence [43]. Symbionts not only aid in nutrient acquisition by plants, but also enhance their defense and tolerance to abiotic and biotic stresses [44]. However, increases in nutrient availability can alter physiological trade-offs among symbiotic microbes in their responses to nutrient enrichment vs. the ability to acquire other soil nutrients, which further negatively affects their host plants [45] (Figure 2). It is predicted that nutrient enrichment may weaken the symbiotic associations between plants and arbuscular mycorrhizal fungi (AMF), as well as between plants and N_2-fixing bacteria, due to reductions in their interdependent resource exchange under nutrient-enriched conditions [24].

Root exudates serve as crucial information carriers that affect the amplitude and direction of plant-microbe symbioses [46]. Root exudates contain a variety of primary metabolites including sugars, amino acids, carboxylates, and proteins, which are substrates for microbe growth and directly influence their biomass and abundance [46]. Additionally, root exudates convey information through secondary metabolites and phytohormones to shift the composition of the microbial community by affecting specific functional taxa [47] (Figure 2). Different host plants generate distinct microbial communities on the root surface and within the root via their unique exudate components [48]. Nevertheless, variations in soil nutrient availability can influence the fitness dynamics between host plants and microbial communities by modifying the composition of secretions [49] (Figure 2). If the fitness equilibrium between plant species and microbes is disturbed, their associations may shift from synergistic to antagonistic, potentially culminating in the gradual suppression of plant growth.

Recent studies have underscored the roles of secondary metabolites and hormones in root exudate mediating root-microbe interactions [40, 49-51]. For example, flavonoids are key signaling compounds for plants to communicate with N_2 fixing rhizobia [46]. Plants release strigolactones (SLs) under phosphorus (P) limitation, which induce symbiosis between roots and AMF [52]. Benzoxazinoids (BXs) in root exudates act as plant defense signals influencing the interactions between plants and pathogens [53]. In addition, emerging evidence suggests that plant gaseous signaling molecules modulated by nutrient availability are involved in mediating plant-microbe associations [54]. For example, high N availability triggers the release of nitric oxide (NO) from roots, which in turn alters microbial community composition [55]. Up to now, a large number of signaling molecules involved in plant-microbe interactions have been identified in model plants and annual crops [40]. However, few studies have characterized the roles of infochemicals released by perennial plants in natural ecosystems, particularly in the context of plant-microbe interactions.

In addition to the indirect effects of root exudates, nutrient enrichment can directly affects microbial biomass, diversity and species composition by increasing nutrient availability or altering soil chemistry [13, 17] (Figure 2). Shifts in microbial communities can, in turn, affect plant growth by changing plant metabolisms [56] (Figure 2). Beneficial microbes, such as plant growth promoting rhizobacteria (PGPR) and endophytic fungi, may stimulate root growth by producing auxin or promoting defensive responses of host plants against pathogens via the production of toxic metabolites [57]. However, under nutrient-enriched conditions, the prevalence of beneficial microbes often diminishes, while pathogen populations tend to increase [17, 21] (Figure 2). If the defensive metabolites produced by beneficial microorganisms are insufficient to counteract the effects of increased pathogen presence, both host plants and their neighboring flora may become more vulnerable to infection, ultimately leading to a gradual decrease in their dominance within the community.

Plant-microbe-herbivore interactions

Nutrient enrichment can significantly influence endogenous primary and secondary metabolites of plants [58; 59], which function as phagostimulants and defensive agents, respectively, to attract or deter to pathogens, insects and other herbivores [60]. Specifically, N enrichment typically results in a reduction in the concentrations of water-soluble carbohydrates, while increasing the levels of proteins and amino acids in plant tissues [58, 61]. Root-feeding herbivores are capable of detecting these primary metabolites as indicators of host plant quality [60]. Due to the interspecific variation in the primary metabolome, plant species with higher nutritional quality under nutrient-enriched conditions may be preferentially targeted by herbivores, thereby elevating their risk of population decline or exclusion from the community.

According to the growth-defense balance hypothesis (GDBH), increased N availability enhances primary metabolism to promote growth, which inevitably inhibits C-based secondary metabolism [62]. For instance, the infertile soil often leads to a greater expression of phenol-based defenses in plants [59], whereas N enrichment negatively regulates the concentrations of total phenols, coumestrol and condensed tannins [62, 63]. A meta-analysis of global experimental data reveals that while elevated N deposition decreases C-based defensive compounds, it increases N-based defensive compounds, such as alkaloids [64]. Nonetheless, the synthesis of these defensive compounds often incurs considerable metabolic costs [65]. Therefore, nutrient enrichment may predispose some plant species to increased herbivory by reallocating resources from costly defensive investments to growth [62]. Conversely, some plants may bolster their resistance to herbivores by augmenting the production of N-based secondary metabolites, such as alkaloids, non-protein amino acids and antimicrobial proteins [66, 67], which can directly impair herbivore growth or disrupt their foraging efficiency (Figure 2).

For root-herbivore interactions, chemical cues in the soil are more important, as soil herbivores lacking visual stimuli likely depend on soluble root exudates and volatile compounds, as well as other gaseous signal molecules to detect roots and locate their hosts [60, 68, 69]. For example, the plant crown daisy (*Chrysanthemum coronarium*), exudes lauric acid from its roots, which suppresses the infestation of tomato roots by nematodes (*Meloidogyne incognita*) [70]. The neurotransmitter L-DOPA, a non-protein amino acid, is released by broad bean (*Vicia faba*) roots in response to aphid (*Acyrtosiphon pisum*) infestation; this compound subsequently induces the production of volatile organic compounds (VOCs) in neighboring conspecific plants, thereby enhancing their attractiveness to aphid parasitoids [71].

VOCs mediate the interactions between plants and herbivores by either directly guiding host selection from a distance or by serving as attractants for the natural enemies of herbivores [72-74]. The spotted knapweed (*Centaurea stoebe*) emits the sesquiterpene (E)-β-caryophyllene into the rhizosphere, which increases the susceptibility of *Taraxacum officinale* to the larvae of *Melolontha melolontha*, a highly polyphagous root feeder, by enhancing carbohydrate and total protein concentrations in roots of *T. officinale* [75]. Herbivory-induced indole in maize plants can diminish parasitoid attraction by modifying the olfactory cues associated with caterpillars [76]. However, the production, release, and composition of these volatiles are also influenced by N levels and are species-specific [77]. Consequently, herbivores may be drawn to particular plant species by tracking these specific volatiles, or their anti-predator defenses may be constrained by the chemical variability of plant tissues, which affects their aggregation behavior on different plant species (Figure 2).

Nutrient availability affects plant-herbivore interactions likely by modulating the persistence of symbiotic microorganisms and the synthesis of microorganism-derived defensive compounds. For instance, fungal endophytes can enhance plant resistance by producing anti-herbivore alkaloids [78]. However, research conducted in grassland ecosystems demonstrated that the addition of N and P increases the performance of native herbivorous insects on tall fescue via reducing the production of alkaloids by endophytic fungi [79]. The colonization of these endophytes into the root system can direct affect the metabolism of their host plants and thereby alter their resistance to insects [80]. Recent research indicates that potato plants reduce the supply hexoses while sustaining the transfer of plant-derived fatty acides to AMF during simultaneous interactions with parasites [81]. Furthermore, soil microbes at the root surface may also influence root-herbivore recognition and foraging patterns by changing or metabolizing root exudates of their hosts [82]. In nutrient-enriched environments, an increase in pathogen abundance combined with plant feeders have synergistic impacts on host plants, which potentially aggravate the damage to plants [83]. Although the earlier studies illustrated the potential of soil microbes to affect root-herbivore interactions, current research efforts have only scratched the surface of this topic, lacking a comprehensive exploration of the physiological and ecological processes that underpin below-ground interkingdom interactions, particularly in the context of global change.

Aboveground herbivore attacks induced by nutrient enrichment [18] trigger plants to synthetize signaling molecules [84]. These signaling molecules are transported from shoots to roots and subsequently released into the soil, triggering a series of belowground cues that modify the soil microbial diversity and composition and influence root herbivore activity [82, 85, 86] (Figure 2). An isotope tracing experiment revealed that aphid infestation disrupts the nutrient exchange between plants and AMF, however, this disruption can be alleviated by the presence of neighboring plants [87]. Soil legacies, resulting from nutrient enrichment-induced changes in soil microbial composition or the metabolites of soil organisms, further exert feedback on plant growth and defense chemistry [22, 88] (Figure 2). The varying sensitivities of plant species to these legacies leads to diverse aboveground responses [89]. For example, nutrient-demanding species can face less effects of plant-soil feedback in a nutrient-rich environment, which confers them greater competitiveness [90]. Several studies have demonstrated the coupling between below- and aboveground cues under conditions of nutrient enrichment [22, 23], ultimately influencing plant community assembly [82].

Over time, belowground interactions among organisms may undergo hierarchical changes (Figure 3). In nutrient-limited environments, plant species coexist though mutual benefits among interkingdoms via positive bidirectional flows of material, energy and information between above- and belowground components. Increases in soil nutrient availability firstly triggers divergent physiological and/or morphological responses of plant individuals and/or microbial functional taxa, which may further change the direction of their interactions from synergistic to antagonistic. As nutrient enrichment progresses, alternations in soil abiotic and biotic properties favor the proliferation of predators, further eliciting root-microbe-herbivore interactions. This scenario could result in negative feedback on the aboveground growth of plant species, thus leading to species reordering or loss. The emergence of aboveground herbivores during the middle or late stages of nutrient enrichment complicates belowground interactions, potentially exacerbating the loss of plant species sensitive to secondary biotic stresses from the community.

Concluding remarks and perspectives

The impacts of N enrichment on plant diversity have been extensively studied for decades across multiple scales, ranging from global and regional levels to the field, community, species and rhizosphere [3-6, 8, 22, 23]. Although numerous mechanisms have been proposed to explain the effects of N enrichment on terrestrial ecosystems (Box 1), there is still a patchy knowledge base limiting our comprehensive understanding of this major global change effect on biodiversity. Multiple lines of evidence suggest that the loss of plant diversity driven by nutrient enrichment is a complex synthesis of interspecific interactions among multiple organisms [18, 21, 23, 90]. However, relatively few studies have explored the contributions of combined eco-physiological processes in mediating interactions among belowground organisms. Therefore, there is an urgent need for in-depth investigation into how nutrient enrichment alters the direction and magnitude of biotic interactions. Elucidating the mechanisms underpinning these interactions is essential for expanding our knowledge of plant diversity decline due to nutrient enrichment. Several questions warrant future research, including: 1) How do plants change the metabolic and morphological traits of their roots when exposed to nutrient-enriched environments? 2) What are the key metabolites produced by plants or soil microbes, and how do these metabolites influence plant performance, neighboring species, and microorganisms? 3) How do nutrient-induced responses impact interactions among organisms, and how do these interactions evolve with the progression of nutrient enrichment? (see Outstanding questions).

In ecosystems, multitrophic interactions are inherently bidirectional: plants have the capacity to modify local physicochemical soil conditions via rhizosphere processes; their associated soil microbiomes, in turn, can influence the local performance of both the plants themselves and neighboring species, thus affecting their survival or extinction. However, the technical challenges associated with monitoring belowground processes pose significant obstacles to elucidating these bidirectional interactions among plants, their neighbors, and other organisms. The implementation of innovative methodologies, such as the rhizobox sampling system for the separation of roots and microbial communities [91], live imaging of roots and microbes [92], molecular techniques [21], and microbial meta-transcriptomics and meta-proteomics [93], promises to illuminate the processes underlying species interactions under nutrient enrichment. Identifying specific infochemicals or key microbial taxa involved in plant community assembly through these methodologies would bridge the gaps between scientific research and ecosystem management. Such insights will be instrumental in achieving the goal of maintaining plant diversity in the face of global environmental changes.

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Box 1. Major hypotheses on plant species loss induced by nutrient enrichment

Light competition: Light competition is characterized by size asymmetry, wherein larger or taller plants acquire more light per unit of biomass compared to their smaller counterparts within a community [7]. Nutrient enrichment exacerbates light competition among plant species by promoting the accelerated growth of larger or taller plants with increasing nutrient availability, which subsequently casts shade on smaller plant species [10]. Empirical evidence from both supplementary light experiments and field grazing experiments designed to mitigate light limitation has demonstrated that light competition contributes to the reduction of plant species richness induced by N enrichment [6, 8, 9]. However, some studies have reported inconsistent findings, indicating that increasing light availability through methods such as tie-backs and clipping does not necessarily alleviate the N-addition-induced species loss [5], suggesting that the biological mechanisms underpinning this ecological phenomenon remain elusive.

Soil legacies: Chronic N deposition causes legacy effects on soil properties, including increased accumulation of dissolved N [12], soil acidification [2], excessive mobilization of toxic metal ions [11, 12], and alterations in microbial biomass, structure, and activity [13, 20]. The relative contributions of changes in soil abiotic and biotic properties induced by N enrichment to the decline in plant diversity vary across different ecosystem types [2, 11, 12]. Nevertheless, these factors can both directly and indirectly affect the growth of plant species and/or functional groups [3, 12]. The divergent responses of individual plants can lead to variation in species relative abundance, ultimately resulting in some plant species thriving while others experience a in abundance within the community.

Secondary stresses: Research on secondary stresses induced by nutrient enrichment in natural ecosystems has been advancing for decades [14, 15]. These secondary stresses, driven by nutrient enrichment, encompass both abiotic and biotic stresses [2, 17, 18, 20]. Nitrogen enrichment influences vegetation dynamics by altering plant sensitivity to climatic fluctuations [15, 16], affecting plant-microbe associations [23, 24], and rendering plants more susceptible to diseases and pests [17, 18, 20].

Box 2 Belowground responses to nutrient enrichment

Nutrient enrichment has a wide range of impacts on soil properties and plant physiological processes (Figure 1). For instance, soil acidification universally occurs with increasing N inputs [2], which subsequently disrupts soil nutrient availability. This disruption is exemplified by the increased chemical availability of toxic metals such as iron (Fe^{3+}), aluminum (Al^{3+}) and manganese (Mn^{2+}), coupled with a reduction in the availability of other essential nutrients including calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) and sodium (Na^{+}) [11, 36]. Soil biotic properties, such as the biomass, richness and composition of microbial communities are influenced by changes in soil chemistry [13, 20] or the indirect effects of nutrient enrichment on physiological processes of plant root systems [39, 46]. Fluctuations of nutrient availability and/or modifications in other soil chemical parameters can directly impact root traits [19] and the composition of root exudates [39] as well as evoke the release of gaseous signaling molecules by plants [55, 77]. These belowground plant responses to nutrient enrichment are further modulated by photosynthetic activity and the allocation of photosynthates from aboveground to belowground parts. These physiological processes integrate to mediate belowground interactions among organisms.

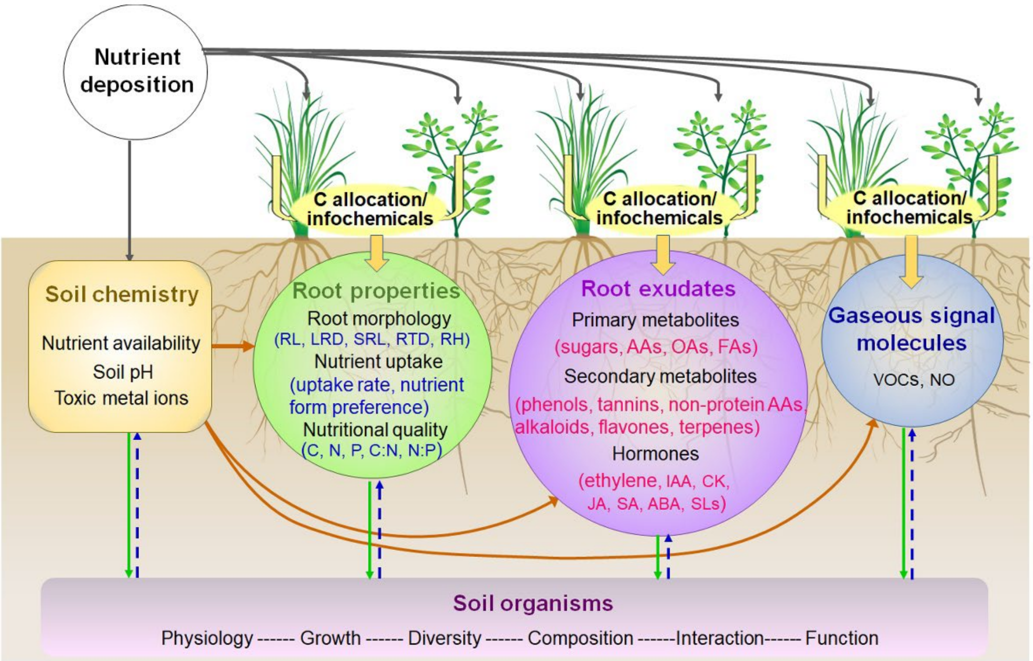
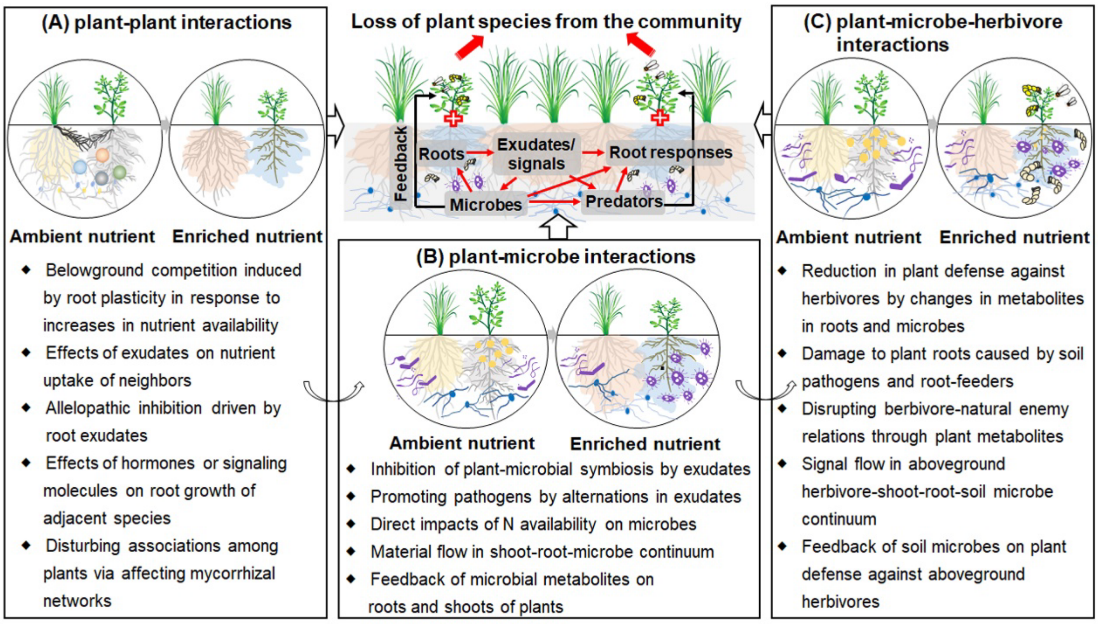


Figure 1. Belowground changes induced by nutrient enrichment. The yellow arrows represent the transport of carbon (C)/nitrogen (N)-containing compounds and signaling molecules including secondary metabolites and phytohormones from shoots to roots. The orange arrows indicate the indirect influences of changes in soil chemistry driven by nutrient enrichment. The green arrows represent the effect of soil chemistry and rhizosphere processes of plants on soil microorganisms, and blue arrows indicate the feedback of microorganisms to plants. RL, root length; LRD, lateral root density; SRL, specific root length, RTD, root tissue density, RH, root hair; C, carbon content; N, nitrogen concentration; P, phosphorus concentration; C:N, ratio of carbon to nitrogen; N:P, ratio of nitrogen to phosphorus; AAs, amino acids; OAs, organic acids; FAs, Fatty acids; IAA, indoleacetic acid; CK, cytokinin; JA, Jasmonic acid; SA, salicylic acid; ABA, abscisic acid; SLs, Strigolactones; VOCs, volatile organic compounds; NO, nitric oxide.



- Belowground competition induced by root plasticity in response to increases in nutrient availability
- Effects of exudates on nutrient uptake of neighbors
- Allelopathic inhibition driven by root exudates
- Effects of hormones or signaling molecules on root growth of adjacent species
- Disturbing associations among plants via affecting mycorrhizal networks

- Inhibition of plant-microbial symbiosis by exudates
- Promoting pathogens by alternations in exudates
- Direct impacts of N availability on microbes
- Material flow in shoot-root-microbe continuum
- Feedback of microbial metabolites on roots and shoots of plants

- Reduction in plant defense against herbivores by changes in metabolites in roots and microbes
- Damage to plant roots caused by soil pathogens and root-feeders
- Disrupting berbivore-natural enemy relations through plant metabolites
- Signal flow in aboveground herbivore-shoot-root-soil microbe continuum
- Feedback of soil microbes on plant defense against aboveground herbivores

Figure 2. Belowground interactions among organisms underlying plant diversity loss driven by nutrient enrichment. Nutrient enrichment triggers eco-physiological responses of multiple organisms and subsequently elicits belowground interspecific interactions including plant-plant, plant-microbe and plant-microbe-herbivore interactions differentially affecting aboveground growth of plant species or functional groups.

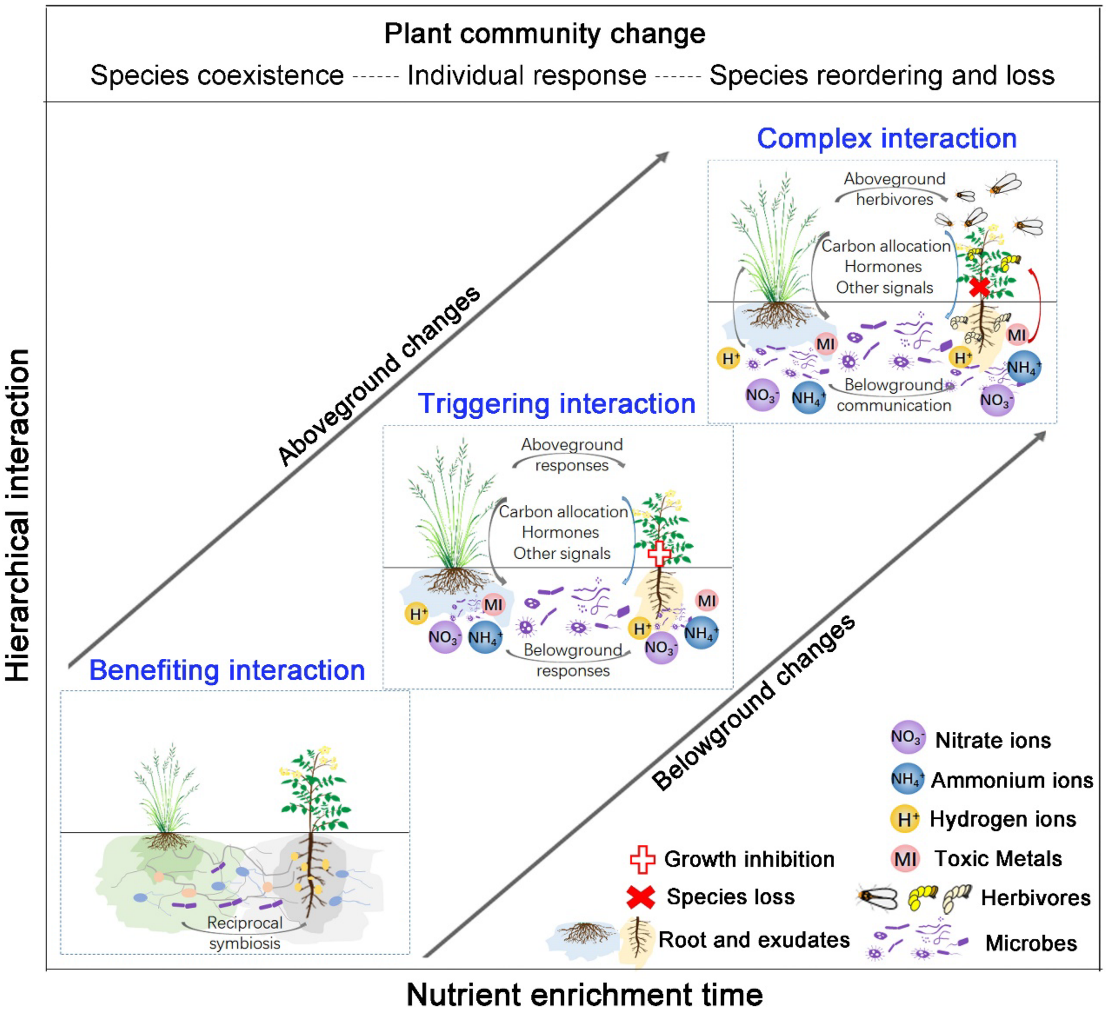


Figure 3. A conceptual scheme illustrating the framework of hierarchical interactions among multiple organisms under conditions of nutrient enrichment. Changes in the plant community induced by nutrient enrichment via hierarchical interactions among multiple organisms from the initial beneficial interactions to antagonistic interactions by individual responses and evolving to complex interactions mediated by multiple abiotic and biotic factors.