Review

Nitrogen Nutrition of Trees in Temperate Forests—The Significance of Nitrogen Availability in the Pedosphere and Atmosphere

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Abstract: Nitrogen (N) is an essential nutrient that is highly abundant as N_2 in the atmosphere and also as various mineral and organic forms in soils. However, soil N bioavailability often limits the net primary productivity of unperturbed temperate forests with low atmospheric N input. This is because most soil N is part of polymeric organic matter, which requires microbial depolymerization and mineralization to render bioavailable N forms such as monomeric organic or mineral N. Despite this N limitation, many unfertilized forest ecosystems on marginal soil show relatively high productivity and N uptake comparable to agricultural systems. The present review article addresses the question of how this high N demand is met in temperate forest ecosystems. For this purpose, current knowledge on the distribution and fluxes of N in marginal forest soil and the regulation of N acquisition and distribution in trees are summarized. The related processes and fluxes under N limitation are compared with those of forests exposed to high N loads, where chronic atmospheric N deposition has relieved N limitation and caused N saturation. We conclude that soil microbial biomass is of decisive importance for nutrient retention and provision to trees both in high and low N ecosystems.

1. Introduction

Following water availability [1–3], access to nitrogen (N) sources is considered to be the main factor limiting the growth and development of plants and, hence, food and biomass production as well as land carbon (C) storage at a global scale [4–7]. This is also evident from the extensive distribution of low N soils across the globe [8]. In such soils, global climate change will further reduce N availability and accelerate N limitation due to reduced precipitation during the vegetation period [9,10] and dilution of bioavailable soil N by the increased growth in response to elevated carbon dioxide (CO_2) [6], provided N deposition from the atmosphere is low [11–15]. In agriculture, N limitation is overcome by the application of inorganic and/or organic fertilizers, amounting to a global inorganic fertilizer use of 108 Mt in 2012 [16]. At a global scale, the production of fertilizer N in conjunction with increased cultivation of N-fixing plants and industrial activities has more than doubled the annual release of reactive N forms to the biosphere [17]. Due to the cascading of mobile reactive N forms across the boundaries of agricultural ecosystems, this anthropogenic perturbation of the global N cycle has regionally increased atmospheric N deposition in forests, with N loads in Europe ranging from 5 to 60 kg N ha^{-1}·year^{-1} [18]. However, in forests, N fertilizer application or large atmospheric N deposition is usually not required for high biomass production and is minute compared with agricultural N use [19], despite a high distribution of forests on marginal soils with low N availability. Since perennial plants such as forest trees are assumed to have evolved on marginal soils, a perennial lifestyle has been proposed to constitute an adaptation to low nutrient availability, including nitrogen [20,21].

Most of the nitrogen in forest soils is fixed in organic compounds such as proteins, lignin, or chitin. These N forms cannot be directly used by plants, but require depolymerization by specialized microorganisms to be converted to largely bioavailable monomeric organic or mineral N forms [22,23]. According to the traditional view, the liberation of nutrients from soil organic matter (SOM) depends on the chemical recalcitrance of SOM compounds, the genetic microbial depolymerization capacity, and the climate-driven enzyme activity. However, recent evidence highlighted that SOM turnover and persistence is also governed by the accessibility of organic matter to decomposer organisms or exo-enzymes. Hence, soil aggregation, sorption/desorption processes and the formation of organo-mineral associations [24,25] are important regulators of SOM decomposition and nutrient liberation. Thus, N availability in soils varies considerably across ecosystem and soil types and is not necessarily related to total soil N content.

The high productivity of forest trees on marginal soils with low N content is not a consequence of a low N demand of forest trees. For example, mature temperate forest stands have an annual nitrogen requirement of about 100 kg N ha^{-1} year^{-1} (Figure 1), which is similar to many agricultural systems [26,27]. Such high amounts of N may become available in forests transiently as a result of accelerated microbial liberation of nutrients when gaps develop due to dying trees or windbreaks, or

due to selective cutting or clear cutting activities in forests [28]. Thus, the major questions are: (i) how is this high N demand met in mature forests on marginal soil with low atmospheric N input? and (ii) which N source(s) become available in the annual growth cycle to meet this high N demand of mature forest trees? These questions are of particular significance when the competition for N between forest trees and microbial N transformation processes and microbial N use in growth and development are considered [12]. Therefore, the present review article summarizes the current knowledge on the processes and fluxes of N distribution between forest trees and microbes with particular emphasis on temperate forest ecosystems on marginal soil with low N content and low atmospheric N input. These processes and fluxes under N limitation are compared with those of forests exposed to high loads of N as observed in patchy landscapes in close proximity to intensively used agricultural and forested areas [29].

2. Distribution and Fluxes of Nitrogen in Marginal Soil

Meeting the high N demand of mature trees on marginal soil is particularly challenging. Forest ecosystems on marginal soil are characterized by a largely closed ecosystem N cycle, *i.e.*, by tightly-coupled microbial mineralization-immobilization turnover, which facilitates effective microbial N retention [12]. Under these conditions, leaching of N, particularly strongly mobile nitrate, into the hydrosphere is negligible. Furthermore, the release of volatile N compounds such as NO, N_2O or N_2, produced during microbial transformation of inorganic and organic N compounds, into the atmosphere is minute (Figure 1). The influx of N into the ecosystem from atmospheric deposition and non-symbiotic biological N fixation by heterotrophic bacteria [30,31], as well as N acquisition from the hydrosphere and its liberation from the geosphere [32] are small, but still can exceed N leaching and volatilization. Hence, the accumulation of N from these N sources can occur, but is slow. Thus, ecosystem N fluxes and N redistribution almost entirely depend on ecosystem internal sources, in particular the decomposition of leaf and root litter as well as decaying microbial biomass and older soil organic matter [12,33]. Since the mean residence time of microbial biomass with a range between days and months [26,34,35] is significantly shorter than the lifespan of leaf and root litter [36–38], microbial biomass turnover is a major driver of N redistribution in N-limited ecosystems. However, our understanding of internal gross nitrogen turnover in N-limited forests is still fragmentary because available studies on the extremely dynamic gross nitrogen mineralization-immobilization turnover have been restricted to single or a few measurement dates. Only for one N-limited forest site, *i.e.*, the Tuttlingen experimental beech forest in southern Germany, were the gross N turnover rates (ammonification, nitrification, microbial inorganic N immobilization, denitrification) determined with sufficient temporal resolution (13 sampling dates between 2002 and 2009) to constrain annual N turnover budgets [35,37,39–44] (Figure 1).
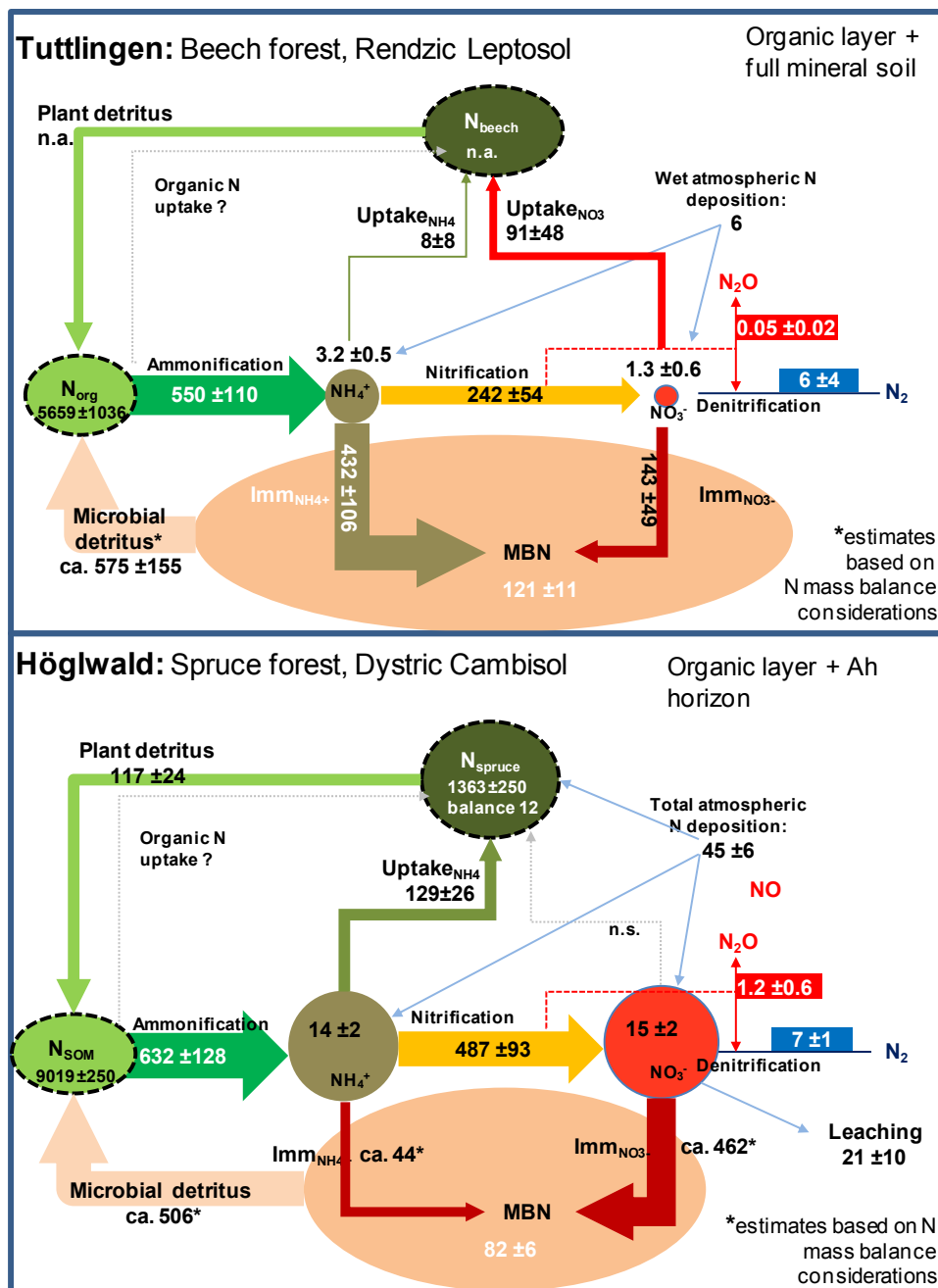
Figure 1. Gross nitrogen fluxes (kg N ha^{-1} year^{-1}) and N pools (kg N ha^{-1}) for two forest ecosystems in southern Germany: The Tuttlingen beech forest on marginal shallow Rendzic Leptosol soil, with low atmospheric N input (upper panel), and the Höglwald forest, a nitrogen-saturated spruce forest on a Dystric Cambisol soil and affected by chronic atmospheric N deposition (lower panel). Note that the thickness of the arrows relates to the size of the process rate, and the pool sizes of ammonium, nitrate and microbial biomass are reflected by the size of the pool signatures. It was not possible to provide size-scaled pool signatures for the N pools in soil organic matter (SOM) and plant biomass (both indicated with black dashed lines), because these pools exceed the labile soil N pools by several orders of magnitude. SOM does not include microbial biomass in this graph. For further information, see text. Ammonium and nitrate uptake was estimated for both forest ecosystems by multiplying the uptake capacities by the fine root biomass [26].

Figure 1 provides a synthesis on annual N turnover in this forest stand based on a compilation of previously published data on gross N turnover rates [35,37,39–43]. This ecosystem shows—despite very high N mineralization and significant nitrification rates—a closed N cycle characterized by competitive partitioning of N between beech trees and microbial N retention pathways so that N loss remains small. This is specifically due to the almost complete partitioning of nitrate to microbial or tree uptake (Figure 1, Tuttlingen: Beech forest, Rendzic Leptosol). Microbial mineralization-immobilization turnover is *ca.* fivefold larger than tree N uptake and plant-mediated internal N turnover, producing an annual microbial detritus >500 kg N ha^{-1}. This means that microbial biomass is processed on average several times a year, recycling and conserving a huge nutrient stock. These N cycle patterns sustain economically and ecologically valuable forests on marginal soils, which are not suitable for traditional agricultural use with herbaceous crops.

Tree uptake rates of amino acids remain difficult to estimate because on the one hand, studies on N uptake capacity using capsules with amino acid solutions attached to soil-free washed root tips show that there is significant uptake capacity [42,43]. On the other hand, recent work based on the injection of double $^{13}C/^{15}N$-labelled amino acids into intact beech-soil systems showed that only ^{15}N and not ^{13}C was recovered in beech [45]. This suggests that either the uptake of intact amino acids was not significant in the presence of microbial competition, or that amino-acid derived C had already been subjected to respiration in the mycorrhizal mantle [46].

A further feature of this N-limited beech stand was the effective closure of the N cycle in the denitrification process, *i.e.*, the removal of reactive N from the biosphere as harmless inert dinitrogen (N_2) rather than nitrous oxide (N_2O) (Figure 1), a potent greenhouse gas and dominant ozone-depleting substance in the stratosphere.

In such forest ecosystems, soil microbial activity provides a huge potential for the recycling and liberation of bioavailable N as well as for microbial competition with plants for N. Redistribution of N between trees and understory plants will largely depend on the competitive strength of these N consumers, with a significant advantage for generalists compared with specialists. As a consequence, the N preferences of tree roots can change from ammonium and amino acids at high N availability to no preference upon N limitation [47], thereby providing a competitive advantage at low N availability compared with more specialized players in the root-microbe system.

Using stable isotope approaches, Guo *et al.* [37,38] showed that leaf litter contributes less to the N nutrition of beech natural regeneration compared with root litter, and that microbial biomass is a much more important sink of N liberated from leaf and root litter compared with beech. Nitrogen from recent leaf litter contributed only a minor amount to the N requirement of beech, indicating that liberation of N from recalcitrant pools of old litter in soil organic matter over years and decades constitutes the dominant N source of beech in N-limited ecosystems. However, the contribution of nutrients recycled from root litter to forest nutrition remains unknown. In this context it has to be considered that microbial nitrate acquisition beyond the actual N demand of the trees for growth, and development and storage constitute an effective means to prevent nitrate leaching and gaseous N losses (Figure 1) from the ecosystem. Recent studies using tree girdling as a tool to unravel the role of C rhizodeposition in N partitioning between beech and free-living microbes showed that microbial-bound N then seems to be used as a transient storage of nutrients [42,47]. Under unfavorable conditions such as drought or reduced carbohydrate exudation of roots and thus reduced C supply to heterotrophic microorganisms,

the transient storage can be abandoned [42,47]. Decaying microbial biomass will then become a new source of nutrients for the beech trees [42,48]. In a similar way "carbon expensive" mycorrhizal fungi with extensive development of rhizomorphal hyphae may be exchanged with "carbon inexpensive" mycorrhizal fungi with reduced development of rhizomorphal hyphae under a C shortage [49]. Apparently, beech trees control ecosystem N cycling and N bioavailability under N limitation, thereby operating under the general principle of "to live and let die".

3. Distribution and Fluxes of Nitrogen in Forests Exposed to High Nitrogen Loads

In forests exposed to high N loads, there are more potential sources for N acquisition compared with low N systems. The demand of plants for N can be met both by root uptake of N originating from pedospheric/hydrospheric sources and by stomatal uptake of reactive N compounds from the atmosphere [50]. Uptake of reactive compounds from atmospheric deposition is largely controlled by stomatal conductance and the concentration gradient of reactive N compounds between the atmosphere and the substomatal cavity. This concentration gradient is often determined by the removal of N from the substomatal cavity into the aqueous solution of the apoplastic space surrounding the substomatal cavity and further on into the symplasm of leaf cells [50–52]. Stomatal closure may be considered a means to down-regulate N influx. However, this down-regulation takes place at the expense of reduced carbon dioxide (CO_2) influx into the leaves, resulting in reduced photosynthetic carbon fixation and hence growth [53]. When the stomatal opening is maintained in the presence of biologically available atmospheric N, this will result in uncontrolled, compulsory N nutrition via the leaves. In the majority of tree species, reduction of N taken up by roots and its assimilation into amino acids takes place exclusively in the roots, and leaves are supplied with amino N by xylem transport [54]. Therefore, influx of atmospheric N via the stomata and its reduction and assimilation in the leaves leads to a complete change in the distribution of N metabolism between leaves and roots and in root-to-shoot allocation of N assimilation products and its metabolites. As a consequence, N deposition can change the distribution of biomass between shoot and roots in favor of the shoot, thereby enhancing drought susceptibility due to enhanced transpiration of the increased shoot biomass [55,56]. Depending on the rate of N influx via the roots plus the shoot and the N demand of the plant, N over-nutrition may be prevented by shoot-to-root interactions [54]. In contrast to the influx of atmospheric N, N uptake from pedospheric/ hydrospheric sources can at least be partially controlled (see below) and can be down-regulated to the extent that atmospheric N contributes to N nutrition [54,57,58]. If high rates of N deposition result in N over-nutrition [29], this is often indicated by extremely high amounts of the N-rich amino acid arginine in phloem and xylem sap [54,59].

At the ecosystem level, long-term N deposition will turn forests from N-limited into N-saturated or even over-saturated systems [60,61]. The term "nitrogen saturation" is defined as a state where either the availability of mineral N exceeds the combined nutritional demands of plants and microbes [62], or where ecosystem N losses approximate or exceed the inputs of N [63]. Hence, nitrogen saturation is connected with a change from closed to open ecosystem N cycling. However, the extent and stage of N saturation needs to be accounted for. At an initial stage, N deposition will result in a more narrow soil C:N ratio, will remove microbial N limitation and will enrich organic matter in N, which will stimulate N mineralization [64,65]. The associated changes in N mineralization and the C:N ratio also alter the

balance of ammonium consuming processes at the expense of microbial immobilization and in favor of nitrification, thus impairing microbial N retention and promoting N losses along hydrological and gaseous pathways [64,66]. In the long-term, chronic N deposition impairs soil microbial activity due to soil acidification and low C availability, thus decelerating soil organic matter decomposition, N mineralization and subsequent N turnover processes, including immobilization, nitrification and denitrification [64,67].

The consequences of chronic atmospheric N deposition for ecosystem N cycling have been analyzed in great detail for a Norway spruce forest in southern Germany surrounded by maize agriculture [26,29,44] (Figure 1). The data in Figure 1 were based on the compilation of Kreutzer *et al.*, 2009 [26], complemented by the gross N turnover dataset of Rosenkranz *et al.*, 2010 [44] such that this new compilation includes much better constrained gross N turnover rates compared with those of Kreutzer *et al.*, 2010 [26].

This forest (the "Höglwald" experimental spruce forest) is characterized by long-term N input from gaseous deposition (10 and 2 kg N ha^{-1} year^{-1} for ammonia and NO$_2$, respectively) and throughfall (18 and 10 kg N ha^{-1} year^{-1} for ammonium and nitrate, respectively) amounting to a total N deposition from the atmosphere to the forest floor of *ca*. 40 kg N ha^{-1} year^{-1} excluding about 4 kg N ha^{-1} year^{-1} taken up by the canopy. About half of the N deposited (21 kg ha^{-1} year^{-1}) is leached into the hydrosphere, almost exclusively in the form of nitrate. In addition, *ca*. 16 kg N ha^{-1} year^{-1} are released into the atmosphere, mostly as nitric oxide (NO) and N$_2$, but to a minor extent also in the form of the potent greenhouse gas N$_2$O [26] (Figure 1). Thus, almost all the N deposited in the spruce forest ecosystem from atmospheric sources is released by leaching and volatilization into the hydrosphere and atmosphere, respectively. Despite these releases, high ecosystem internal N fluxes were observed. Figure 1 provides a synthesis of published annual N fluxes of this ecosystem [26,44]. Spruce trees were estimated to acquire 129 kg N ha^{-1} year^{-1} from pedospheric/hydrospheric NH$_4^+$ and 4 kg N ha^{-1} year^{-1} from atmospheric sources, but most of the N retrieved is lost annually in the form of leaf and root litter (117 kg N ha^{-1} year^{-1}). However, uptake from pedospheric/hydrospheric N sources may constitute an underestimation, since only inorganic N uptake was considered and organic N uptake that is thought to be a significant N source of trees [44,46] was neglected. Gross microbial mineralization-immobilization turnover in the Höglwald forest was about five times higher than tree N uptake and plant-mediated internal N turnover. Hence, microbial detritus (*ca*. 500 kg N ha^{-1} year^{-1}) constituted a much stronger N source of polymeric soil organic matter than spruce litter. Nitrogen bound in microbial biomass was significantly lower (82 kg N ha^{-1}) than microbial N immobilization or microbial N detritus formation, indicating a high turnover of this N pool with a mean residence time of about two months. Despite the relatively high N losses of this forest compared with low N natural ecosystems, it needs to be noted that annual gross nitrification rates (almost 500 kg N ha^{-1} year^{-1}) as well as microbial nitrate immobilization rates (*ca*. 460 kg N ha^{-1} year^{-1}) exceed annual N losses by about an order of magnitude. In view of this dominant role of microbial nitrate immobilization in N loss pathways, we conclude that microbial biomass turnover is the most important process mediating redistribution and retention of N at the ecosystem level in N over-saturated forest ecosystems [26,44,65].

4. Regulation of N Acquisition and Distribution in Trees

Nitrogen acquisition by trees and the significance of root N uptake in the biogeochemical cycle is ultimately regulated at the whole plant level. Nitrogen taken up from atmospheric sources by leaves and not used inside the leaves, as well as N taken up from pedospheric/hydrospheric sources by roots and not used inside the roots are both loaded into an N pool cycling inside the whole plant by phloem and xylem transport. This cycling of N includes bidirectional exchange not only between the xylem and phloem in the leaves and the roots, but also along the long-distance transport path [54,68]. Glutamine, the most abundant long-distance transport form of N in many tree species, also seems to be the most prominent N compound subjected to this bidirectional exchange. However, the exchange of N may also include metabolic interconversion of amino compounds [54]. The cycling pool of plant N serves whole-plant distribution of N stored in the bark and wood of stems and roots (deciduous trees) or the leaves and roots (evergreen trees) upon mobilization for the spring growth of leaves and for fructification. Furthermore, the cycling N pool also constitutes the transport path used for filling up these storage pools of N during annual growth [69–71]. In addition, it provides N for growth and development of all meristematic tissues at the advantage of immediate N availability in close proximity to these tissues. Thus, the cycling plant N pool mediates swift N supply of any part of the plant throughout the year. The size of this cycling plant N pool may change depending on N nutrition, and this pool may even be used for N storage, particularly in the form of arginine, an amino acid with high N content [59]. Mobilization of N from this storage pool may provide N faster than the mobilization from storage proteins in bark and wood [70,71]; therefore, it may be of particular significance to meet N requirements in response to environmental changes. The processes regulating the loading and unloading of the cycling N pool have currently only been studied for seasonal storage and mobilization in poplar [70,71]. Therefore, the mechanisms responsible for the up- and down-regulation of the size of this pool in response to environmental changes, including nutrient availability, constitute an exciting area of future research.

When N acquisition from pedospheric/hydrospheric and atmospheric sources as a whole exceeds the N demand of trees for growth, development, and defense, the cycling pool of N will be expanded initially by the accumulation of glutamine. This accumulation seems to result in a repression of nitrate uptake at the transcriptional or post-transcriptional level [72–74]. However, the mechanism(s) of how trees sense whole plant N status and transmit this information into changes in root uptake remain poorly understood. Whole plant N status may be integrated by cycling amino acids such as glutamine [75], but nitrate may also be involved indirectly [76,77]. Provided cycling glutamine constitutes a systemic signal, the down-regulation of nitrate uptake would require homeostasis between the cycling pool of glutamine and the glutamine pool at the site of nitrate uptake as a means to monitor the whole plant N status by the roots. Recently, a cellular mechanism of glutamine-sensing has been identified that seems to be widespread in the plant kingdom [78]. The plastid-localized PII signaling proteins can sense and integrate metabolic signals by conformational changes, thereby exerting control at all levels of metabolic regulation, including transport activity, metabolic reactions and gene expression. This protein was shown to control the key-enzyme of the ornithine synthesis pathway, N-acetyl-L-glutamate kinase, leading to arginine and polyamine synthesis [78]. It may therefore be assumed that PII signaling proteins can sense high glutamine levels and initiate arginine synthesis under these

conditions. However, it remains unclear how glutamine sensing by these plastid-localized proteins reduces nitrate uptake via the root plasmalemma at a post-transcriptional or transcriptional level.

Repression of the expression of the high affinity nitrate transporter gene NRT2.1 by amino acids such as glutamine was found to be mimicked by the expression of transcription factors LBD37/38/39 [79]. In addition, an evolutionary conserved component of the RNA polymerase II complex, HN19-At1WS1, was shown to repress the transcription of root NRT2,1 in *Arabidopsis* [80]. Evidence for the action of similar mechanisms in trees remains to be seen. The release of repression of the transcription of nitrate transporters also requires a systemic signal communicating that whole plant N demand for actual growth and development exceeds N acquisition by the roots. It has been suggested that a cycling pool of cytokinins mediates such a feed-forward control in trees [73,74] and that the concerted action of feedback control by cycling glutamine and feed-forward control by cycling cytokinins regulate nitrate acquisition at the whole plant level [54,81]. However, this assumption requires further research using trees with different N nutritional states. Down-regulation of expression and activity has also been observed for ammonium transporters under a high plant N status [82], but the molecular mechanisms involved have not been reported. The significance of N nutrition for the activity and expression of root amino acid transporters in trees has not been elucidated.

Under N limitation, N availability for a particular tree will be determined by competition at the forest ecosystem level. The competitors include old growth trees, natural regeneration, other herbaceous and perennial understory species, as well as free-living microorganisms [12]. Tree-microbe competition for N occurs at several stages of the soil N cycle: (1) When plants take up monomeric organic compounds such as amino acids, they compete with the diverse microbes taking up dissolved organic nitrogen and performing ammonification and heterotrophic nitrification using organic N sources; (2) When plants take up ammonium, they reduce substrate availability for autotrophic nitrification and heterotrophic microbial ammonium immobilization; (3) Taking up nitrate, plants compete with microbial denitrification, microbial nitrate immobilization, and dissimilatory nitrate reduction to ammonium [12,23,27,83].

Little is known about root-to-root interactions in N acquisition between competitors for N in forests [43,84], and information on microbial-root interactions has only recently been established (e.g., [42,84,85]). In this context, microbial NO produced in significant amounts during nitrification and denitrification seems to be of particular significance [86]. Rhizospheric NO was shown to modulate the uptake of N compounds by tree roots [85,87,88]. Apparently, NO of microbial origin in the soil is sensed by the roots and functions as a signal that determines the competitive strength of N acquisition by the roots relative to microbial use. The mechanism of interaction of microbial NO with N uptake processes of the roots does not seem to include changes in the transcription of N transporters; activation of transporter proteins by phosphorylation/dephosphorylation has been suggested for NO stimulated N uptake by roots, as previously found for the interaction of NO produced inside plant cells with various cellular processes [89]. However, it remains unclear whether NO produced inside root cells can also interact with N uptake and if the action of extracellular and intracellular NO is mediated by similar processes [86].

5. Conclusions

The high N demand of temperate forest ecosystems on marginal soil relies almost exclusively on ecosystem internal sources. In this context, free-living soil microorganisms are responsible for both nutrient liberation and competition against trees and their associated mycorrhizal fungal symbionts. Internal ecosystem microbial N mineralization-immobilization turnover exceeds plant-mediated internal ecosystem N-cycling loops by approximately one order of magnitude. Thus, the soil microbial biomass represents a potential plant nutrient reservoir of considerable importance, and there is increasing evidence that temperate forest trees exert a direct influence on the availability of microbial-bound N for plant uptake. Chronic atmospheric N deposition relieves N limitation and microbial competition for N, resulting in mineral N accumulation in soil and ecosystem N losses along hydrological and gaseous pathways in the range of the N input rates. However, microbial N retention largely dominates over N loss and remains an important pathway of nutrient retention in N-saturated forest ecosystems. Reduced microbial competition for ammonium can result in trees taking up ammonium rather than nitrate.

While our understanding of the regulation of N uptake and distribution in trees has improved, a thorough biogeochemical quantification and understanding of soil microbial liberation, turnover and uptake of bioavailable N has thus far only been achieved for very few forest ecosystems and therefore deserves further attention in order to obtain a better understanding of the N cycling patterns in the plant-soil-microbe system of temperate forests under a changing climate.

Acknowledgments

Author Contributions

Heinz Rennenberg and Michael Dannenmann jointly analyzed the literature and data and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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