Effects of Nitrogen Forms on the Growth and Development of Trees

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Abstract Nitrate nitrogen and ammonium nitrogen are the main nitrogen forms absorbed by trees from soil, and they have significantly different physiological regulation effects on trees; trees can also absorb some soluble organic nitrogen compounds, such as urea and amino acids. Trees supplied with single ammonium nitrogen or nitrate nitrogen will have higher photosynthesis, and the promotion effect of mixed nitrogen sources on tree photosynthesis is stronger. Enzymes play an important role in the metabolism of trees. The key enzymes of nitrogen metabolism include nitrate reductase, *etc.*, which affect the metabolism of trees through different responses of key enzymes to various nitrogen forms. The input of different nitrogen forms changes the content of mineral elements in trees and then affects the growth of trees. Different nitrogen forms have significant differences in the growth and metabolic processes of trees, which in turn have different physiological effects on plants. Several key issues in the future research on nitrogen nutrition and physiology of trees are discussed.

Key words Nitrogen form, Growth, Biomass, Photosynthesis, Nitrogen metabolism

1 Introduction

As the most demanded mineral nutrient during plant growth and development, nitrogen can participate in protein, nucleic acid and cell wall composition and the synthesis of organic molecules such as enzymes, hormones, and vitamins, and is a basic element that participates in plant secondary metabolism and signal transduction substances[3]. It has an important physiological regulation function in the process of plant life activities. Plants require more nitrogen than other elements. The main function of nitrogen is to promote plant vegetative growth, improve photosynthetic capacity, and ensure its normal growth and development. At the same time, nitrogen is also the main substance that plays a catalytic role in the physiological metabolism of plants. Except that some plants fix the nitrogen in the atmosphere as a nitrogen source by nitrogen-fixing microorganisms, most plants absorb and use organic and inorganic nitrogen in the soil[1-2]. The main nitrogen forms absorbed by plants are nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N). They can also absorb some soluble organic nitrogen compounds, such as urea and amino acids. Different forms of nitrogen have significant differences in the effect on plant growth and physiological metabolic processes, which in turn have different physiological effects on plants. At present, the research on the effects of nitrogen on plants is mainly focused on crops, and there is very little research on trees. However, it has attracted attention in recent years and has made certain progress. Based on the different effects of nitrogen forms on different tree species, the effects of nitrogen and its forms on the growth and development of trees based on the retrieval of China National Knowledge Network (CNKI) database resources were analyzed to deeply understand and exert the potential physiological and ecological functions of trees, further enrich the theory of tree nutrition management and promote the sustainable development of forestry production.

2 Species of trees involved

At present, there are many studies on the effects of nitrogen forms on plants, but most of them are on the effects of crop growth, and there are few studies related to the effects on trees. In recent years, the effects of nitrogen forms on trees have attracted attention, and some progress has been made. In China, the effect of nitrogen forms on the growth of citrus roots was first studied in 1990, which is the beginning of the study on the effect of nitrogen forms on the growth and development of trees. Table 1 shows the changes in the number of Chinese documents on the effects of nitrogen forms on the growth and development of trees. A total of 58 papers have been published so far, of which the number was the largest in 2019, up to 10. The number of documents is currently increasing year by year. At present, the main tree species studied are tea trees, citrange and Pingyi sweet tea, and most of the research objects are economic tree species. Table 2 is a summary of the existing research on the effects of nitrogen forms on the growth and development of different trees. There are 16 tree species. The number of related documents on tea trees and tea-oil trees is the largest, and there are also documents on rare tree species distributed in the north such as ginkgo and red pine.

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Table 1 Changes in the number of Chinese documents on the effects of nitrogen forms on the growth and development of trees

Year	Number of Chinese documents	Proportion//%
1991 – 2000	0	0
2001 – 2005	0	0
2006 – 2008	7	12.1
2009 – 2012	8	13.8
2013 – 2015	9	15.5
2016 – 2018	16	27.6
2019 – 2021	18	31.0
Total	58	100.0

3 Effects on growth and biomass

Nitrate nitrogen and ammonium nitrogen are the main forms of nitrogen absorbed by plants. Plants have different responses to different forms of nitrogen nutrition in the growth environment. Different forms of nitrogen have more significant effects on various morphological indicators of plants. Sun Minhong's research on citrange shows that total nitrate-nitrogen cultivation is better than total ammonium-nitrogen cultivation, and citrange is a plant that likes nitrate nitrogen[3]. Ma Jian *et al.* found that loquat likes ammonium obviously, and the ammonium nitrogen absorbed by loquat roots is more than nitrate nitrogen[4].

Table 2 Summary of the existing research on the effects of nitrogen forms on the growth and development of different trees

No.	Latin name of plants	Genus and family	Quantity of documents	Published year
1	*Malus hupehensis* var. *mengshanensis* G. Z. Qian & W. H. Shao	*Malus*, Rosaceae	5	2007, 2008,2014, 2010
2	*Ginkgo biloba* L.	*Ginkgo*, Ginkgo family	3	2007, 2019
3	*Camptotheca acuminata* Decne.	*Camptotheca*, Nyssaceae	3	2008, 2019
4	*Camellia sinensis* (L.) O. Ktze.	*Camellia*, Camptotheca	9	2009, 2010, 2013, 2018, 2019, 2020
5	*Morus alba* L.	*Morus*, Moraceae	4	2010, 2015, 2017, 2010
6	*Juglans regia* L.	*Juglans*, Juglandaceae	3	2013, 2020, 2021
7	*Cunninghamia lanceolata* (Lamb.) Hook.	*Cunninghamia*, Taxodiaceae	4	2014, 2018, 2020
8	Citrange	*Poncirus*, Rutaceae	6	2015, 2016, 2017
9	*Camellia oleifera* Abel.	*Camellia*, Camptotheca	3	2016, 2019
10	*Larix gmelinii* (Rupr.) Kuzen.	*Larix*, Pinaceae	1	2017
11	*Populus* × *xiaohei* T. S. Hwang & Liang in Bull. Bot. Res.	*Populus*, Salicaceae	2	2018
12	*Hovenia acerba* Lindl.	*Hovenia*, Rhamnaceae	1	2018
13	*Betula platyphylla* Sukaczev	*Betula*, Betulaceae	1	2019
14	*Pinus koraiensis* Siebold & Zucc.	*Pinus*, Pinaceae	1	2019
15	*Cinnamomum bodinieri* Lévl.	*Cinnamomum*, Lauraceae	1	2019
16	*Quercus mongolica* Fisch. ex Ledeb.	*Quercus*, Fagaceae	1	2021

Seen from the mechanism of nitrogen absorption, ammonium nitrogen is more easily absorbed by plants, while absorbing nitrate nitrogen consumes more energy. Compared with a single nitrogen form, when nitrate nitrogen and ammonium nitrogen coexist, its utilization efficiency is higher. Many scholars believe that when ammonium nitrogen and nitrate nitrogen are mixed in a certain proportion, the effect is better than that of single nitrogen application. The application of nitrogen fertilizer at the seedling stage is one of the important measures for cultivating strong seedlings. The suitable nitrogen forms and proportions of different plants are different, and improper fertilization may cause the growth and development of seedlings to be inhibited[5].

Studies have shown that some trees grow best when the ratio of nitrate nitrogen to ammonium nitrogen in mixed nitrogen is 1. The test results of *Cinnamomum bodinieri* Lévl. show that when the ratio of NH_4^+-N to NO_3^--N is 5∶5, the growth of the stems, leaves and roots of the seedlings is the largest, and the root vigor is the highest; as the ratio of NH_4^+-N to NO_3^--N is 3∶7 and 7∶3, the growth of the stems, leaves and roots of the seedlings becomes smaller, and the root vigor becomes lower; under the conditions of no NH_4^+-N or no NO_3^--N at all, the seedlings have small leaves, thin and weak stems, few roots, low root vigor[6]. Different ratios of nitrogen forms obviously affect walnut seedlings' height, ground diameter, and biomass. When the ratio of NH_4^+-N to NO_3^--N is 50∶50, the plant height, ground diameter and biomass of walnut seedlings are the highest. The application of a single form of nitrogen is not conducive to the growth of walnut seedlings. In production, nitrate and ammonium nitrogen fertilizers should be mixed in an appropriate ratio to supplement the nitrogen of walnut seedlings[7]. It is found that different nitrogen forms have obvious promotion effects on the plant height, stem thickness and leaf number of citrange seedlings. The application of mixed nitrogen is more conducive to the growth of the above-ground part of the seedlings than the single form of nitrogen, and the effect is the best when the ratio of nitrate nitrogen to ammonium nitrogen is 5∶5, followed by 3∶7; for the single form of nitrogen, the total nitric acid culture is better than the total ammonium culture[8].

Studies have shown that the ratio of ammonium nitrogen to nitrate nitrogen in mixed nitrogen, 75∶25 or 25∶75, is most beneficial to the growth and development of some trees. The research on *Acacia mangium* × *A. au-riculiformis* Cunn. ex Bench found that the NH_4^+∶NO_3^- ratio of 75∶25 is most beneficial to the growth of seedlings[9]. With the increase of nitrate nitrogen proportion, the plant height, stem thickness, branch number and biomass of Hovenia acerba tend to rise. Among them, the branch number and whole plant biomass in treatment 25∶75 are significantly higher

than other treatments[10]. When the ratio of ammonium nitrogen to nitrate nitrogen is 75∶25, the growth status of *Rosa roxburghii* seedlings is the best, and the biomass is the largest; the content of various nutrients in the body is significantly increased. However, in the case of a single supply of nitrate nitrogen or ammonium nitrogen, it will inhibit the growth[11]. The study on male *Populus tomentosa* show that the ratio of ammonium nitrogen to nitrate nitrogen is 25∶75, the rhizome ratio increases significantly, which significantly promotes the thickness of leaves and the growth of fine roots[12].

Plants mainly absorb nitrate nitrogen and ammonium nitrogen, but can also absorb some soluble organic nitrogen compounds, such as urea and amino acids. Amide nitrogen is conducive to the growth of plant height, stems and leaves of *Betula platyphylla* seedlings, while nitrate nitrogen is conducive to root growth.

Since the roots of seedlings growing in a container do not be damaged during the emergence of seedlings, amide nitrogen fertilizer should be the first choice when adding nitrogen to *B. platyphylla* seedlings growing in a container[13]. In the case of single amino acid fertilization, arginine has the best effect on promoting the growth of seedlings, followed by glutamic acid, while glycine is the worst; in the case of combined fertilization of amino acids, the combination of arginine fertilization has good growth of seedlings, and the combination of glycine fertilization has poor growth of seedlings[14]. Different amino acid fertilization treatments have no obvious effect on the nitrogen content in various organs of *Populus nigra* seedlings. Guo Yafen *et al.* used a hydroponic experiment and set up different organic nitrogen fertilizers (glycine, glutamic acid, and lysine) to supply *Larix olgensis* and *Pinus koraiensis*. It is found that the growth of seedlings applied with glycine and glutamic acid grow was better than that with lysine[15-16].

4 Effects on photosynthesis

Photosynthesis is the basis of all material metabolism in nature, including a series of complex processes of photophysical, photochemical and biochemical transformations. Studies have shown that nitrogen forms can affect plant photosynthesis, and then affect plant growth and development. Chlorophyll is one of the basic elements of plant physiological metabolism and photosynthetic assimilation[17].

Different plants have a bias in the absorption of nitrogen forms. If they are supplied with single ammonium nitrogen or nitrate nitrogen, plants will have higher photosynthesis. Under the same nitrogen level, the chlorophyll content and net photosynthetic rate in the treatment of nitrate nitrogen are higher, which promotes the growth of young apple trees, and especially the indicators of young apple trees under the control concentration are the best or better[18]. The total chlorophyll content and net photosynthetic rate of *Camptotheca acuminata* in nitrate nitrogen treatment are higher than those in ammonium nitrogen treatment[19]. NO_3^--N has a great influence on the formation of chlorophyll b in citrange, and affects the total chlorophyll content by influencing the formation of chlorophyll b. *Fraxinus mandshurica* Rupr. has a preference for NO_3^--N. When NH_4^+-N and NO_3^--N are mixed and applied, the net photosynthetic rate of leaves increases as the ratio of NO_3^--N rises, and the biomass and other indicators also increase significantly[20].

Most studies show that mixed nitrogen sources have a stronger promotion effect on plant photosynthesis. Li Haixia *et al.* found that compared with nitrate nitrogen, ammonium nitrogen has a more significant promotion effect on the photosynthesis of *Quercus mongolica* seedlings, and the mixture of equal amounts of nitrate nitrogen and ammonium nitrogen is more conducive to the overall photosynthetic performance of *Q. mongolica* seedlings[21]. Chen Ming *et al.* proposed that the photosynthesis ability of *Camellia oleifera* seedlings in the treatment of ammonium and nitrate nitrogen is the strongest[22]. Han Haozhang *et al.* suggested that the chlorophyll content in the stems, leaves and roots of *Cinnamomun bodinieri* seedlings is the highest when the ratio of NH_4^+-N to NO_3^--N is 5∶5, and becomes lower as the ratio is 3∶7 or 7∶3[6]. Different ratios of nitrogen forms obviously affect the chlorophyll content of walnut seedling leaves. The chlorophyll content in the leaves of walnut seedling is the highest when the ratio of NH_4^+-N to NO_3^--N is 50∶50, and the growth indicators of walnut seedlings in the single nitrogen form treatments (NH_4^+-N/NO_3^--N) are lower than in the treatments with two forms of nitrogen[7]. Chen Yongliang *et al.* found that the order of the effects of different nitrogen forms on the net photosynthetic rate of *Pinus koraiensis* seedlings is as follows from large to small: the mixture of equal amounts of NO_3^--N and NH_4^+-N, single supply of NH_4^+-N, and single supply of NO_3^--N[23]. Tian Yanan *et al.* found that when the ratio of NH_4^+-N to NO_3^--N is 1∶3, the chlorophyll content in the leaves of *Pyrus betulaefolia* seedlings is higher than that in the treatment with the ratio of 3∶1[24].

5 Effects on nitrogen metabolism and mineral nutrition

The process of nitrogen assimilation is particularly important for plant growth and development. Inorganic nitrogen must be assimilated into organic nitrogen such as glutamate and glutamine before it can be further utilized by plants. Enzymes play an important role in the metabolism of plants. The key enzymes for nitrogen metabolism include nitrate reductase (NR), glutamate synthase (GOGAT) and glutamine synthetase (GS), *etc.*, and have always been the starting point for scholars to study plant nitrogen metabolism. Xu *et al.* found that the activity of GOGAT in plant leaves is closely related to the activity of NR and GS, indicating that in the metabolism of nitrate nitrogen, the activities of GOGAT, GS and NR are synergistic; the activity of GS and NR is related to the amount of substrate that GOGAT acts on, which in turn also affects the activity of GOGAT; NR is the first enzyme to assimilate nitrate nitrogen, both an inducible enzyme and a rate-limiting enzyme[25]. Sun Minhong *et al.* proved that the NR activity of the leaves and roots of *Citrus sinensis* × *Poncirus trifoliate* seedlings increases with the extension of culture time, and the NR activity of the leaves is higher than that of the roots[8]. Wang Rui

et al. found that the activity of GS and NR of *Camellia oleifera* seedlings reaches the maximum when *m* (NH_4^+-N): *m* (NO_3^--N) is 5∶5, and the activity of GOGAT is up to the maximum as *m* (NH_4^+-N): *m* (NO_3^--N) is 10∶0; the correlation analysis results also show that there is no significant correlation between GOGAT activity and GS and NR activity, because GOGAT activity requires GS and NR to provide substrates as well as NADH coenzyme[26]. Hu Guoce *et al.* proposed that the gene expression of GS and GOGAT in tea leaves is highest when the ratio of ammonium nitrogen to nitrate nitrogen is 2∶2, and the gene expression of GS and GOGAT is higher when the ratio of ammonium nitrogen to nitrate nitrogen was 3∶1[27].

Due to the input of nitrate nitrogen or ammonium nitrogen, there are big differences in the content of mineral elements in plants. When the proportion of ammonium nitrogen in the nutrient solution is 50% and 70%, the content of N, P, Ca, Fe, Zn, and Cu in the leaves of *Rosa roxburghii* significantly increases, while the content of B and Mo reduces as the proportion of ammonium nitrogen increases[11]. With the continuous increase of ammonium nitrogen concentration, the content of elements such as Ca and Mg in citrus leaves decreases significantly, while the content of P and Fe increases significantly. The change of mineral element content has a significant impact on the quality and yield of citrus[28]. The absorption test results of N, P, and K nutrients in *Larix gmelinii* seedlings showed that amino acid nitrogen can effectively increase the mass fraction of N, P, and K nutrients in the seedlings; ammonium sulfate inhibits the absorption of K, and calcium nitrate inhibits the absorption of P[29]. Zhang Jian *et al.* used DKW as the basic medium formula to adjust the ratio of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in macronutrients. Macronutrients K and Mg and trace elements Mn, Cu, B may play an important role in the rooting of *Juglans sigillata* seedlings[30].

6 Outlook

Nitrate nitrogen and ammonium nitrogen are the main nitrogen forms absorbed by trees from soil, and they have significantly different physiological regulation effects on trees; trees can also absorb some soluble organic nitrogen compounds, such as urea and amino acids. Trees supplied with single ammonium nitrogen or nitrate nitrogen will have higher photosynthesis, and the promotion effect of mixed nitrogen sources on tree photosynthesis is stronger. Enzymes play an important role in the metabolism of trees. The key enzymes of nitrogen metabolism include nitrate reductase, *etc.*, which affect the metabolism of trees through different responses of key enzymes to various nitrogen forms. The input of different nitrogen forms changes the content of mineral elements in trees and then affects the growth of trees. The current research on the effects of nitrogen on plants is mainly focused on crops, and there are few related studies on trees, which has attracted attention and has made some progress in recent years. However, the research is not deep enough, and is still concentrated on analysis of the effects of nitrogen forms on trees by conventional physiological methods. The molecular mechanisms of nutrient absorption and transport are less studied, and the understanding of the function of nitrogen transporters is limited. Although many reports have confirmed that the mixed application of ammonium nitrogen and nitrate nitrogen has a positive effect on trees, the coupling mechanism of two forms of nitrogen and the mechanism of how to promote tree growth are still unclear. The characteristics and action rules of two different forms of nitrogen should be deeply analyzed to further clarify their regulation mechanism on trees; the changes in tree-related gene expression caused by different forms of nitrogen should be further clarified, and the gene function should be further analyzed to understand the mechanism of the effects of nitrogen forms on trees; combined with modern omics technology, the molecular basis of nitrogen affecting the growth, development and resistance of trees is analyzed at the protein and metabolism levels, and the physiological and ecological effects of nitrogen are analyzed in depth.

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