J Ginseng Res 44 (2020) 627–636

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research Article

Appropriate nitrogen application enhances saponin synthesis and growth mediated by optimizing root nutrient uptake ability

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ARTICLE INFO

Article history:
Received 9 October 2018
Received in Revised form
5 April 2019
Accepted 11 April 2019
Available online 18 April 2019

Keywords:
growth dynamics
maximum growth rate
nitrogen
root architecture
saponin synthesis

ABSTRACT

Background: Cultivation of medicinal crops, which synthesize hundreds of substances for curative functions, was focused on the synthesis of secondary metabolites rather than biomass accumulation. Nutrition is an important restrict factor for plant growth and secondary metabolites, but little attention has been given to the plasticity of nutrient uptake and secondary metabolites synthesis response to soil nitrogen (N) change.
Methods: Two year-field experiments of Sanqi (*Panax notoginseng*), which can synthesize a high level of saponin in cells, were conducted to study the effects of N application on the temporal dynamics of biomass, nutrient absorption, root architecture and the relationships between these parameters and saponin synthesis.
Results: Increasing N fertilizer rates could improve the dry matter yields and nutrient absorption ability through increasing the maximum daily growth (or nutrient uptake) rate. Under suitable N level (225 kg/ha N), Sanqi restricted the root length and surface and enhanced the root diameter and N uptake rate per root length (NURI) to promote nutrient absorption, but the opposite status of Sanqi root architecture and NURI was found when soil N was deficient. Furthermore, increasing N rates could promote the accumulation of saponin in roots through improving the NURI, which showed a significant positive relationship with the content of saponin in the taproots.
Conclusion: Appropriate N fertilizer rates could optimize both root architecture and nutrient uptake efficiency, then promote both the accumulation of dry matter and the synthesis of saponins.

1. Introduction

Sanqi [*Panax notoginseng* (Burk.) F. H. Chen], a member of the Araliaceae family, is widely used as a medicinal herb [1,2]. Triterpenoid saponins (ginsenosides) are the primary bioactive secondary metabolites present in Sanqi plants [3]. More than 20 different ginsenosides have been identified in Sanqi [1,4]. Owing to their significant effects against cancer and cardiovascular disease [4,5], ginsenosides are widely used in pharmaceuticals, for industrial applications, for cosmetics, for agriculture, and for food markets. Because the need for ginsenosides has increased, more than 0.5 million hectares of Sanqi are being sown annually in China. Obtaining high biomass yields and ginsenoside contents is important in Sanqi plantations.

Nitrogen (N) is one of the main nutrients required for plant growth and is essential for the synthesis of amino acids, proteins and enzymes, and so on. [6]. N nutrient availability is dynamic and changes during the course of plant growth [7,8]. However, most relevant studies have focused only on the effects of N application on Sanqi biomass at the end of growth [9–11]. Studying plant dynamic

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https://doi.org/10.1016/j.jgr.2019.04.003
p1226-8453 e2093-4947/$ – see front matter

growth can provide a better picture of how soil N influences plant growth and nutrient uptake throughout the course of the growing season [12] and can improve our understanding of biomass and nutrient accumulation. By explicitly modeling Sanqi biomass and nutrient uptake via parameters that are biologically interpretable, we can understand the dynamic effects of N fertilizer applications on Sanqi growth.

In addition to plant growth, secondary metabolites are also affected by soil nutrient availability. Previous studies have shown that suitable nutrient supplies are required for normal accumulation of plant secondary metabolites [13]. For example, the synthesis of betaine, carotenoids, and flavone in *Lycium barbarum* is significantly correlated with N fertilizer levels, and the proper amount of N application (600-900 kg hm^{-2}) is beneficial to the formation and accumulation of those secondary metabolites [14]. In addition, soil nutrients can also alter the variety of secondary metabolites. Under N-deficient conditions, non-N secondary metabolites such as terpenoids and phenols can accumulate, whereas nitrogenous secondary metabolites such as alkaloids and cyanogenic glycosides can accumulate under N-sufficient conditions [15]. Soil fertility was shown to affect both the quantitative amount and qualitative composition of saponins in *Bupleurum chinense* [16]. All these findings resulted from the plasticity of plant responses to soil N conditions. Ensuring the appropriate N application to establish the optimal relationship between yield and saponins is important to Sanqi production.

Root architecture and physiology are the basis of nutrient and water absorption of plants and play an important role in the effects of soil nutrients on plant secondary metabolites [17,18]. Many studies have shown that increases in root length density (RLD) result in increased root surface area, which provides more exposed area for nutrient uptake [19]. Moreover, the RLD distribution is sensitive to changes in the supply and distribution of inorganic nutrients in the soil [20]. For example, when the soil N supply was adequate, intercropped wheat presented a greater RLD and occupied a larger soil volume to capture nutrients; when the soil N was either deficient or abundant, intercropped wheat roots exhibited a reduced extent of both root distribution and RLD to maximize aboveground growth [6]. In addition, root physiology, which is more carbon efficient than root architecture, can greatly contribute to the nutrient absorption ability of species [21]. All of these findings were due to the phenotypic plasticity of plant root responses to varying soil N [22]. Phenotypic plasticity is the property of a given genotype that produces different physiological or morphological phenotypes in response to different environmental conditions [23]. This ability can help plants adapt to various soil conditions, increase nutrient uptake, and alter secondary metabolite production. Therefore, analyzing the relationship between root growth and soil nutrients, especially in response to N fertilizer application, is important for understanding the mechanism underlying the effects of N on Sanqi growth, nutrient uptake, and saponin synthesis.

In the present study, we aim to (1) identify the suitable N fertilizer rates that resulted in the optimal growth, nutrient uptake, and saponin content of Sanqi plants; (2) address how N fertilizer affects biomass and nutrient uptake dynamics; and (3) explore the changes in root morphology and physiology in response to varying N fertilizer applications and their relationship with saponin synthesis.

2. Materials and methods

2.1. Study site and experimental design

Field work was carried out at the experimental station of Yunnan Agricultural University, Xundian County, Yunnan, China (103.13°E, 25.67°N; altitude of 1880 m), in 2015 and 2016. Sanqi plants were grown in soil supplemented with one of the following N contents in 2015: 0, 56, 113, 225, and 450 kg N/ha (i.e., N0, N56, N113, N225, and N450, respectively). In 2016, we optimized the N fertilizer rates based on the results from 2015: 0, 56, 113, and 225 kg N/ha (i.e., N0, N56, N113, and N225, respectively). The experiment was conducted as a randomized complete block design with three replications (blocks) per treatment. The area of each plot was 1.5 m × 8 m = 12 m^2. The field soil consisted of soil, perlite, and sand (3:1:1) [2015/2016: pH: 7.65/7.75; electrical conductivity: 580/520 μS/cm; soil bulk density: 0.59/0.58 g cm^{-3}; soil specific gravity: 2.33/2.25 g cm^{-3}; soil porosity: 75%/73%; and nutrient contents: 118.53/110.25 mg/kg available N, 295.10/280.80 mg/kg available phosphorus (P), and 664.36/631.45 mg/kg available potassium (K), respectively]. Healthy seeds were surface sterilized with 1% sodium hypochlorite for 5 min, washed three times with sterilized water, and sown in each plot (5 cm × 5 cm). The planting density of Sanqi was 400 plants m^{-2}. The seeds were sown on 7 January and emerged on 10 March. Then, the greenhouse was shaded with a polyethylene net that allowed 10% light transmission to imitate the natural conditions for Sanqi growth. When the seed germination reached 90% on 15 April, fertilizer was periodically applied between April and August. The N, P (P_2O_5), and K (K_2O) fertilizers were supplied as urea (46.4%), calcium superphosphate (16%), and potassium sulfate (50%), respectively, to each plot. The rates of P_2O_5 (180 kg/ha) and K_2O (270 kg/ha) were the same in all treatments. Every month, each experimental plot received 20% of the total fertilizer.

2.2. Sample collection and determination

The Sanqi plants were sampled approximately at 3-week intervals after seedling emergence. The plants were sampled on 7 May, 6 June, 6 July, 25 July, 13 August, 3 September, 16 September, 2 October, 17 October, 1 November, and 16 November. All plants within a 0.5 m long × 1.5 m wide area in each plot were sampled at each sampling time. The roots were carefully washed and air dried, after which the aboveground shoots and belowground roots were separated. We subsequently separated the belowground roots into taproot and fibrous root parts. All samples were then divided into four different groups. One group was for determining plant biomass and nutrient uptake, and the dry weight was measured after the plant samples had dried. Another group was for determining nutrient absorption, as described below. The third group was also used to analyze the roots, as described below, and the fourth group was for determining the saponin content in the Sanqi roots.

The fibrous root fractions were scanned using an Epson Perfection V850 Pro scanner (Epson Inc., Beijing, China), and the total root length, root diameter, root surface area, and root volume of the root samples in each N treatment were analyzed with WinRHIZO™ software (Régent Instrument Inc., Québec, Canada). The N uptake rate per root length (NURI) at three sampling times was calculated using the following formula [24]:

$$\mathrm{NURI} = \frac{(\mathrm{N2} - \mathrm{N1})}{(\mathrm{R2} + \mathrm{R1})/2}$$

where N1 and N2 represent the N acquisition of plants at the start and end of the sampling interval, respectively, and R1 and R2 represent the total root length of plants in the start and end of the sampling interval, respectively. The aboveground shoot and the belowground root of oven-dried samples were digested in a mixture with H_2SO_4 and H_2O_2 [25], and N, P, and K concentrations in the plant dry matter of the aboveground shoot and the belowground root were measured by micro-Kjeldahl procedure,

Table 1
Effect of Nitrogen (N) rates on the biomass of Sanqi (*Panax notoginseng*)

Years	Treatments	Unit area (kg/ha)				Unit plant (mg/plant)			
		Aboveground dried biomass	Taproot dried biomass	Fibrous dried biomass	Root shoot ratio, R/S	Aboveground dried biomass	Taproot dried biomass	Fibrous dried biomass	Root shoot ratio, R/S
		(kg/ha)	(kg/ha)	(kg/ha)		(mg/Plant)	(mg/Plant)	(mg/Plant)	
2015	N0	216.43 ± 16.97b	758.68 ± 58.9b	65.43 ± 4.78ab	3.81 ± 0.1a	65.72 ± 6.57b	230.29 ± 22.25b	19.79 ± 1.35ab	3.81 ± 0.1a
	N56	142.63 ± 19.54c	512.93 ± 50.25c	41.77 ± 2.27c	3.94 ± 0.2a	41.29 ± 5.13b	148.68 ± 12.37c	12.13 ± 0.53c	3.94 ± 0.2a
	N113	270.06 ± 7.96b	892.4 ± 31.51b	77.76 ± 4.31a	3.6 ± 0.21a	78.23 ± 2.69b	258.47 ± 9.61b	22.54 ± 1.42a	3.6 ± 0.21a
	N225	444.15 ± 25.29a	1077.88 ± 21.24a	68.77 ± 4.24ab	3.42 ± 0.23a	103.05 ± 7.51a	332.79 ± 6.83a	15.92 ± 0.94bc	3.42 ± 0.23a
	N450	242.81 ± 26.18b	841.01 ± 93.18b	57.04 ± 5.29b	3.74 ± 0.44a	80.56 ± 15.07b	275.83 ± 43.22b	18.84 ± 3.15ab	3.74 ± 0.44a
2016	N0	215.56 ± 33.29a	332 ± 57.84a	46.33 ± 9.79a	1.74 ± 0.05a	69.74 ± 4.18a	107.32 ± 9.55b	14.8 ± 1.52a	1.74 ± 0.05a
	N56	185.11 ± 6.26a	271.78 ± 15.89a	34.11 ± 4.04a	1.65 ± 0.05a	70.22 ± 3.89a	102.65 ± 3.72b	12.93 ± 1.59a	1.65 ± 0.05a
	N113	178.89 ± 16.96a	291.67 ± 55.99a	38.22 ± 6.29a	1.81 ± 0.2a	74.93 ± 2.4a	120.64 ± 16.55ab	15.9 ± 1.76a	1.81 ± 0.2a
	N225	187.89 ± 12.67a	343.45 ± 39.16a	38 ± 3.38a	2.02 ± 0.12a	74.7 ± 1.6a	136.11 ± 10.96a	15.09 ± 0.77a	2.02 ± 0.12a

Mean ± standard errors calculated from three biological replicates are shown. Different letters indicate significant differences among the five treatments by ANOVA, Duncan's multiple range tests at $P < 0.05$.
ANOVA, analysis of variance.

vanadomolybdate method, and flame photometry, respectively. Nutrient uptake was calculated as the product between aboveground and belowground biomass and its nutrient concentration. Nutrient uptake was used for logistic growth function fitting mass and its nutrient concentration.

2.3. Logistical model(growth model and the nutrient model)

The values of eleven biomass samples and nine N, P, and K uptake data samples in response to different N fertilizer rate treatments from the seed stage until plant death or harvest were all fitted to logistic models using ordinary least squares via the following equations [7,12].

$$\mathrm{Mt} = \frac{\mathrm{K}}{1 + \exp\left(r*(t\mathit{Imax} - t)\right)} \tag{1}$$

$$\mathrm{NUt} = \frac{\mathrm{NUmax}}{1 + \exp\left(r*(t\mathit{Imax} - t)\right)} \tag{2}$$

The growth curve used Eq. (1), where Mt (mg/plant) is the aboveground and belowground dry matter weight per unit plant of Sanqi seedling grown under different N rate treatments at t days after seeding throughout the course of the growing season, K (mg/plant) represents the maximum biomass, r (day^{-1}) is the initial per capita growth rate (dMt/dt × 1/Mt), and t_{Imax} (day) is the time of the maximum instantaneous growth rate. The nutrient uptake curve used Eq. (2), where NUt (mg/plant) (kg/ha) is the nutrient (N, P, or K) uptake per unit ground area of plant aboveground and belowground biomass at t days after Sanqi seedling emergence. The nutrient uptake was based on the area actually occupied by the aboveground and belowground plant parts. NUmax (mg/plant) represents the asymptotic maximum cumulative nutrient uptake per unit plant of the aboveground and the belowground plant parts, r (day^{-1}) is the relative nutrient uptake rate (dNUt/dt × 1/NUt), and t_{Imax} (day) is the time needed to achieve the maximum daily nutrient uptake rate. All these parameters were estimated using the Slogistic1 procedure of OriginPro 8 software (OriginLab Corporation, Northampton, MA, USA).

The instantaneous growth rate (mg/plant/day) and the daily nutrient uptake rate (μg/plant/day) can be derived by Eqs. (3) and (4), respectively, as follows:

$$\frac{\mathrm{dMt}}{\mathrm{dt}} = \mathrm{rMt}\left(1 - \frac{\mathrm{Mt}}{\mathrm{K}}\right) \tag{3}$$

$$\frac{\mathrm{dNU}t}{\mathrm{d}t} = r\mathrm{NU}t\left(1 - \frac{\mathrm{NU}t}{\mathrm{NUmax}}\right) \tag{4}$$

The instantaneous growth rate peaks at Mt=K/2; therefore, the maximum instantaneous growth rate, $\mathit{Imax} = r\mathrm{K}/4$, occurs at time t_{Imax}. The maximum daily nutrient uptake rate occurs at NUt = NUmax/2. As a result, the maximum daily nutrient uptake rate is as follows: $\mathit{Imax} = r\mathrm{NUmax}/4$, which occurs at time t_{Imax}.

2.4. Analysis of saponins content

To identify the effects of different N fertilizer rates on the accumulation of saponins as the Sanqi plants grew, nine individual types of saponins, including R1, Rg1, Re, Rf, Rb1, Rg2, Rh1, Rd, and Rg3, within the taproots and fibrous roots were qualified by Ultra Performance Liquid Chromatography (UPLC) in accordance with the method of [2]. 1 g root powder from dried Sanqi taproot or fibrous root tissue was transferred to a 15 mL tube and ultrasonically extracted by 4 mL methanol (MeOH):H_20 (80:20) at 40°C for 40 min. The tube was then centrifuged for 5 min (12,000 g, 4°C). The extraction procedure was performed three times. The solutions from the three extractions were combined and evaporated to dryness under a vacuum at 40°C. The dry residue was then redissolved in 100% MeOH to a concentration of 0.1 g/mL and passed through a 0.22 μm nylon membrane filter (JTSF0311, Tianjin Jinteng Experiment Equipment Co., Ltd.). The filtered solution was stored at 4°C until further determination.

A Shimadzu Nexera X2 UHPLC system (Shimadzu, Japan) equipped with an LC-30AD × 2 UPLC pump (Shimadzu, Japan), an SPD-M20A diode array detector (Shimadzu, Japan), and an SIL-30AC autosampler (Shimadzu, Japan) was used to perform the UPLC analysis and to determine the content of the nine individual types of saponins and the crude ginseng saponin. The UPLC separations were performed using a Shimadzu C18 Shim-pack XR-ODS column (2.0 mm i.d. × 75 mm, 1.6 μm). A multistep gradient with an initial injection volume of 10 μl and a flow rate of 0.4 mL/min was used for all separations. The solvent system consisted of the following linear gradient of solvent A [acetonitrile (MeCN)] and solvent B (0.1% phosphoric acid in water): from 1% to 18% A for 0.0 to 16.0 min, from 18% to 33% A for 16.0 to 30.0 min, from 33% to 38% for 30.0 to 32.0 min, from 38% to 50% for 32.0 to 34.0 min, holding at 50% for 6 min, and from 55% to 18% for 34.0 to 45.0 min. The column temperature was maintained at 30°C, and the chromatograms were recorded at 203 nm. MeOH and MeCN (HPLC grade) were purchased from MeckChina Co., Inc. (Germany). Ultrapure water was

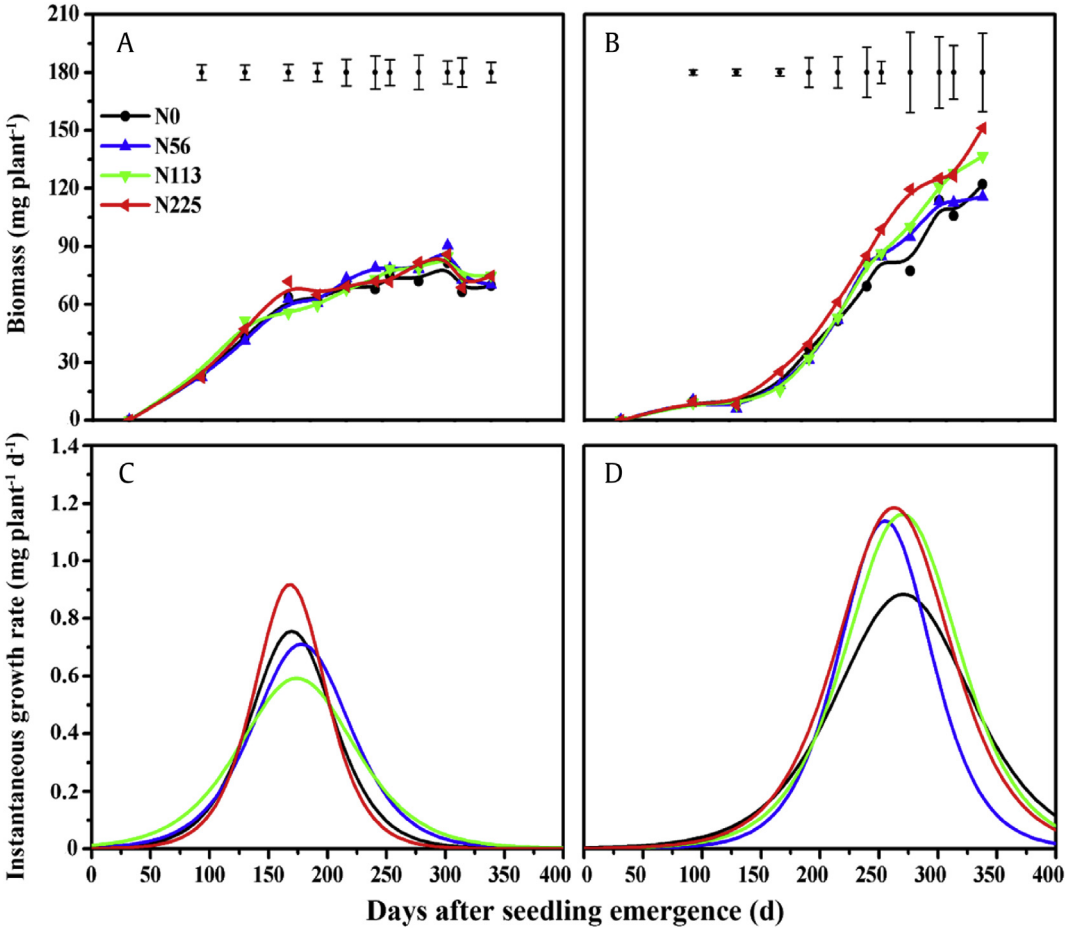

Fig. 1. Biomass growth of sanqi (*Panax notoginseng*) per plant in soil treated with different rates of nitrogen (N). (A) and (B) are the aboveground biomass growth and instantaneous growth rates, respectively. (C) and (D) are the belowground biomass growth and instantaneous growth rates, respectively. Each symbol is the mean of three replicates. N0, N56, N113, and N225 represent 0, 56, 113, and 225 kg N/ha.

generated with a Milli-Q ultrapure water system (MeckChina Co., Inc., Germany). The nine standard types of individual ginsenosides (R1, Rg1, Re, Rf, Rb1, Rg2, Rh1, Rd, and Rg3; purity≥98%; Guizhou Dida Biological Technology Co.) were each dissolved in a small volume of MeOH and then diluted in distilled water to a concentration of 1.0 mg/L. Distilled water containing the same concentration of MeOH (1.0%) was used as a control. The ginsenosides in the samples were identified by comparing the results to those of authentic ginsenoside standards. The concentration of ginsenosides in the samples was quantified using standard curves that showed the linear relationships between the peak areas and the concentrations.

2.5. Statistical analysis

SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for general statistical analyses. The effects of N applications at different rates on the four parameters (r, NU_{max}, t_{Imax}, and I_{max}) related to plant nutrient uptake were analyzed. To identify significant differences between treatments, mean separations among the treatments were analyzed by one-way analysis of variance and Duncan's multiple range test ($P < 0.05$). We used linear regression to analyze the relationships between the saponin contents of the taproot and fibrous roots and the morphology of the roots and the NURI, respectively. The goodness of fit of all the regression equations was determined by P-values and the coefficients of determination (R^2).

3. Results

3.1. N rates affect the dynamic growth of Sanqi shoot and root

Two years (2015 and 2016) of field data showed that the N fertilizer rates affected the growth of Sanqi. In terms of per unit area or per plant, the N_{225} treatment yielded the greatest shoot and taproot biomass in 2015 (Table 1). Similar results were observed concerning the effects of N fertilizer rates on taproot biomass per plant in 2016, but the shoot biomass did not significantly differ between different N applications (Table 1). Increased N fertilizer rates also increased the root:shoot ratio of Sanqi in 2016 but had no effect in 2015 (Table 1). Moreover, N application showed no significant fibrous root biomass or the root:shoot ratio (Table 1).

Sanqi growth fit the logistic function well (aboveground: $0.945 \leq$ adjusted $R^2 \leq 0.961$, $P < 0.05$; belowground: $0.971 \leq$ adjusted $R^2 \leq 0.994$, $P < 0.05$). The initial stage [100-320 days after sowing (DAS)] of shoot growth was close to exponential, after which the growth stopped after 320 DAS; in contrast, the root growth was slow during the initial stage (100-180 DAS) but was exponential after 180 DAS (Fig. 1A and B). Increased N application significantly increased the initial growth rate (r) and the maximum growth rate (I_{max}) of Sanqi shoot biomass per plant (maximum values were achieved in the N_{225} treatment) but did not affect the time of maximum growth rate (t_{Imax}) or maximum biomass (K) (Fig. 1; Table S1). Moreover, the roots of each Sanqi plant produced greater maximum biomass as the N application

increased, but there were no significant effects on the other parameters (Fig. 1C and D; Table S1). The Sanqi plants achieved the maximum shoot and root growth at 170 days and 265 days, respectively (Table S1).

3.2. N rates affect the dynamic nutrient uptake of Sanqi shoot and root

The N, P, and K uptake in the shoots and roots per Sanqi plant under different N fertilizer rates fit the logistic model (aboveground N: mean adjusted $R^2 = 0.812$, $0.730 \leq$ adjusted $R^2 \leq 0.890$, $P < 0.05$; belowground N: mean adjusted $R^2 = 0.929$, $0.886 \leq$ adjusted $R^2 \leq 0.966$, $P < 0.05$; aboveground P: mean adjusted $R^2 = 0.743$, $0.623 \leq$ adjusted $R^2 \leq 0.813$, $P < 0.05$; belowground P: mean adjusted $R^2 = 0.937$, $0.918 \leq$ adjusted $R^2 \leq 0.953$, $P < 0.05$; aboveground K: mean adjusted $R^2 = 0.795$, $0.764 \leq$ adjusted R^2 0.860, $P < 0.05$; belowground K: mean adjusted $R^2 = 0.940$, $0.912 \leq$ adjusted $R^2 \leq 0.970$, $P < 0.05$) (Fig. 2, Fig. 3).

The N and P uptake by Sanqi shoots increased exponentially from 0-210 DAS and then slowed or stopped, but the K uptake continued to increase until 310 DAS (Fig. 2A, C, E). The N, P, and K uptake in the roots per Sanqi plant continued to increase from emergence until the end of the season, after which the accumulation increased rapidly after 210 DAS (Fig. 2B, D, F). The dynamic accumulation of nutrient uptake per area of Sanqi plant shoot and root also exhibited a similar tendency (Fig. S1).

N application rate affected the nutrient uptake (N, P, and K) per Sanqi plant root (Fig. 2). The maximum N, P, and K uptake ($NU_{max\text{-}N}$, $NU_{max\text{-}P}$, and $NU_{max\text{-}K}$, respectively) and the corresponding maximum daily nutrient rates (*Imax–N*, *Imax–P*, and *Imax–K*, respectively) of the Sanqi roots significantly increased as the N fertilizer rate increased (Fig. 3B, D, F; Table S2). There was no difference in r or t_{Imax} of the Sanqi roots in response to different N applications (Fig. 3B, D, F; Table S2). Moreover, increased N application significantly increased only the $NU_{max\text{-}N}$ per plant but had no effect on the $NU_{max\text{-}P}$ or $NU_{max\text{-}K}$ of the Sanqi shoots (Fig. 3A, C, E; Table S2).

3.3. Response of root system architecture to the N fertilizer rate

The N fertilizer rate significantly affected the root architecture throughout the entire tested growth period of the Sanqi plants. On 6 July, there were no significant differences in root length, surface area, volume, or diameter per plant between any N fertilizer rates (Fig. 4; Fig. S2). On 16 September and 16 November, all root architecture parameters (root length, surface area, diameter, and

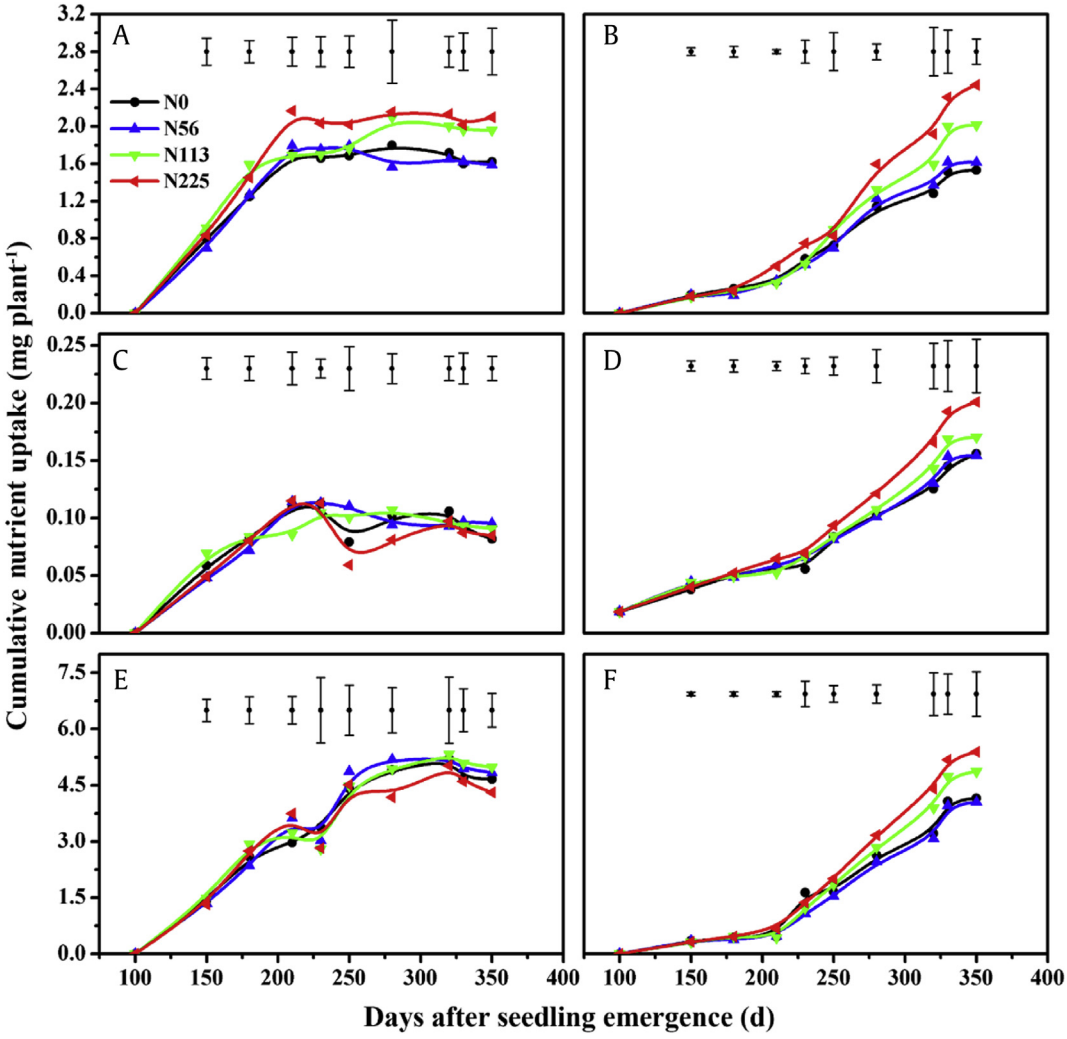

Fig. 2. Trajectories of cumulative N, P, and K uptake by Sanqi (*Panax notoginseng*) per plant in soil treated with different rates of nitrogen (N). Aboveground trajectories of cumulative N (A), P (C), and K (E) uptake. Belowground trajectories of cumulative N (B), P (D), and K (F) uptake. Each symbol is the mean of three replicates. N0, N56, N113, and N225 represent 0, 56, 113, and 225 kg N/ha.

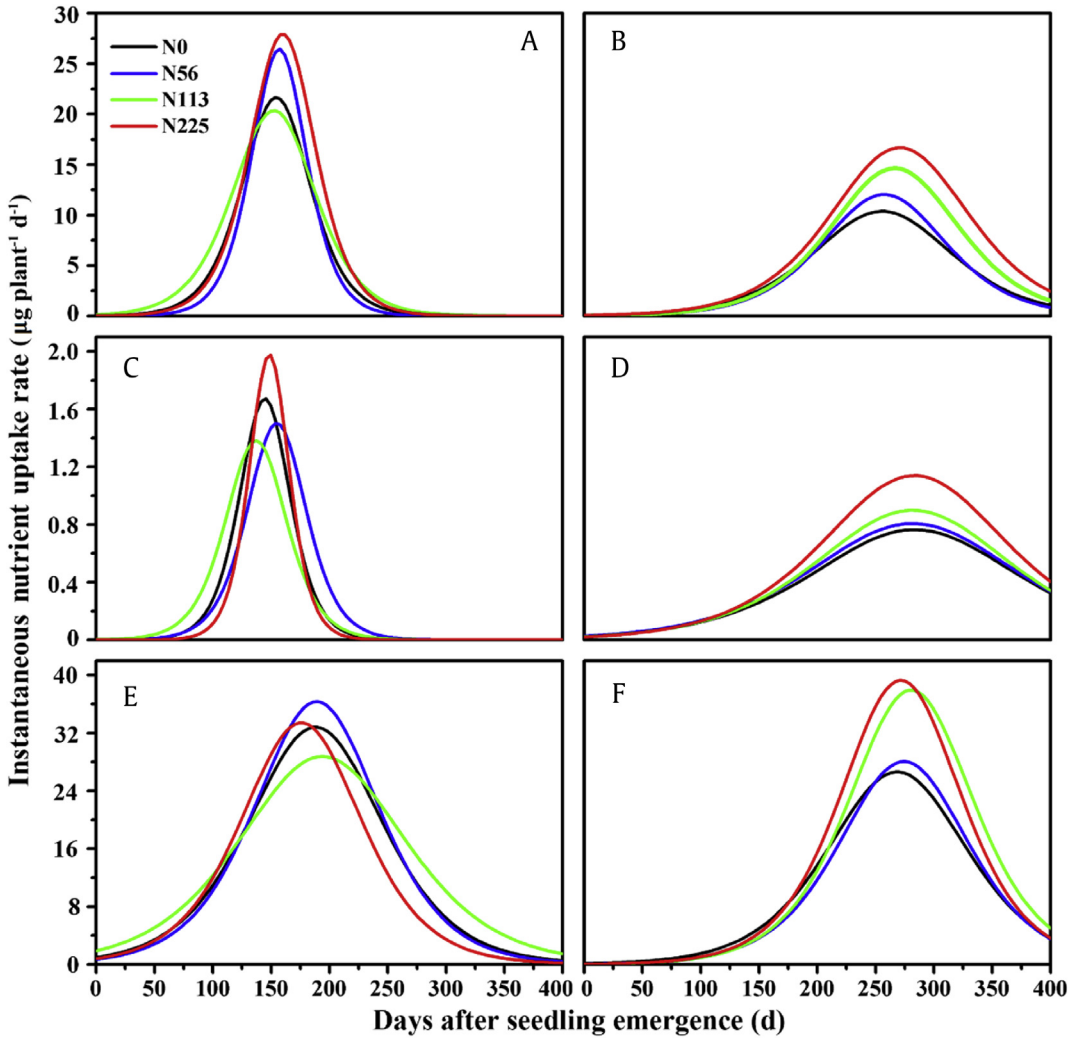

Fig. 3. Daily N, P, and K uptake by Sanqi (*Panax notoginseng*) per plant in soil treated with different rates of nitrogen (N). Aboveground daily N (A), P (C), and K (E) uptake. Belowground daily N (B), P (D), and K (F) uptake. Each symbol is the mean of three replicates. N0, N56, N113, and N225 represent 0, 56, 113, and 225 kg N/ha.

volume) were greater than those on 6 July. Increased N fertilizer rates significantly reduced the root length and increased the root diameter per Sanqi plant, but there were no significant differences in root surface area or volume per plant among different N fertilizer rates (Fig. 4). In addition, the root surface area and volume per area significantly decreased as the N fertilizer rate increased on 16 November, which differed from the results on a per-plant basis (Fig. S2).

During 0-120 DAS, the NURI in the N_{225} treatment was significantly larger—by 31.5–42.1%—than that in the other N fertilizer treatments (N_0, N_{56}, and N_{113}), regardless of whether the value was on a per-unit-area or per-plant basis (Table 2). During 121-189 DAS, the NURI exhibited similar trends as exhibited during 0-120 DAS: increased N fertilizer rates significantly promoted the NURI per unit area and per plant (Table 2). During 190-310 DAS, there was no significant difference in the NURI among the different N fertilizer rates (Table 2).

3.4. N fertilizer rates regulate saponin accumulation in Sanqi roots

To investigate the effects of N fertilizer rates on ginsenoside accumulation, the contents of the total ginsenosides and nine active ginsenosides in the Sanqi fibrous roots and taproots under five N fertilizer rates were tested. The data showed that increased N fertilizer rates could significantly increase the content of ginsenosides in both the taproots and fibrous roots (Fig. 5A). The content of total ginsenosides under N-sufficient conditions (N_{113} and N_{225}) was significantly higher than that under N-deficient condition (N_0 and N_{56}) (Fig. 5A). In addition, the ratio of ginsenosides in the taproot and fibrous roots also increased as the N fertilizer rate increased (Fig. 5B). In addition, the total content of ginsenosides (R1, Rg1, Re, Rb1, Rg2, Rh1, Rd, and Rg3) per Sanqi plant taproot under N-sufficient conditions (N_{113} and N_{225}) was significantly higher than that under N-deficient conditions (N_0 and N_{56}) (Fig. S3).

3.5. The nutrient absorption capacity of roots affects the saponins synthesis

The content of ginsenosides in the taproots exhibited a significant positive linear relationship with the NURI (per plant and per unit area) during 121-189 DAS (Fig. 6A, C) but exhibited no significant relationship with the NURI during 0-120 DAS or 190-309 DAS. In contrast, there was a significant negative linear relationship between the content of fibrous root saponins and the NURI (per plant and per unit area) during 0-120 DAS, but there was no significant relationship during 121-189 DAS or 190-309 DAS (Fig. 6B, D). In addition, there were significant positive linear relationships

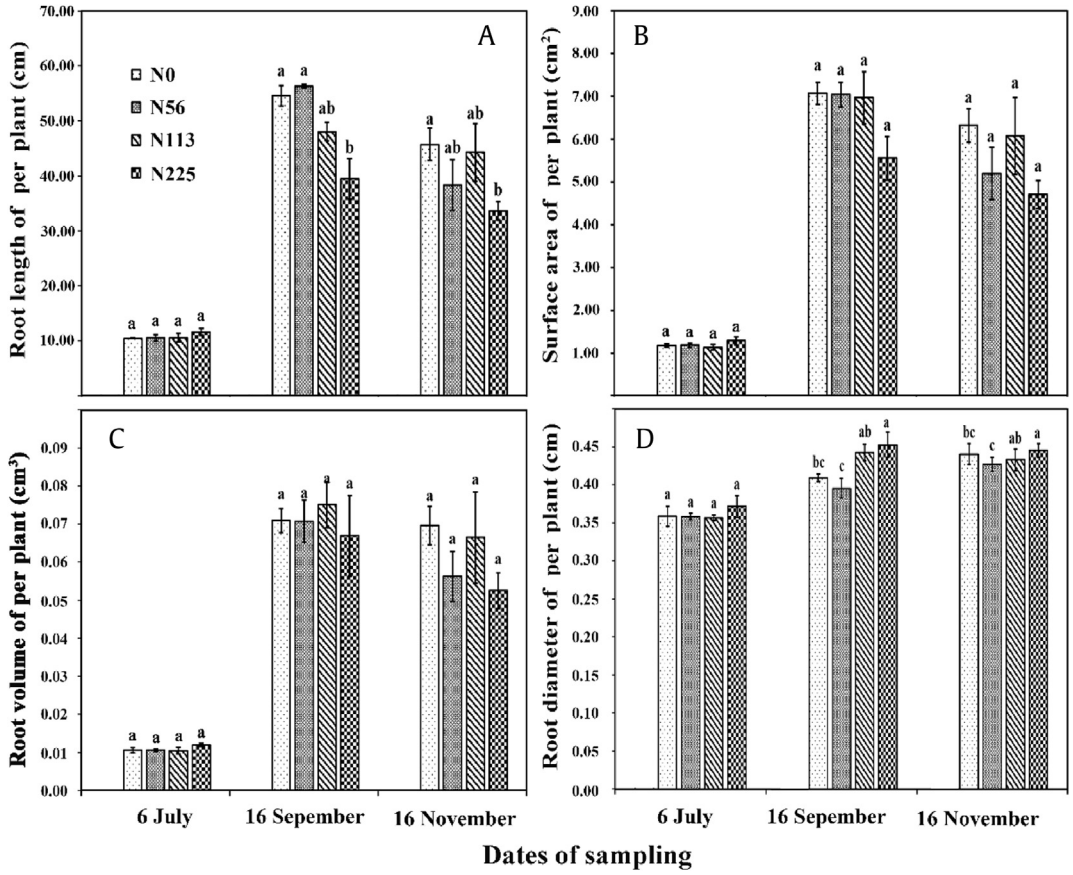

Fig. 4. Root morphology of sanqi (*Panax notoginseng*) per plant at three sampled dates (6 July, 16 September, and 16 November) among different nitrogen application rates. (A) Root length. (B) Root surface area. (C) Root volume. (D) Root doameter. N0, N56, N113, and N225 represent 0, 56, 113, and 225 kg N/ha. All data are presented as the mean ± standard errors (SEs) calculated from three biological replicates. Bars indicate SE, and different letters indicate significant differences among the treatments by Duncan's multiple range tests at $P < 0.05$.

between the content of fibrous root saponins and root length, root surface area, and root volume ($P < 0.05$), and the R^2 value and slope of the regression equation of the root length were greater than those of the root surface area and volume (Fig. S4B, N, F). However, there were no significant relationships between the content of taproot saponins and fibrous root length, surface area, or volume (Fig. S4A, C, E).

4. Discussion

Our main goal in this study was to explore the effects of N fertilizer rates on the growth of and the synthesis of active compounds in the medicinal herb Sanqi. We found that root morphology and physiology can be adjusted to optimize the absorption of nutrients and that suitable levels of N stimulate the accumulation of saponins in the taproots.

In this study, the taproot biomass of Sanqi plants increased as the N fertilizer rate increased; maximum biomass was attained under 225 kg N/ha in both years (Table 1), indicating that soil N concentration could alter the tuber growth of Sanqi plants. These results are in accordance with those of Ou et al. [26], who reported that 165-270 kg N/ha applications resulted in the greatest Sanqi root yields. Increased N fertilizer rates promoted the shoot biomass of Sanqi plants in 2015 but had no effect in 2016 (Table 1). This result might be attributed to the interference of extreme weather (snow in January and high temperature in July) in 2016. Sanqi seedlings may allocate increased amounts of carbohydrates only to the roots when faced with severe environmental conditions and overall tend to grow in suitable environments. The lower yield of

Table 2
Nitrogen uptake rate per Root length of Sanqi (*Panax notoginseng*) at growth stages with five N rates

Nitrogen application	Per area (mg /m. d)			Per plant (mg / m. d)		
	0–120d	121–189d	190–309d	0–120d	121–189d	190–309d
N0	5.436 ± 0.617b	2.929 ± 0.413b	3.759 ± 2.235a	0.054 ± 0.006b	0.035 ± 0.003c	0.013 ± 0.006a
N56	5.547 ± 0.404b	3.812 ± 0.482ab	2.006 ± 1.222a	0.055 ± 0.004b	0.038 ± 0.002bc	0.014 ± 0.003a
N113	5.134 ± 0.472b	5.127 ± 0.309a	1.798 ± 1.306a	0.051 ± 0.005b	0.049 ± 0.005b	0.026 ± 0.013a
N225	7.295 ± 0.352a	5.08 ± 0.466a	6.391 ± 1.105a	0.073 ± 0.004a	0.061 ± 0.003a	0.039 ± 0.006a

Mean ± standard errors calculated from three biological replicates are shown. Different letters indicate significant differences among the five treatments by ANOVA, Duncan's multiple range tests at $P < 0.05$.
ANOVA, analysis of variance.

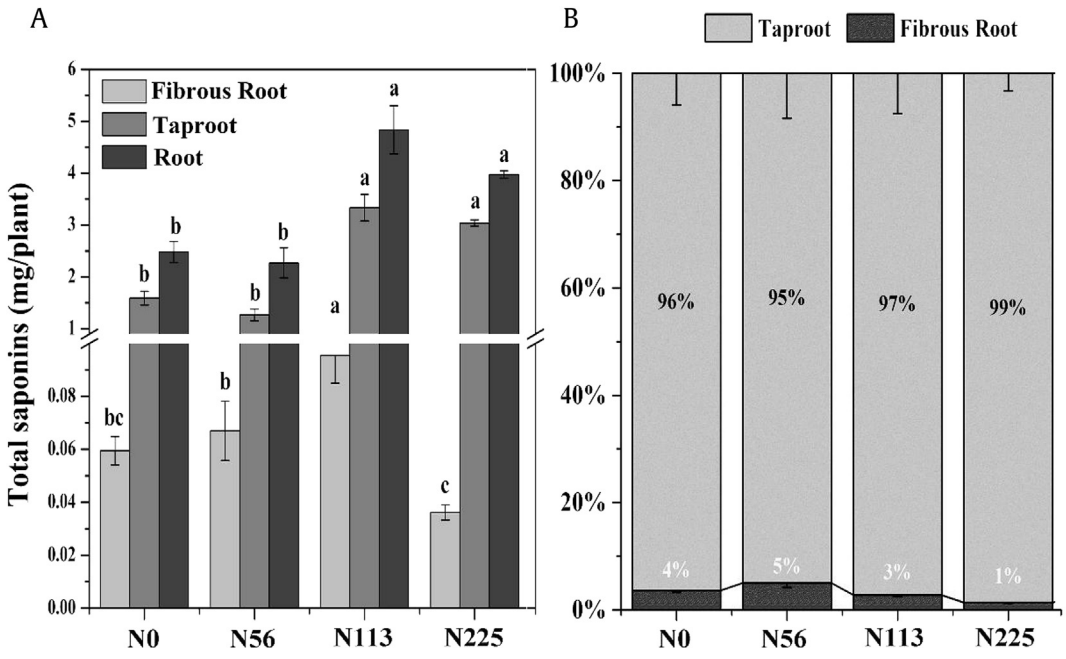

Fig. 5. The total saponin contents of Sanqi (*Panax notoginseng*) per plant among different nitrogen application rates. (A) Total saponin content. (B) The proportion of total saponins. N0, N56, N113, and N225 represent 0, 56, 113, and 225 kg N/ha. All data are presented as the mean ± standard errors (SEs) calculated from three biological replicates. Bars indicate SE, and different letters indicate significant differences among the treatments by Duncan's multiple range tests at $P < 0.05$.

Sanqi seedlings in 2016 than in 2015 also supports this hypothesis (Table 1). Hence, 225 kg N/ha may be the optimum N application for Sanqi plant growth.

The temporal or spatial niche of species is important for resource use in terms of plant growth [27,28]. In our research, Sanqi plants exhibited maximal aboveground growth during the early growth stage (t_{lmax}: approximately 170 DAS), after which they

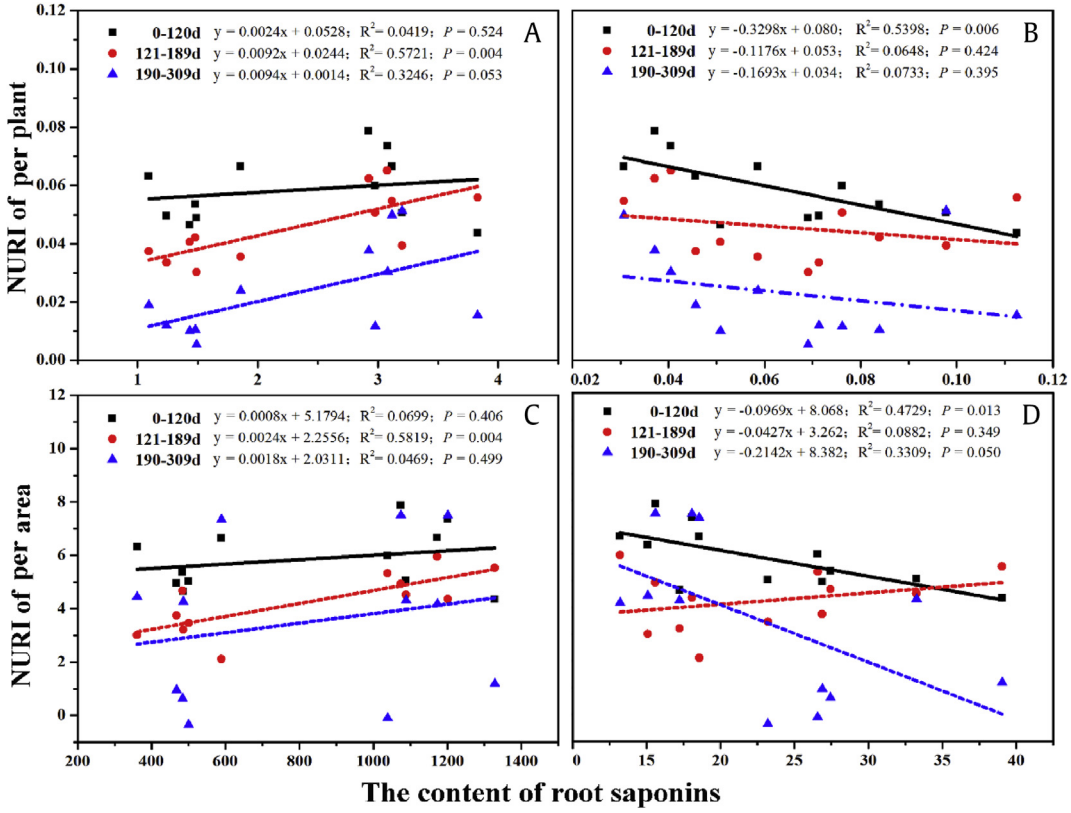

Fig. 6. Correlation between the content of root saponins and NURI of Sanqi (*Panax notoginseng*). (A) per plant for taproot. (B) fibrous root. (C) per area for taproot. (D) fibrous root.

entered into an exponential belowground growth phase (t_{Imax}: approximately 265 DAS) (Fig. 1A and B; Table S1). These results highlight the unique strategy of the biomass allocation of both aboveground and belowground Sanqi plant parts and are in accordance with the results of Cui and Wang [29], who reported that the growth rate of Sanqi plants peaked before August and from August to December in terms of aboveground biomass and belowground biomass, respectively. Similar results concerning the allocation of nutrients (*N*- t_{Imax}: approximately 155/260 DAS, *P*-t_{Imax}: approximately 145/280 DAS, *K*- t_{Imax}: approximately 185/280 DAS) between the shoots and roots also support this finding (Fig. 2, Fig S1; Table S1). In *Panax ginseng* cultivation, the biomass allocation between aboveground and belowground parts is similar to that in Sanqi plants [30]. This biomass allocation strategy may be a common characteristic of root-type medicinal perennials. In addition, the N and P uptake of Sanqi shoots stopped after 210 DAS, and K uptake continued to increase until 300 DAS, which coincided with the taproot swelling period (Fig. 2, Fig. S1), indicating a key role of K uptake during Sanqi taproot growth. Many studies have shown that increased K uptake could promote both enzymatic activity of starch synthase and tuber swelling in tuberous crop species [31,32]. Therefore, the continuous uptake of K in roots may be the basis of increased photosynthetic product translocation from shoots to roots during the swelling process of Sanqi tuberous roots.

The I_{max} theory states that, compared with plants with relatively slow growth rates, plants with relatively fast growth rates (greater capacity for resource capture) tend to survive longer and are more competitive [33]. In our research, soil N applications increased the maximum capacity for biomass/nutrients (K/NU_{max}); the maximum growth rate for biomass/nutrients (I_{max}); and N, P, and K uptake of Sanqi taproots but had no effect on *r* or t_{Imax} (Figs. 1 and 3; Table S1, Table S2). These results indicated that the soil N concentration regulated the growth and nutrient uptake dynamics of Sanqi taproots mainly by altering the maximum accumulation rate of biomass and nutrients. Similar results were reported by Zhang et al. [34], who found that intercropped maize exhibited greater I_{max} values and maximum biomass under 225 kg N/ha compared with 0 kg N/ha. The I_{max} theory states that plants with relatively fast growth rates (greater capacity for resource capture) tend to be superior competitors [33]. In our other study, the survival rate of Sanqi plants under N_{225} was significantly greater than that under other N applications [35]. Hence, greater survivability of Sanqi seedlings under optimal N fertilization may play a key role in the rapid Sanqi seedling growth and strong nutrient uptake ability. In addition, N application had little effect on Sanqi shoot growth and nutrient uptake but did affect the maximum N uptake. This result was in accordance with the previously mentioned hypothesis in which taproot growth and nutrient uptake had priority over those of the shoots.

Plant roots growing in soil exhibit highly plastic responses to surrounding environmental changes [36]. In our research, the root length, surface area, and volume of the Sanqi plants increased under N-deficient conditions, whereas the diameter decreased; however, the opposite results occurred under N-sufficient conditions (Fig. 4; Fig. S2; Fig S5). The results indicate that the root growth of Sanqi plants was sensitive to soil available N concentrations. A field study of wheat and maize intercropping also showed that decreased N applications could promote RLD, but the root distribution decreased in response to excessive N applications [6]. Other studies have also shown that an optimal N supply to seedlings increases carbon partitioning to roots, accelerates root growth, and results in greater total root length [20,37]. These consequences in terms of the root growth of Sanqi plants in response to various N applications are indicative of the phenotypic plasticity of root distribution, which has generally been characterized by efficient resource acquisition via optimized root growth and distribution under different soil resource conditions [20,36]. In addition, plants can respond to soil nutrients by altering their root architecture (morphological plasticity) and by increasing the uptake capacity of their roots exposed to high nutrient concentrations (physiological plasticity) [38,39]. In our research, the NURI of Sanqi significantly increased in response to increased N applications (Table 2). This result reflects the typical physiological plasticity of Sanqi roots in response to soil N applications and is likely the basis for adequate nutrient absorption of Sanqi plants under optimum N fertilization (N_{225}), in which root length, surface area, and root volume decreased. Robinson [40] also reported that nutrient uptake per unit root usually increases when the roots of nutrient-deprived plants are supplied with nutrients. Combined with the effects of N application on biomass and nutrient uptake mentioned previously, the root length of the Sanqi plants decreased, and greater amounts of photosynthate were allocated to tuberous roots under optimum N fertilization, which promoted thick root growth to ensure adequate nutrient absorption.

In plants, the synthesis of specific active compounds is regulated by environmental factors [41–43]. In the present study, the synthesis of saponins in Sanqi significantly differed in response to various N applications and was maximal in response to N_{113} and N_{225} (Fig. 5), indicating that soil N could alter the synthesis of saponins. Ibrahim et al. [44] also reported that N levels significantly impacted the production of total phenolics and flavonoids in *Labisia pumila* Benth. N fertilizers also significantly increased the content of alkaloids in *Lupinus albus* [45], and Kang [14] also reported that major secondary metabolites including betaine, carotenoids, and flavones in *L. barbarum* varied in response to different application amounts of N. Moreover, the NURI of Sanqi plants was positively correlated with the synthesis of saponins in tuberous roots (Fig. S4), indicating that the nutrient absorption ability of fibrous roots is an important factor for the synthesis of saponins. This positive relationship may be derived from two aspects: (1) a direct relation between N absorption and the synthesis of saponins and (2) the increased N absorption ability of Sanqi fibrous roots may affect the uptake of other microelements, which affects the synthesis of saponins. Zhou et al. [46] reported that increasing N levels could reduce the contents of trace elements (Ca, Mn, Cu, and Zn) in different parts of *Allium fistulosum* L. plants. Trace element accumulation can affect the levels of secondary metabolites in crop species such as carrots, onions, and potatoes [47]. In addition, there was negative correlation between the NURI and the synthesis of fibrous root saponins. This result may be due to the reduction in root length and volume, which showed the opposite tendency with the NURI in response to various N applications. The negative relationship between root morphology and saponin content supported this hypothesis (Fig. S4).

5. Conclusions

The present study shows that Sanqi could adjust its root growth for nutrient absorption according to the soil N status. When soil N was sufficient, Sanqi exhibited reduced root length and surface and enhanced root diameter and NURI to improve nutrient absorption ability. The higher maximum accumulation rate of biomass and nutrients were the main reason of greater biomass and nutrient uptake of Sanqi taproot. Meantime, increasing N application also promoted synthesis of saponins, which showed important relationship with NURI and root architecture.

Overall, on the basis of soil N concentration, root morphology and physiology can be modified to optimize the absorption of nutrients, and this plasticity also affects the synthesis of saponins.

Understanding the relationship between root morphology, nutrient availability, and the synthesis of secondary metabolites may help us obtain additional active compounds from roots by managing N levels in medicinal herb production systems.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was financed by the National Key R&D Program of China (Project No. 2017YFD0201601-5, SQ2017YFC170021-02 and 2018YFD0201100) and the Natural Science Foundation of China (31601682), the Major Programme for Biomedicine of Yunnan Province (Project No. 2016ZF001), and the Young and Middle-aged Academic and Technical Leaders Reserve Programme in Yunnan Province (2017HB024).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.04.003.

References

[1] Wang T, Guo R, Zhou G, Zhou X, Kou Z, Sui F, Li C, Tang L, Wang Z. Traditional uses, botany, phytochemistry, pharmacology and toxicology of *Panax notoginseng* (Burk.) F.H. Chen: a review. J Ethnopharmacol 2016;188:234–58.
[2] Yang M, Zhang X, Xu Y, Mei X, Jiang B, Liao J, Yin Z, Zhen J, Zhao Z, Fan L, et al. Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. PLoS One 2015;10:e0118555.
[3] He Y, Song SY, Yong-Hua XU, Gao YG, Zhang LX. Research progress on triterpenoid saponins in *Panax ginseng* and its ecological effects. Chinese Tradi Herb Drugs 2017;48:1692–8.
[4] Chung AS, Park KM. Chapter 4. Anticancer and antineurodegenerative effects of ginsenosides. Stud Nat Prod Chem 2016;50.
[5] Leung KW, Wong ST. Pharmacology of ginsenosides: a literature review. Chin Med 2010;5:20.
[6] Liu Y, Zhang W, Sun J, Li X, Christie P, Li L. High morphological and physiological plasticity of wheat roots is conducive to higher competitive ability of wheat than maize in intercropping systems. Plant Soil 2015;397:387–99.
[7] Trinder C, Brooker R, Davidson H, Robinson D. Dynamic trajectories of growth and nitrogen capture by competing plants. New Phytol 2012;193:948–58.
[8] Trinder CJ, Brooker RW, Robinson D. Plant ecology's guilty little secret: understanding the dynamics of plant competition. Func Ecol 2013;27:918–29.
[9] Sun Y, Chen Z, Wei M. Effects of various nitrogen fertilizers on the yield and quality of *Panax notoginseng*. Soil Fertil Sci China 2008;26:22–5.
[10] Wang C, Chen Z, Sun Y. Effect of different proportion fertilizing on the growth and yield of *Panax notoginseng*. Res Pract Chinese Med 2007;21:5–7.
[11] Wei M, Sun Y, Huang T. Effects on the growth and the contents of saponin with different level of nitrogen applied in *Panax notoginseng*. Res Pract Chinese Med 2008;22:17–20.
[12] Andersen M, Hauggaardnielsen H, Jacob W, Eriksteen J. Competitive dynamics in two- and three-component intercrops. J Appl Ecol 2007;44:545–51.
[13] Gaude N, Brehelin C, Tischendorf G, Kessler F, Dormann P. Nitrogen deficiency in Arabidopsis affects galactolipid composition and gene expression and results in accumulation of fatty acid phytylesters. Plant J 2007;49:729–39.
[14] Kang JH. Study on the relationship between the main secondary metabolites and polysaccharide in fruits of *Lycium barbarum* at different application amounts of nitrogen. J Anhui Agr Sci 2008;36:16008–10.
[15] Gershenzon J. Changes in the levels of plant secondary metabolites under water and nutrient stress. In: Timmermann BN, Steelink C, Loewus FA, editors. Phytochemical adaptations to stress. Boston: Springer; 2002. p. 273–320.
[16] Szakiel A, Pączkowski C, Henry M. Influence of environmental biotic factors on the content of saponins in plants. Phytochem Rev 2011;10:493–502.
[17] Fan W, Yang H. Effect of soil type on root architecture and nutrient uptake by roots of young apple rootstocks. Acta Horticulturae 2011:885–90.
[18] Forde B, Lorenzo H. The nutritional control of root development. Plant Soil 2001;232:51–68.
[19] Yan HT, Beyrouty CA, Norman RJ, Gbur EE. Nutrient uptake relationship to root characteristics of rice. Plant Soil 1995;171:297–302.
[20] Gruber BD, Giehl RF, Friedel S, von Wiren N. Plasticity of the Arabidopsis root system under nutrient deficiencies. Plant Physiol 2013;163:161–79.
[21] Mou P, Jones RH, Tan Z, Bao Z, Chen H. Morphological and physiological plasticity of plant roots when nutrients are both spatially and temporally heterogeneous. Plant Soil 2013;364:373–84.
[22] Sultan SE. Phenotypic plasticity for plant development, function and life-history. Trends Plant Sci 2001;5:537–42.
[23] Pigliucci M, Hayden K. Phenotypic plasticity is the major determinant of changes in phenotypic integration in Arabidopsis. New Phytol 2001;152:419–30.
[24] Hunt R. Plant growth analysis. Suffolk: The Lavenham Press; 1982.
[25] Page AL, Miller RH, Keeney DR. Methods of soil analysis. Part 2: chemical and microbiological properties. 2nd ed. Madison: ASA and SSSA; 1982. p. 643–98.
[26] Ou X, Yang Y, Guo L, Zhu D, Liu D. Effect of organic-inorganic N sources on growth, Npk nutrients and secondary metabolites of *Panax notoginseng* (Burk.) F. H. Chen. Emir J Food Agr 2017:629–38.
[27] Brokaw N, Busing RT. Niche versus chance and tree diversity in forest gaps. Trends Ecol Evol 2000;15:183.
[28] Hauggaard-Nielsen H, Jensen ES. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. Field Crop Res 2001;72:185–96.
[29] Cui X, Wang c. Study on the growth and dry matter accumulation dynamics of *Panax notoginseng*. Chinese Herb Med 1991;9:9–11.
[30] Liu Z, Li S, Sun Y, Xiu L, Zhang H, Lu j. Dynamic changes of dry matter accumulation and distribution of ginseng. Spec Wild Econ Anim Plant Res 1994;4:32–5.
[31] Lindhauer MG, Fekete MARD. Starch synthesis in potato (*Solanum tuberosum*) tubers: activity of selected enzymes in dependence of potassium content in storage tissue. Plant Soil 1990;124:291–5.
[32] Wang S, Yu Z, Wang D. Effect of potassium on sucrose content of flag leaves and starch accumulation of kernels in wheat. Acta Phytoecologica Sinica 2003;27:196–201.
[33] Grime JP. Plant strategies and vegetation processes. Biologia Plantarum 1979;23:254.
[34] Zhang W, Liu G, Sun J, Zhang L, Weiner J, Li L. Growth trajectories and interspecific competitive dynamics in wheat/maize and barley/maize intercropping. Plant Soil 2015;397:227–38.
[35] Wei W, Yang M, Liu Y, Huang H, Ye C, Zheng J, Guo C, Hao M, He X, Zhu S. Fertilizer N application rate impacts plant-soil feedback in a sanqi production system. Sci Total Environ 2018;633:796–807.
[36] Croft SA, Hodge A, Pitchford JW. Optimal root proliferation strategies: the roles of nutrient heterogeneity, competition and mycorrhizal networks. Plant Soil 2012;351:191–206.
[37] Giehl RFH, Gruber BD, Von Wirén N. It's time to make changes: modulation of root system architecture by nutrient signals. J Exp Bot 2014;65:769–78.
[38] Cahill JF, Mcnickle GG, Haag JJ, Lamb EG, Nyanumba SM, Clair CCS. Plants integrate information about nutrients and neighbors. Science 2010;328:1657.
[39] Orians CM, Babst B, Zanne AE. 17 – Vascular constraints and long distance transport in dicots. Vasc Transport 512 Plant 2005:355–71.
[40] Robinson D. The responses of plants to nonuniform supplies of nutrients. New Phytol 1994;127:635–74.
[41] Arena ME, Postemsky PD, Curvetto NR. Changes in the phenolic compounds and antioxidant capacity of *Berberis microphylla* G. Forst. berries in relation to light intensity and fertilization. Sci Hortic 2017;218:63–71. Amsterdam.
[42] Virjamo V, Sutinen S, Julkunen-Tiitto R. Combined effect of elevated UVB, elevated temperature and fertilization on growth, needle structure and phytochemistry of young Norway spruce (*Picea abies*) seedlings. Global Change Biol 2014;20:2252–60.
[43] Woodrow P, Ciarmiello LF, Annunziata MG, Pacifico S, Iannuzzi F, Mirto A, D'Amelia L, Dell'Aversana E, Piccolella S, Fuggi A, et al. Durum wheat seedling responses to simultaneous high light and salinity involve a fine reconfiguration of amino acids and carbohydrate metabolism. Physiol Plantarum 2016;159:290.
[44] Ibrahim MH, Jaafar HZ, Rahmat A, Rahman ZA. The relationship between phenolics and flavonoids production with total non structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under high CO_2 and nitrogen fertilization. Molecules 2011;16:162–74.
[45] Ciesiołka D, Gulewicz P, Martinezvillaluenga C, Pilarski R, Bednarczyk M, Gulewicz K. Products and biopreparations from alkaloid-rich lupin in animal nutrition and ecological agriculture. Folia Biol-Prague 2005;53:59–66.
[46] Zhou L, Yue Q, Zhao L, Cui R, Liu X. Effect of nitrogen level on growth and metabolism of Allium fistulosum L. In: International Conference on Energy, Environment and Sustainable Development; 2018.
[47] Flemotomou E, Molyviatis T, Zabetakis I. The effect of trace elements accumulation on the levels of secondary metabolites and antioxidant activity in carrots, onions and potatoes. Food Nutr Sci 2011;2:1071–6.