

Root Morphology and Secretion of two subtropical tree species to $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ Deposition

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Abstract

Background: Both NH_4^+ and NO_3^- are capable of greatly influencing plants' growth and biomass. However, the belowground responses of subtropical trees to either NH_4^+ or NO_3^- deposition remain poorly understood. Here, we discuss how these two forms of N deposition can affect root development, and experimentally analyzed how they could impact nitrogen and phosphorus absorption in two types (broadleaved with a fibrous root system vs. conifer with a tap root system) of subtropical tree species.

Results: In a greenhouse in southern China, 1-year-old *S. superba* and *P. massoniana* seedlings grown on P-limited and P-normal soil were treated with NaNO_3 and NH_4Cl solutions of 0, 80, and 200 $\text{kg N ha}^{-1} \text{ year}^{-1}$, corresponding to the control, N80, and N200 groups, respectively. Root phenotype characteristics and metabolism ability were measured after 8 months of growth. The results showed that the root morphology and physiology variables differed significantly between the two species under different N and P treatments. Although *S. superba* had a larger quantity of roots than *P. massoniana*, both its root growth rate and root absorption were respectively lower and weaker. N addition differentially affected root growth and activity as follows: (1) NO_3^- -N80 and NH_4^+ -N80 increased root growth and activity of the two species, but NH_4^+ -N80 led to thicker roots in *S. superba*; (2) NO_3^- -N200 and NH_4^+ -N200 had inhibitory effects on the roots of *P. massoniana*, for which NH_4^+ -N200 led to thinner and longer roots and even the death of some roots; and (3) NH_4^+ -N could promote metabolic activity in thicker roots (> 1.5 mm) and the NO_3^- -N was found to stimulate activity in thinner roots (0.5–1.5 mm) in the fibrous root system having a larger quantity of roots, namely *S. superba*. By contrast, NO_3^- -N and NH_4^+ -N had an opposite influence upon functioning in the tap root system with a slender root, namely *P. massoniana*.

Conclusion: We conclude *P. massoniana* has a much higher root absorption efficiency; however, nitrogen deposition is more beneficial to the root growth of *S. superba*.

Background

The root is a vital organ in terrestrial plants that can fix the plant into the soil and absorb nutrients and water from the soil to promote its growth, especially via fine roots [1]. Much research has shown that root morphology and activity can greatly impact nutrient absorption, yet nutrition can also shape the roots' phenotype and influence its physiological activity [1]. For example, when the soil is rich in nutrients, the lateral root (LR) and total root length undergo increased growth, whereas in barren soil root growth is poor [1]. This contrasts with studies drawing the opposite conclusion, such when the total root volume and root secretions are decreased in rich soil [1]. These contradictory findings are mainly due to the nutrient elements' type, forms, distribution, concentrations, and the species studied [1].

Nitrogen (N) and phosphorus (P) are the two fundamentally important elements in plants and both directly influence root growth [8,10–11]. In the subtropics, however, soil P is usually present at low levels, particularly in forest soils in China [1]. To overcome this limitation, plants growing in this region usually have a specialized root morphology and secretion mechanism to promote their phosphorus acquisition efficiency (PAE); the latter defined as the ability of the plant to acquire P from the soil [11]. For example, depending on the species, the plant will increase the root base branch angle and density of its lateral roots and root hairs to form a shallow root system, or increase the clustering of roots and colonization by arbuscular mycorrhiza (AM) or ectomycorrhizal (ECM) fungi, to increase its PAE [1]. Recent research suggests that ECM fungi are most beneficial to plants in N-limited environments, while AM fungi dominate in P-limited environments [17]. At the same time, because the rate of atmospheric deposition of nitrogen (N) is high and accelerating in subtropical forests of southern China [1], the nitrogen acquisition efficiency (NAE) of plants could also be seriously affected. Zhu et al. reported an average deposition flux of N in China of $13.69 \pm 8.69 \text{ kg ha}^{-1} \text{ a}^{-1}$ [1] and this deposition trend is expected to increase in future decades, such that the production of reactive N will reach 270 Tg N a^{-1} by 2050 [1]. Furthermore, nitrogen oxide (NO_x) and ammonia (NH_3), the major pollutants contributing to N deposition, may be transformed into a variety of secondary pollutants, such as nitrate (NO_3^-) and ammonium (NH_4^+), of which NH_4^+ -N is the predominant form and NO_3^- -N is the largest increased by the accelerating rates of N deposition in China [21]. All of them could variously affect plants' growth and alter their roots' phenotype and its physiological activity [1]. In particular, evidence suggests ammonium stimulates LR branching and nitrate stimulates LR elongation [25]. Nevertheless, either dysplasia or physiological dysfunction of the root system will severely affect plant growth and development [1].

Schima superba and *Pinus massoniana* are the predominantly cultivated tree species in secondary subtropical forest stands and plantations in southern China. Generally, *S. superba* is a broadleaf tree species with a well-developed fibrous-root system, while *P. massoniana* is a coniferous tree species with a tap-root system. Both trees are well known for their ability to grow and thrive under barren forest soil [1]. Previous research has shown these two species could be affected by not only P and N concentrations but also N forms, and species-specific responses in terms of their aboveground growth state have been observed [28]. For example, *P. massoniana* is better suited to a low-P environment, whereas *S. superba* benefits from more N addition, especially of NH_4^+ . A moderate addition of NO_3^- could also promote the aboveground growth of both species, but a high dose would inhibit *P. massoniana*. Our previous study had shown that the biomass and leaf N and P contents of plants are affected by different N forms in low-P versus high-P soil [28]. In this study, we investigated the effects of N forms, along with N and P levels, on root length, volume, surface area, average diameter, fine roots, root- and exudates-acid phosphatase, oxalic acid, PAE, and NAE. Results of this study provide detailed guidance for managing plant growth under realistic NO_3^- -N and NH_4^+ -N deposition.

Results

Root morphology and physiology under NH_4^+ or NO_3^- treatment

Root morphology and physiology differed significantly between the P and species, and the thicker roots' length, nutrient absorption, and physiological activity were significantly influence by the added N (Table 1, Fig. 1). Although the root quantity of *S. superba* was higher than that of *P. massoniana*, the former's root growth rate was lower than the latter's (Table 2, Fig. 1). For example, on average, root volume was increased by 47.1% and 19.4% under the low-P and high-P soil, respectively, in *S. superba* after the N addition, yet it was respectively increased by 82.9% and 62.6% in *P. massoniana* compared with N0. Both the PAE and NAE were higher in *P. massoniana* than *S. superba* (Table 2, Fig. 1). However, the increased rate of PAE and NAE depended on both species identity and the N forms' addition at different levels. For example, the PAE of *S. superba* and NAE of *P. massoniana* respectively increased by 73.4% and 57.9% when averaged over the two N forms and their addition levels, thus exceeding the PAE increased rate of 58.9% for *P. massoniana* and the NAE increased rate of 39.4% for *S. superba*.

Table 1
Three-way ANOVA for the root morphological and physiological variables of *Schima superba* and *Pinus massoniana* between the nitrogen (N) and phosphorus (P) treatments (n = 120 plants).

	F-value						
	P	N	Species	P · N	P · Species	N · Species	P · N · Species
RL	96.15**	1.58	19.72**	1.11	0.24	1.85	1.68
SA	79.69**	1.96	14.17**	1.05	0.02	1.71	1.19
AD	5.22*	3.71**	0.41	3.02*	20.60**	3.13*	4.12**
RV	60.72**	2.24	9.65**	0.97	0.42	1.58	0.84
Tips	25.70**	0.85	103.83**	0.88	0.36	1.94	1.67
Forks	61.86**	2.31	21.38**	1.77	0.26	1.45	1.16
0 < L ≤ 0.5/cm	97.90**	1.54	30.41**	1.23	0.83	1.97	2.05
0.5 < L ≤ 1.0/cm	79.46**	1.46	1.52	0.96	0.65	1.37	0.97
1.0 < L ≤ 1.5/cm	37.68**	1.63	5.59*	1.18	0.00	1.12	0.48
1.5 < L/cm	56.99**	2.72*	3.73*	1.62	0.09	2.24	0.91
PAE	437.83**	4.60**	95.49**	5.11**	19.99**	6.11**	1.72
NAE	487.81**	8.16**	142.40**	13.88**	30.83**	4.79**	1.21
RAPase	11.65**	4.73**	47.33**	1.67	9.69**	3.29*	1.31
SAPase	6.45*	13.64**	8.46**	0.54	15.14**	0.77	6.73**
OX	57.57**	83.16**	193.79**	21.51**	142.83**	24.98**	17.27**

Table 2
The root morphology of *Schima superba* and *Pinus massoniana* under different N and P treatments. (Means with S.D. in parentheses, n

P treatment	N treatment	RL /cm	SA /cm ²	AD /mm	RV /cm ³	Tips	Forks	0.0 < L ≤ 0.5 /cm	0. /c
LP	<i>S. superba</i>								
	N0	174.8(71.2)b	30.7(14.2)ns	0.55(0.05)ab	0.44(0.24)ns	656.7(272.9)ns	205.4(117.8)ns	138.5(13.7)b	30
	NO ₃ ⁻ -N80	233.3(76.5)ab	38.8(14.7)ns	0.53(0.04)b	0.52(0.24)ns	733.9(272.3)ns	267.7(98.9)ns	187.9(19.5)ab	35
	NO ₃ ⁻ -N200	288.3(104.8)a	48.5(18.9)ns	0.54(0.07)b	0.66(0.32)ns	970.2(395.0)ns	348.2(165.4)ns	233.1(23.9)a	44
	NH ₄ ⁺ -N80	236.9(91.0)ab	44.0(19.3)ns	0.58(0.06)ab	0.66(0.34)ns	951.6(422.1)ns	312.1(164.9)ns	184.5(18.9)ab	42
	NH ₄ ⁺ -N200	215.6(132.1)ab	43.7(33.8)ns	0.61(0.09)a	0.72(0.67)ns	974.9(718.2)ns	288.0(242.2)ns	163.9(17.9)b	39
	<i>P. massoniana</i>								
	N0	78.1(22.4)b	12.91(4.9)b	0.52(0.07)ab	0.17(0.08)b	170.8(53.3)ns	73.3(34.2)b	58.1(9.6)b	17
	NO ₃ ⁻ -N80	176.7(60.9)a	29.60(11.0)a	0.53(0.06)ab	0.40(0.17)a	278.2(95.9)ns	175.2(58.9)a	133.3(13.2)a	36
	NO ₃ ⁻ -N200	146.8(54.9)ab	25.61(9.8)ab	0.55(0.03)a	0.36(0.14)ab	262.0(122.3)ns	152.0(84.8)ab	109.3(11.9)ab	30
NH ₄ ⁺ -N80	107.8(36.7)ab	19.60(6.8)ab	0.58(0.04)a	0.28(0.10)ab	187.8(57.9)ns	117.8(57.4)ab	76.4(9.5)b	25	
NH ₄ ⁺ -N200	128.3(36.0)ab	18.85(5.6)ab	0.47(0.01)b	0.22(0.07)ab	216.0(51.9)ns	108.2(46.2)ab	107.3(10.4)ab	19	
HP	<i>S. superba</i>								
	N0	485.1(202.2)ab	79.1(36.3)ab	0.51(0.04)b	1.03(0.54)b	1286.7(474.5)ab	570.9(311.7)ab	389.5(36.1)ab	73
	NO ₃ ⁻ -N80	410.2(266.6)b	70.1(44.3)b	0.55(0.04)ab	0.95(0.58)b	958.7(468.6)bc	490.4(358.8)b	314.9(32.0)b	75
	NO ₃ ⁻ -N200	518.7(317.4)ab	92.7(63.1)ab	0.56(0.04)a	1.32(1.01)ab	1096.1(531.5)abc	615.8(449.1)ab	388.3(37.5)ab	97
	NH ₄ ⁺ -N80	387.7(107.8)b	65.4(18.4)b	0.54(0.03)ab	0.88(0.02)b	857.6(254.2)c	450.1(184.8)b	300.2(18.2)b	68
	NH ₄ ⁺ -N200	677.8(301.5)a	122.2(65.7)a	0.56(0.05)a	1.77(0.05)a	1402.7(393.3)a	914.5(484.9)a	509.4(39.1)a	12
	<i>P. massoniana</i>								
	N0	276.1(125.9)ns	47.64(21.57)ns	0.55(0.04)ns	0.66(0.31)ns	403.0(149.6)b	235.3(112.6)b	201.4(25.4)ns	61
	NO ₃ ⁻ -N80	435.9(174.5)ns	75.3(27.93)ns	0.56(0.05)ns	1.04(0.36)ns	753.7(336.7)a	491.7(196.2)a	325.9(38.0)ns	88
	NO ₃ ⁻ -N200	325.4(116.3)ns	62.7(24.08)ns	0.61(0.04)ns	0.97(0.40)ns	498.5(154.9)ab	371.2(152.6)ab	223.3(21.5)ns	80
NH ₄ ⁺ -N80	423.8(106.1)ns	78.8(22.79)ns	0.59(0.05)ns	1.17(0.40)ns	587.5(169.1)ab	451.3(135.4)ab	291.4(26.2)ns	10	
NH ₄ ⁺ -N200	381.3(94.2)ns	72.7(15.83)ns	0.61(0.03)ns	1.11(0.21)ns	539.0(112.5)ab	498.8(128.3)a	268.9(28.1)ns	89	

Low-P soil

The root morphology and physiology variables of the two tree species were all positively stimulated by N (Fig. 1). For example, under the low-P treatment, NO₃⁻ could increase root growth by 157.5% and root activity by 143.6% in *P. massoniana* (Table 2); in *S. superba*, the corresponding increases were 48.7% and 106.9%. When the level of NO₃⁻ increased from 80 to 200 kg N ha⁻¹ year⁻¹, the root morphology of *S. superba*, such as root length, surface area, and volume,

continued to expand (growth ratio increased nearly 32.0%), but that of *P. massoniana* grew slower (growth ratio decreased nearly 31.2%). The high NO₃⁻ level began to have inhibitory effects on the roots of *P. massoniana*, especially the fine roots (Table 2). Compared with NO₃⁻, an increased NH₄⁺ concentration could promote the root volume and quantity of *S. superba*, whose roots became much thicker (AD growth ratio increased from 5.5–9.5%; RL growth ratio decreased from 35.6–23.3%). By contrast, applying NH₄⁺-N200 inhibited the root growth of *P. massoniana*, whose roots became thinner and longer (AD growth ratio decreased by 11.9% to -10.2%; the RL growth ratio increased from 38.2–64.3%).

Although the root morphology of *P. massoniana* was poor under the low-P treatment, its PAE and NAE were 8.3 and 3.3 times higher than those of *S. superba*, respectively, and NO₃⁻ promoted greater root absorption of both species (Table 3, Fig. 1). NH₄⁺ increased the sensitivity of *P. massoniana* to P, which diminished its PAE and NAE. Generally, under an increased N concentration, the root activity of *S. superba* was reduced, but the NO₃⁻ increased the OX content 4.8-fold to promote root absorption, whereas the NH₄⁺ enhanced the secretion to promote the PAE, such as the SApase increasing 1.2-fold (Table 3). Compared with *S. superba*, the NO₃⁻-N200 increased the root secretion of *P. massoniana*, such as that of SApase increasing 1.6-fold and OX increasing 8.5-fold; they were all higher than activity under the NO₃⁻-N80 treatment. However, both the PAE and NAE were lower under NO₃⁻-N200 than NO₃⁻-N80 (Table 3). The NH₄⁺-N200 significantly decreased the PAE and NAE of *P. massoniana*.

Table 3
Root physiology of *Schima superba* and *Pinus massoniana* under different N and P treatments. (Means with S.D. in parentheses, n = 6 plant samples.)

P treatment	N treatment	RAPase	SAPase	OX	PAE	NAE
LP	<i>S. superba</i>					
	N0	41.6(47.2)bc	2.59(0.19)ab	30.7(20.1)b	0.03(0.01)b	1.08(0.07)c
	NO ₃ ⁻ -N80	105.8(92.2)a	2.39(0.41)ab	30.6(14.5)b	0.09(0.06)a	1.66(0.48)b
	NO ₃ ⁻ -N200	51.2(33.6)abc	2.25(0.72)b	147.9(63.8)a	0.10(0.03)a	2.37(1.12)a
	NH ₄ ⁺ -N80	85.8(85.7)ab	2.17(1.39)b	52.6(14.5)b	0.04(0.02)b	1.36(0.55)bc
	NH ₄ ⁺ -N200	15.5(11.8)c	3.07(0.72)a	27.9(15.6)b	0.04(0.02)b	1.14(0.25)bc
	<i>P. massoniana</i>					
	N0	4.2(3.6)d	1.57(0.08)d	14.2(8.9)e	0.25(0.05)d	3.51(1.34)d
	NO ₃ ⁻ -N80	9.3(6.2)c	1.48(0.12)e	17.0(6.5)d	0.66(0.12)a	8.33(2.55)a
	NO ₃ ⁻ -N200	3.1(2.9)e	2.45(0.55)a	121.1(50.1)a	0.54(0.24)b	6.76(2.32)b
	NH ₄ ⁺ -N80	45.1(30.9)a	1.65(0.10)c	67.9(17.2)b	0.50(0.17)c	6.63(1.45)c
	NH ₄ ⁺ -N200	13.8(10.1)b	2.19(0.86)b	18.7(7.7)c	0.09(0.03)e	2.33(1.13)e
HP	<i>S. superba</i>					
	N0	218.0(186.6)a	1.73(0.28)b	128.9(10.1)c	0.84(0.49)b	9.68(4.94)b
	NO ₃ ⁻ -N80	155.6(128.1)ab	1.59(0.22)b	41.9(24.3)d	1.09(0.53)b	9.47(3.65)b
	NO ₃ ⁻ -N200	108.8(69.2)bc	2.57(0.97)a	334.5(78.9)a	1.16(0.92)b	11.44(7.98)b
	NH ₄ ⁺ -N80	186.4(84.7)ab	1.34(0.19)b	79.3(34.4)d	0.77(0.28)b	8.51(4.43)b
	NH ₄ ⁺ -N200	20.1(12.2)c	2.29(0.75)a	206.6(45.7)b	1.85(0.72)a	20.18(7.26)a
	<i>P. massoniana</i>					
	N0	14.4(9.6)b	1.93(0.13)b	4.4(2.3)e	1.60(0.46)ns	16.79(4.73)b
	NO ₃ ⁻ -N80	6.4(3.3)e	1.65(0.15)d	6.6(2.8)d	2.54(0.60)ns	25.06(6.62)ab
	NO ₃ ⁻ -N200	12.4(5.7)d	1.64(0.09)e	27.8(13.2)b	1.82(0.47)ns	18.70(4.42)b
	NH ₄ ⁺ -N80	47.2(29.4)a	1.74(0.22)c	7.3(3.3)c	2.31(0.90)ns	24.08(8.50)ab
	NH ₄ ⁺ -N200	12.9(9.6)c	3.01(0.96)a	80.9(30.2)a	2.12(0.39)ns	29.31(4.16)a

High-P soil

The two tree species grow better under high-P soil with less nutrient stress (Fig. 1). The 80 kg N ha⁻¹ year⁻¹ was an N level evidently favorable to *S. superba*, despite its root growth and secretion decreasing by 13.0% and 30.0%, respectively, since its PAE and NAE remained higher than that of N0 (Tables 2 and 3). When the N level increased to 200 kg N ha⁻¹ year⁻¹, the roots grew fast and their secretion was also increased. The root growth of *P. massoniana* was increased by 62.2% under 80 kg N ha⁻¹ year⁻¹ but it decreased by 46.0% under 200 kg N ha⁻¹ year⁻¹ (Table 2).

Both root growth and activity were increased by NO₃⁻ to a lesser extent under high-P than low-P soil. Although the NO₃⁻-N200 increased the OX concentration 6.3-fold in *P. massoniana*, its PAE and NAE were lower and root activity weakened (Table 3). The NH₄⁺-N200 did not produce the same inhibition effect as the NO₃⁻-N200 in *P. massoniana*, instead increasing its SApase, OX, PAE, and NAE by 1.6-, 18.4-, 1.3-, and 1.7-fold compared with N0, respectively.

The relationship between root morphology, secretion, and absorption

The root PAE and NAE were always positive correlated with root morphology and secretion. However, under low-P soil and N addition treatment, the correlations of PAE and NAE with root morphological variables weakened, especially under the NH₄⁺ addition (Fig. 1). For example, the correlation coefficient of PAE with the root length of *S. superba* was $r = 0.22$ ($p = 0.38$) and $r = 0.30$ ($p = 0.23$), respectively, under the N0 and NO₃⁻-N80 treatments in low-P soil, yet it was $r = 0.02$ ($p = 0.94$) under NH₄⁺-N80. High-P soil significantly increased the correlation strength between the PAE and NAE with root morphological variables. The fine root ($D \leq 1.0$ mm) portion was significantly correlated with PAE and NAE, especially when the N level increased to N200. Under the N80 treatment in high-P soil, the PAE and NAE generally had weak relationships with root morphology. Compared to *S. superba*, both PAE and NAE were much more positively correlated with root secretion of *P. massoniana* in low-P soil, and the moderate N addition strengthened this relationship (Fig. 1).

We used these 15 root variables in a PCA, whose results showed that PC1 mainly encompassed the root morphological characteristics, while PC2 mainly represented root absorption, with PC3 corresponding to root secretion (Fig. 2). Phosphorus and species significantly influenced the relationship between root absorption and root morphology (Fig. 2B). At a higher P level, the roots grew faster and absorbed more N and P, with the root quantity higher in *S. superba* than in *P. massoniana*. However, the root absorption was stronger in *P. massoniana* than *S. superba*. The root growth and absorption of *S. superba* responded positively to N addition, whereas *P. massoniana* only performed positively under a moderate N level (N80) and N form, which depended on the P level (Fig. 2B). The relationship between root absorption and secretion was more complex and influenced more by N addition (Fig. 2C). At a higher P level, moderate N addition could promote root secretion and absorption in *P. massoniana*; however, root secretion was poor in *S. superba*. Although a higher N addition, especially NH₄⁺, promoted root secretion in *P. massoniana* this did not increase its root absorption, which remained similar to that in low-P soil (Fig. 2C).

Discussion

The trees *S. superba* and *P. massoniana* are both well known for their ability to grow and thrive under adverse environmental conditions, especially low-P soil. Research has shown that *S. superba* forms a fibrous root system and *P. massoniana* forms a tap root system^[27-28]. Our previous study demonstrated that *P. massoniana* was more suited to low-P soil than *S. superba*^[28]. In the present study, we found the root phenotypic and physiological characteristics of *P. massoniana* had significantly lower values than those of *S. superba*; however, the PAE and NAE of *P. massoniana* significantly exceeded that of *S. superba* (Tables 2 and 3). This may be due to the ectomycorrhizal fungi and molecular mechanism of higher P efficiency *in vivo* of *P. massoniana*^[1]. Work by Zhang et al. revealed four members of the Pht1 phosphate transporter protein family encoding phosphate transporters in Masson pine (PmPTs), whose up-regulated genes could improve P nutrition^[31]. By contrast, *S. superba* survives in barren soil by increasing its root quantity and exudates to promote both its PAE and NAE^[11].

Nitrogen addition led to the coarsening of roots and a greater root surface area (Table 2). For example, compared with N0, the average AD and SA increased 4.4% and 19.6% in *S. superba* and 5.4% and 58.2% in *P. massoniana*, respectively, when N was added. The thickening and increasing surface area of roots was more pronounced in *P. massoniana*, driven by roots with a diameter over 1.0 mm (Fig. 3). With an increasing N level, the $D = 0-0.5$ mm roots grew fast in *P. massoniana*, and their N absorption was correspondingly higher (growth ratio of PAE, GRP_{*P. massoniana*} = 80.8%, GRN_{*S. superba*} = 101.8%; growth ratio of NAE, GRN_{*P. massoniana*} = 71.1%, GRN_{*S. superba*} = 50.7%) when compared with N0; however, the $D > 0.5$ mm roots grew faster in *S. superba* and their P absorption was higher. This pattern in the results indicated that N addition promotes fine root growth and their absorption of elements depends on tree species identity. Although the rate of root growth was not faster in *S. superba* than in *P. massoniana*, the former's nutrient absorption was increased and the N addition enhanced its P utilization^[1]. The fine root fraction differed between the two species, in that for the 0-0.5 mm root, the proportion was 74.9-80.9%, with an average of 77.9% in *S. superba*, whereas it was 68.6-83.7%, with an average of 73.4% in *P. massoniana* (Table 2, Fig. 3). By contrast, for the 0-1.5 mm range roots, their proportions were stable overall (*S. superba*: 96.4-98.7%, 97.6%; *P. massoniana*: 96.6-99.3%, 97.8%). This was mainly affected by different N forms and we describe this situation using a root growth model for the two tree species under low-P soil conditions when N is added (Fig. 4). Our results suggested NH₄⁺ could affect the relationship between the > 1.5 mm roots and nutrient absorption in *S. superba*, whereas the relationship between > 0.5 mm roots and nutrient absorption was affected by NH₄⁺ in *P. massoniana*, whose height growth aboveground was much more apparent (Table 4, Fig. 4). Compared with NH₄⁺, we find that NO₃⁻ had an opposite effect on the relationship between fine roots and absorption, and the stem diameter growth aboveground was more notable^[28]. We speculate that NH₄⁺ could promote thicker root activity and that NO₃⁻ may have stimulated the thinner root activity in the fibrous root plants with a larger quantity of roots, whereas NO₃⁻ and NH₄⁺ have the opposite function in a tap root system with slender roots.

Table 4
Pearson correlation coefficients between the fine root ratio and nutrient absorption in *Schima superba* and *Pinus massoniana*.

		0.0–0.5 mm	0.5–1.0 mm	1.0–1.5 mm	>1.5 mm	<1.5 mm
<i>S. superba</i>						
NH ₄ ⁺	PAE	-0.379	0.004	0.138	0.702	-0.811*
	NAE	-0.373	-0.003	0.139	0.699	-0.810*
NO ₃ ⁻	PAE	-0.775	0.601	0.670	0.810*	-0.824*
	NAE	-0.703	0.497	0.613	0.847*	-0.868*
<i>P. massoniana</i>						
NH ₄ ⁺	PAE	-0.703	0.620	0.574	0.854*	-0.875*
	NAE	-0.671	0.574	0.581	0.866*	-0.866*
NO ₃ ⁻	PAE	-0.369	0.060	0.324	0.845*	-0.741
	NAE	-0.372	0.056	0.333	0.856*	-0.754

There was a negative relationship between the growth of roots 0–0.5 mm in diameter and both PAE and NAE after the N addition in both species. The roots consume over 50% carbon fixation on root consumption, including new root growth, ion uptake and transport, and maintenance processes, for which the maintenance of respiration is linearly related to root fresh weight¹¹. When *S. superba* is growing in its preferred P and N environment, it decreases its proportion of 0–0.5 mm roots in the root system and remains in a low-consumption mode^[8,11,34-]. For *P. massoniana*, this negative relationship was likely caused by abiotic stress and the death of the 0–0.5 mm roots (Table 2).

The root-secreted acid phosphatase and other organic acids may promote P absorption, and they may also influence the roots' physiological activity. Since oxalic acid is the most abundant organic acid in the two tree species, we chose to study this acid herein. N addition increased the acid phosphatase and oxalic acid secretion in *P. massoniana*, and this may be an important reason for why this plant's PAE was enhanced. The *S. superba* plants use oxalic acid for main auxiliary absorption (data not published), and only the highest N level addition was able to augment its secretion (Table 3). This result suggested that *S. superba* was mainly through the active absorption combined with root morphological changes.

Conclusions

Increasing the N and P concentrations in soil promotes the root growth and secretion in young *P. massoniana* and *S. superba*. The root quantity was larger in *S. superba* but the root growth rate and absorption were stronger in *P. massoniana*. The root growth and absorption of *S. superba* are better suited to N addition, while *P. massoniana* fares better under moderate N addition. Different N forms influenced the relationships between fine root proportion and both PAE and NAE. NH₄⁺ promoted thicker root (> 1.5 mm) activity whereas NO₃⁻ stimulated thinner root (0.5–1.5 mm) activity in the fibrous root system plants with a larger quantity of roots. By contrast, NO₃⁻ and NH₄⁺ have opposite effects on a tap root system with slender roots. The results revealed contrasting effects of different nitrogen forms on the root growth between conifers and broad-leaved trees species, which has implications for their forest operation and management.

Methods

Experimental conditions

Seeds of *S. superba* and *P. massoniana* were collected from seedling station, who undertook the formal indentation and deposition, in Zhejiang province, China. The experiment research was complied with institutional and national guidelines. All field studies were performed in accordance with the local legislation in China and complied with the convention on the trade in endangered species of Wild Fauna and Flora. The experiment and sampling undertaken in this study were conducted in our long-term cooperation base, which was National Masson Pine base of Laoshan Forest Farm in Zhejiang province, China. They gave us the permissions on arranging the experiment and sampling.

The simulation experiment was conducted as described in Zhang et al., whose design consisted of two species (*S. superba* and *P. massoniana*), crossed with two levels of soil P (low-P = 1.1 and high-P = 25 mg P.kg⁻¹), three levels of N (N0 = 0, N80 = 80, and N200 = 200 kg N ha⁻¹ year⁻¹), and two forms of N (NH₄⁺ and NO₃⁻)^[28]. Seeds of *S. superba* and *P. massoniana* were pregerminated on a seedbed containing normal forest soil (on 20 March 2013). About a month later, when the seedlings were 2–3 cm tall, two seedlings per container were transplanted into plastic containers (18 cm in diameter, 25 cm tall; on 20 April 2013). These seedlings grew in a greenhouse without climate control in Zhejiang Province, China, where they were watered every other day. Only one seedling was retained in each container, to ensure a one seedling/container planting density throughout the experiment. With 12 replicates per treatment combination, this experiment used a total of n = 240 individual plants (2 levels of P · 2 species · 5 tests for each N form · 12 replicates).

The added N, NH₄Cl, and NaNO₃, was sprayed into each treatment plot (1.1 m · 0.9 m, 24 containers per plot) once a month, for 6 months, beginning when the leaves had started to bud. All seedlings were watered with distilled water until their harvest during the first week of November 2013. Pests and weeds were controlled manually.

Measurements

At their harvest (mid November 2013), six plants from each treatment were randomly selected and the biomass of their roots, stems, and leaves was determined [28]. The roots were carefully separated from the soil and rinsed with water for later measurements, and these seedlings from each treatment were placed in a black-out flask container with 100 ml of deionized water. After 12 h, the root exudates were collected and vacuum concentrated at 40 °C using a Rotavapor (RV10, IKA, Germany); these solutions were used for HPLC (High Performance Liquid Chromatography) to determine each plant sample's organic acid contents. This HPLC analysis was carried out using an Atlantis dC18 column (4.6 mm · 250 mm; 5 µm) (Atlantis C18, Waters, USA), for which the parameters were set as follows: column temperature, 35 °C; sample size, 30 µl; working flow rate, 1 ml/min; detector, PAD; wavelength, 210 nm; mobile phase, diammonium hydrogen phosphate (pH = 2.4) (GB/T5009.157-2003). The root- and exudates-acid phosphatase (Apase) activity was measured using the methods of McLanchlan [1]. Briefly, 0.4 g of fresh root was ground in 5 ml of ice-distilled water, then centrifuged at 10000 · g at 4 °C for 15 min, from which 0.1-ml supernatant (or 2-ml exudate solution) was mixed with 3 ml of 0.5 g L⁻¹ 4-nitrophenyl phosphate disodium salt hexahydrate, and 37 °C dark-reacted for 30 min (or 2 h for exudates-APase activity). To end the reaction, 1 ml of 2 mol L⁻¹ NaOH was applied, then the sample's OD value was recorded at 405 nm.

Root images were acquired using a scanner. Morphological and architectural parameters of the roots, such as root length and root volume, were quantified using computer image analysis software (WinRhizo Pro STD1600⁺, Regent Inc., Canada). The plant samples were dried at 105 °C for 1 h, and then at 80 °C for 48 h, after which they were cooled in a desiccator filled with silicon dioxide gel until they reached a constant mass, which was recorded as the biomass. Total nitrogen and phosphorous content were determined using the standard Kjeldahl method [1] and the Mo-Sb antispectrophotography method [1], respectively.

Statistical analysis

Six of the twelve plants from each treatment were randomly selected for determining root length (RL), surface area (SA), average diameter (AD), volume (RV), tips, forks, length classification separate by diameter (0.0 < L ≤ 0.5, 0.5 < L ≤ 1.0, 1.0 < L ≤ 1.5, 1.5 < L), PAE (Phosphorus acquisition efficiency), NAE (Nitrogen acquisition efficiency), root acid phosphatase (RAPase), secretion acid phosphatase (SAPase), and oxalic acid (OX) variables. The data were analyzed using the SAS statistical program (v8, SAS Institute, Cary, NC, USA). All variables were normally distributed and did not require any transformation. Three-way ANOVA was performed at a significance level of 0.05, with N form and N levels as the independent factors. Next, all 15 variables' data were standardized by the Z-score and run in a PCA (principal component analysis) implemented in SPSS (PASW Statistics v18, Polar Engineering and Consulting, USA). Tukey's HSD test was used to determine whether significant (*P* < 0.05) differences in means existed between the treatments.

Abbreviations

AD: average diameter; Apase: acid phosphatase; HPLC: High Performance Liquid Chromatography; NAE: Nitrogen acquisition efficiency; OX: oxalic acid; PAE: Phosphorus acquisition efficiency; PCA: principal component analysis; RAPase: root acid phosphatase; RL: root length; RV: root volume; SA: surface area; SAPase: secretion acid phosphatase.

Declarations

Ethics approval and consent to participate

The experiment research was complied with institutional and national guidelines. All field studies were performed in accordance with the local legislation in China and complied with the convention on the trade in endangered species of Wild Fauna and Flora.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The plant specimens used in our study are not an endangered species.

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Authors' contributions

RZ and ZZ conceived and designed the study. RZ and YW performed the experiments. RZ wrote the paper. RZ and ZZ reviewed and edited the manuscript. All authors read and approved the manuscript.

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Figures

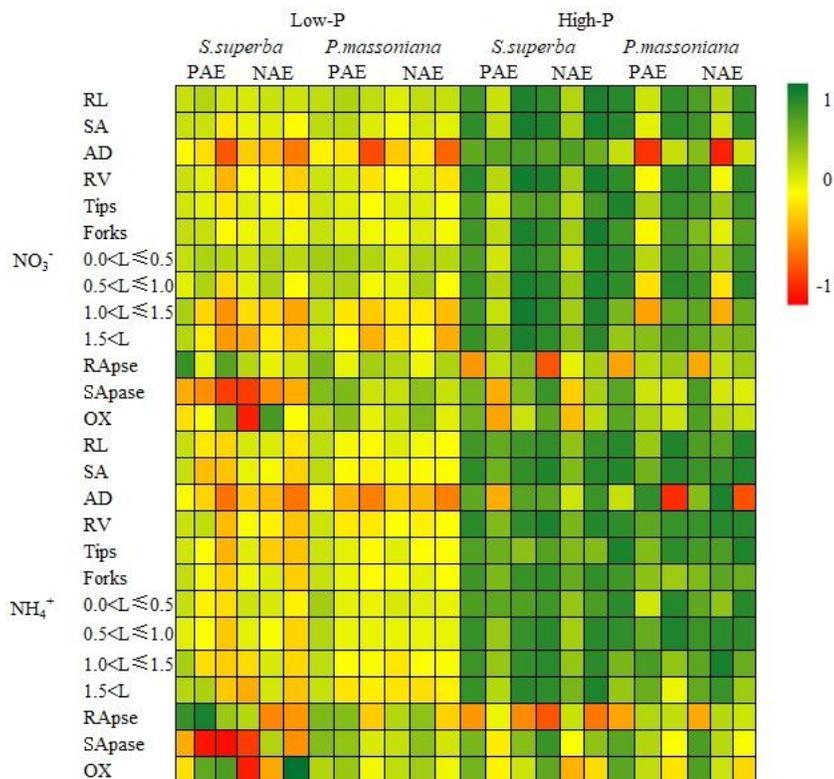


Figure 1

Heat map of the correlation index between phosphorus acquisition efficiency (PAE), nitrogen acquisition efficiency (NAE), and root morphology and secretion of the two studied tree species (*Schima superba* and *Pinus massoniana*).

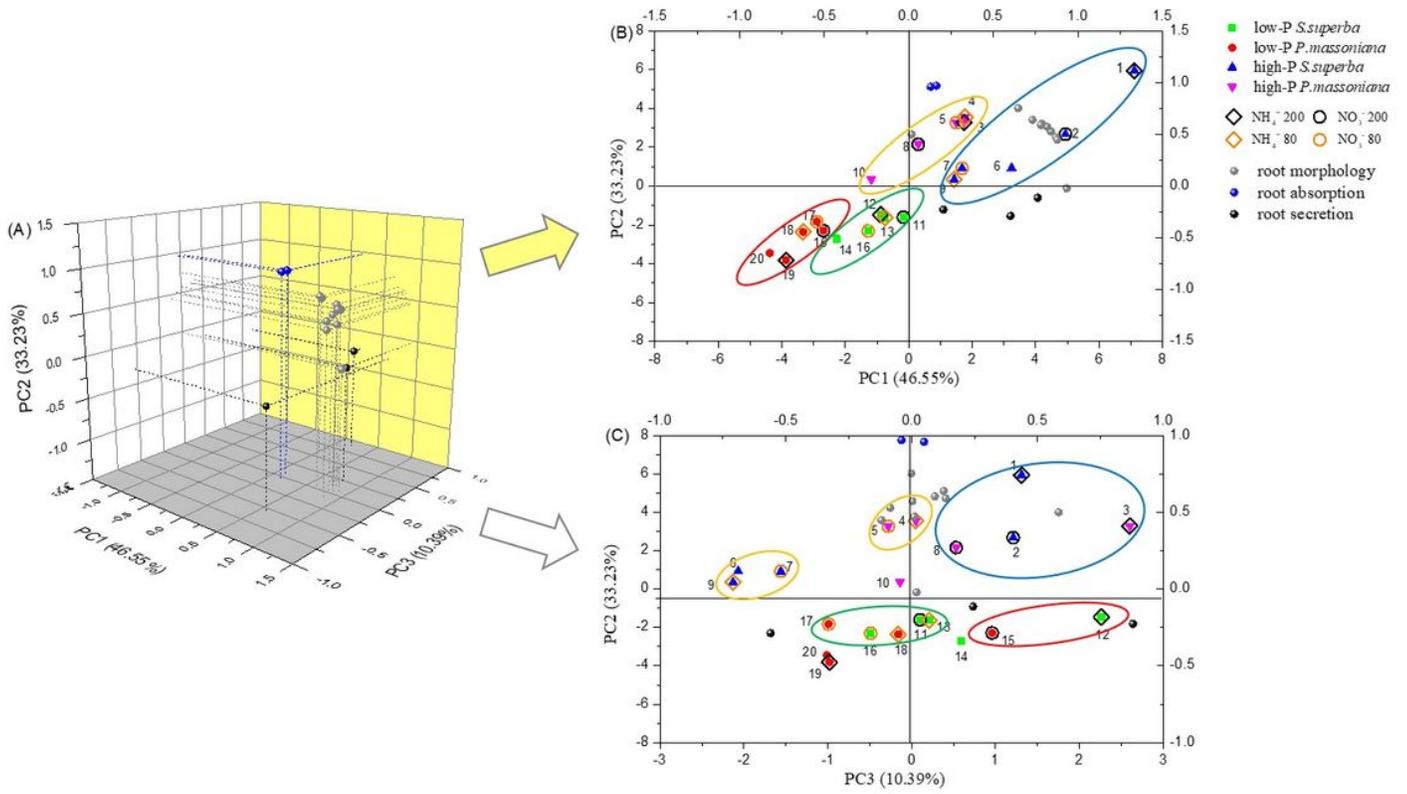


Figure 2
 PCA (principal component analysis) results based on the 15 root variables (listed in Table 1) of the two studied tree species (*Schima superba* and *Pinus massoniana*). Left: 3-D loadings of the rotated component (A); right: 2-D biplots on PC1–PC2 (B) and PC2–PC3; (C) plane showing the overlapping scores' loadings.

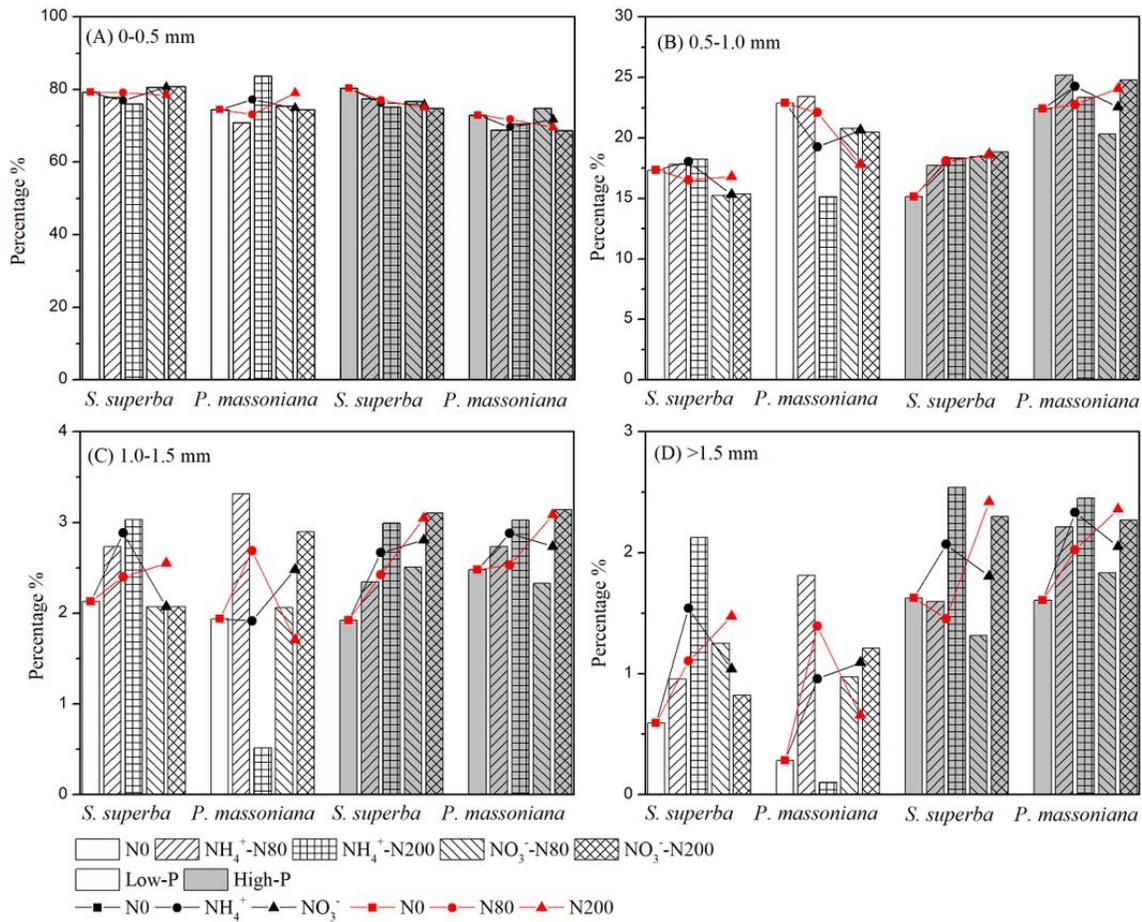


Figure 3
 Effects of nitrogen (N) addition on seedling fine root proportion of the two studied tree species (*Schima superba* and *Pinus massoniana*). The proportion of root length for roots (A) with a diameter of 0–0.5 mm; (B) with a diameter of 0.5–1.0 mm; (C) with a diameter of 1.0–1.5 mm; (D) with a diameter over 1.5 mm. The column is a percentage value, calculated as fine root length divided by total root length. The black dots are average values under the N forms added; the red dots are average value under the N levels used.

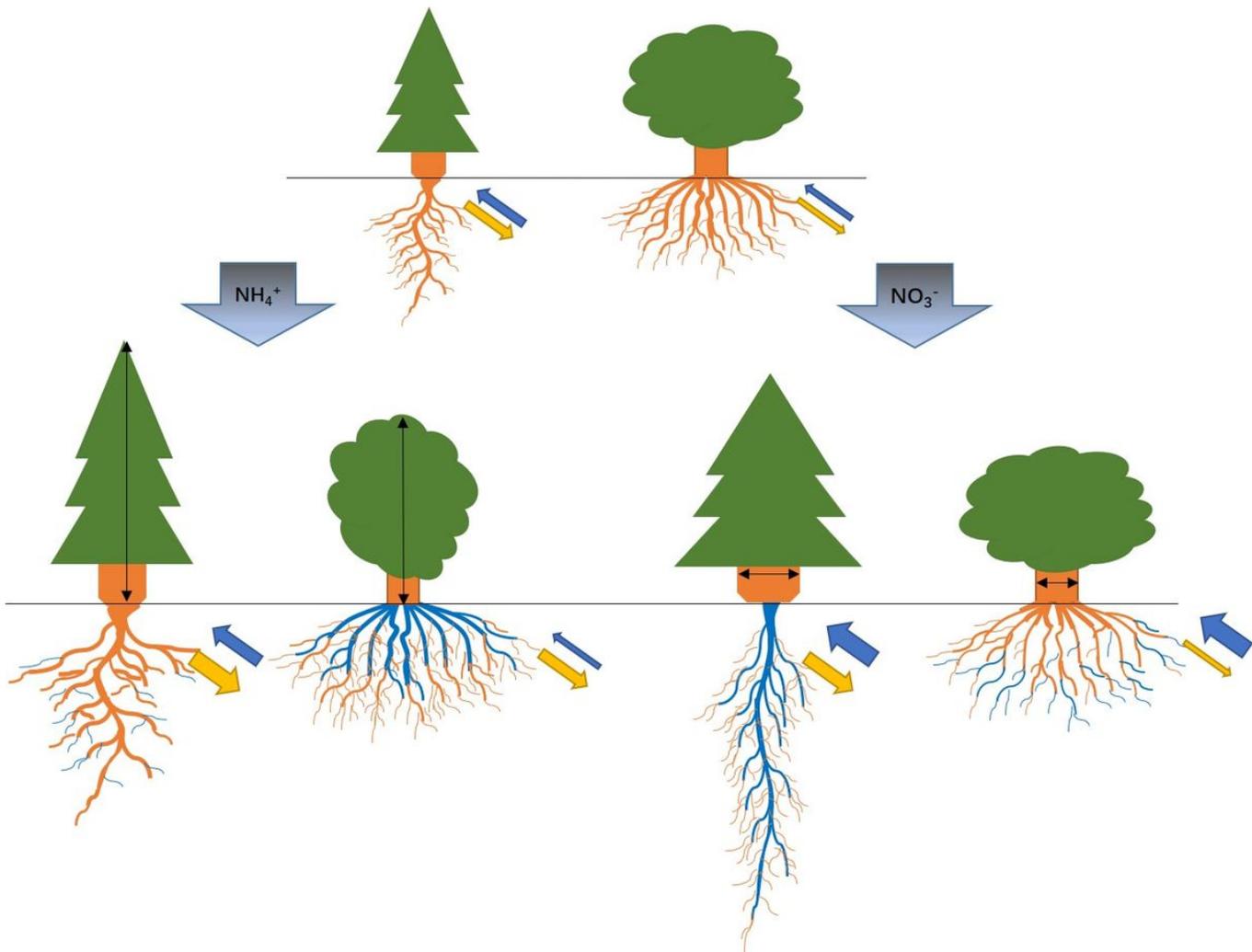


Figure 4

Growth model of the two studied tree species (*Schima superba* and *Pinus massoniana*) under nitrogen (N) deposition. Blue arrow is nutrient absorption; yellow arrow is the released secretion; an arrow's thickness indicates its strength; an orange root is the original one, while a blue root is the one strong correlated with absorption under N deposition. The NH_4^+ deposition led to thicker roots of both species but a large quantity of *S. superba* roots; small (0–0.5 mm diameter) roots contribute more to nutrient absorption in *P. massoniana* while large (>1.5 mm diameter) roots contribute more to nutrient absorption in *S. superba*. The NO_3^- deposition led to thinner roots in the two species and large quantity of *P. massoniana* roots; large (>1.5 mm diameter) roots contribute more to nutrient absorption in *P. massoniana* whereas the small (0–0.5 mm diameter) roots contribute more to nutrient absorption in *S. superba*. The black double arrow indicates the aboveground growth state. The NH_4^+ deposition influences vertical growth and the NO_3^- deposition influence radial growth in the trees.