



Full Length Article

Short-term Nitrogen (N)-Retranslocation within *Larix olgensis* Seedlings is Driven to Increase by N-deposition: Evidence from a Simulated ¹⁵N Experiment in Northeast China

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Abstract

Urban areas are key sources of reactive atmospheric nitrogen (N). Urban forests are moving towards a state of “N-saturation” due to N-deposition. However, the possible future-trend of inherent N-cycling in trees in response to urbanization-related N-deposition is not well known. With regard to the contradiction of current studies on the exogenous N-addition on endogenous N-retranslocation (NR) in the month- or season-long term, we treated 15-month old Changbai larch (*Larix olgensis* Henry.) seedlings with ¹⁵N-labeled ammonium sulfate supplied at rates of 0 (control), 30 (F30), 105 (F105), and 315 (F315) mg ¹⁵N seedling⁻¹ to simulate N-deposition at the accumulatively future amounts driven by urbanization. Seedlings were harvested nine days after treatment to determine their response of NR. Results revealed that N-deposition had a significant short-term effect on N-derived from plant (NDFP) in needles, wherein NDFP rose with the increase of ¹⁵N-deposition intensity. In fine root (in diameter of 0-1 mm) NDFP in the F315 treatment was statistically higher than that in other treatments. There was a mean amount of 1.26 mg of N-retranslocated into needles, which accounted for 19% of total N-content. Results in the present study, together with the other ones we summarized, could be concluded that short-term NR in Changbai larch tended to increase in response to urbanization-related N-deposition. © 2014 Friends Science Publishers

Keywords: Urbanization; Urban-to-rural gradient; Fine root; N-remobilization; ¹⁵Nitrogen

Introduction

Urbanization is accelerating worldwide (Chen *et al.*, 2010). The cities are expected to grow 2.5 times in area by 2030, consuming some 1 million square kilometers, or 1.1% of the total land area of countries (Angel *et al.*, 2005). Urban areas are key sources of reactive atmospheric nitrogen (N) and experience enhanced deposition from the by-products of fossil fuel combustion (Rao *et al.*, 2013). In urban and suburban areas, forests are moving rapidly towards a state of “N-saturation” due to much greater wet and dry atmospheric N-deposition than rural areas (Chen *et al.* 2010). As a response, a series of changes have been determined, such as higher litter N concentration (Nikula *et al.*, 2010), greater microbial biomass N content (Chen *et al.*, 2010), and increased N-mineralization and N-nitrification (Baxter *et al.*, 2002; Chen *et al.*, 2010; Cusack, 2013), greater foliar N-accumulation and growth of trees (Chen *et al.*, 2010; Kuang *et al.*, 2011; O’Brien *et al.*, 2012; Searle *et al.*, 2012; Cusack, 2013; Liu *et al.*, 2013). However, it still reminds unclear enough about the possible future-trend of stored N-cycling within trees in response to probably increased urbanization-related N-deposition.

Nitrogen demand for tree growth is met by not only exogenous N-uptake but also endogenous N-retranslocation (NR) (Bazot *et al.*, 2013; Rehman *et al.*, 2013). The former influences new organ growth and the latter is critical for meeting initial sink demands of newly planted plants (Wei *et al.*, 2012a). As an exogenous N-source, N-deposition may have a null effect on NR in trees because NR was concluded to be driven by N-storage but not current N-supply (Millard and Grelet, 2010). In contrast, some findings disproved this viewpoint (Salifu and Timmer, 2003; Ueda *et al.*, 2012; Wei *et al.*, 2012a). In response to N-deposition, although results of increased exogenous N concentrated in tree leaves suggested the weakened retranslocating demand of stored N for growth (Chen *et al.*, 2010; Kuang *et al.*, 2011), direct evidence of determination of NR is quite insufficient, which hinders us to predict future response of inherent N-cycling within trees to urbanization-related N-deposition.

In order to better detect the direct impact of N-deposition on NR, trees may probably need to be examined in a short-term because the month-long or season-long term effect of exogenous N addition on endogenous N-cycling may be interfered by significant soil N-leaching (Xiong *et al.*, 2010) and gross N-cycling (Ueda *et al.*, 2012).

In a short term, e.g. within 24 h, although photosynthetic and growth may not show any evident changes, exogenous N-addition can cause significant influence on endogenous N-cycling because synthesis of proteins from inorganic N occurs when internal N-cycling has been triggered (Warren *et al.*, 2003). The short-term N-addition could not be absorbed thoroughly by tree seedling roots, therefore results of N-derived from plant (NDFP) in seedling organs in pot-culture experiments may be under estimated (Warran *et al.*, 2003; Metcalfe *et al.*, 2011). The short-term response of forest system to N-deposition are drawing more and more research attention in recent years (Savva and Berninger, 2010; Tu *et al.*, 2011; Nave and Curtis, 2011; Rossi *et al.*, 2013). Considering that most studies referred to by Millard and Grelet (2010) and relevantly others (Zein *et al.*, 2011; Ueda, 2012; Jordan *et al.*, 2012; Bazot *et al.*, 2013) were performed in a longer term, a study about short-term response of inherent N-cycling in trees may replenish or even disprove current conclusions.

In China, fast economic growth has contributed to rapidly increasing emission of sulfur dioxide (SO₂) and N-oxides (NO_x) since the 1980s when the open-door policy and economic reforms were implemented (Kuang *et al.*, 2011). China and other economies are facing a continuing challenge to reduce emissions of reactive-N, N-deposition and their negative effects on human health and the environment (Liu *et al.*, 2013). In the present study, we determined the effect of simulated N-deposition on NR in 15-month-old Changbai larch (*Larix lgensis* Henry) seedlings in a semi-controlled environment in a short term during summer in Northeast China. Two questions require resolutions: (i) will N-retranslocation within Changbai larch seedlings respond to N-deposition in a short term, and (ii) will N-retranslocation increase or decrease along a N-deposition gradient? The resolutions of these two questions are necessary to better understand the future trend of N-cycling in forests affected by urbanization related N-depositions. Results of this study will supply Chinese government with theoretical evidence when making the policy to cope with N-deposition, and the contribution to the mechanism of short-term relationship between exogenous N and NR would supply an international scope for better understanding the possible future-trend of N-cycling in trees in response to urbanization-related N-deposition.

Materials and Methods

Study Site and Plant Materials

Study site: The study was conducted in Jiangmifeng nursery (43°45'N, 126°45'E), Jilin City, Jilin Province, Northeast China (Fig. 1), which locates at the suburban side of Jilin City and was undergoing urbanization. Locally annual precipitation is 650-750mm with summer rainfall accounts for 60% of the annual precipitation. The annual air temperature is 3-5°C, with a mean early growing season

temperature of 4-9°C. The texture of soils from 0-20cm in depth was a sandy loam with 55 % sand, 30% silt, and 15% clay. Average soil pH was 6.30, NH₄⁺-N was 6.93 mg kg⁻¹, NO₃⁻-N was 10.35 mg kg⁻¹. More soil properties and specific determination methods could be found in Wei *et al.* (2012a, b) and Duan *et al.* (2013).

Plant materials: Changbai larch (*Larix lgensis* Henry) is a typical larch species locally in temperate China, which had a significant response of NR to exogenous N-supply in a month-long term during summer (Wei *et al.*, 2012a). Changbai larch seedlings were chosen as materials because they can easily fulfill the determination at the whole-tree level and they can be comparable with results of other relevant studies employing tree seedlings as materials (Warren *et al.*, 2003; O'Brien *et al.*, 2012; Searle *et al.*, 2012; Ueda, 2012). Seeds of Changbai larch were collected by employees of Xiaobeihu Forest Station (44°03' N, 128°28' E) in a local wild stand in Heilongjiang Province, Northeast China. After collection, seeds were transported to the nursery and stored at 0-4°C, where they were soaked in 5% potassium permanganate (W/W) solution for 24 h and stratified for 5 d at 0-4°C, then on 3 May 2009, sowed at a density of 700 seeds m⁻² in a nursery bed. In mid June 2009, germinated seedlings were thinned to about 550 seedlings m⁻². On 12 October 2009, seedlings were harvested then moved into cold storage until 13 April 2010, when over-winter seedlings were transplanted into another nursery beds. More specific details about nursery bed shaping, seedling culture, and transplanting manipulation can be found in Wei *et al.* (2012a, b), Duan *et al.* (2013), and Wei *et al.* (2013).

On 18 July 2009, forty seedlings with a uniform size were chosen from nursery beds. Eight seedlings chosen from them were measured for their initial height and root collar diameter, which were 13.4 cm and 2.6 mm, respectively. After washing the roots free from soils, another thirty-two seedlings were planted into pots (top diameter × height × bottom diameter: 18 cm × 18 cm × 16 cm) filled with acid washed sand. One pot contained one seedling. All pots were placed on the open-air ground in the nursery at 1m × 1m spacing which is the planting density for local afforestation of Changbai larch (Duan *et al.*, 2013).

Simulated ¹⁵N Deposition Treatment

Nitrogen deposition was simulated by supplying ¹⁵N-labeled solutions to Changbai larch to be distinguished from pre-experiment N-storage within them. Distilled-water-solute ammonium sulfate [(NH₄)₂SO₄] was labeled with N-enriched to 10.11 atom% ¹⁵N (SRICI, Shanghai, China), and supplied at rates of 0 (control), 30 (F30), 105 (F105), and 315 (F315) mg ¹⁵N seedling⁻¹ delivered as two solution applications on 25 July and 1 August 2010. N was labeled by ¹⁵N to be distinguished from the stored N. Ammonium sulfate was chosen to simulate urbanization-related deposition because in China N from ammonium is the

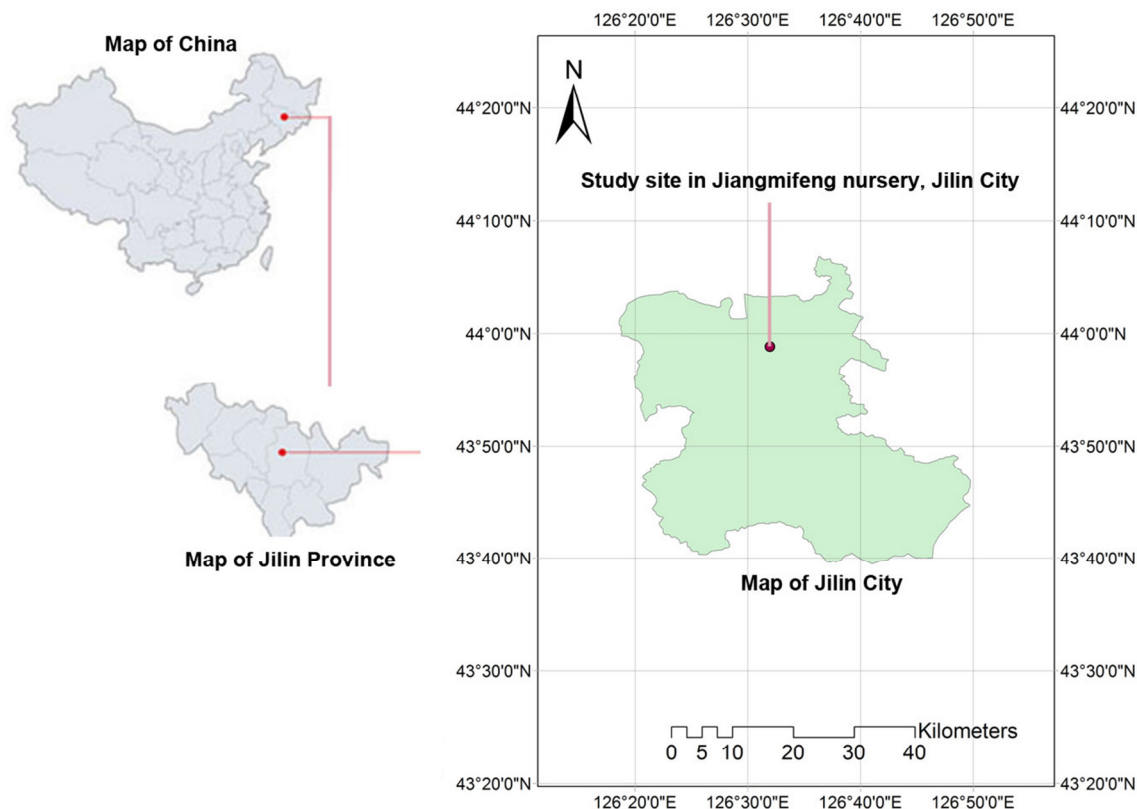


Fig. 1: Study site in Jiangmifeng Nursery, Jilin City, Northeast China

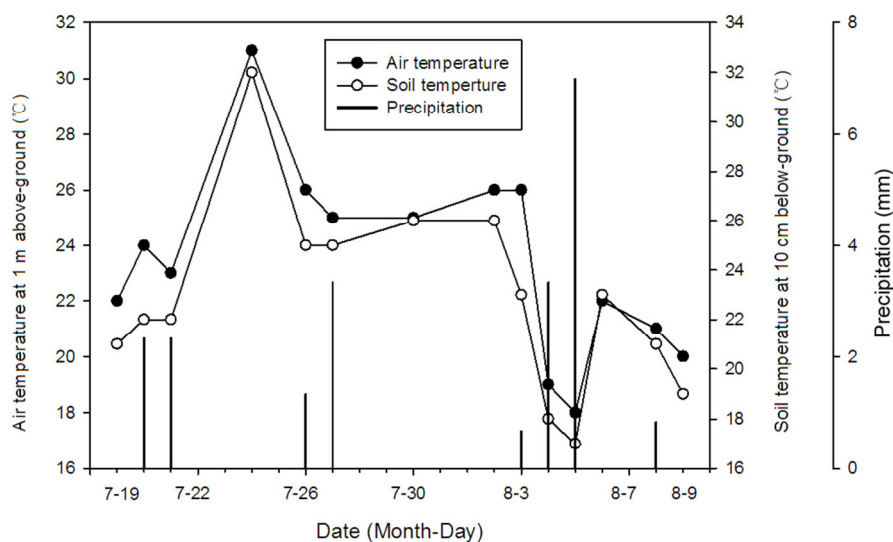


Fig. 2: Environment conditions during the experiment

dominant form of N in bulk deposition (Liu *et al.*, 2013) and fast economy development has resulted in increasing emission of sulfur dioxide (Kuang *et al.*, 2011). The method of irrigating with N-enriched solutions to simulate N-deposition employed by Tu *et al.* (2011) and Metcalfe *et al.*

(2011) were followed. Because pots containing seedlings were placed at 1 m × 1 m spacing (see above), each of potted seedlings in the control treatment received deposited N to the area of 0.001 m². In Northeast China, annual N-deposition was evaluated to be 7.5-14.5 kg N ha⁻¹ (Song and

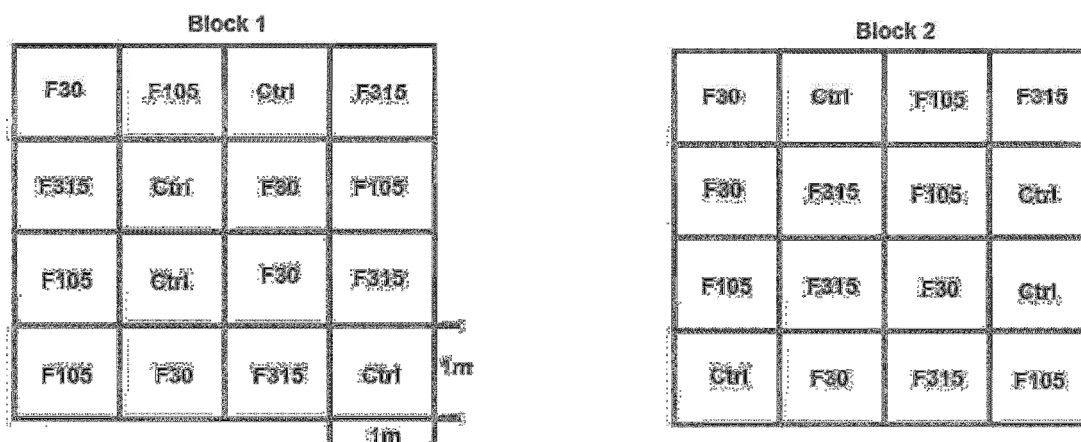


Fig. 3: Experiment design of simulated ¹⁵N-deposition treatments at rates of 0 (Ctrl), 30 (F30), 105 (F105), and 315 mg ¹⁵N seedling⁻¹ (F315) distributed into two blocks located in Jiangmifeng Nursery, Jilin City, Northeast China. Within each block, one treatment was replicated for 4 pots. Each pot contained one 15-month-old Changbai larch (*Larix olgensis* Henry) seedlings as an experimental unit. All pots were randomly placed at 1m × 1m spacing within each of the blocks. Therefore, there were totally eight replicates for each treatment

Liu, 2013; Yu *et al.*, 2011), therefore simulated N-deposition level in the F30 treatment was comparable to the sum of N-deposition in the future 20 years, and that in the F105 and F315 treatments were performed to study the response of newly planted Changbai larch seedlings to N-deposition that may be driven by urbanization locally in the future. N-depositions were performed in sunny days during the experiment to avoid being diminished by rains. Weather conditions during the experiment are shown in Fig. 2. Only the soils were irrigated to avoid ¹⁵N contamination of shoots (Warren *et al.*, 2003).

Seedling Harvest and N-determination

On 10 August 2010, when seedlings had nearly finished the primary shoot extension, all seedlings were harvested and transported on ice to the Laboratory, where seedling roots were washed free from sand, and then seedlings were sliced at cotyledon scars and divided into partitions of shoot and root. Shoots were divided into needles, current stems (light-green and soft stem), and old stems (dark-brown and woody stems). Roots were divided into coarse (>2 mm in diameter), medium (1-2 mm in diameter), and fine (<1 mm in diameter) roots. Root dry mass was measured after oven dried for 48 h at 70°C. Dried plant samples were ground with a Wiley mill and determined for ¹⁵N-abundance using a FLASH 2000 NC Analyzer (EA 1112 HT-MAT253, Thermo Scientific®, U.S.A.). Total N-content was determined by an automatic N-analyzer (UDK 152 automatic N-analyzer, VELP Co., Usmate [MB], Italy), as described by Wei *et al.* 2012 (b) and Duan *et al.* (2013).

Statistical Calculation and Analysis

The ¹⁵N atom % was calculated from:

$$\delta^{15}N = \frac{{}^{15}\text{Natom}\%_{\text{sample}} - {}^{15}\text{Natom}\%_{\text{std.}}}{{}^{15}\text{Natom}\%_{\text{std.}}} \quad (1)$$

Where $\delta^{15}N$ is the ¹⁵N ratio of sample, ¹⁵Natom %_{treatment} and ¹⁵N atom %_{std.} are the N isotope ratios of the treated sample and standard (0.366, adapted by Warren *et al.*, 2003). N derived from fertilizer (NDFP) was calculated from the equation (Warren *et al.*, 2003):

$$\%NDFP = \frac{{}^{15}\text{Natom}\%_{\text{treatment}} - {}^{15}\text{Natom}\%_{\text{control}}}{{}^{15}\text{Natom}\%_{\text{labeled fertilizer}} - {}^{15}\text{Natom}\%_{\text{std.}}} \times 100\% \quad (2)$$

Where, ¹⁵N atom%_{control} is ¹⁵N atom % in controlled sample. ¹⁵N atom%_{labeled fertilizer} is ¹⁵N atom% in (¹⁵NH₄)₂SO₄ of 10.11. Amount (mg) of NDFP is calculated as:

$$NDFP = \%NDFP \times TN_{\text{final}} \quad (3)$$

Where TN_{final} is total N-content (mg) in seedling organs finally after ¹⁵N-deposition treatment (determined on seedlings sampled on 10 August 2010). Amount (mg) of N-derived from plant (NDFP) was calculated as:

$$NDFP = TN_{\text{final}} - NDFP - TN_{\text{initial}} \quad (4)$$

Where TN_{initial} is total N-content (mg) in seedling organs initially before ¹⁵N deposition treatment (determined on seedlings sampled on 18 July 2010). % NDFP is calculated as:

$$\%NDFP = \frac{NDFP}{TN} \times 100\% \quad (5)$$

All data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Tests for normality and constant variance were performed and no transformations were necessary. The experiment was conducted as a random unit design with half units for one treatment separated into either block. One seedling was a unit. An one-way analysis of variance (one-way ANOVA) was performed for each of the

seedling organ components of needles, current stem, old stem, coarse root, medium root, and fine roots to detect the significance of effects of ^{15}N -deposition treatments on total N-content (TN), NDFP and NDFP wherein. Another one-way ANOVA was performed to compare the difference among the seedling organ components. When the effects were indicated to be significant by ANOVA through the general linear model (GLM), means ($\pm\text{SE}$) were ranked according to Tukey's student zed range test at $\alpha = 0.05$.

Results

Response of Biomass in Seedling Organs to ^{15}N -deposition

On 18 July 2010, initial biomass was greatest in needles and least in current stem and medium root (Table 1). On 10 August 2010, the after-treatment biomass followed the similar gradient among organs like the ones before treatment, but net biomass increment was greatest in coarse root. Although the value of net biomass increment in medium root was negative, it did not differ from others statistically (Table 1). Simulated ^{15}N -deposition affected biomass in organs of current stem (Fig. 4), wherein the F30 and F105 treatments resulted in greater biomass compared to the control.

Response of TN_{final} and NDFP in Seedling Organs to ^{15}N -deposition

Simulated N-deposition had a significant effect on both TN_{final} and NDFP in organs of Changbai larch seedlings ($P < 0.0001$). Both TN_{final} and NDFP increased with the rate of ^{15}N -deposition treatment (Fig. 4). In needles, old stem and fine root, the F315 treatment resulted in greater TN_{final} than the control treatment ($P = 0.0025$, $P = 0.0215$, $P < 0.0001$, respectively). Seedlings in the F315 treatment had greater NDFP than those in the control treatment in all seedling organs (Needles, $P < 0.0001$; Current stem, $P = 0.0153$; Old stem, $P < 0.0001$; Coarse root, $P < 0.0001$; Medium root, $P = 0.0004$; Fine root, $P < 0.0001$). In coarse root and fine root, the F105 treatment resulted in greater NDFP than the control treatment.

Both TN_{final} and NDFP were different among seedling organs (Fig. 5). Either TN_{final} or NDFP was highest in needles or fine root ($P < 0.0001$, $P = 0.0082$, respectively). TN_{final} was greater in old stem than in current stem and medium root, but no difference was detected for NDFP in seedling organs except needles and fine root.

Response of NDFP in seedling organs to ^{15}N -deposition

^{15}N -deposition did not affect NDFP in current stem ($P = 0.5909$), old stem ($P = 0.1361$), coarse root ($P = 0.7468$), and medium root ($P = 0.1929$) (Table 2). However, ^{15}N -deposition had a significant effect on NDFP in needles ($P = 0.0332$) and fine root ($P = 0.0002$), and needle NDFP

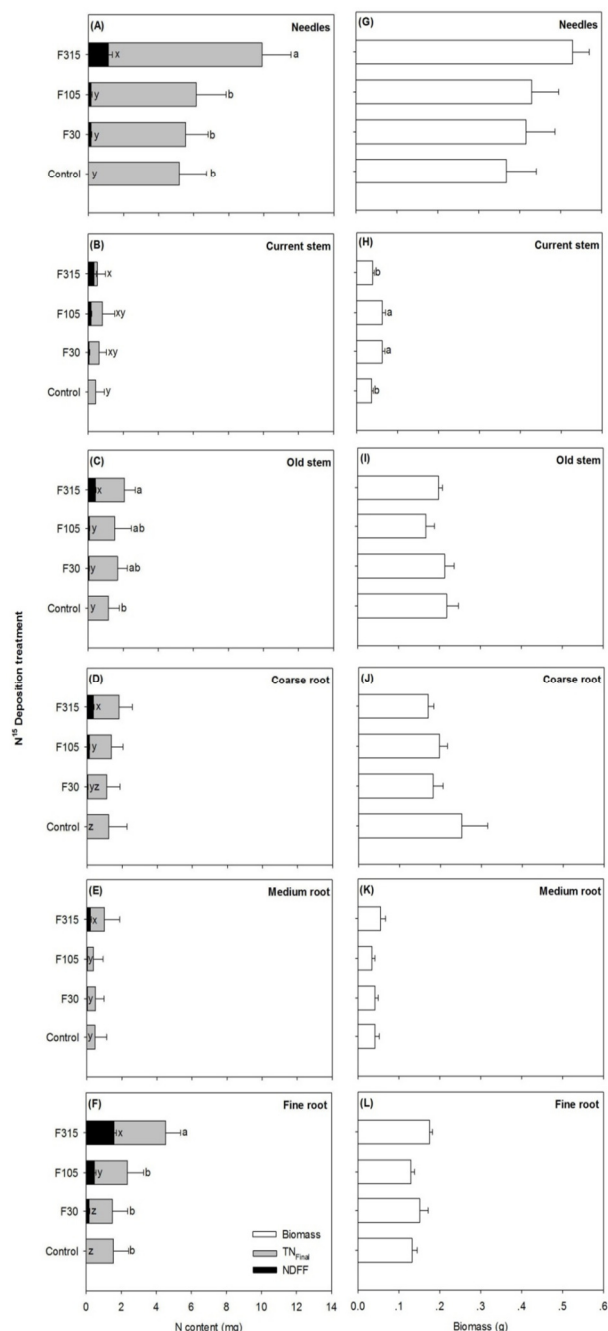


Fig. 4: Total N-content finally after N-deposition treatment (TN_{Final}), N-derived from fertilizer (NDFP) (left) and biomass (right) in needles (A, G), current stem (B, H), old stem (C, I), coarse root (D, J), medium root (E, K), and fine root (F, L) investigated on 10 August in 15-month old Changbai larch seedlings, which received simulated ^{15}N -deposition treatments at rates of 0 (Control), 30 (F30), 105 (F105), and 315 mg ^{15}N seedling $^{-1}$ on 25 July and 1 August 2010. Results are presented as mean \pm SE. Different letters indicate statistically differences among ^{15}N treatments according to Tukey's studentized range test at $\alpha = 0.05$

Table 1: Biomass (g) of needles, current stem, old stem, coarse root, medium root and fine root in 15-month old Changbai larch (*Larix olgensis* Henry) seedlings before and after the ¹⁵N-deposition treatment (determined on 18 July and 10 August 2010, respectively). Values before N-deposition treatment are the average of 8 replicates ($n=8$) \pm one standard error, while those after N-deposition treatment are the average of average of 32 replicates ($n=32$) from the pool of 4 treatments \pm one standard error

Organ	Before N-deposition treatment	After N-deposition treatment	Net biomass increment
Needles	0.41 \pm 0.03a	0.44 \pm 0.03a	0.03 \pm 0.03b
Current stem	0.03 \pm 0.00c	0.05 \pm 0.00c	0.02 \pm 0.00b
Old stem	0.18 \pm 0.00b	0.20 \pm 0.01b	0.02 \pm 0.01b
Coarse root	0.10 \pm 0.00bc	0.20 \pm 0.02b	0.10 \pm 0.02a
Medium root	0.05 \pm 0.00c	0.04 \pm 0.00c	-0.01 \pm 0.00b
Fine root	0.15 \pm 0.00b	0.17 \pm 0.01b	0.02 \pm 0.01b

Values before N-deposition treatment are the average of 8 replicates ($n=8$) \pm one standard error, while those after N-deposition treatment are the average of average of 32 replicates ($n=32$) from the pool of 4 treatments \pm one standard error. Different letters indicate significant difference according to Tukey's studentized range test at $\alpha=0.05$

Table 2: N-derived from plant (NDFP) (mg) in needles, current stem, old stem, coarse root, medium root and fine root of 15-month old Changbai larch seedlings subjected to ¹⁵N-deposition treatments at rates of 0 (Control), 30 (F30), 105 (F105) and 315 (F315) mg ¹⁵N seedling⁻¹

Seedling organ	¹⁵ N-deposition treatment			
	Control	F30	F105	F315
Needles	0.17 \pm 0.85b	0.37 \pm 0.59ab	0.98 \pm 1.00ab	3.74 \pm 1.09a
Current stem	-0.05 \pm 0.09	0.09 \pm 0.07	0.01 \pm 0.18	0.12 \pm 0.05
Old stem	-0.07 \pm 0.32	0.03 \pm 0.10	-0.50 \pm 0.12	0.07 \pm 0.11
Coarse root	-0.11 \pm 0.39	-0.26 \pm 0.20	-0.08 \pm 0.14	0.12 \pm 0.17
Medium root	-0.31 \pm 0.15	-0.32 \pm 0.08	-0.43 \pm 0.10	0.02 \pm 0.22
Fine root	-0.12 \pm 0.26b	-1.35 \pm 0.23b	-0.80 \pm 0.24b	0.25 \pm 0.19a

Values are the average of 8 replicates ($n=8$) \pm one standard error. Different letters in a horizontal line indicate significant difference among N-deposition treatments for one seedling organ according to Tukey's studentized range test at $\alpha=0.05$

Table 3: Ratios of N-derived from plant (NDFP) to TN_{initial} and TN_{final} in needles, current stem, old stem, coarse root, medium root and fine root in Changbai larch seedlings

Seedling organs	NDFP/TN _{initial}	NDFP/TN _{final}
Needles	26.18 \pm 9.99a	19.64 \pm 7.49a
Current stem	-4.57 \pm 11.17ab	-3.68 \pm 9.00ab
Old stem	-7.19 \pm 6.14ab	-7.11 \pm 6.07ab
Coarse root	-6.21 \pm 8.84ab	-6.00 \pm 8.54ab
Medium root	-33.59 \pm 9.88b	-44.78 \pm 13.18c
Fine root	-28.12 \pm 5.81b	-30.29 \pm 6.26bc

Values are the average of average of 32 replicates ($n=32$) from the pool of 4 treatments \pm one standard error. Different letters indicate significant difference according to Turkey test at 0.05 level

tended to rise with the increase of ¹⁵N-deposition intensity. The F315 treatment resulted in greater NDFP than the

control samples in needles and fine root NDFP in the F315 treatment was greater than that in the F30 and the F105 treatments, values of which were negative (Table 2).

Values of NDFP in all seedling organs was negative except for that in needles indicated N was retranslocated from roots upwards to the neighboring organ one by one until needles, wherein the NDFP value was positive (Fig. 5). Ratios of NDFP to TN_{initial} and to TN_{final} were different among seedling organs ($P<0.0001$; Table 3). The ratio of NDFP to TN_{initial} in needles was higher than in medium root and fine roots, and the ratio of NDFP to TN_{final} in needles was higher than that in medium root (Table 3).

Discussion

Locally in mid Jilin Province wet N-deposition in summer accounts for 75% of annual N-deposition (Song and Liu, 2013) due to abundant rainfalls (see Materials and Methods section). In summer, we found a significant effect of simulated N-deposition on NR in Changbai larch seedlings (Table 2), which clearly answers our first question. Ueda *et al.* (2012) indicated that the validity of exogenous N-addition to endogenous N-retranslocation was seasonal-dependent. As summarized in Table 4, the null responses found in current studies may be due to the post-N-addition effect were determined too early in spring (Millard and Neilson, 1989; Millard and Proe, 1991; Jordon *et al.*, 2012) when demand of exogenous N-uptake for new growth has not been fully recovered; or too late (Millard and Proe, 1992; Grelet *et al.*, 2001; Cheng and Fuchigami, 2002; Wei *et al.*, 2012a; Ueda *et al.*, 2012) when the utilization of currently absorbed N was covered by intensive inherent N-cycling. Otherwise, a post-N-addition sampling in fall may result in the influence of spring N-addition to be diminished (Wei *et al.*, 2012a). In our study, the sampling time was within period when Changbai larch was undergoing the intensively growing period, when the heavy demand for new growth in Changbai larch seedlings drove N to be obtained from both endogenous NR and exogenous N-uptake (Fig. 5).

As for NR and biomass accumulation, although the results of short term effect of N-deposition on NR appeared to disagree with Millard and Grelet (2010) and other findings (Zein *et al.*, 2011; Jordan *et al.*, 2012), the conclusions of these studies, however, were not comparable with ours, because our results were obtained in a much shorter term than the former ones. Besides NDFP, simulated N-deposition treatment also resulted in significant response of NDFP and biomass mainly in current stem (Fig. 4). Using seedlings as materials as well, Warren *et al.* (2003) reported the significant short term effect of N pulse on Douglas-fir (*Pseudotsuga menziesii*), but indeed they did not talk much about NR. Through rates of simulated N-depositions to a bamboo plantation, Tu *et al.* (2011) reported that short-term N-deposition increased carbon (C) sequestration by

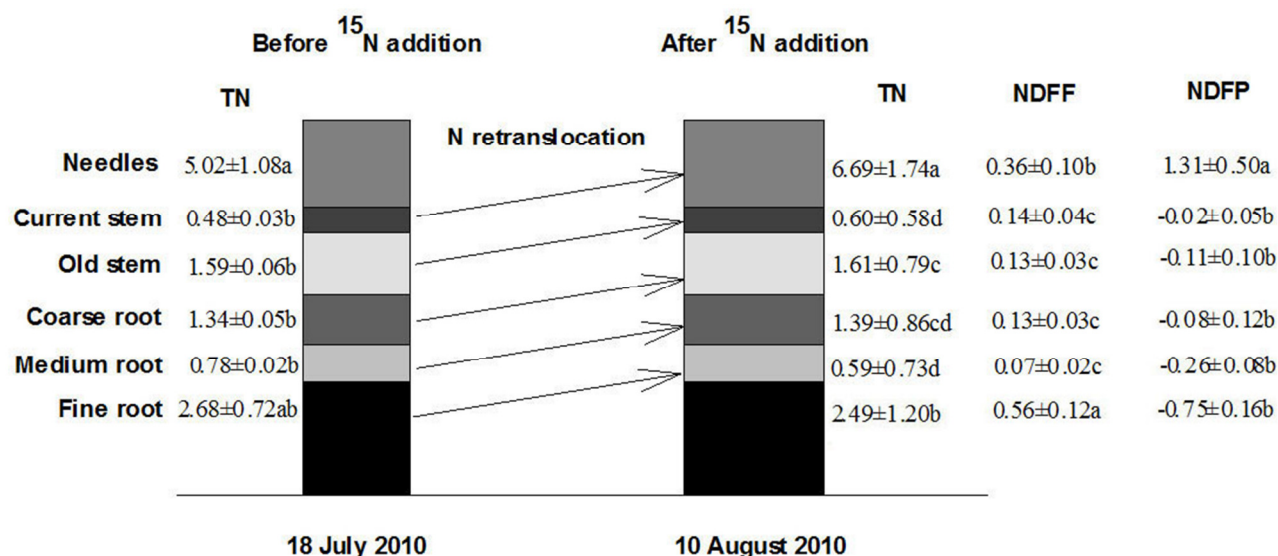


Fig. 5: Total N-content (TN) in needles, current stem, old stem, coarse root, medium root, and fine root investigated on 18 July and TN, N-derived from fertilizer (NDFF) (mg), and N-derived from plant (NDFP) (mg) investigated on 10 August in 15-month old Changbai larch (*Larix olgensis* Henry) seedlings. Simulated ¹⁵N-deposition treatments were applied to seedlings on 25 July and 1 August 2010. Values before N-deposition treatment are the average of 8 replicates ($n=8$) \pm one standard error, while those after N-deposition treatment are the average of average of 32 replicates ($n=32$) from the pool of 4 treatments \pm one standard error. Different letters in a column indicate statistically differences among seedling organs according to Tukey's studentized range test at $\alpha = 0.05$. Arrows indicate the direction of N-retranslocation from initial N-storage organ on 18 July towards the final N organ on 10 August 2010. Among all seedling organs, the value of NDFP was determined to be positive only in needles, indicating N-retranslocation from bottom organs upwards to the neighbor organ until needles

Pleioblastus amarus plantation system though soil C emission was stimulated as well. Savva and Berninger (2010) considered N-deposition could increase tree growth in a short term in boreal forests in Eurasia, but actually the growth of trees there declined due to accompanied sulphur deposition. In contrast, Nave and Curtis (2011) suggested that N-deposition was not likely to have any significant short-term effects on forest biomass N-content or NPP in *Populus tremuloides* – *Pinus strobes* forest mesocosms. Additionally, Rossi *et al.* (2013) failed to find the short term effect of N-deposition on root vitality and ectomycorrhizae presence in black spruce (*Picea mariana*) stands of the boreal forest of Quebec. In spite of the significant effect of N-deposition on NR in Changbai larch seedlings, future work is suggested to test this effect in mature trees, which will reveal the more realistic response of forest system to N-deposition.

Because increased N-deposition is one of the results driven by urbanization, our results about the biomass response to increased rates of N-deposition (Fig. 4) can be comparable with others with respect to urbanization. Searle *et al.* (2012) and O'Brien *et al.* (2012) clearly revealed the enhancing effect of urbanization on biomass accumulation in red oak (*Quercus rubra* L.) seedlings and adult red-cedar (*Thuja plicata*) trees, respectively. Compared to the study of

Searle *et al.* (2012) there were less significant responses of biomass found for Changbai larch seedling organs (Fig. 4), which was probably attributed to the shorter research term in our study. In current stem of Changbai larch seedlings, the decreased biomass in the F315 treatment relative to that in the F30 and F105 treatments indicated a potential depress of future urbanization related N-deposition on photosynthetic twigs. This disagreed with the results of obtained by Searle *et al.* (2012) possibly because their field experiment along a rural-urban gradient transect contained more involved environmental-factors (such as temperature) rather than N-deposition.

Surprisingly, we found that NR in needles was driven to rise by simulated N-deposition at increased rates in a short term, and needle NDFP in the F315 treatment was significantly greater than that in the control set (Table 2). These results answer our second question, suggesting that aggravated urbanization-related N-deposition in future would stimulate the short-term demand of NR for new growth in Changbai larch. Similar results have also been reported by Nambiar and Fife (1991), Crane and Banks (1992), Marmann *et al.* (1997), and Salifu and Timmer (2003) (Table 4). This can be explained by increased N-demand following stimulated growth. In contrast, some reports also documented the prohibitive effect of current N-

Table 4: Studies on N-retranslocation in trees

Reported study	Tree material	N-treatment	Time of determination on N-retranslocation	Effect of current N-supply on N-retranslocation
Nambiar and Fife 1991	Radiate pine (<i>Pinus radiata</i> D. Don) trees	¹⁴ N-fertilizations in springs of 2 nd , 3 rd , 4 th , and 5 th years	Summers in 4 th and 5 th years; Spring-summings in 5 th and 6 th years	Enhance
Millard and Neilson 1989	M26 apple (<i>Malus domestica</i> Borkh.) rootstock	¹⁵ N in April in the 2 nd year	September in the 2 nd year	Null
Millard and Proe 1991	Sycamore (<i>Acer pseudoplatanus</i> L.) seedlings	¹⁵ N throughout the 1 st year, and ¹⁴ N since March of 2 nd year	May in the 2 nd year	Null
Millard and Proe 1992	Three-year-old clonal cuttings of <i>Picea sitchensis</i> (Bong.) Carr.	¹⁵ N throughout the 1 st year, and ¹⁴ N since March of 2 nd year	Throughout the 2 nd year	Null
Crane and Banks 1992	Radiate pine (<i>Pinus radiata</i> D. Don) trees	¹⁴ N-fertilizations in 1 st and 2 nd years	Throughout 4 years	Enhance
Munson et al. 1995	White pine (<i>Pinus strobus</i> L.) and white spruce (<i>Piceaglauca</i> (Moench) Voss) trees	Annual ¹⁴ N-fertilization for 4 years	Four years after plantation establishment	Enhance for white pine Reduce for white spruce
Marmann et al. 1997	Ash (<i>Fraxinus excelsior</i>) trees	¹⁵ N-fertilization throughout the 1 st year	May in the 2 nd year	Enhance
Imo and Timmer 2001	Black spruce (<i>Picea mariana</i> (Mill.) BSP) seedlings	¹⁴ N-loading in the 1 st year, and field ¹⁴ N-fertilization in June of 2 nd year	October in the 2 nd year	Reduce
Salifu and Timmer 2001	Black spruce seedlings	¹⁴ N-loading in the 1 st year, and pot ¹⁴ N-fertilization in May of 2 nd year	July and September in the 2 nd year	Reduce
Grelet et al. 2001	N. Mature ramets of <i>Vaccinium myrtillus</i> and <i>Vaccinium vitis-idaea</i>	¹⁴ N-fertilization throughout the 1 st year, and ¹⁵ N-addition in May in 2 nd year	Throughout the 2 nd year	Null
Cheng and Fuchigami 2002	M26 apple trees	¹⁴ N-throughout the 1 st year, and ¹⁵ N from April to June in the 2 nd year	June in the 2 nd year	Null
Salifu and Timmer 2003b	Black spruce seedlings	¹⁴ N-loading in the 1 st year, and pot ¹⁵ N-fertilization in May of 2 nd year	July and September in the 2 nd year	Enhance
Dong et al. 2004	Poplar (<i>Populus trichocarpa</i> × <i>P. deltoides</i>) stocks	¹⁴ N-fertilization throughout the 1 st year, and ¹⁵ N-addition from April to July in 2 nd year	July in the 2 nd year	Reduce
Wei et al. 2012a	Changbai larch (<i>Larixolgensis</i> Henry) seedlings	¹⁴ N-fertilization in the 1 st year, and ¹⁴ N-fertilization at transplant in April of 2 nd year	July and September in the 2 nd year	Reduce in July; Null in September
Ueda et al. 2012	<i>Quercus serrata</i> Thunb. ex. Murray saplings	¹⁵ N was added during the early and late growing seasons	After ¹⁵ N was added, one to three months later in early growing season; four weeks later in late growing season	The effect of N-fertility in the current growing season was larger in the late phase than in the early phase
Jordan et al. 2012	One-year-old peach root-stocks (<i>Prunus persica</i> Batsch. cv. GF305)	¹⁴ N-fertilization in fall of the 1 st year and ¹⁵ N-addition in spring of the 2 nd year	29 May in the 2 nd year	Null

supply on NR (Imo and Timmer, 2001; Salifu and Timmer, 2001; Ueda, 2012). From current studies it can be concluded that when exogenous N-availability is low NR would be stimulated to compensate the N-demand for sink growth. Our results, however, underlined that in a short term exogenous N-input by deposition actually stimulated NR, although it seemed that urban forests would not need more NR in future because they are believed to move rapidly towards a state of “N-saturation” (Chen et al., 2010).

The increased NDFE, as well as NR, in Changbai larch needles in response to N-deposition suggested the strengthened demand of N for photosynthetic assimilation stimulated by urbanization-related N-addition in a short term. Thus, seedling demand of NR for new growth during intensively growing period was imperious to some extent and can't be ignorable (Fig. 5; Warren et al., 2003). Searle et al. (2012) indicated that urban-grown red oak seedlings allocated more growth to leaves than did rural-grown seedlings, resulting in 10-fold greater photosynthetic area. However, Searle et al. (2012) did not detect any difference in photosynthetic capacity of foliage per unit

area in red oak seedlings. At the community level, O'Brien et al. (2012) found that urbanization did not enhance seedling recruitment. For trees exposed to N-deposition, current evidence failed to link the short-term individual response to the community performance, which limits us to more deeply understand the undetected phenomenon. The mechanism that short-term demand for carbon assimilation “pulled” stored N out of reserves to be retranslocated towards photosynthetic organs of tree seedlings subjected to N-deposition is suggest to draw more attention at the community level.

The most surprising result in our study occurred in fine root, where in values of NDFE were negative for control, F30, and F105 treatments (Table 2). This suggested some N was retranslocated out of fine roots, which corroborate the findings of Nambiar (1987) and Gordon and Jackson (2000) regarding a little retranslocation of N from senescent fine roots in trees. In another study, Bausenwein et al. (2001) ever found N-retranslocated out of fine roots of *Rumex acetosa*. In contrast, when the intensity of N-deposition caught the highest rate for Changbai larch (i.e., the F315

treatment), the value of NDFP was positive. Here, NDFP was not able to represent NR anymore, because N is unlikely to be retranslocated from other organs, such as coarser to finer roots. Otherwise, positive value of NDFP in fine root can be attributed to the N-resorption of fine root from other associated organisms, such as soil biota or symbionts (Gordon and Jackson, 2000). Before the experiment commencement, Changbai larch seedlings were obtained from nursery soils, wherein roots were likely to be inoculated considering that (arbuscular-, ecto- and ericoid) mycorrhizal fungi constitute a large part of the fine-root volume of most plants – up to 40% for ectomycorrhizas and 80% for ericoid mycorrhizas (Freschet *et al.*, 2010). Additionally, like the results of NDFP, among N-deposition treatments only NDFP in the F315 treatment increased significantly compared to that in the control set. Hence, the surmised explanation of resorption in the F315 treatment was reasonable when it met the N-demand of needles, and the F315 treatment had a significant “disturbance” on fine-root N-recycling. However, more evidence is essentially needed in future investigations about effect of N-deposition on N-recycling, especially NR, in fine roots of larger and mature trees.

Nitrogen-derived from plant has been reported to account for 68-83%, 15-35%, 78-96%, and 26-53% of the TN in new shoot growth of young black walnut (*Juglan nigra*) (Salifu *et al.* 2009), black spruce (Salifu and Timmer, 2003), lodge pole pine (*Pinus contorta* Dougl. var. *latifolia* Enelm) (Amponsah *et al.*, 2004), and citrus (*C. sinensis*) (Martínez-Alcántara *et al.*, 2011), respectively. In our study, NR accounted for 19% of TN in needles of Changbai larch seedlings (Table 4), which is lower than most the current studies. However, our results were comparable with the month-long term research on Changbai larch seedlings, wherein the ratio of NDFP to TN in new shoots ranged from 5-20% (Wei *et al.*, 2010a). For deciduous trees, perennial organs of stem and root were found to be the sites where N is retranslocated from for deciduous tree species in longer-term studies (Millard and Proe, 1991; Marmann *et al.*, 1997; Duan *et al.*, 2013). At the whole-plant level, there was a mean amount of about 1.26 mg N being retranslocated from perennial organs to needles in growing Changbai larch seedlings in days (Fig. 5). This result is much lower than that in other studies (at least about 3 mg per plant; Table 4), which may be due to the short term of experiment.

In this study, NDFP was calculated in a shorter spell than TN change, i.e. NDFP was calculated from 1 August to 10 August, but the TN change was calculated as the difference from 18 July to 10 August. Therefore, the NDFP was probably overestimated. However, all seedlings underwent the same condition throughout the experiment; hence the overestimated result did not impair the trend of seedling responses to N-deposition treatments. Our results revealed that simulated N-deposition had a significant effect on NR, which was found to occur from N reserves in perennial organs, upwards through newly growing stems,

eventually towards needles. This disagreed with the conclusion of some former studies, but still concurs with some other ones. With the increase of simulated N-deposition rates, NDFP in needles was found to increase as well. In fine root, however, N-deposition in the F30 and F105 treatments did not result in any significant responses of NDFP compared to that in the control treatment (no N-deposition was performed), and values of NDFP in these three treatments were negative. However, the value of NDFP in fine root in the F315 treatment was positive, which was also statistically higher than the other three ones.

In conclusion, our results complement current knowledge about possible future trend of short-term inherent N-cycling in temperate larch species in response to urbanization-related N deposition. The short-term effect of rates of exogenous N input on endogenous NDFP contributed to a better understanding of NR in trees. In spite of present and the previous findings, there still exists a gap between the tree responses to N deposition at the individual level and those at the community level.

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