Nursery fertilization affected field performance and nutrient resorption of *Populus tomentosa* Carr. ploidy levels

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Nutrient resorption (NuR) is an important nutrient conservative strategy but little information is available about the effect of nursery fertilization on NuR in the field. In this study, diploid and triploid one-year-old plants of *Populus tomentosa* Carr. were fertilized with 9 g N per plant, and non-fertilized plants as control. Initial functional attributes, i.e., height, diameter, stem mass, mineral nutrients and non-structural carbohydrate (NSC) levels of each tissue, were measured before planting. Field performance (survival, total height, diameter, stem volume and their growth, leaf nutrient status, and NuR) were measured in the field. Compared to control, 9 g N per plant was benefit for plant growth, mineral nutrients and NSC accumulation of diplodids, but declined plant size of triploids before planting. While in the field, fertilization effect on plant size was inversed for each ploidy level. Nursery fertilization increased nitrogen resorption efficiency (NRE) of triploids and decreased phosphorus resorption efficiency (PRE) of both ploidy levels. Initial plant size were the most effective parameters predicting field performance and NuR. Furthermore, NRE was multi-elements controlled as indicated by the correlation of N and P in green and senesced leaves, while PRE was only positively correlated with P in green leaves. However, there was no relationship between field growth and NuR. This study deepened our understanding of NuR from the perspective of artificial managements, for instance nursery fertilization.

Keywords: Nursery Fertilization, Nutrient Resorption, Leaf Nutrient Status, Plant Growth, Initial Functional Attributes, Ploidy Levels

Introduction
Nutrient resorption (NuR) from senesced leaves to living tissues is an essential nutrient conservation strategy to diminish nutrient dilution from litter decomposition and alleviate the dependence upon soil fertility, and it is generally characterized as nutrient resorption efficiency (NuRE – Aerts 1996, Gutzt et al. 2012, Brant & Chen 2015). Globally, 62.1% nitrogen (N) and 64.9% phosphorus (P) are resorbed in terrestrial plants (Vergutz et al. 2012), and contributed to 31% and 40% of plant demands in N and P, respectively (Cleveland et al. 2013). Numerous studies have addressed the factors affecting NuR (Yuan & Chen 2009, Vergutz et al. 2012, Brant & Chen 2015, Yuan & Chen 2015), indicating leaf nutrient status as one of the most direct impact factors (Wright & Westoby 2003, Kobe et al. 2005). Kobe et al. (2005) reported a negative relationship between nutrient status (in green and senesced leaves) and NuRE by analyzing 92 publications with 297 perennial species of different life-forms. Vergutz et al. (2012) also confirmed the relationship through a global meta-analysis of 86 studies with ~1000 data points with different plant types. However, some studies reported that NuR was positive or neutral related with leaf nutrient status (Aerts 1996, Yan et al. 2016, Sohrt et al. 2018). The relationship between leaf nutrient status and NuR still need to be examined with specific species or genotypes.

Leaf N concentration (based on mass) also correlates with plant growth. For instance, high leaf N concentration generally combines with great photosynthesis as chlorophylls and photosynthetic enzymes are enriched in N (Masclaux-Daubresse et al. 2010), and consequently, more assimilated C is allocated to growth (Reich et al. 1997, Reich 2014). On the other hand, growth exerts a feedback regulation on leaf N status through modifying nutrient demands (Lambers et al. 2008, Masclaux-Daubresse et al. 2010), which might further affect NuR. Nonetheless, limited attention has been given to the links between NuR and growth; some studies illustrated a positive relationship between growth and NuR (Crane & Banks 1992, Nambari & Fife 1991, Zhang et al. 2015). Fortier et al. (2017) also showed that hybrid poplar with the most productive clones were more proficient in NuR. Whereas, Pasche et al. (2002) reported that N retranslocation from mature leaves do not constitute a net source supporting shoot growth of *Rhododendron ferrugineum* L. (Ericaceae). Salehi et al. (2013) found that *Populus deltoides* Marsh. cv. “Harvard” showed the largest morphological traits with the lowest NuRE.

For newly transplanted seedlings, the leaf nutrient status and growth also closely linked to initial functional attributes modified by nursery fertilization (Grossnickle 2012, Li et al. 2014, Oliet et al. 2013, Villar-Salvador et al. 2013). Villar-Salvador et al. (2004) showed that high N fertilization improved *Quercus ilex* L. early establishment and growth in the field. Oliet et al. (2009) highlighted that seedling size advantages due to nursery fertilization in *Pinus halepensis* Mill. persisted 7 years after out-plantation. Additionally, the largest and nutrient-rich seedlings also showed the highest survival rate after 7 years. Fu et al. (2017) reported that *Pinus tabulaeformis* Carr. fer-
Table 1 - Soil nutrient status at the nursery and in the field.

| Soil layer | Depth (cm) | N (mg kg⁻¹) | P (mg kg⁻¹) | K (g kg⁻¹) |
|------------|------------|-------------|-------------|------------|
| Nursery    | 0-30       | 363.5       | 714.0       | 4.0        |
|            | 30-60      | 446.8       | 280.1       | 8.3        |
| Field      | 0-30       | 287.3       | 195.1       | 7.3        |

Field experimental design

On March 31, 2018, one-year-old plants were transplanted to afforestation. The experimental design in the field was completely randomized with four treatments and four replicates. Each replicate with 15 plants were planted in five rows and three lines, resulting in 240 plants in total. Plants were planted in mechanically dug planting hole (diameter: 0.5 m; depth: 0.3 m) with 3 × 3 m spacing. Irrigation and weeding were performed manually three times during the growing season.

Plant sampling and chemical analysis

Measurements of initial functional attributes

On November 23, 2017, five plants per replicate (20 plants per treatment, 80 plants totally) were randomly sampled to determine height, diameter, stem mass, mineral nutrients and non-structural carbohydrates (NSC) levels as initial functional attributes. Root systems were washed to remove the growing medium and excised from the stem. Each plant part was oven-dried at 65 °C until steady weight and stem mass was determined. The stems and roots were pooled separately for the five plants of each replicate, ground and sieved through a 0.25-mm mesh. Approximately 0.2 g of each subsample was wet-digested in a sulphuric acid-hydrogen peroxide mixture using a block digester, followed by mineral nutrient analysis (Lowther 1980). Nitrogen was determined using standard Kjeldahl digestion with water distillation on a distillation unit (UDK-152®, VELP Scientifica, Italy). Phosphorus (P) was determined using the molybdenum blue method (Allen 1974) with a UV-visible spectrophotometer (Agilent 8453®, Waldbronn, Germany). Potassium (K) was determined using atomic emission spectroscopy (SpectraAA 220® Atomic Absorption Spectrometer, Varian Inc., Washington, DC, USA).

Approximately 0.1 g of subsamples was extracted with 80% ethanol at 80 °C for carbohydrate analysis (Wang & Huang 2015). Concentrations of starch and soluble sugar were determined, respectively, using amylose hydrolysis or anthrone colorimetry. Final measurements were taken with a UV-visible spectrophotometer. Total NSC concentration of each tissue was soluble sugar plus starch concentration.

Measurements of field performance and NuR

Green and senesced leaves were collected from five plants of each replicate. At growth peak (August 20, 2018), forty leaves were collected from middle southern position of each replicate. Senesced leaves were collected when dry and yellow (November 23, 2018). All leaves were oven-dried at 65 °C for 48 h, ground and sieved through a 0.25-mm mesh. Leaf N and P concentrations were measured and then, nutrient resorption efficiency (NuRE, %)
Field performance and nutrient resorption affected by nursery fertilization

Statistical analysis
The effect of ploidy levels and fertilization and their interaction on initial functional attributes (height, diameter, stem mass, mineral nutrients and NSC levels), field performance (survival, total height, diameter and stem volume, growth of height, diameter and stem volume, as well as leaf nutrient status), as well as NuRE were assessed using two-way ANOVA; for initial functional attributes, block served as a main factor. When ANOVA assumptions were not met, data were transformed. When ANOVA results showed a significant effect, a Fisher LSD test was carried out for multiple comparisons among treatments (α = 0.05). A generalized nonlinear model for binomial distribution and a logit link function were carried out to analyze the effects of ploidy levels and fertilization regime on survival. Statistical analyses were performed using SPSS® 19.0 (IBM, Chicago, IL, USA). Graphs and the linear regression among initial functional attributes, leaf nutrient status, growth, and NuRE were obtained using SigmaPlot® v. 12.5 (Systat Software, San Jose, CA, USA).

Results

Functional attributes before planting

Plant size
Regardless of fertilization, plant height of diploids was lower than that of triploids (ploidy levels and fertilization interaction: F = 0.99, p = 0.346 – Fig. 1A). There was an interactive effect between ploidy levels and fertilization on diameter (F = 64.3, p < 0.001) and stem mass (F = 69.1, p < 0.001 – Fig. 1B, Fig. 1C). Compared to controls, fertilization significantly increased diameter and stem mass of diploid plants by 6.0% and 21.2%, respectively, but decreased diameter and stem mass of triploid plants by 12.9% and 18.4%, respectively. Consequently, in comparison to triploids, diameter and stem mass of diploids were inferior to no fertilized plants, but superior to plants treated with 9 g N of N.

Mineral nutrients
Ploidy levels and fertilization affected stem nutrient concentration independently (ploidy levels and fertilization interaction, F = 3.28, p = 0.104 for N concentration; F = 2.03, p = 0.188 for P concentration; F = 1.35, p = 0.275 for K concentration). Compared to control plants, fertilization significantly decreased stem N concentration by 20.2% in triploid plants, but significantly increased stem P and K concentration by 38.5% and 39.4% in diploid plants, respectively (Fig. 2A-C). Meanwhile, stem nutrient concentrations of triploids were higher than triploids in all fertilization treatments. Ploidy levels and fertilization significantly interactive affected only root N concentration (F = 110, p < 0.001, Fig. 2D). In comparison to control plants, fertilization significantly increased root N concentration by 13.3% in diploid plants, but no significant effect was detected on triploids. Root P


Fig. 1 - Effects of ploidy levels and fertilization on height (a), diameter (b) and stem mass (c) of one-year-old *Populus tomentosa* Carr. plants before field planting. Bars marked with different letters differ significantly in each variable (mean ± SE, Fisher LSD test, α = 0.05, n = 4).

Fig. 2 - Effects of ploidy levels and fertilization on stem N (a), P (b), K (c) concentration and root N (d), P (e), K (f) concentration of one-year-old *Populus tomentosa* Carr. plants before outplanting. Bars marked with different letters differ significantly in each variable (mean ± SE, Fisher LSD test, α = 0.05, n = 4).
and K concentration were unaffected by fertilization, but P concentration of diploid plants was lower than triploids at each fertilization treatment (ploidy levels and fertilization interaction, F=0.88, p=0.372 for P concentration; F=0.38, p=0.552 for K concentration, Fig. 2-E-F).

**Non-structural carbohydrate levels**

Compared to control, fertilization with 9 g N per plant significantly increased stem NSC concentration by 22.8% in diploids, but no significant effect was detected on triploids (Tab. 2). Nonetheless, there was no significant difference in stem NSC concentration between diploid and triploid plants (Tab. 2). The increase in stem NSC concentration observed in diploid plants was mainly attributed to the increase in starch concentration (45.6%), as indicated by the significant combined effect between ploidy levels and fertilization (Tab. 2). However, there was no significant effect on stem soluble sugar concentration (Tab. 2).

Root NSC concentration was affected by ploidy levels and fertilization independently (Tab. 2). In all fertilization treatments, diploid plants had lower soluble sugar and NSC concentration than triploids. Compared to control plants, triploid plants fertilized with 9 g N showed a significant decrease (-17.2%) in starch concentration.

**Field performance and nutrient resorption**

**Plant growth and survival rate**

At the end of growing season in the field, ploidy levels and fertilization showed a sig-
significant interactive effect on plant size, except for height (Tab. 5 in Supplementary material). For diploids, fertilization significantly increased total height (+8.5%) and decreased total diameter of plants (-6.48%) compared to controls. Therefore, no significant differences in total stem volume were found between fertilization treatments. For triploid plants, total height, diameter and stem volume were increased by 4.29%, 7.81% and 21.0%, respectively, in response to fertilization. Consequently, total diameter and stem volume of triploids were higher than diploids fertilized with 9 g N per plant. Furthermore, total height of triploid plants was higher than that of diploids at each fertilization treatment.

Ploidy levels and fertilization interaction had no significant effect on height growth (F=0.05, p=0.833, Fig. 3A) or survival (χ²=0.182, p=0.666), but their combined effect was significant on diameter (F=19.4, p=0.004) and stem volume growth (F=20.6, p=0.001 – Fig. 3B-C). For diploid plants, fertilization significantly decreased diameter growth by 28.8% and, consequently, stem volume reduced by 16.3%, as compared to control plants. For triploids, the significant increase (+19.9%) of stem volume growth in fertilized plants was mainly related to the marginally increase of diameter growth in comparison to control plants.

Leaf nutrient concentration and nutrient resorption efficiency

The interaction between ploidy levels and fertilization had a significant effect on leaf nutrient concentrations, including NₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑEdward 2015: 16-23
(Tab. S2 in Supplementary material). Furthermore, leaf nutrient concentration was also related with field growth, as indicated by the positive correlation of diameter growth with $N_{\text{P}}$ and $P_{\text{GR}}$ (Tab. S3 in Supplementary material).

**Discussion**

*Effect of nursery fertilization on initial functional attributes and field performance*

Our results showed different responses to fertilization of one-year-old plants with different ploidy levels in *Populus tomentosa* Carr. In response to no fertilization, triploid plants showed higher height, diameter, stem mass and root NSC levels, but lower mineral nutrient status (except for root P and K concentration) than diploid plants before planting (Fig. 1, Fig. 2, Tab. 2). However, triploid plants grown in the field kept superior performances only regarding total height (Tab. S1 in Supplementary material). Total diameter and stem volume, as well as their growth and nutrient status of green and senesced leaves for diploid plants were similar or even higher than those found in triploid plants (Fig. 3, Fig. 4, Tab. S1 in Supplementary material), implying that diploids with small size but enriched mineral nutrient status performed better than triploid plants in the field. After fertilization with 9 g N per plant, diploid plants increased their diameter and stem mass, as well as mineral nutrients (stem P and K, and root N concentration) and stem NSC levels. Contrastingly, triploids had reduced diameter and stem mass, as well as stem N and root starch concentrations before planting (Fig. 1, Fig. 2, Tab. 2). Consequently, diploid plants were superior in plant size and mineral nutrient status (except for root P and K concentration) but inferior in root NSC levels in comparison to triploids. In the field, fertilization of diploid plants with 9 g N decreased the diameter and the stem volume growth, as well as $N_{\text{GR}}$ and $P_{\text{GR}}$, but the opposite responses in total height and diameter led to non-difference of total stem volume between fertilization treatments. While for triploid plants, fertilization with 9 g N significantly enhanced diameter and stem volume growth, $N_{\text{GR}}$ and $P_{\text{GR}}$, as well as total height, diameter and stem volume. Therefore, triploid plants were larger in size and growth than diploids, suggesting that the lower initial plant size in triploid plants was reversed in the field, and root NSC levels might contribute to this variation.

Under our experimental condition, nursery fertilization affected initial functional attributes, which in turn affect field performance of poplar seedlings. At each fertilization treatment, NRE and PRE of diploid plants were higher than those of triploids except for NRE at 9 g N per plant. Vergutz et al. (2012) reported that mean values of NRE and PRE of temperate deciduous species were 57.6% and 54.1%, respectively. Yan et al. (2018) showed that mean values of NRE and PRE in woody species were 48.4% and 53.3%, respectively. Under our experimental conditions, NRE was $43.0-50.9\%$, which in line with global mean values, but PRE ($14.5-39.1\%$) was lower. Furthermore, the observed values were much higher than other Poplar species or clones for NRE (12.09-18.93%) but similar or lower for PRE (32.46-39.63%) – Salehi et al. (2013), suggesting that nutrient resorption is species- or even ploidy-level specific. Further, the difference of environmental conditions, time of collecting leaves, plant age or measurements methods might also contribute to the observed difference (Brant & Chen 2015).

We investigated the effect of nursery fertilization on nutrient resorption in the field, as well as its relationship with field leaf nutrient status and growth in Chinese white poplar. Under our experimental conditions, fertilization with 9 g N per plant provided benefits in terms of initial plant size, mineral nutrients and NSC levels before planting, but impaired field performance of diploid plants. While for triploids, the initial lower size of plants can be reversed in the field. Nursery fertilization increased NRE of triploids and decreased PRE of both ploidy levels. In all the fertilization treatments, NRE and PRE of diploid plants were higher than those of triploids except for NRE at 9 g N per plant. Vergutz et al. (2012) reported that mean values of NRE and PRE of temperate deciduous species were 57.6% and 54.1%, respectively. Yan et al. (2018) showed that mean values of NRE and PRE in woody species were 48.4% and 53.3%, respectively. Under our experimental conditions, NRE was $43.0-50.9\%$, which in line with global mean values, but PRE ($14.5-39.1\%$) was lower. Furthermore, the observed values were much higher than other Poplar species or clones for NRE (12.09-18.93%) but similar or lower for PRE (32.46-39.63%) – Salehi et al. (2013), suggesting that nutrient resorption is species- or even ploidy-level specific. Further, the difference of environmental conditions, time of collecting leaves, plant age or measurements methods might also contribute to the observed difference (Brant & Chen 2015).

Our results showed that NRE was positively related to $N_{\text{GR}}$, $P_{\text{GR}}$ and $P_{\text{AN}}$ but negatively associated with $N_{\text{AN}}$ (Tab. 4), suggesting that NRE is a multi-elements controlled, while PRE was only positively related with $P_{\text{GR}}$ (Tab. 4). The relationship was consistent with the findings reported by several studies (Zhou et al. 2016, Zeng et al. 2017, Ji et al. 2018), but differed from others (Aerts 1996, Kobe et al. 2005, Vergutz et al. 2012). Species-specificity, plant age, environmental conditions, time of collecting leaves or different measurement methods might underlie such contrasting evidence (Brant & Chen 2015). Furthermore, the close relationship between diameter growth and $N_{\text{P}}$ or $P_{\text{GR}}$ in the field (see Tab. S3 in Supplementary material) confirmed the positive correlation between N, and growth (Reich et al. 1997, Reich 2014). However, there was no relationship between field growth and NuR, consistently with previous studies (Harvey & Van Den Driessche 1999, Pasche et al. 2002) and implying that field growth is not dependent factor for NuR. The different result with other studies (Salehi et al. 2013, Zhang et al. 2015) might be attributed to variation in plant age, experimental conditions and/or methods (Brant & Chen 2015). Furthermore, initial functional attributes were correlated with NuR (Tab. 4, Tab. S2 in Supplementary material). Plant size was negatively related with both NRE and PRE, while mineral nutrients correlated with PRE, and NSC levels correlated with NRE, respectively. Considering previous studies reporting that initial morphological traits were mostly correlated with field performance (Oliet et al. 2009, Villar-Salvador et al. 2013), our results suggest that the initial plant size is the most important parameters impacting field performance in Chinese white poplars, also combined with NuR.

**Conclusion**

We investigated the effect of nursery fertilization on nutrient resorption in the field, as well as its relationship with field leaf nutrient status and growth in Chinese white poplar. Under our experimental conditions, fertilization with 9 g N per plant provided benefits in terms of initial plant size, mineral nutrients and NSC levels before planting, but impaired field performance of diploid plants. While for triploids, the initial lower size of plants can be reversed in the field. Nursery fertilization increased NRE of triploids and decreased PRE of both ploidy levels in the field. Along with the relationship between initial functional attributes and NuR (i.e., plant size was negatively related with both NRE and PRE), our results showed that initial plant size was the most effective parameter in predicting both field performance and NuR. Furthermore, NuR (especially NRE) closely correlates with leaf nutrient status, while no relationship with growth was found in the field. Our results fill the gap in understanding NuR in terms of artificial managements, for instance nursery fertilization. Further study are needed to optimize the fertilization regimen in different field conditions and nutrient resorption with long-term monitoring.
Field performance and nutrient resorption affected by nursery fertilization

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Conflict of Interest
The authors declare that they have no conflict of interest.

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SUPPLEMENTARY MATERIAL

Tab. S1 - Mean ± SE, F and p values of total height, diameter and stem volume with fertilization in diploid and triploid one-year-old Populus tomentosa Carr. at the end of growing season.

Tab. S2 - F, p, and R² values of linear regression (y = ax+b) between field growth and nutrient resorption efficiency of one-year-old Populus tomentosa Carr.

Tab. S3 - F, p, and R² values of linear regression (y = ax+b) between field growth and leaf nutrient status of one-year-old Populus tomentosa Carr.

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