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Relationship between photosynthetic-water and nitrogen use efficiencies in young *Pinus taeda* L. trees at two contrasting sites

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Abstract

Background: Nitrogen and water are important limiting factors to forest productivity. At the plant level, there is contrasting empirical evidence about the trade-off between water use efficiency (WUE) and nitrogen use efficiency (NUE).

Methods: A study was conducted on 3-year-old loblolly pine (*Pinus taeda* L.) trees sampled at two contrasting sites (Virginia Piedmont (VA) and North Carolina Coastal Plain (NC)) in the southeastern United States. We investigated the leaf-level relationship between water and nitrogen use efficiency (i.e., WUE_{ins} versus PNUE). Both parameters were measured in the fall, three years after outplanting.

Results: WUE_{ins} and PNUE were higher at VA than NC. At both sites, WUE_{ins} increased from August to November, while PNUE showed a consistent decline for the same period only at NC, with no clear pattern observed at VA. The Pearson's coefficient of correlation (r) between WUE_{ins} and PNUE was negative (r=0.50) at VA in August, positive (r=0.77) at NC in October, and insignificant for the other measurement dates.

Conclusions: Regardless of the site, at the young stage of stand development, WUE_{ins} and PNUE were mostly uncoupled, although it can be speculated that a transient trade-off between these two variables can be expected depending on soil water rather than nitrogen availability.

Keywords: Instantaneous water use efficiency, photosynthetic nitrogen use efficiency, loblolly pine

Introduction

Photosynthetic water (WUE) and nitrogen use efficiency (PNUE) are important variables typically assessed in tree ecophysiological studies because water and nitrogen are the most limiting resources in many ecosystems (Reich et al. 1989; Galloway et al. 2003). These variables may be useful for the selection of desirable traits associated with N acquisition and the selection of genotypes most suitable for individual physiological components (Li et al. 1991). At the leaf level, these parameters can be derived from instantaneous measurements of leaf gas exchange and nitrogen concentration. WUE and PNUE measure the amount of carbon assimilated by photosynthesis per water and nitrogen unit, respectively (Field et al. 1983), and are considered interdependent traits (Plett et al. 2020). Both variables are related to stomatal conductance, and as stomatal closure has major effects on transpiration than on photosynthesis, it does not affect the foliar nitrogen concentrations, which ultimately increases WUE and decreases PNUE (Broeckx et al. 2014; Patterson et al. 1997).

The relationship between internal mesophyll conductance and photosynthesis determines the

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variations of PNUE and how this trait is related to WUE (Warren & Adams 2006). Renninger et al. (2015) mentioned that a trade-off between WUE and PNUE may occur when there is spatial segregation of water and nitrogen (e.g., water available at higher depths than nitrogen), which may alter the uptake of one resource at the expense of another. This trade-off has been observed in evergreen species (Field et al. 1983), deciduous species (Broeckx et al. 2014; Reich et al. 1989), annual crops (Cabrera-Bosques et al. 2007), and grasses (Gong et al. 2010). It has also been observed in deep-rooted species, such as pines, which may exhibit higher WUE and lower NUE compared to shallow-rooted species (Renninger et al. 2015). Arguably, these variables may not be related under optimal resource availability (Broeckx et al. 2014; Palmroth et al. 2013). This suggests that resource-use efficiency depends on the most limiting resource (Reich et al. 1989).

Nitrogen is one of the main factors limiting loblolly pine productivity in the southern United States (Fox et al. 2007). However, reductions in the precipitation are also projected in the future in the western edge of the species range, which might compromise further gains in productivity (Maggard et al. 2016). One of the approaches to increase productivity under this type of scenario is properly matching specific genotypes (i.e., varieties) with management prescriptions, but the growth responses will depend on soil resources and sites characteristics (Yáñez et al. 2015). In this study, we use two 3-year-old loblolly pine stands located at two sites of contrasting plant productivity in the southeastern United States to investigate the relationship between instantaneous water use efficiency (WUE_{ins}) and photosynthetic nitrogen use efficiency (PNUE). Both sites exhibit high differences in tree growth at young ages, which has been reported to be more related to limitations of soil moisture than nitrogen content (Yáñez et al. 2015; Yáñez et al. 2017). Thus, we hypothesised that a potential tradeoff of WUE and PNUE for the species in these sites is mostly driven by the soil moisture than by the nitrogen availability. Understanding the trade-off between proxies of NUE and WUE may guide the design of silvicultural systems that could optimise both water and nitrogen use in sites with different productivity and potentially threatened by increased periods of drought.

Methods

Study sites and tree selection

This study was conducted in two field trials established in 2009 in the southern U.S. Yáñez et al. (2015) described details of the study sites and trials. Briefly, one site was located in the North Carolina Coastal Plain (NC), at the Bladen Lakes State Forest (34° 49' 49.63''N, 78° 35' 18.52''W) in the native range of loblolly pine, while the other site was located at the Virginia Piedmont (VA) at the Reynolds Homestead Forest Resources Research Center (36° 38' 35.32''N, 80° 09' 18.84''W) in an area where loblolly pine grows successfully although outside the native range of the species. The planting material and study design were the same at the two sites and corresponded to a factorial combination of six genetic entries, two silvicultural intensities and four clonal varieties, and one open pollinated (OP) and one control mass pollinated (CMP) family (Yáñez et al. 2015). This study was originally designed to assess the potential productivity of loblolly pine genotypes under different silvicultural intensities and planting densities. The sites represent two contrasting edaphoclimatic areas where loblolly pine is planted. The soil at the VA site was a well-drained Fairview Series (sandy clay loam, kaolinitic, mesic Typic Kanhapludults), while the soil at the NC site was a poorly drained Rains series (fine sandy loam, siliceous, semiactive, thermic Typic Paleaquults). Table 1 shows the soil characteristics at the study sites (slope and physical properties obtained from websoilsurvey.se.egov.usda.gov). The annual average temperature and precipitation at VA are 13 °C and 1,159 mm, respectively, while at NC, those values are 16.9 °C and 1,171 mm, respectively (from NOAA online weather data http://sercc.com/nowdata.html).

Photosynthetic water and nitrogen use efficiency

Three years after planting (the year 2012), we selected a subsample of 36 healthy trees at each site, representing the variation in tree size at both sites. Selected trees were established at a planting density of 1,235 trees ha^{-1} (3.66 between rows and 2.21 m within rows). Leaf-level physiology and nitrogen content were measured in the months of August, October, and November. At each measurement date, three fully-sun exposed fascicles were randomly selected in the upper third of the crown. Fascicles selected were fully expanded and taken from the last current year foliage. Light-saturated photosynthetic rate (A_{sat}, at 1,600 μmol $m^{-2}s^{-1}$) and transpiration (E, mmol $m^{-2}s^{-1}$) were measured between 9.00 and 13.00 hrs. (local time) using a portable photosynthesis system (LI-6400, LiCor Inc., Lincoln, NE, USA). Gas exchange measurements were carried out on detached fascicles avoiding overlapping. The instantaneous water use efficiency (WUE_{ins}) was determined as the A_{sat} to E ratio (Medrano et al. 2015). Block temperature and relative humidity in the chamber were set at ambient conditions,

TABLE 1: Main soil characteristics at the study sites (0-15 cm).

Site	Slope (%)	AWC (cm cm^{-1})	K_{sat} (cm h^{-1})	pH	N (mg k^{-1})	P (mg k^{-1})	K (mg k^{-1})	CEC (meq/100 g)
VA	2-15	0.13	3.2	4.5	0.050	3.1	31.8	4.1
NC	0-2	0.14	8.6	4.3	0.047	2.3	23.3	6.1

Note: AWC is available water capacity, K_{sat} is saturated hydraulic conductance, and CEC is cation exchange capacity.

while the carbon dioxide concentration was set to 385 μmol mol^{-1}. Afterward, in the laboratory, the sampled fascicles were oven dried, weighed, and ball-milled to a fine powder. Specific Leaf Area (SLA) was determined as the leaf area to mass ratio. Nitrogen concentration (N) was measured by dry combustion with a VARIO MAX CN Analyzer (Elementar, Hanau, Germany). Then, the photosynthetic nitrogen use efficiency (PNUE, μmol CO_2 mol^{-1} N s^{-1}) was determined as the A_{sat} to N ratio (Guo et al. 2016) and adjusted by SLA.

Statistical analysis

To assess the variation of each variable over time at each specific site, we run a one-way repeated measures analysis with date as the main factor. Temporal correlation among residuals were accounted for using the unstructured function of PROC MIXED of SAS version 9.4 (SAS Institute, Cary, NC, USA). Post-hoc mean comparisons were based in the Tukey's mean comparison method. Additionally, we calculated the mean and coefficient of variations (CV) for WUE_{ins} and PNUE on gas exchange traits. The relationship between WUE_{ins} and PNUE was explored by site and measurement date using the Pearson's coefficient of correlation. Significant differences were considered at an alpha level of 0.05.

Results and Discussion

Water and nitrogen are the main factors limiting the productivity of many ecosystems. Thus, improving the use efficiency of those scarce resources is essential to improve the productivity of commercial species such as loblolly pine. In this study, WUE_{ins} increased from August to November (from 2.3 to 6.8 μmol mmol^{-1} at NC and from 3.5 to 7.6 μmol mmol^{-1} at VA), which is due to a higher decline rate in E relative to A_{sat} through the autumn months (Table 2). During the studied period, the mean temperature at the sites decreased from 26 to 9 °C at NC and from 23 to 7 °C at VA, which likely had a greater effect on the transpiration than photosynthetic rates. Similarly, Tang et al. (2003) showed decreasing air temperature, light intensity, and leaf transpiration from the summer to winter, being that gradient less pronounced for photosynthetic rates for loblolly pine. In contrast, PNUE decreased at NC during the same study period, but a higher decline (38%) was observed from October to November (Table 2). PNUE decreased by 47% from October to November at VA, while the PNUE value was intermediate between these two extremes during August (Table 2). A drastic decrease in PNUE from October to November for both sites might be explained by a higher decline in the photosynthetic rate during this period (due to low light intensity and temperature) relative to the small decline of foliar N associated with the starting of nutrient remobilisation (Yáñez et al. 2017).

Moreover, for a particular site and measurement date, we observed a moderate variation among trees in both WUE_{ins} and PNUE (Table 2). Coefficients of variation for WUE_{ins} varied from 12.7% to 25.3% at NC, and from 12.5% to 39.4% at VA, whereas for PNUE ranged from 15.2% to 39.9% at NC, and from 18.3% to 28.5% at VA. The within-site variability of these parameters could be associated with the spatial variability of water and nitrogen at the study sites, which may also vary with time. The topography is irregular at the VA site (slopes of 2 to 15%, Table 1), with different grades of erosion typical of Piedmont sites, likely creating gradients in the soil water and nutrient retention. In contrast, the soil at the NC site is poorly drained (slope of 0 to 2%) with variations in the microtopography, thus with variations in the water table (Yáñez et al. 2015).

Both sites were similar in N content and available water capacity (Table 1), and according to Yáñez et al. (2017) the trees at both sites were not N deficient (foliar nitrogen > 1.1 %), thus the lower soil moisture at VA during summer and the excess of water at NC likely

TABLE 2: *P*-values for the analysis of variance per site (NC = North Carolina Coastal Plain, VA = Virginia Piedmont) on light-saturated photosynthetic rate (A_{sat}), transpiration (E), nitrogen concentration (N), instantaneous water use efficiency (WUE_{ins}), and photosynthetic nitrogen use efficiency (PNUE). The coefficient of variation (CV) is presented for WUE_{ins} and PNUE. At each site, different letters within a column indicate significant differences according to the Tukey's mean comparison test.

Site	Month	A_{sat} (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	N (%)	WUE_{ins}		PNUE	
					Mean (μmol CO_2 mmol^{-1} H_2O)	CV (%)	Mean (μmol mol^{-1} N s^{-1})	CV (%)
NC	August	6.1 a	2.7 a	1.5 a	2.3 c	12.7	165.5 a	15.2
	October	5.2 b	1.4 b	1.3 b	3.5 b	25.3	137.2 b	38.0
	November	3.5 c	0.5 c	1.3 b	6.8 a	20.1	86.8 c	39.9
VA	August	4.7 b	1.4 a	1.4 a	3.5 c	12.5	137.2 b	28.5
	October	6.7 a	1.4 a	1.4 a	5.2 b	21.5	198.8 a	18.3
	November	3.6 c	0.6 b	1.2 b	7.6 a	39.4	106.4 c	22.3
	Effect							
	Date	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	

limited growth at both sites. The results of this study showed that the relationship between PNUE and WUE_{ins} varied between sites and measurement dates (Figure 1). The Pearson's coefficient of correlation (r) between WUE_{ins} and PNUE was negative (r=0.50) at VA in August, positive (r=0.77) at NC in October, and non-significant for the other measurement dates. At the VA site, the negative relationship between PNUE and WUE_{ins} found in August might be explained by the spatial segregation of resources mentioned by Renninger et al. (2015). This month is the end of summer; thus, less water is available in the soil to meet the plant requirements. Moreover, Yáñez et al. (2015) reported a higher presence of weeds at the VA than NC (particularly *Rubus* spp.). This likely increased

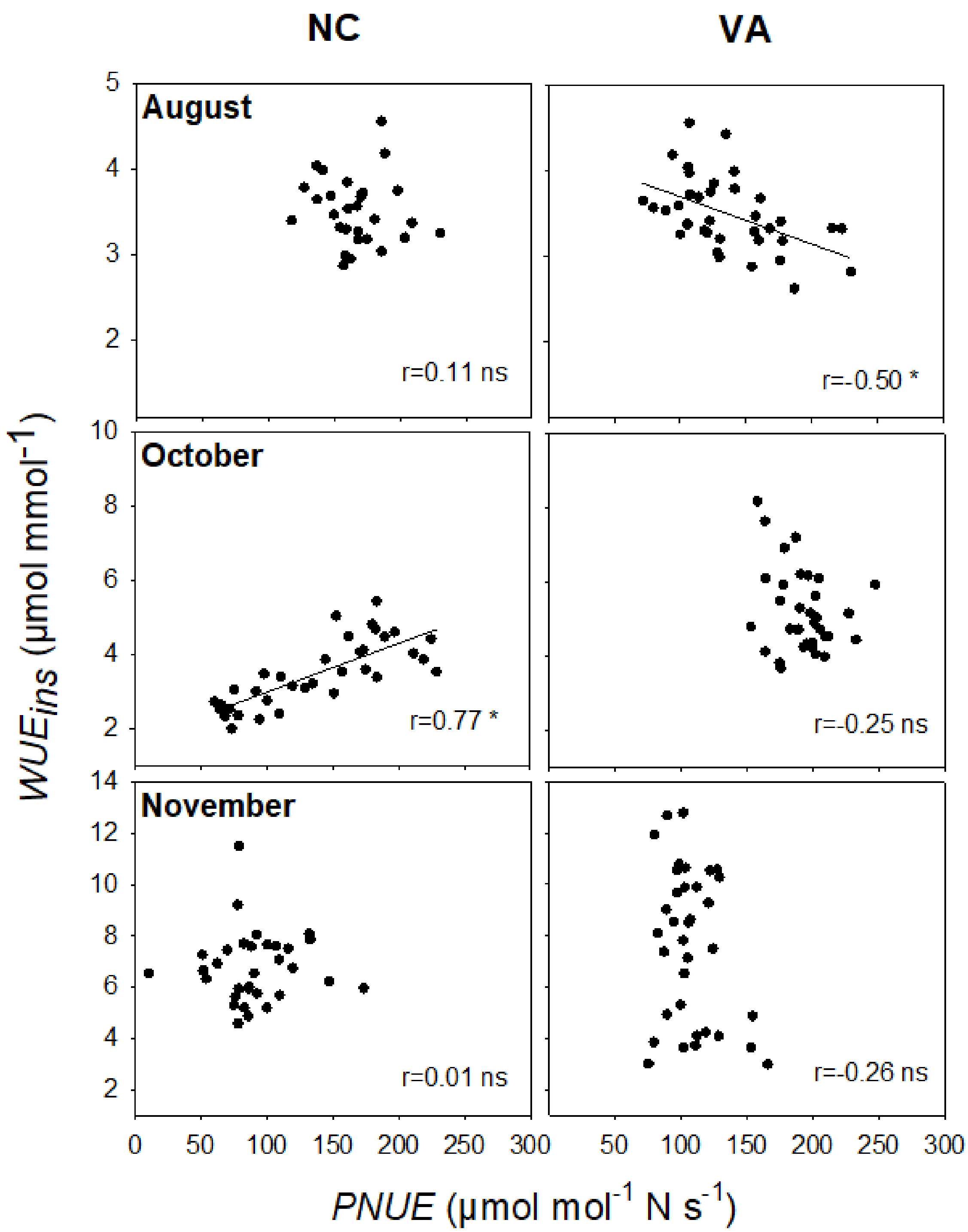

FIGURE 1: Relationship between instantaneous water use efficiency (WUE_{ins}) and photosynthetic nitrogen use efficiency (PNUE) for each site (NC = North Carolina Coastal Plain, VA = Virginia Piedmont) and date. * and ns denotes significant and non significant correlations at an alpha level of 0.05, respectively.

the water stress (i.e., stomatal closure) of loblolly pine trees at VA, and triggered the trade-off between WUE_{ins} and PNUE. Stomatal closure due to water stress has a faster-decreasing effect on stomatal conductance and transpiration than photosynthesis, with no impact in foliar nitrogen in the short-term, which explains the trade-off between WUE_{ins} and PNUE (Warren & Adams 2006). The lack of significant relationships between WUE_{ins} and PNUE in August and November at NC and October and November at VA suggests that neither water nor nitrogen were simultaneously limiting at those dates (Palmroth et al. 2013; Soolanayakanahally et al. 2009). While a trade-off between WUE and NUE has been found in loblolly pine (Palmroth et al. 2013) and other species (Renninger et al. 2015; Dijkstra et al. 2016), the coefficient of correlations between those parameters are moderate to low. In our study, foliage acclimated quickly within the chamber (less than 3 min), and stomatal conductance did not depress within this measurement time. However, we recommend verifying this acclimation period in future studies since foliage water status seems to have a higher effect on WUE versus PNUE trade-off than nitrogen.

Conclusions

The trade-off between WUE_{ins} and PNUE at VA site in the summer months was mainly attributed to the low water availability at that date, which agrees with our hypothesis. As nitrogen and water were probably not strongly limiting factors for growth at both sites during the measurement period, WUE_{ins} and PNUE were mostly uncoupled. However, a trade-off may appear as the demand for soil resources increases with stands aging or under severe drought conditions. Thus, further research is needed to understand the nature of these relationships and their applicability in developing specific site-silvicultural management to obtain maximum WUE in increasing drought-prone sites in the U.S.

List of abbreviations

WUE = water use efficiency, NUE = nitrogen use efficiency, WUE_{ins} = instantaneous water use efficiency, PNUE = photosynthetic nitrogen use efficiency, A_{sat} = light-saturated photosynthetic rate, E = transpiration, VA = Virginia Piedmont site, NC = North Carolina Coastal Plain site, AWC = available water capacity, K_{sat} = saturated hydraulic conductance, CEC = cation exchange capacity, N = Nitrogen, P = phosphorus, K = potassium, r = Pearson's coefficient of correlation, CV = coefficient of variation, NOAA = National Oceanic and Atmospheric Administration.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

MY participated in the design of the study, data collection, performed the statistical analysis and drafted the earlier version of the manuscript. SE and JO helped to draft the manuscript. CM and EM commented the final version of the manuscript. All authors read and approved the final manuscript.

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