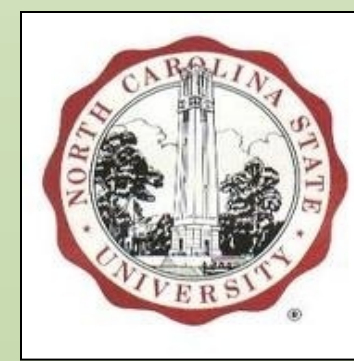


Isotopic estimates of water use efficiency: a high-throughput α -cellulose extraction method for softwood and its application

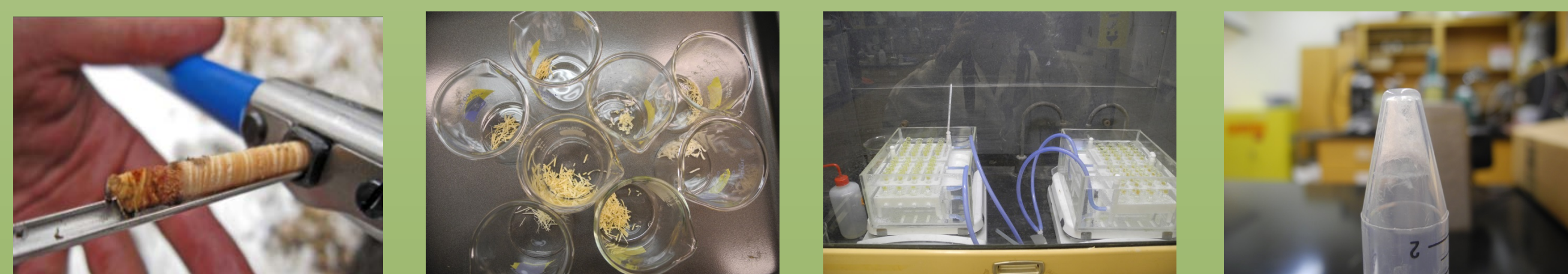


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Introduction

Wood stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) offer insights about plant water source, water availability and water use efficiency, which are informative for predicting the sensitivity of plants to projected future precipitation patterns. We recently developed a new and more time-efficient method for high-throughput α -cellulose extraction from resinous softwood for the purposes of PINEMAP-wide application. Here we tested the method with wood of loblolly pine from 5 geographic locations in SE US and of 5 other wide-spread coniferous species (black spruce, Fraser fir, Douglas-fir, Norway spruce, and ponderosa pine) with contrasting resin profiles. The calculated intrinsic water use efficiency (iWUE) and its relationships with environmental variables are also reported.



Materials and methods

Both new and traditional methods are used to extract α -cellulose from the latewood samples of the following species. Extracted α -cellulose are sent to Cornell Stable Isotope Laboratory for isotopic analysis..

Species	Location	The year of latewood sampled	number of replicates
Loblolly pine (<i>Pinus taeda</i>)	Clarke County, Georgia, USA	2010 (wet year), 2008 (dry year)	10
Loblolly pine (<i>P. taeda</i>)	Washington County, North Carolina, USA	2006 (wet year), 2008 (dry year)	3
Loblolly pine (<i>P. taeda</i>)	Buckingham County, Virginia, USA	2002 (dry year)	3
Loblolly pine (<i>P. taeda</i>)	McCurain County, Oklahoma, USA	2011 (dry year)	3
Loblolly pine (<i>P. taeda</i>)	Alachua County, Florida, USA	2004 (wet year)	3
Norway spruce (<i>Picea abies</i>)	Elva, Estonia	2011(dry year)	3
Fraser fir (<i>Abies fraseri</i>)	Boone County, North Carolina, USA	2008(dry year)	3
Ponderosa pine (<i>Pinus ponderosa</i>)	Klamath County, Oregon, USA	1994(dry year)	3
Douglas-fir (<i>Pseudotsuga menziesii</i>)	Klamath County, Oregon, USA	1994(dry year)	3
Black spruce (<i>Picea mariana</i>)	Saskatchewan, Canada	Multiple years	3

iWUE is calculated with $\delta^{13}\text{C}$ values of the extracted α -cellulose ($\delta^{13}\text{C}_{\text{plant}}$)

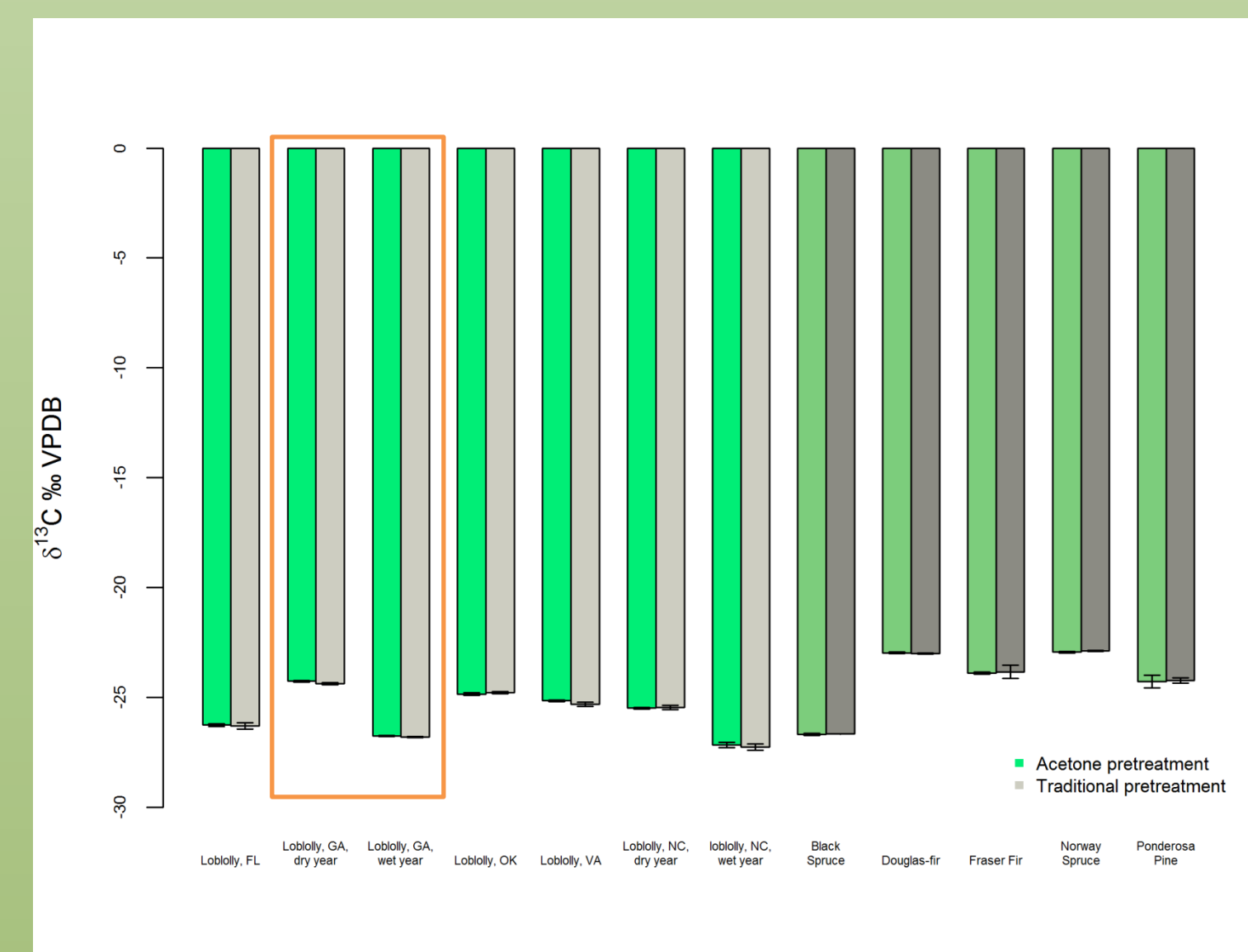
$$iWUE = 0.625 * c_a \left[1 - \frac{\delta^{13}\text{C}_{\text{plant}} - \delta^{13}\text{C}_{\text{air}} + a}{b - a} \right]$$

where a is the discrimination against $^{13}\text{CO}_2$ during diffusion through the stomata (-4.4%), b is the net discrimination due to carboxylation (-27%), c_a is ambient CO_2 concentrations, and $\delta^{13}\text{C}_{\text{air}}$ is the isotopic signature of the air.

Comparison of two methods: results of isotopic signatures

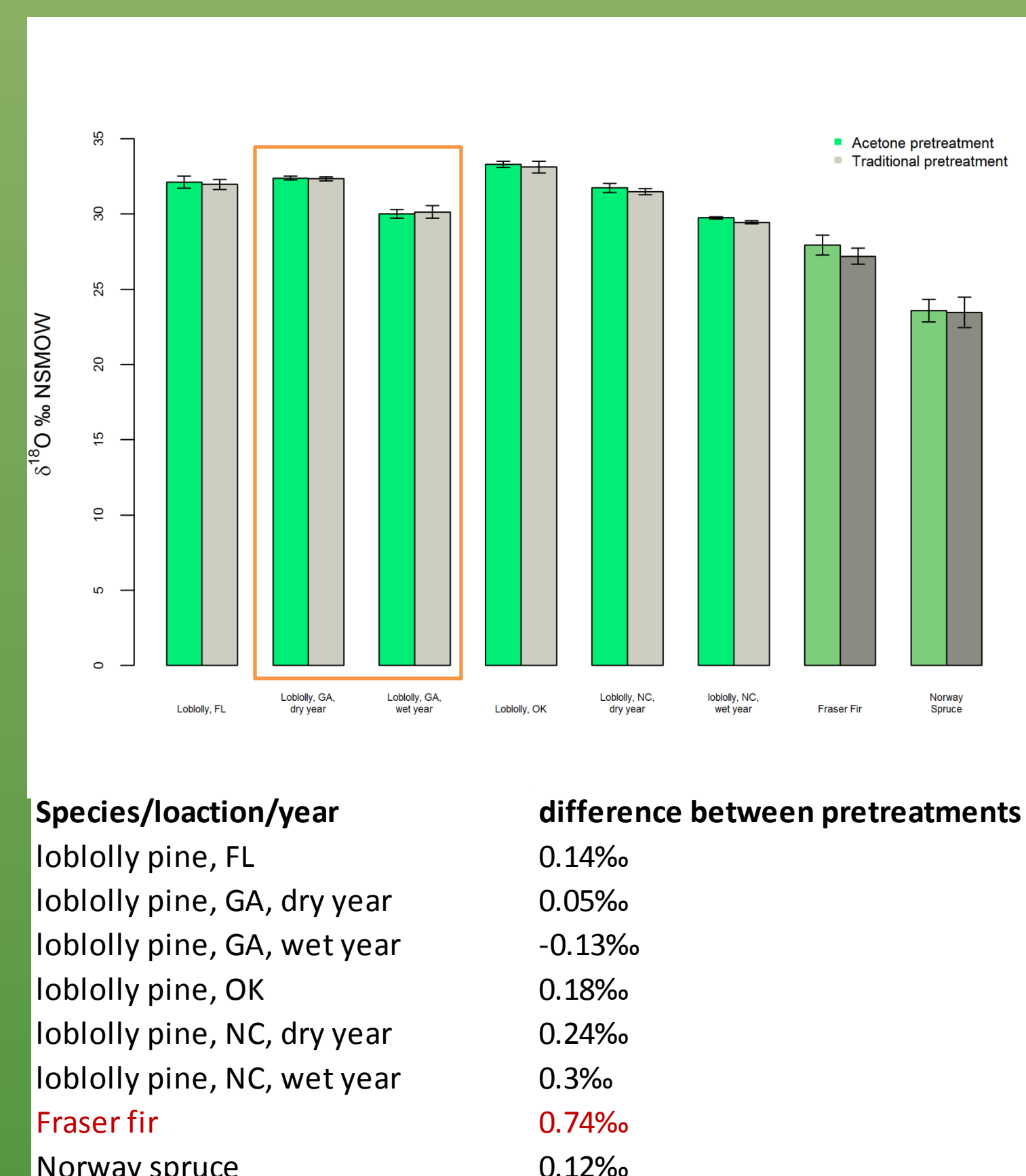
$\delta^{13}\text{C}$

- After data transformation, a paired t-test indicates that there is no significant difference between the $\delta^{13}\text{C}$ values obtained from the new and traditional methods (p-value=0.51) for different groups of samples.
- Two-way ANOVA of $\delta^{13}\text{C}$ values from wood samples of loblolly pine taken from Georgia (n=10) indicates that the main effect of pretreatment is significant (p-value <0.01). However, the 95% confidence intervals of $\delta^{13}\text{C}$ values between two methods are within the precision of the isotope ratio mass spectrometry (IRMS) method used ($\pm 0.2\%$ for carbon):
 - Dry year: 0.05 ‰ ~0.18 ‰
 - Wet year: 0.01 ‰ ~0.09 ‰



$\delta^{18}\text{O}$

- No paired t-test was done because data is not complete.
- Most of the difference of $\delta^{18}\text{O}$ values between two methods are within the precision of IRMS method used ($\pm 0.3\%$ for oxygen) except fraser fir.
- Two-way ANOVA of $\delta^{18}\text{O}$ values for wood samples of loblolly pine taken from Georgia (n=10) indicates that the main effect of pretreatment is not significant (p-value =0.92).

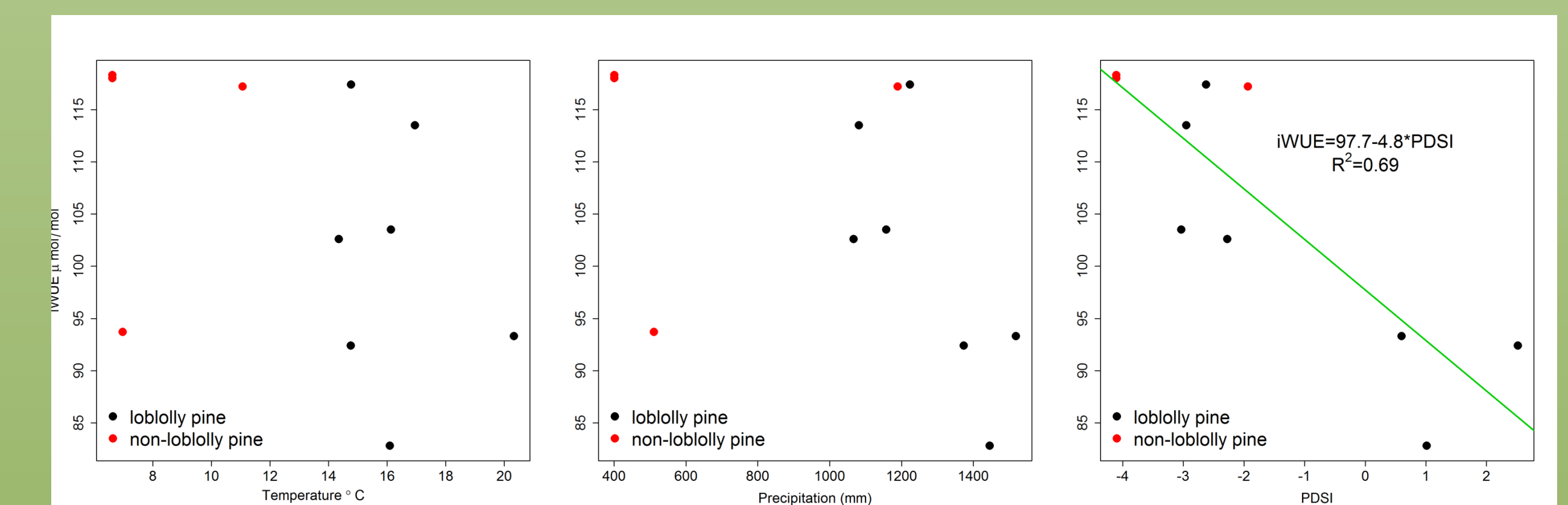


Therefore, comparing to the traditional methods, the new technique was equally effective for most species tested

Isotopic estimates of water use efficiency

$\delta^{13}\text{C}$ values can be converted to intrinsic water use efficiency (iWUE) with other parameters. The values of iWUE calculated from $\delta^{13}\text{C}$ values of latewood produced in wet and dry years differ significantly ($\Delta=25.0 \mu\text{mol/mol}$, $p<0.001$, $n=10$).

The iWUE of loblolly pine from 5 locations across SE US correlates with annual temperature and precipitation. The iWUE of the 5 tested species also exhibits good correlation with Palmer Drought Index (PDSI) during the year of interest.



In summary, the resolution of the modified method is sufficient for quantifying the expected changes in tree water use under the ranges of natural rainfall variability and the drought effects imposed in the Tier III installations.

Future work

Tree cores of loblolly pines are obtained from Tier II sites across southeast US. Wet and dry years would be obtained from local climate data and α -cellulose would be extracted from latewood produced in corresponding years. After conversion from isotopic values to water use efficiency, a relationship between WUE and environmental variables would be established.

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