**New Phytologist Supporting Information:**

**Article Title:** miR156-mediated changes in leaf composition lead to altered photosynthetic traits during vegetative phase change
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**Table S1.** Linear fit between photosynthetic rates and leaf composition traits depicted in figure 5.

| Species | Traits | Slope | *y*-intercept | $r^2$ | *p*-value |
|---|---|---|---|---|---|
| *P. tremula x alba* | $A_{sat}$ Area vs. SLA | -0.021 | 20.41 | 0.167 | <0.0001 |
| | $A_{sat}$ Area vs. N (g $m^{-2}$) | 0.1911 | -2.948 | 0.637 | <0.0001 |
| *A. thaliana* | $A_{sat}$ Area vs. SLA | -0.0046 | 9.049 | 0.709 | <0.0001. |
| | $A_{sat}$ Area vs. N (g $m^{-2}$) | 0.02324 | 2.867 | 0.629 | <0.01 |
| *Zea mays* | $A_{sat}$ Mass vs. SLA | 0.0027 | 0.0119 | 0.485 | <0.0001 |
| | $A_{sat}$ Mass vs. N (g $g^{-1}$) | 0.425 | -0.108 | 0.503 | <0.0001 |

**Table S2.** Additional leaf traits for adult, juvenile and juvenilized leaves of *P. tremula x alba*, *A. thaliana* and *Zea mays. P*-values determined by one-way ANOVA with developmental stage as the effect variable and corrected for multiple testing using false-discovery rate. Student's *T*-test was conducted on traits where $p < 0.05$, means significantly different from each other depicted by different lowercase letters.

| Trait | Species | Developmental Stage | Mean ± SE | N | df | *p*-value |
|---|---|---|---|---|---|---|
| $Chl_{a+b}$ ($\mu g\ mg^{-1}\ FW$) | *P. tremula x alba* | Adult | 12.35 ± 1.01 | 20 | 3 | 0.5179 |
| | | Juvenile | 15.02 ± 1.58 | 13 | | |
| | | Juvenilized-40 | 19.27 ± 3.38 | 20 | | |
| | | Juvenilized-78 | 21.72 ± 6.59 | 20 | | |
| | *A. thaliana* | Adult | 1.65 ± 0.29 | 6 | 2 | 0.3691 |
| | | Juvenile | 2.23 ± 0.49 | 6 | | |
| | | Juvenilized | 3.93 ± 1.63 | 5 | | |
| | *Zea mays* | Adult | 34.99 ± 3.65 | 33 | 2 | 0.2069 |
| | | Juvenile | 28.84 ± 4.98 | 34 | | |
| | | Juvenilized | 51.69 ± 13.71 | 32 | | |
| Chl a:b ratio | *P. tremula x alba* | Adult | 1.17 ± 0.05 a | 20 | 3 | <0.001 |
| | | Juvenile | 0.93 ± 0.06 b | 13 | | |
| | | Juvenilized-40 | 0.99 ± 0.03 b | 20 | | |
| | | Juvenilized-78 | 0.99 ± 0.02 b | 20 | | |
| | *A. thaliana* | Adult | 1.76 ± 0.07 | 6 | 2 | 0.1227 |
| | | Juvenile | 1.47 ± 0.08 | 6 | | |
| | | Juvenilized | 1.46 ± 0.11 | 5 | | |
| | *Zea mays* | Adult | 0.97 ± 0.05 a | 33 | 2 | <0.01 |
| | | Juvenile | 1.24 ± 0.04 b | 34 | | |
| | | Juvenilized | 1.20 ± 0.05 b | 32 | | |
| $V_{cmax}$ Mass* ($\mu mol\ g^{-1}\ s^{-1}$) | *P. tremula x alba* | Adult | 1.59 ± 0.20 | 15 | 3 | 0.2641 |
| | | Juvenile | 2.04 ± 0.21 | 12 | | |
| | | Juvenilized-40 | 2.35 ± 0.44 | 13 | | |
| | | Juvenilized-78 | 1.57 ± 0.30 | 14 | | |
| | *A. thaliana* | Adult | 0.002 ± 0.0001 a | 7 | 2 | <0.05 |
| | | Juvenile | 0.005 ± 0.0010 b | 7 | | |
| | | Juvenilized | 0.003 ± 0.0002 a | 6 | | |
| | *Zea mays* | Adult | 1.66 ± 0.12 a | 5 | 2 | <0.05 |
| | | Juvenile | 2.46 ± 0.08 b | 6 | | |
| | | Juvenilized | 1.76 ± 0.25 a | 6 | | |
| $J_{max}$ Mass* ($\mu mol\ g^{-1}\ s^{-1}$) | *P. tremula x alba* | Adult | 2.47 ± 0.31 | 15 | 3 | 0.1790 |
| | | Juvenile | 3.49 ± 0.33 | 12 | | |
| | | Juvenilized-40 | 3.65 ± 0.67 | 13 | | |
| | | Juvenilized-78 | 2.39 ± 0.48 | 14 | | |
| | *A. thaliana* | Adult | 0.004 ± 0.0006 a | 7 | 2 | <0.05 |
| | | Juvenile | 0.012 ± 0.0024 b | 7 | | |
| | | Juvenilized | 0.006 ± 0.0006 a | 6 | | |
| | *Zea mays* | Adult | 9.55 ± 0.82 | 5 | 2 | 0.0897 |
| | | Juvenile | 14.34 ± 0.60 | 6 | | |
| | | Juvenilized | 9.22 ± 1.70 | 6 | | |
| $\Phi$ | *P. tremula x alba* | Adult | 0.06 ± 0.002 | 19 | 3 | 0.9793 |
| | | Juvenile | 0.06 ±0.003 | 14 | | |
| | | Juvenilized-40 | 0.06 ± 0.007 | 20 | | |
| | | Juvenilized-78 | 0.06 ± 0.003 | 20 | | |
| | *A. thaliana* | Adult | 0.05 ± 0.006 | 6 | 2 | 0.4135 |
| | | Juvenile | 0.12 ± 0.041 | 7 | | |

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|---|---|---|---|---|---|---|
| | | Juvenilized | 0.11 ± 0.026 | 7 | | |
| | *Zea mays* | Adult | 0.06 ± 0.001 | 35 | 2 | 0.0592 |
| | | Juvenile | 0.07 ± 0.003 | 37 | | |
| | | Juvenilized | 0.07 ± 0.001 | 32 | | |
| LCP ($\mu mol\ m^{-2}\ s^{-1}$) | *P. tremula x alba* | Adult | 9.91 ± 1.14 a | 19 | 3 | <0.05 |
| | | Juvenile | 9.80 ± 1.27 a | 14 | | |
| | | Juvenilized-40 | 5.09 ± 1.51 b | 20 | | |
| | | Juvenilized-78 | 5.94 ± 1.17 b | 20 | | |
| | *A. thaliana* | Adult | 5.10 ± 3.90 | 6 | 2 | 0.3443 |
| | | Juvenile | 16.21 ± 4.06 | 6 | | |
| | | Juvenilized | 16.90 ± 6.59 | 7 | | |
| | *Zea mays* | Adult | 15.59 ± 1.23 | 35 | 2 | 0.2326 |
| | | Juvenile | 12.72 ± 1.20 | 37 | | |
| | | Juvenilized | 14.58 ± 0.77 | 32 | | |
| Fv/Fm | *P. tremula x alba* | Adult | 0.79 ± 0.006 a | 20 | 3 | <0.05 |
| | | Juvenile | 0.74 ± 0.012 b | 14 | | |
| | | Juvenilized-40 | 0.75 ± 0.011 b | 20 | | |
| | | Juvenilized-78 | 0.76 ± 0.011 b | 20 | | |
| | *A. thaliana* | Adult | 0.77 ± 0.008 | 6 | 2 | 0.7133 |
| | | Juvenile | 0.77 ± 0.006 | 7 | | |
| | | Juvenilized | 0.76 ± 0.008 | 7 | | |
| | *Zea mays* | Adult | 0.76 ± 0.004 | 32 | 2 | 0.4904 |
| | | Juvenile | 0.75 ± 0.004 | 37 | | |
| | | Juvenilized | 0.76 ± 0.003 | 31 | | |
| Fv'/Fm' | *P. tremula x alba* | Adult | 0.47 ± 0.019 | 19 | 3 | 0.5592 |
| | | Juvenile | 0.46 ± 0.010 | 14 | | |
| | | Juvenilized-40 | 0.44 ± 0.015 | 19 | | |
| | | Juvenilized-78 | 0.45 ± 0.009 | 19 | | |
| | *A. thaliana* | Adult | 0.51 ± 0.006 | 6 | 2 | 0.3191 |
| | | Juvenile | 0.51 ± 0.012 | 7 | | |
| | | Juvenilized | 0.53 ± 0.008 | 7 | | |
| | *Zea mays* | Adult | 0.47 ± 0.009 a | 32 | 2 | <0.05 |
| | | Juvenile | 0.44 ± 0.010 b | 37 | | |
| | | Juvenilized | 0.47 ± 0.006 a | 32 | | |
| $\Phi$ PSII | *P. tremula x alba* | Adult | 0.13 ± 0.010 a | 19 | 3 | <0.01 |
| | | Juvenile | 0.12 ± 0.010 ab | 14 | | |
| | | Juvenilized-40 | 0.09 ± 0.007 c | 19 | | |
| | | Juvenilized-78 | 0.11 ± 0.007 bc | 21 | | |
| | *A. thaliana* | Adult | 0.11 ± 0.014 | 6 | 2 | 0.3191 |
| | | Juvenile | 0.08 ± 0.010 | 7 | | |
| | | Juvenilized | 0.09 ± 0.011 | 7 | | |
| | *Zea mays* | Adult | 0.17 ± 0.004 | 32 | 2 | 0.3662 |
| | | Juvenile | 0.17 ± 0.007 | 37 | | |
| | | Juvenilized | 0.18 ± 0.008 | 32 | | |
| NPQ | *P. tremula x alba* | Adult | 3.42 ± 0.94 | 19 | 3 | 0.2641 |
| | | Juvenile | 5.30 ± 0.89 | 14 | | |
| | | Juvenilized-40 | 4.72 ± 0.80 | 19 | | |
| | | Juvenilized-78 | 5.84 ± 0.82 | 21 | | |
| | *A. thaliana* | Adult | 5.76 ± 1.07 a | 6 | 2 | <0.05 |
| | | Juvenile | 0.43 ± 0.49 b | 6 | | |
| | | Juvenilized | 3.92 ± 0.94 a | 7 | | |
| | *Zea mays* | Adult | 2.96 ± 0.52 | 32 | 2 | 0.6679 |
| | | Juvenile | 2.95 ± 0.48 | 37 | | |
| | | Juvenilized | 3.83 ± 0.56 | 32 | | |

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| ETR ($\mu mol\ m^{-2}\ s^{-1}$) | *P. tremula x alba* | Adult | 87.45 ± 6.32 a | 19 | 3 | <0.01 |
| | | Juvenile | 81.91 ± 5.86 a | 14 | | |
| | | Juvenilized-40 | 60.08 ± 4.45 b | 19 | | |
| | | Juvenilized-78 | 69.60 ± 4.62 ab | 21 | | |
| | *A. thaliana* | Adult | 48.15 ± 6.21 | 6 | 2 | 0.3191 |
| | | Juvenile | 35.17 ± 3.83 | 7 | | |
| | | Juvenilized | 40.45 ± 4.68 | 7 | | |
| | *Zea mays* | Adult | 135.18 ± 3.37 | 32 | 2 | 0.3662 |
| | | Juvenile | 132.85 ± 5.60 | 37 | | |
| | | Juvenilized | 143.49 ± 6.02 | 32 | | |
| ETR Mass ($\mu mol\ g^{-1}\ s^{-1}$) | *P. tremula x alba* | Adult | 3.22 ± 0.23 a | 18 | 3 | <0.01 |
| | | Juvenile | 4.33 ± 0.30 b | 14 | | |
| | | Juvenilized-40 | 2.96 ± 0.25 a | 19 | | |
| | | Juvenilized-78 | 3.12 ± 0.18 a | 21 | | |
| | *A. thaliana* | Adult | 2.04 ± 0.33 | 6 | 2 | 0.0876 |
| | | Juvenile | 3.83 ± 0.57 | 7 | | |
| | | Juvenilized | 2.57 ± 0.33 | 7 | | |
| | *Zea mays* | Adult | 6.63 ± 0.20 a | 32 | 2 | <0.001 |
| | | Juvenile | 8.87 ± 0.47 b | 25 | | |
| | | Juvenilized | 9.81 ± 0.35 b | 32 | | |
| $R_{day}$ ($\mu mol\ m^{-2}\ s^{-1}$) | *P. tremula x alba* | Adult | -0.78 ± 0.22 | 21 | 3 | 0.9793 |
| | | Juvenile | -0.77 ± 0.14 | 14 | | |
| | | Juvenilized-40 | -0.84 ± 0.17 | 19 | | |
| | | Juvenilized-78 | -0.89 ± 0.18 | 20 | | |
| | *A. thaliana* | Adult | -1.20 ± 0.44 | 6 | 2 | 0.4135 |
| | | Juvenile | -1.41 ± 0.68 | 7 | | |
| | | Juvenilized | -0.23 ± 0.57 | 7 | | |
| | *Zea mays* | Adult | -0.86 ± 0.13 | 31 | 2 | 0.2351 |
| | | Juvenile | -0.42 ± 0.12 | 19 | | |
| | | Juvenilized | -0.84 ± 0.20 | 32 | | |
| $g_s$ *High Light* ($mol\ m^{-2}\ s^{-1}$) | *P. tremula x alba* | Adult | 0.195 ± 0.020 a | 19 | 3 | <0.0001 |
| | | Juvenile | 0.200 ± 0.021 a | 14 | | |
| | | Juvenilized-40 | 0.087 ± 0.013 b | 20 | | |
| | | Juvenilized-78 | 0.083 ± 0.010 b | 20 | | |
| | *A. thaliana* | Adult | 0.103 ± 0.012 | 6 | 2 | 0.1999 |
| | | Juvenile | 0.060 ± 0.016 | 6 | | |
| | | Juvenilized | 0.098 ± 0.013 | 7 | | |
| | *Zea mays* | Adult | 0.164 ± 0.004 | 35 | 2 | 0.4109 |
| | | Juvenile | 0.186 ± 0.012 | 37 | | |
| | | Juvenilized | 0.173 ± 0.007 | 32 | | |
| $g_s$ *Low Light* ($mol\ m^{-2}\ s^{-1}$) | *P. tremula x alba* | Adult | 0.010 ± 0.015 a | 19 | 3 | <0.001 |
| | | Juvenile | 0.156 ± 0.020 b | 14 | | |
| | | Juvenilized-40 | 0.068 ± 0.008 a | 20 | | |
| | | Juvenilized-78 | 0.073 ± 0.008 a | 20 | | |
| | *A. thaliana* | Adult | 0.032 ± 0.002 | 6 | 2 | 0.3191 |
| | | Juvenile | 0.030 ± 0.012 | 6 | | |
| | | Juvenilized | 0.052 ± 0.011 | 7 | | |
| | *Zea mays* | Adult | 0.044 ± 0.004 a | 35 | 2 | <0.01 |
| | | Juvenile | 0.066 ± 0.007 b | 37 | | |
| | | Juvenilized | 0.042 ± 0.003 a | 32 | | |

*$V_{cmax}$ Mass and $J_{max}$ Mass in *A. thaliana* and *Z. mays* were calculated using the mean SLA for the corresponding leaf number/developmental stage

**Table S3**. Statistical results for leaf traits depicted in figures 2, 3, 6 and 7. *P*-values determined by one-way ANOVA with developmental stage as the effect variable and two-way ANOVA with leaf position and genotype as the effect variables. All *p*-values corrected for multiple testing using false-discovery rate. Developmental stages are adult, juvenile and juvenilized; genotypes are wild-type and miR156 overexpressors and leaf positions are 2-11 in *Z. mays* and 10, 15, 20 and 25 in *P. tremula x alba*. Leaf position is shown to have an effect on a trait independent of developmental stage when $p < 0.05$ for Leaf position but not for Leaf position x Genotype.

| Trait | Species | Effect | df | *p*-value |
|---|---|---|---|---|
| $A_{sat}$ Area | *P. tremula x alba* | Developmental Stage | 3 | <0.0001 |
| | | Leaf Position | 1 | <0.01 |
| | | *Leaf Position x Genotype* | 1 | <0.0001 |
| | *A. thaliana* | Developmental Stage | 2 | <0.05 |
| | *Zea mays* | Developmental Stage | 2 | 0.0733 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | 0.1282 |
| $A_{sat}$ Mass | *P. tremula x alba* | Developmental Stage | 3 | <0.0001 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | <0.05 |
| | *A. thaliana* | Developmental Stage | 2 | 0.1227 |
| | *Zea mays* | Developmental Stage | 2 | <0.0001 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | <0.0001 |
| SLA | *P. tremula x alba* | Developmental Stage | 3 | <0.0001 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | <0.05 |
| | *A. thaliana* | Developmental Stage | 2 | <0.001 |
| | *Zea mays* | Developmental Stage | 2 | <0.0001 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | <0.01 |
| Mass-based Leaf Nitrogen | *P. tremula x alba* | Developmental Stage | 3 | <0.01 |
| | | Leaf Position | 1 | <0.001 |
| | | *Leaf Position x Genotype* | 1 | 0.1467 |
| | *A. thaliana* | Developmental Stage | 2 | 0.2781 |
| | *Zea mays* | Developmental Stage | 2 | <0.0001 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | <0.0001 |
| Area-based Leaf Nitrogen | *P. tremula x alba* | Developmental Stage | 3 | <0.0001 |
| | | Leaf Position | 1 | 0.1641 |
| | | *Leaf Position x Genotype* | 1 | <0.01 |
| | *A. thaliana* | Developmental Stage | 2 | <0.01 |
| | *Zea mays* | Developmental Stage | 2 | 0.1524 |
| | | Leaf Position | 1 | 0.2501 |
| | | *Leaf Position x Genotype* | 1 | 0.3258 |
| $A_{low\ light}$ Area | *P. tremula x alba* | Developmental Stage | 3 | 0.7808 |
| | | Leaf Position | 1 | 0.6630 |
| | | *Leaf Position x Genotype* | 1 | 0.6630 |
| | *A. thaliana* | Developmental Stage | 2 | 0.6363 |
| | *Zea mays* | Developmental Stage | 2 | <0.01 |
| | | Leaf Position | 1 | 0.2501 |
| | | *Leaf Position x Genotype* | 1 | 0.7828 |
| $A_{low\ light}$ Mass | *P. tremula x alba* | Developmental Stage | 3 | <0.05 |

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|---|---|---|---|---|
| | | Leaf Position | 1 | 0.3532 |
| | | *Leaf Position x Genotype* | 1 | 0.1075 |
| | *A. thaliana* | Developmental Stage | 2 | <0.05 |
| | *Zea mays* | Developmental Stage | 2 | <0.0001 |
| | | Leaf Position | 1 | <0.0001 |
| | | *Leaf Position x Genotype* | 1 | 0.3258 |
| $V_{cmax}$ | *P. tremula x alba* | Developmental Stage | 3 | 0.1742 |
| | | Leaf Position | 1 | 0.1131 |
| | | *Leaf Position x Genotype* | 1 | 0.0684 |
| | *A. thaliana* | Developmental Stage | 2 | 0.7371 |
| | *Zea mays* | Developmental Stage | 2 | 0.0963 |
| $J_{max}$ | *P. tremula x alba* | Developmental Stage | 3 | 0.0690 |
| | | Leaf Position | 1 | <0.05 |
| | | *Leaf Position x Genotype* | 1 | 0.0996 |
| | *A. thaliana* | Developmental Stage | 2 | 0.7133 |
| | *Zea mays* | Developmental Stage | 2 | <0.05 |