CHAPTER II SUPPLEMENTAL MATERIAL

Analysis of maternal effects
While we cannot conclusively eliminate maternal effects, there are two lines of evidence that suggest they are minimal in this system. First, if maternal effects are responsible for explaining variation in plant size, then we should expect to see inconsistent patterns of annual growth diameter between 2013 - 2016 measurements (Roach and Wulff 1987). Based on our results, we find consistent evidence that annual growth diameter varies among populations across nearly all measurement years (Main text Fig. 2b), and that the soil N gradient in the field consistently and positively correlates with annual growth diameter for each measurement year (Main text Fig. 3). Together, these findings strongly suggest maternal effects play a minimal role in determining the expression of annual growth diameter in the common garden.

Second, maternal effects could also be detected with a relationship between maternal tree size and the initial size of cuttings collected in the field, or by affecting growth rates in the common environment, both of which might cause a correlation between initial size and the internode diameter of new growth. To test for potential maternal effects in this manner, we measured basal diameter change (final – initial diameter spanning 193 days) for replicate genotypes from seven of the populations across the provenances (Provenance 1: Blue River, Oak Creek; Provenance 2: San Miguel River, San Juan River; Provenance 3: Ogden Canyon Creek, Weber River, and Snake River) to determine if maternal tree size affected 1) initial basal diameter or 2) basal diameter growth. Two generalized linear models (GLMs) were constructed with DBH as a fixed factor and initial basal diameter or basal diameter change as the responses to determine if maternal size enhanced the initial size or growth response of cuttings in the common garden. Additionally, we constructed a GLM with initial basal diameter as a fixed effect and internode diameter as the response to test whether initial cutting size affected internode diameter of new growth. Based on our results, *P. angustifolia* exhibits no clear maternal effects on plant size. Initial basal diameter of cuttings in the common garden was not determined by DBH (p = 0.26, $r^2 = 0.003$; Fig. S2a). Similarly, maternal size did not influence basal diameter growth of cuttings (p = 0.34, $r^2 = 0.004$; Fig. S2b), and internode diameter was unrelated to the initial size of cuttings (p = 0.59, $r^2 = 0.001$; Fig. S3).

Overall, the alternate hypothesis that maternal effects are responsible for greenhouse trait variation is unlikely since cuttings were grown for multiple years in the common environment and plant growth was measured in four separate years. These results are consistent with conclusions from a recent study that found little evidence for maternal effects in *P. angustifolia* (Holeski et al. 2013). We also found no evidence to support alternative explanations that larger maternal trees had any effect on the initial size or diameter growth of cuttings, or that the initial diameter of cuttings predicted internode diameter, indicating that our standardization of measurements on areas of new growth in the greenhouse provided the most accurate survey of genetic variation in plant growth possible in this study. Together, these results support the contention that *Populus* size in our study is genetically based.
**Analysis of environmental filtering**

It is possible that seedlings are sensitive to small-scale spatial heterogeneity in soil characteristics (e.g., resource quality; Tilman 1982) and offspring that land in higher resource patches survive with greater frequency. To investigate this potential effect of environmental filtering as an alternative explanation for our results, we examined the paired distribution of tree to interspace response ratios in soil traits across all populations. If trees do not condition soils but primarily survive in higher quality soil patches, we expect there should be little to no variation in response ratios (i.e., predominately positive). We calculated the observed mean response ratio and compared this to a null distribution that was generated from our data using a Monte Carlo randomization and resampling approach. Here, the null distribution is the density of response ratio observations between randomly paired tree-interspace comparisons from 10,000 simulations. In the absence of environmental filtering, we expect that our observed mean response ratio will not be different from the null model, suggesting that any paired tree-interspace differences are not due to a soil heterogeneity bias but instead reflect plant-soil conditioning.

We observed variation in soil N response ratios with approximately one-third of effects being negative (soil nutrients beneath trees were lower than interspace levels; Fig. S4 and S5a). Additionally, our observed response ratios were not significantly different from a null distribution using Monte Carlo analysis (p = 0.49; Fig. S5b). This provides evidence against environmental filtering and, instead, that plant-soil conditioning is more likely the mechanism behind spatial differences in soil traits.

### Table 1. Stepwise model selection results of environmental factors that predict the genetic cline in *Populus angustifolia* annual growth diameter.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter ~ Carbon + Nitrogen + pH + MAT + AP + Latitude</td>
<td>-7.2</td>
</tr>
<tr>
<td>Diameter ~ Carbon + Nitrogen + pH + MAT + AP</td>
<td>-14.5</td>
</tr>
<tr>
<td>Diameter ~ Nitrogen + pH + MAT + AP</td>
<td>-21.1</td>
</tr>
<tr>
<td>Diameter ~ Nitrogen + MAT + AP</td>
<td>-26.0</td>
</tr>
<tr>
<td>Diameter ~ Nitrogen + AP</td>
<td>-27.6</td>
</tr>
<tr>
<td><strong>Diameter ~ Nitrogen</strong></td>
<td><strong>-27.4</strong></td>
</tr>
<tr>
<td>Diameter ~ 1</td>
<td>-23.0</td>
</tr>
</tbody>
</table>

MAT = mean annual temperature. AP = annual precipitation. Bold model was selected as best because it is slightly better and simpler (contains less factors) than the Nitrogen + AP model for predicting variation in annual growth diameter. Note that soil factors are from interspace locations.
Supplementary Figure Legends

Figure S1. Tree size in the field does not predict initial size or growth rates of cuttings in the common garden. A) Diameter at breast height (DBH) did not predict the initial basal diameter of cuttings in the common garden. B) Basal diameter change of cuttings (final - initial diameter spanning 193 days of growth) was not influenced by DBH, suggesting that the size of maternal trees does not determine initial steckling size or growth rates in the common garden.

Figure S2. Internode diameter is not determined by the initial size of cuttings in the common garden. Measurements of internode diameter on areas of new growth were not related to initial basal diameter measurements of stecklings, indicating variation in plant size is genetically controlled.

Figure S3. *P. angustifolia* diameter is influenced by genetics. A) Diameter at breast height (DBH; field) predicted variation in annual growth diameter (common garden) across all measurement years, indicating these growth traits are relatable for the purposes of our study. Symbols depict population mean values ± 1 standard error and the shaded area represents 95% confidence intervals.

Figure S4. Soil nitrogen (N) is greater beneath *P. angustifolia* trees relative to paired interspaces. Bars represent soil location means ± 1 standard error.

Figure S5. Soil nitrogen (N) response ratios by population shows variation in standardized differences between tree and interspace environments. Observations of tree-interspace total soil N response ratios with positive responses in grey (i.e., higher N beneath trees than interspace), negative responses in black, and respective numbers of observations for each positive/negative response.

Figure S6. Analysis of soil nitrogen (N) response ratios shows no effect of environmental filtering. A) All observations of tree-interspace total soil N response ratios with positive responses in grey (i.e., higher N beneath trees than interspace), negative responses in black, and the red line indicating the mean response (0.219). Approximately two-thirds of observations found tree soils had higher N levels than interspace samples. B) Monte Carlo analysis shows the observed soil N response ratio between paired tree-interspace samples (red line) is not different than simulated soil N response ratios after randomizing and resampling unpaired soil N observations.
Figure S1.
Figure S2.
Figure S3.
Figure S4.
Figure S6.