

(A) 0h 2h 4h 8h 12h 24h 48h

3
0
-3

Upregulated

(B) P/Ca EX SCW CD

4
0
-4

Upregulated after 2h

Downregulated

Upregulated after 4h

Upregulated after 8h
Upregulated after 12h



Downregulated after 2h

Downregulated after 4h

Downregulated after 8h

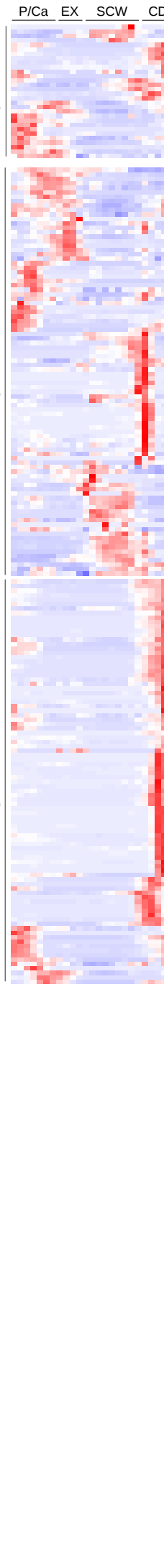


Figure S1 Time bin analysis where DEGs were grouped based on their first appearance after nitrate treatment. (A) Heatmaps for genes of each time bin according to their variance stabilized expression values (scaled for each gene) obtained from RNA-seq. (B) Heatmaps for genes of each time bin according to their $\log_2(\text{TPM}+1)$ -transformed expression values (scaled for each gene) during cambial growth and wood development in Tree1 of the AspWood database (Sundell et al., 2017). P/Ca, phloem/cambium; EX, expanding xylem; SCW, secondary cell wall formation; CD, cell death. Gene annotations are based on the closest Arabidopsis gene from TAIR. See Table S1E for details.

Sundell D, Street NR, Kumar M, Mellerowicz EJ, Kucukoglu M, Johnsson C, et al. AspWood: high-spatial-resolution transcriptome profiles reveal uncharacterized modularity of wood formation in *Populus tremula*. The Plant Cell. 2017;29:1585-1604.

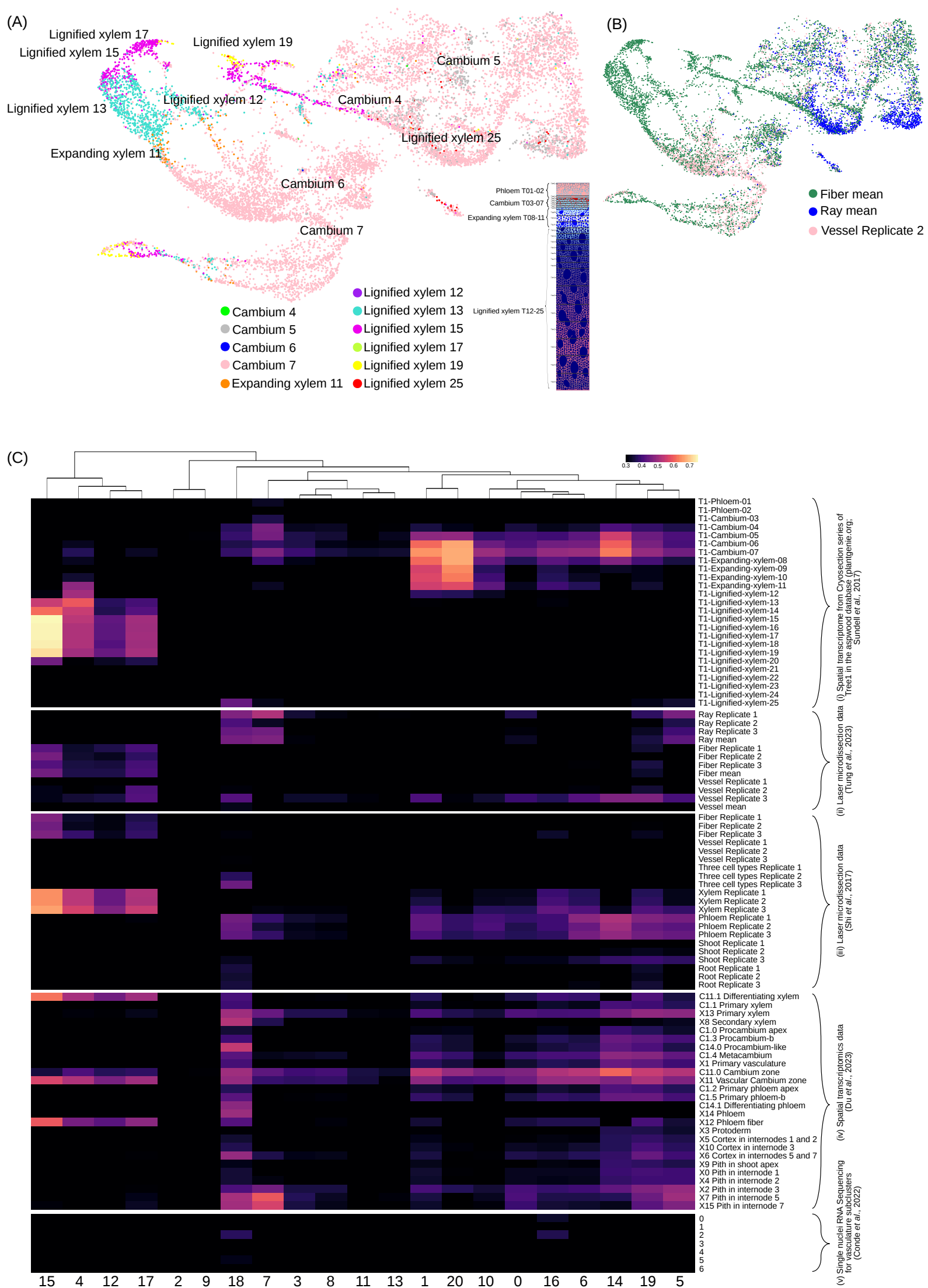


Figure S2 Correlation of clusters identified from our scRNA-seq experiment with reported spatial and cellular transcriptomes in *Populus* species. (A) Correlation dotplot of cells from our cell clusters with Tree1 in the Aspwood database (Sundell et al., 2017). (B) Correlation dotplot of cells from our cell clusters with transcriptome profiles of laser capture microdissected (LCM) xylem rays, fibers, and vessels from *P. trichocarpa*, reported in Tung et al. (2023). Note that for the vessels, we show only replicate 2 that most likely is free from other cell types. (C) Correlation heatmap of our scRNA-seq clusters (below) to various cellular and spatial profiles available in the public domain. (i) Correlation to RNA-seq data from Aspwood sections from Tree 1 where sections 1 and 2 consist of phloem, 3-7 vascular cambium, 8-11 expanding xylem and the rest lignified xylem. Vessel cell death is estimated to occur in section 18 and fiber cell death in section 24. Section 25 comprises exclusively of the living rays. (ii) Correlation to RNA-seq data of three biological replicates of each of the xylem cell type (fiber, vessel and ray) captured by LCM and their respective means (Tung et al., 2023). (iii) Correlation to RNA-seq data of various xylem cells and tissues captured by LCM (Shi et al., 2017). (iv) Correlation to clusters obtained from spatial transcriptome profiles from shoot apex till the 7th internode of wood by Du et al. (2023). (v) Correlation to clusters obtained from single nuclei RNA-seq from vasculature of the shoot apex (Conde et al., 2022). Gene expression data utilized in the analysis are $\log_2(\text{TPM}+1)$ -transformed expression values.

1. Conde D, Triozzi PM, Pereira WJ, Schmidt HW, Balmant KM, Knaack SA, et al. Single-nuclei transcriptome analysis of the shoot apex vascular system differentiation in *Populus*. *Development*. 2021;149:dev200632.
2. Du J, Wang Y, Chen W, Xu M, Zhou R, Shou H, et al. High-resolution anatomical and spatial transcriptome analyses reveal two types of meristematic cell pools within the secondary vascular tissue of poplar stem. *Mol Plant*. 2023;16:809-828.
3. Shi R, Wang JP, Lin Y-C, Li Q, Sun Y-H, Chen H, et al. Tissue and cell-type co-expression networks of transcription factors and wood component genes in *Populus trichocarpa*. *Planta*. 2017;245:927-938.
4. Sundell D, Street NR, Kumar M, Mellerowicz EJ, Kucukoglu M, Johnsson C, et al. AspWood: high-spatial-resolution transcriptome profiles reveal uncharacterized modularity of wood formation in *Populus tremula*. *The Plant Cell*. 2017;29:1585-1604.
5. Tung C-C, Kuo S-C, Yang C-L, Yu J-H, Huang C-E, Liou P-C, et al. Single-cell transcriptomics unveils xylem cell development and evolution. *Genome Biol*. 2013;24:3.

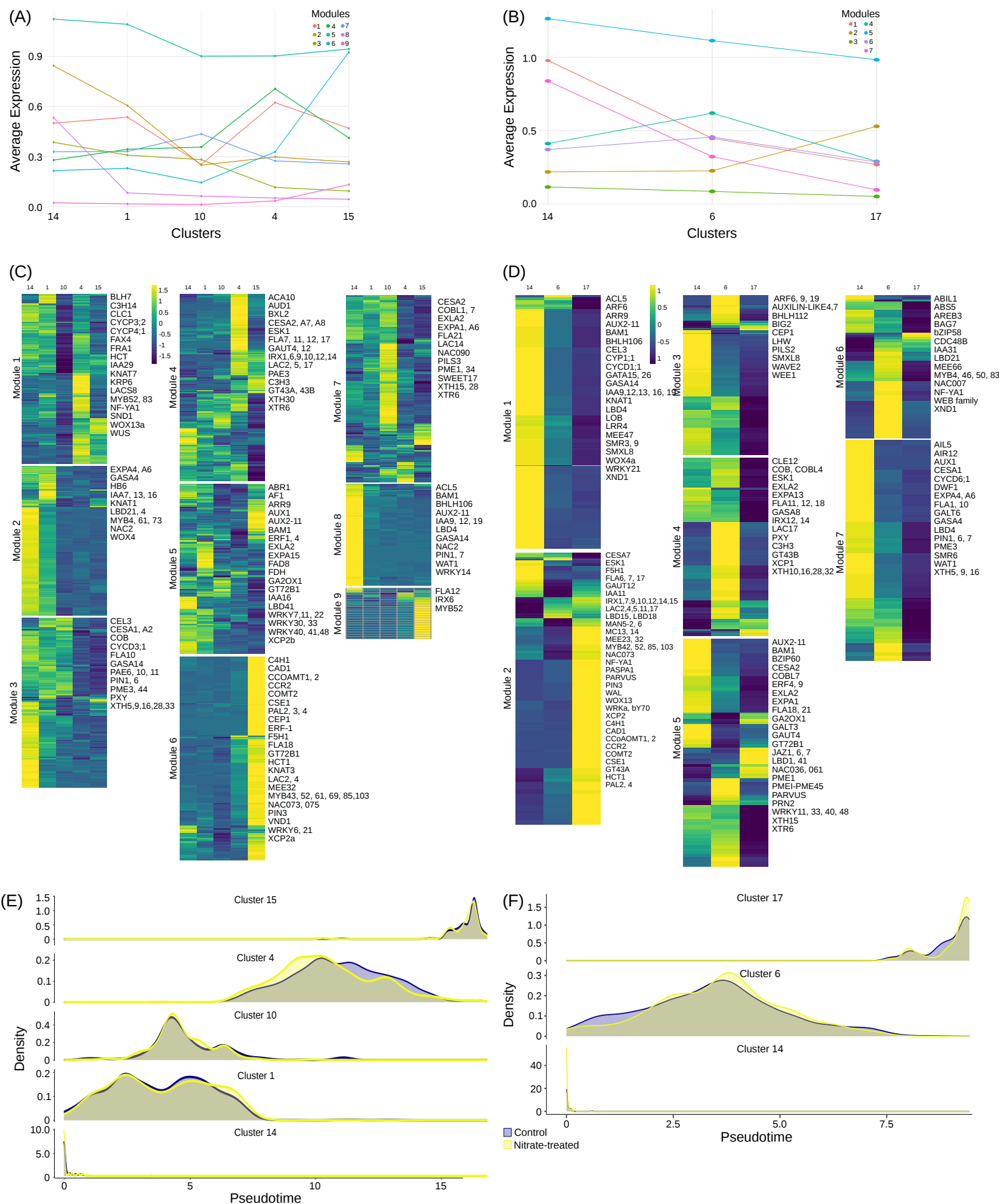


Figure S3 Pseudotime developmental trajectory of xylem vessels and fibers. (A-B) Monocle was used to identify modules of genes that co-vary across pseudotime during fiber (A) and vessel (B) cell differentiation. (C-D) Heatmap of pseudotime-dependent genes in each module during fiber (C) and vessel (D) differentiation. Example of genes in each module are shown. See Table S2G for full set of genes in each module. (E-F) Effect of nitrate treatment on the developmental trajectory. Density plot showing fiber (E) and vessel (F) developmental trajectories for control and nitrate-treated samples over pseudotime.