Supporting Information

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SI Methods

Transcript profiling details of the thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 15 s, primer annealing at 57 °C for 30 s, and extension at 72 °C for 30 s, for total of 45 cycles. The presence of only a single product/reaction was confirmed by analysis of the dissociation curves. The iCycleriQ software version 3.0 (Bio-Rad) was used to calculate the first significant fluorescence signal above noise, the threshold cycle (C_T). When necessary, the threshold level was adjusted manually to cross the exponential portion of the amplification curves for all reactions being compared on the 96-well plate. The PCR efficiencies of each amplicon were determined by using pooled cDNA samples from all samples in 4-fold serial dilutions. The relative transcript levels were calculated as follows: $100{,}000 \times E^{\mathrm{CrControl}}/E^{\mathrm{CrTarget}}$, in which E is the amplification efficiency, thus normalizing the target gene expression to the reference gene (generic ribosomal 18S) expression.

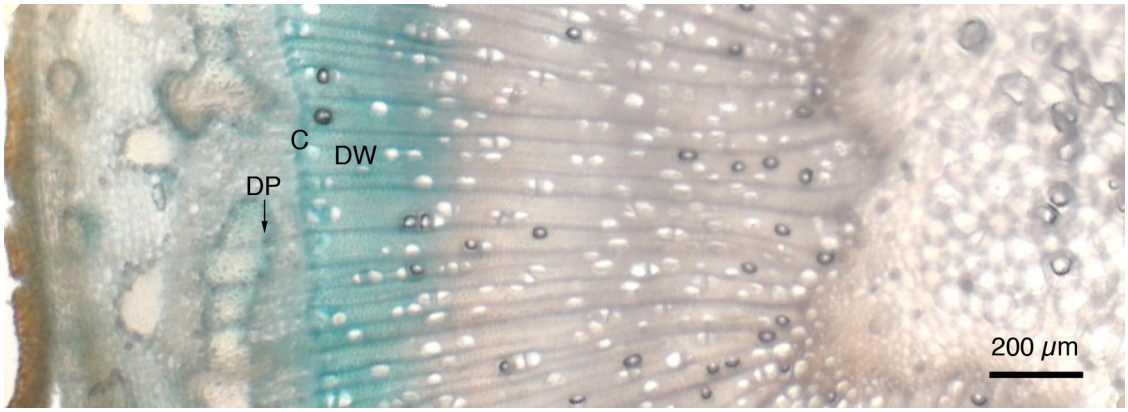

Fig. S1. GUS reporter expression patterns of the LMX5 promoter in *Populus* stem tissues. The LMX5 promoter preferentially directs expression to the cambial meristem (C), developing wood cells (DW), and developing phloem fibers (DP). Vascular strands in elongating shoot and root and in petioles and leaves also showed GUS staining, but no GUS staining was observed in the apical meristems. Four independent transgenic trees were analyzed for GUS activity, and all showed a similar expression pattern. A representative expression pattern from internode 25 (counted from top) of a greenhouse-grown tree is illustrated.

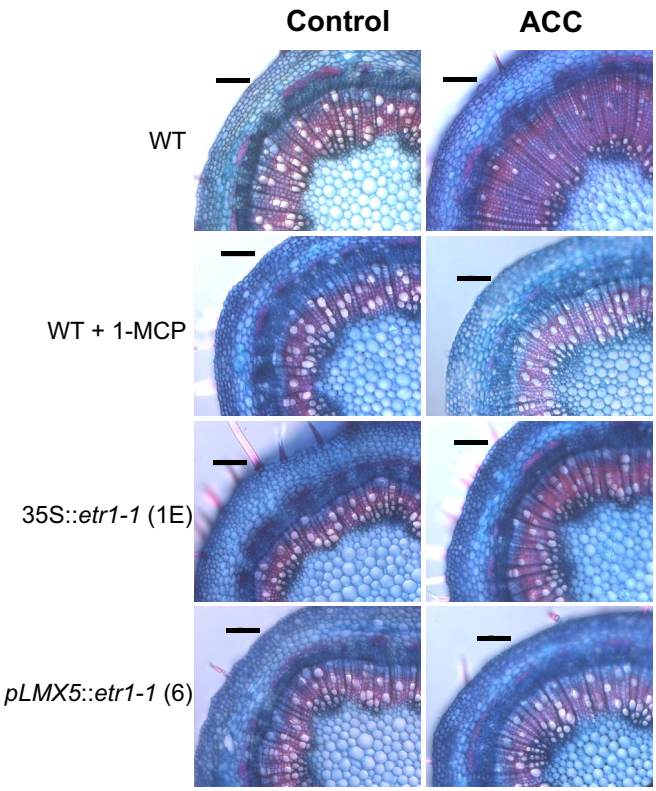

Fig. S2. Response in xylem anatomy to water (control) and ACC of wild-type, 1-MCP-treated wild-type, and transgenic 35S::*etr1-1* and pLMX5*:etr1-1* lines. Xylem anatomy in response to 100 μM ACC was used to screen for ethylene insensitivity in primary transgenic lines expressing *etr1-1* under the 35S and LMX5 promoters. The figure shows a typical response in a wild-type tree, a tree treated with 1-MCP (positive control), and from a transgenic line representing each promoter. (Scale bars: 100 μm.) Trees were grown in vitro. Treatments were done when the trees had reached a height of 5 cm, and trees were sampled 12 days after the treatment. Sections were sampled from the reference internode, in which all xylem was formed under the influence of ACC.

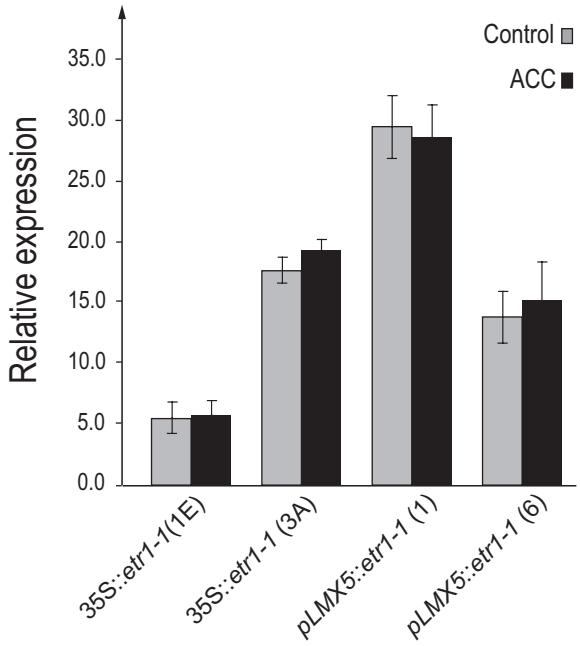

Fig. S3. Real-time qPCR analysis of At*etr1-1* in 35S*:etr1-1* and pLMX5*:etr1-1* transgenic lines after water (control) and ACC treatment. Transgenic trees expressing At*etr1-1* under the 35S (lines 1E and 3A) or LMX5 (lines 1 and 6) promoters were grown in vitro and treated with either water or 100 μM ACC. Treatment was done at a height of 5 cm, and trees were sampled 12 days after treatment. Each sample consisted of pooled internodes from 3 independent trees. Mean $\pm$ SD of 3 technical replicates is shown. Data are normalized to 18S ribosomal RNA gene. No transcript was amplified from wild-type trees that were included as a negative control.

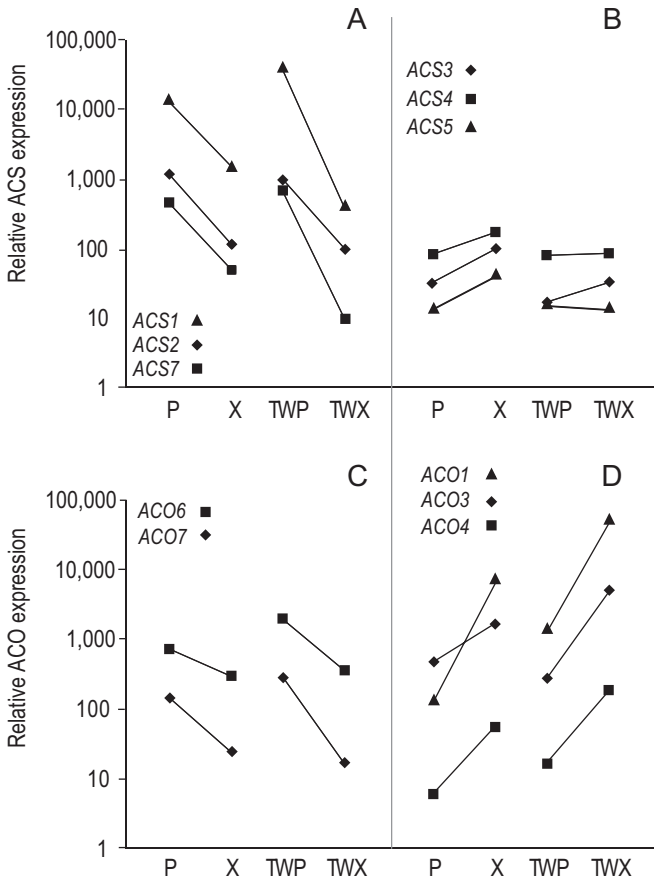

Fig. S4. qPCR analysis of *Populus ACS*s and *ACO*s in upright and leaned trees. (*A*) The 3 most abundant *ACS*s were highly expressed in phloem/cambium tissues. (*B*) Three low-abundant *ACS*s showed small differences between phloem/cambium and developing xylem tissues. (*C*) Two *ACO*s showed higher expression in phloem/cambium tissues than in developing xylem tissues. (*D*) Three *ACO*s were preferentially expressed in developing xylem, including the highly abundant *ACO1*. Trees were grown in greenhouse to a height of 2 m. Transcripts were analyzed with gene-specific primers in pooled tissues of phloem and cambium (P) or developing xylem (X) sampled from 5 upright trees and from 5 TW-forming tissues of trees that had been leaned for 3 weeks. Mean value is calculated from 3 technical replicates (SD not larger than symbol). Data are normalized to 18S ribosomal RNA gene. Gene numbering for *ACS/ACO* gene family is according to existing *Populus tremula* × *tremuloides* National Center for Biotechnology Information accessions and U.S. Department of Energy Joint Genome Institute *Populus trichocarpa* annotations.

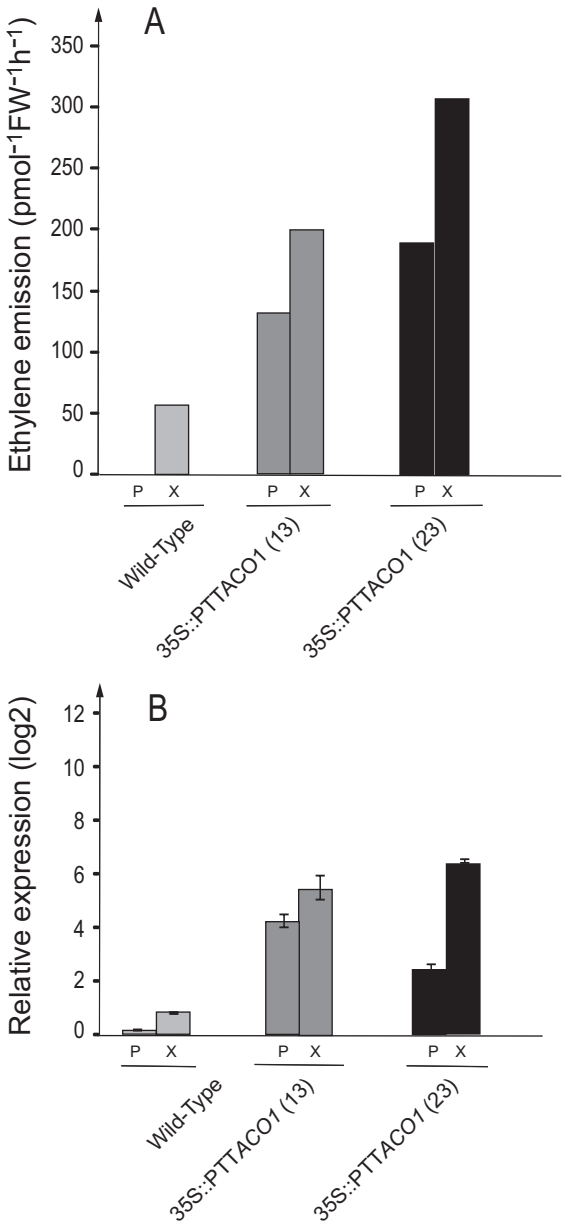

Fig. S5. ACO activity (*A*) and Ptt*ACO1* expression (*B*) in wild-type and transgenic 35S::*ACO1* trees. In wild-type trees, expression of Ptt*ACO1* and ACO activity is primarily seen in developing xylem. Transgenic 35S::*ACO1* trees exhibited an increase in ACO activity and Ptt*ACO1* transcript level in both developing xylem and cambium/phloem tissues. Trees were grown in a greenhouse to a height of 2 m. ACO activity was measured in pooled tissues of cambium and phloem (P) or developing xylem (X) scraped from 5 trees. Ptt*ACO1* abundance was measured in the same samples by qPCR. Mean ± SD of 3 technical replicates is shown. Data are normalized to 18S ribosomal RNA gene.

>LMX5/A055P19U

ACTCGTCTTCGTGCACCAAGATGCAAAAGCTGGAATGAGAATGCGCTAAATGCGAAAAGACAGAGAGAGCGAATAAAT
CGTGCAAAAAAAGGAGTGGGGTGGGTAACGGGTTGAGCTAGAAGAAGAAAAGGGACAAGTGCACTTTAGGAGGGGGGC
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CAACTTAAAAACTAAAACTGCAGGATGGGTAGCTATCCAAGAATGATTAATAAACGATTTTAACAAAGAACTACGTTT
TTTGTAAACTTAATTTTGATGGGCATGTAGTTAACAAGTATTTGTCATTGATCAATTCAAGAGCCATGTCTGCATCAT
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CATGAAATTAGTCCCCTAGTGGAGCTAATTCTACTCACTCCATATTTACTCATTCCACTATATAACGCCCTCAACGAC
CATCCTCAAAGCAACCCAAACACCTTCTTCTCTTCTCTTTCTCCCGGAAA**ATG**GCCGGTTTGATTTCAAGATCA
GTTCCTTGTGCAATCCTGGTAGTCTTGTGCACGGTGGTGCCCATTTTGGCTAAAGATTACACTGTAGGAGATACTTCA
GGCTGGGCAATTGGTATGGATTATAGCACCTGGACTAGTGGCAAGACCTTTTCAGTTGGCGACAGCCTTGGTGAGTCC
CTTTCTCTCAGTACACGTTACTGATCAAGATTAGTACTTGATTTACTAATCAGGTTTCTGTTTAAATATATAATCTAG
TGTTTAACTACGGAGGAGGCCACACGGTGGATGAAGTGAGAGCCAGTGACTACAGCACATGCACTACAGGCAATGCAA
TCACTTCAGATAGCAGTGGTGCTACCACAGTAGCCCTCAAGACTGCCGGAACCCATTATTTCATTTGTGGTGTTCCTG
GCCACTGTGGGAGTGGCATGAAGGTTTCAGTCACTGTTGCAGCAGCAGGATCGAGCACAAGTCCCTCCTCCTCAGGAA
CTCCATCTTCTGATGGCACTACCACTTCTCCGGCCGGTAGTAATGTCACCAATTACAAGCCTTCATCCAACAACGTAC
CCGATTCATCCTTAGGGATCAATATTTCCCCATTTGTGGCTTTAGCCGGTACTTGTGTTGCCGTTTTTGTGATGGGTT
TCTCATGA

Fig. S6. The gene sequence of LMX5 promoter region obtained with GenomeWalker Universal Kit upstream of the EST clone A055P19. Promoter region is underlined, and the primer sites for promoter cloning and vector constructions are double-underlined. y, T or C; r, A or G. The 5′ UTR and predicted protein coding sequence are highlighted with gray. The predicted translational start codon is marked with bold font.

Table S1. *Populus* trees treated with 1-MCP or expressing A*tetr1-1* have reduced sensitivity to ACC-induced inhibition of diameter growth of fibers and vessel elements

Trees (line) and treatment	Fiber diameter, μm	Vessel element diameter, μm
WT plus H_2O	18.4 ± 0.4	33.3 ± 0.9
WT plus ACC	17.2 ± 0.3*	27.8 ± 0.7***
WT plus H_2O plus 1-MCP	17.5 ± 0.4	34.9 ± 0.8
WT plus ACC plus 1-MCP	17.6 ± 0.4	35.3 ± 1.0
35S::*etr1-1* (1E) plus H_2O	14.7 ± 0.6	32.6 ± 1.3
35S::*etr1-1* (1E) plus ACC	14.6 ± 0.5	29.9 ± 0.9
35S::*etr1-1* (3A) plus H_2O	14.7 ± 0.4	32.4 ± 0.9
35S::*etr1-1* (3A) plus ACC	15.7 ± 0.8	32.4 ± 1.2
pLMX5::*etr1-1* (1) plus H_2O	14.6 ± 0.4	31.1 ± 1.4
pLMX5::*etr1-1* (1) plus ACC	14.0 ± 0.5	32.8 ± 2.0
pLMX5::*etr1-1* (6) plus H_2O	14.8 ± 0.5	34.9 ± 0.9
pLMX5::*etr1-1* (6) plus ACC	15.9 ± 0.2	34.6 ± 0.2

Wild-type control trees, transgenic trees expressing *Atetr1-1* under control of the 35S (lines 1E and 3A) or LMX5 (lines 1 and 6) promoters, and wild-type trees treated with 1-MCP were grown in vitro and treated with either water or 100 μM ACC. The inhibition of ACC on fiber and vessel element diameter growth was nullified in transgenic and 1-MCP-treated trees, demonstrating their ethylene insensitivity for these processes. Treatments were done when the trees had reached a height of 5 cm, and trees were sampled 12 days after the treatment. Xylem cell width was measured in macerated xylem from the reference internode, in which all xylem was formed under the influence of ACC. For each tree, 20 vessel elements and 30 fibers were measured, and a mean value for each tree was calculated. This mean value from each individual tree was used to calculate a mean ± SE of between 8 and 15 independent trees. Statistically significant differences (Student's *t* test) between water- and ACC-treated trees within genotype (or within 1-MCP-treated trees) are indicated by *, $P < 0.05$; ***, $P < 0.001$.

Table S2. Primer sequences used in cloning procedures

Gene/target	Primer	Product size, bp
LMX5 promoter	5′-GATGCAAAAGCTGGAATGAG (plus a 5′ BglII site) 5′-GAGAAGAAGGTGTTTGG (plus a 5′ SalI site)	1,807
Cassette reading frame B	5′-AAGGTGATCAATCAACAAGTTTG (5′ BclI) 5′-CCGAGCTCATCAACCACTTT (5′ SacI)	1,731
Genomic *etr1-1*	5′-*att*B1-GTTAAACCCAACCAATTTTGAC 5′-*att*B2-TGTTGTTGTTCTACTAAGCGGC	3,595

For every primer pair, the upper one is the forward primer and the lower one the reverse primer. The added restriction sites are underlined.

Table S3. Primer sequences used in all real-time qPCR analyses

Gene	Real-time qPCR primer	Product size, bp
Ribosomal 18S RNA	5′-TCAACTTTCGATGGTAGGATAGTG	145
	5′-CCGTGTCAGGATTGGGTAATTT	
etr1-1	5′-AGCCATTCCCCGACATTCAA	98
	5′–GGTGTACAAGAAGTCCCTTCGTCA	
ACS1	5′-TGGAAAGCCTATGACACTGAT	132
	5′-GGAGGCTTTTGGATTCTTCTT	
ACS2	5′-GATGGATGGAAAGCTTACGAT	140
	5′-ATGGAGGCTTTGGGATGTT	
ACS3	5′-GAGTCATGGCTGGCTAAGAA	138
	5′-TTCTGCCATGAATTCTACCAA	
ACS4	5′-TGGCTTGCTAATAACCAGGA	129
	5′-AGCCATGAATTCCACCAA	
ACS5	5′-GCTGGAAGGCACACGAT	130
	5′-GCTTCTGAATTCTGTTCCAAGTA	
ACS6	5′-TCATCTAATCCATCAGGAGTCAT	128
	5′-CTAAACCCTGGCGATCCTTT	
ACS7	5′-GCTGGAAAGCCTATGACAGTA	134
	5′-GGAGGCTTTTGGGTTGTT	
ACS8	5′-GAATCCATATCACGATGTGCAT	134
	5′-CACCATTCTTCTTCAACCCAA	
ACO1	5′-GATAACGAGTGGCCATCAA	158
	5′-CCTTCTCCATCATTGAATGCTT	
ACO2	5′-TTGCGACATCTCCCCAT	147
	5′-GACTACCGCTAGATCCACAGAA	
ACO3	5′-CGCCATCTCCCTAAGTCAA	185
	5′-GGACTACCGTTTGATCCATAGAA	
ACO4	5′-TTGTCATCAATACCGGTGAT	155
	5′-TGCTGGTGCAGGAGCTA	
ACO5	5′-CCAACATCCAACATCAATGAA	143
	5′-ATGTAATCCTTGTCCAGTCCAA	
ACO6	5′-CAACCATGGAGGTGATCAA	152
	5′-TGCTAGCCACCATCTCCTT	
ACO7	5′-CCAACCATGGAGAAGATTGAA	154
	5′-CCACCATTTCCTTGAACCTT	

For the every primer pair, the upper one is forward primer and the lower one is the reverse primer. Gene numbering for *ACS/ACO* gene family is according to existing *Populus tremula* × *tremuloides* National Center for Biotechnology Information accessions and U.S. Department of Energy Joint Genome Institute *Populus trichocarpa* annotations. The genome mining for the *P. trichocarpa ACS/ACO* gene family was conducted by a BLAST search using *Arabidopsis*, rice, and tomato *ACS/ACO* genes and their domain variants as an electronic probe.