

**Supplementary materials to:**

**Nitrogen metabolism of two contrasting poplar species in acclimation to limiting nitrogen availability**

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**Supplementary Table S1. Primers used for qRT-PCR.**

Gene model	Accession number	Gene Name	Closest AGI	Primer-Forward	Primer-Reverse	PCR efficiency (%)
a		<i>AMT1;2</i>	AT4G13510	5'-GATGGCATTATAAAGGCATCAAA-3'	5'-GAAGAAGGCGTACGTGAATGA-3'	101
Potri.009G045200 <sup>b</sup>	KC256927 <sup>Pp</sup> , KC256928 <sup>Pg</sup>	<i>AMT1;6</i>	AT1G64780	5'-TTCAGGTGAGGCGGGAGT-3'	5'-CCAAAGCGCCAATGATACAG-3'	104
Potri.006G102800 <sup>b</sup>	KC256925 <sup>Pp</sup> , KC256926 <sup>Pg</sup>	<i>AMT2;1</i>	AT2G38290	5'-GTTATAGGTTGGAACGTGGTGT-3'	5'-TCAATATTACATGCGTCAAGATTC-3'	104
Potri.003G111500 <sup>b</sup>	KC256933 <sup>Pp</sup> , KC256934 <sup>Pg</sup>	<i>NRT1;1</i>	AT1G12110	5'-CTAAACCAAGGGAGGCTCCATGAT-3'	5'-CCCAACACAAAAGTAGGCGAAAAG-3'	100
Potri.012G070700 <sup>b</sup>	KC256935 <sup>Pp</sup> , KC256936 <sup>Pg</sup>	<i>NRT1;2</i>	AT1G69850	5'-TCTTTGGTAGCAACTTGAACAA-3'	5'-TCTCTCTCTCTCTCGTCTCCCT-3'	100
Potri.009G008500 <sup>b</sup>	KC256937 <sup>Pp</sup> , KC256938 <sup>Pg</sup>	<i>NRT2;4B</i>	AT5G60770	5'-AATAGAGGAAGGGAATGGCTG-3'	5'-TGAGGTTGTCCCGAATGATAG-3'	98
Potri.009G008600 <sup>b</sup>	KC256939 <sup>Pp</sup> , KC256940 <sup>Pg</sup>	<i>NRT2;4C</i>	AT1G08090	5'-CAGTCCCACAGATAACAAC-3'	5'-CTTCCCCTACAACGATTTTC-3'	98
Potri.015G085000 <sup>b</sup>		<i>NRT3;1B</i>	AT5G50200	5'-TCATAGCCTCTTCTTCTACCTTTCC-3'	5'-CCACCTTTCAATACTTGTCGG-3'	105
Potri.015G085100 <sup>b</sup>		<i>NRT3;1C</i>	AT5G50200	5'-AGAGGTCTCAGTGAAGCGAACAAG-3'	5'-CGCAAATACAAACGCAATTATCAT-3'	105
Potri.005G172400 <sup>c</sup>	KC256931 <sup>Pp</sup> , KC256932 <sup>Pg</sup>	<i>NR</i>	AT1G37130	5'-ATCATCGGATCGGAGAGTTGG-3'	5'-GACGGT-GCTAGTTGGCGTATAG-3'	99
Potri.004G140800 <sup>d</sup>		<i>NiR</i>	AT2G15620	5'-ACAAGTTGCCGATATTGGGTTTCAT-3'	5'-CCTCTATCACCCGTCGTAGTCTTG-3'	98
Potri.017G131100 <sup>c</sup>	DQ855559	<i>GS1;3</i>	AT5G37600	5'-GGCGCAGACCAAGCTTCTC-3'	5'-GCGAAGTGACAGATTTAGGATTGC-3'	100
Potri.010G029100 <sup>c</sup>	DQ855560	<i>GS2</i>	AT5G35630	5'-ATCAGGTGGTCCCAGTGTG-3'	5'-GCGAAGTGACAGATTTAGGATTGC-3'	95
Potri.016G036900 <sup>c</sup>	DQ855561	<i>Fd-GOGAT</i>	AT5G04140	5'-AACCCTAAAGGCATCAGACTCAG-3'	5'-AGTAAAGCAGGTCCATCCAAG-3'	101
Potri.012G011700 <sup>c</sup>	KC256929 <sup>Pp</sup> , KC256930 <sup>Pg</sup>	<i>NADH-GOGAT</i>	AT5G53460	5'-GGTGTGTGGATATTCCTCTG-3'	5'-TCAGATGCGGCGACAACCC-3'	95
Potri.013G058300 <sup>d</sup>		<i>GDH</i>	AT5G18170	5'-ATTTATGCTAACTCAGGAGGCGTT-3'	5'-GAGGAGGAAACTAGGGCAATACAT-3'	102
Potri.018G006000 <sup>d</sup>		<i>VHA1;1</i>	AT2G24520	5'-CCGCTCAGACCAACTATCTT-3'	5'-TCCTCTGGGCTTGAATGGTAG-3'	96
Potri.018G090300 <sup>d</sup>		<i>VHA2;2</i>	AT2G18960	5'-GGCTCCAACCTGAGACAG-3'	5'-AGCACAGCCCTTCTCTCCA-3'	104
e		<i>Actin 2/7</i>		5'-CCCATTGAGCACGGTATTGT-3'	5'-TACGACCACTGGCATACAGG-3'	101

a: The gene name was from Selle A et al (2005) The high affinity poplar ammonium importer PttAMT1.2 and its role in ectomycorrhizal symbiosis. *New Phytologist* 168, 697–706. However, we re-designed primers for qRT-PCR;

b: From Li H et al (2012) N-fertilization has different effects on the growth, carbon and nitrogen physiology, and wood properties of slow- and fast-growing *Populus* species. *Journal of Experimental Botany* 63, 6173-6185;

c: From Dluzniewska P et al (2007) Nitrogen uptake and metabolism in *Populus × canadensis* as affected by salinity. *New Phytologist* 173, 279-293;

d: These primers were designed by ourselves based on the database of phytozome *Populus trichocarpa* v3.0;

e: From Brunner AM et al (2004) Validating internal controls for quantitative plant gene expression studies. *BMC Plant Biology* 4:14;

Pp: Genes cloned from *P. popularis*; Pp: Genes cloned from *P. alba × P. glandulosa*.

**Supplementary Table S2.** Mineral nutrients ( $\mu\text{mol g}^{-1}$  DW) in roots and leaves of *P. popularis* (Pp) and *P. alba*  $\times$  *P. glandulosa* (Pg) exposed to 10, 100 or 1000  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . Data indicate means  $\pm$  SE (n = 6). Different letters behind the values in the same column indicate significant difference. *P*-values of the ANOVAs of species, N treatment (N) and their interaction are indicated. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; \*\*\*\*:  $P < 0.0001$ ; ns: not significant.

Species	Tissues	N treatment ( $\mu\text{M}$ )	P	S	Na	K	Ca	Mg	Mn	Fe	Al
Pp	Roots	10	506.9 $\pm$ 82.8 a	102.1 $\pm$ 0.64 d	61.8 $\pm$ 9.6 a	333.9 $\pm$ 2.9 c	1545.9 $\pm$ 232.1 b	342.0 $\pm$ 16.6 d	6.68 $\pm$ 0.8 b	103.7 $\pm$ 8.6 b	136.6 $\pm$ 7.2 c
	Roots	100	730.0 $\pm$ 72.9 b	78.6 $\pm$ 0.66 ab	63.2 $\pm$ 4.2 a	266.2 $\pm$ 5.6 b	1330.2 $\pm$ 134.6 ab	338.1 $\pm$ 18.6 c	7.8 $\pm$ 0.9 b	175.2 $\pm$ 4.9 c	146.8 $\pm$ 6.4 c
	Roots	1000	645.2 $\pm$ 64.6 ab	82.4 $\pm$ 3.38 bc	72.0 $\pm$ 8.1 a	171.5 $\pm$ 16.0 a	1302.1 $\pm$ 14.7 ab	251.8 $\pm$ 4.4 b	6.8 $\pm$ 0.7 b	87.5 $\pm$ 1.8 b	143.4 $\pm$ 5.0 c
Pg	Roots	10	715.1 $\pm$ 52.3 b	87.8 $\pm$ 5.55 c	82.5 $\pm$ 8.1 a	345.8 $\pm$ 5.7 d	1559.4 $\pm$ 187.5 b	290.5 $\pm$ 3.8 c	4.3 $\pm$ 0.4 a	43.9 $\pm$ 6.2 a	48.6 $\pm$ 6.7 a
	Roots	100	697.6 $\pm$ 66.1 ab	79.0 $\pm$ 1.3 abc	70.2 $\pm$ 0.8 a	342.8 $\pm$ 8.2 d	1041.0 $\pm$ 106.6 a	191.9 $\pm$ 11.1 a	3.8 $\pm$ 0.3 a	33.4 $\pm$ 1.9 a	50.6 $\pm$ 2.5 a
	Roots	1000	693.8 $\pm$ 56.6 ab	72.6 $\pm$ 2.0 a	74.8 $\pm$ 8.1 a	259.2 $\pm$ 10.3 b	1063.2 $\pm$ 81.3 a	167.4 $\pm$ 0.42 a	3.8 $\pm$ 0.1 a	35.8 $\pm$ 5.8 a	80.1 $\pm$ 5.4 b
Pp	Leaves	10	100.2 $\pm$ 4.6 a	70.9 $\pm$ 1.2 a	20.93 $\pm$ 1.0 a	460.2 $\pm$ 0.9 a	254.6 $\pm$ 14.0 cd	164.4 $\pm$ 8.9 ab	0.8 $\pm$ 0.1 bc	1.8 $\pm$ 0.1 a	3.6 $\pm$ 0.4 a
	Leaves	100	112.0 $\pm$ 6.8 ab	79.6 $\pm$ 2.0 ab	21.5 $\pm$ 0.9 a	508.3 $\pm$ 19.6 ab	256.1 $\pm$ 5.6 cd	178.0 $\pm$ 7.4 b	0.9 $\pm$ 0.2 c	2.3 $\pm$ 0.3 ab	4.0 $\pm$ 0.7 a
	Leaves	1000	112.1 $\pm$ 5.1 ab	73.7 $\pm$ 3.9 ab	23.0 $\pm$ 1.3 a	504.6 $\pm$ 13.3 ab	157.9 $\pm$ 6.5 a	140.6 $\pm$ 4.4 a	0.6 $\pm$ 0.1 ab	2.1 $\pm$ 0.1 a	5.1 $\pm$ 0.9 ab
Pg	Leaves	10	109.6 $\pm$ 6.8 ab	93.3 $\pm$ 4.8 bc	19.7 $\pm$ 1.2 a	508.3 $\pm$ 33.2 ab	277.2 $\pm$ 7.9 d	184.6 $\pm$ 17.9 b	0.5 $\pm$ 0.2a	2.6 $\pm$ 0.1 b	8.7 $\pm$ 0.3 c
	Leaves	100	123.8 $\pm$ 11.8 b	114.9 $\pm$ 13.5 c	25.3 $\pm$ 2.0 a	621.2 $\pm$ 42.0 c	231.7 $\pm$ 5.7 c	181.2 $\pm$ 15.5 b	0.4 $\pm$ 0.1 a	2.0 $\pm$ 0.1 a	6.3 $\pm$ 0.6 bc
	Leaves	1000	124.8 $\pm$ 8.4 b	107.1 $\pm$ 9.2 c	22.8 $\pm$ 5.4 a	579.3 $\pm$ 44.8 bc	200.2 $\pm$ 11.7 b	155.6 $\pm$ 12.3 ab	0.6 $\pm$ 0.1 ab	2.6 $\pm$ 0.2 b	7.5 $\pm$ 0.3 bc
P-Values	Roots	Species	ns	**	ns	****	ns	****	****	****	****
	Roots	N	ns	***	ns	****	*	****	ns	****	*
	Roots	Species $\times$ N	ns	ns	ns	**	ns	*	ns	****	*
	Leaves	Species	ns	***	ns	**	ns	ns	***	*	****
	Leaves	N	ns	ns	ns	*	****	*	ns	ns	ns
	Leaves	Species $\times$ N	ns	ns	ns	ns	**	ns	**	**	*

**Supplementary Table S3.** PCA of physiological parameters of both poplar species exposed to 10, 100 and 1000  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ .

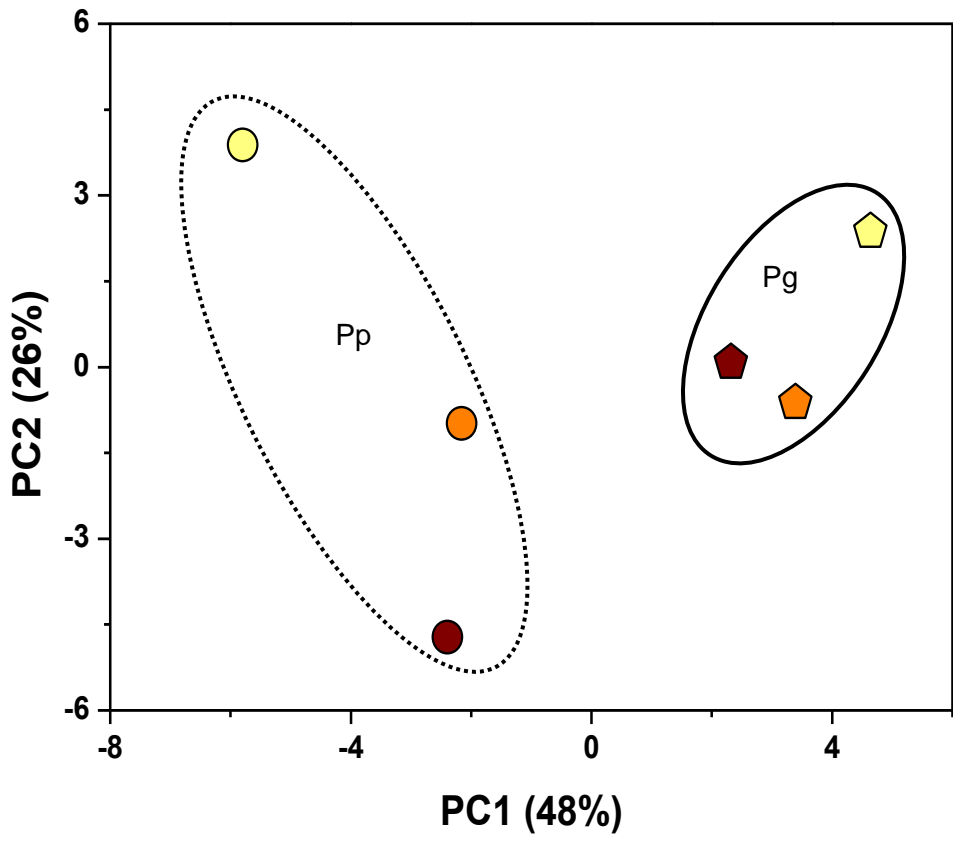
Variables	PC1	PC2	PC3	PC4	PC5	PC6
Root biomass	-0.137	-0.020	-0.124	-0.235	-0.139	0.012
Primary root length	-0.107	0.111	-0.155	0.060	0.018	-0.063
Total root length	-0.108	-0.027	-0.166	0.277	0.052	-0.061
Fine root root length	-0.155	-0.059	-0.217	0.057	-0.016	0.036
Fine root to total root length ratio	-0.157	0.039	-0.125	0.101	0.012	0.082
Root average diameter	0.176	0.024	0.062	-0.086	0.066	-0.060
Root surface area	0.015	-0.146	-0.298	0.079	0.132	0.031
Fine root surface area	-0.158	0.028	-0.116	0.153	0.008	0.083
Root volume	0.165	-0.027	-0.051	-0.053	0.100	-0.126
Leaf dry weight	0.018	-0.066	-0.013	0.118	-0.097	0.307
Specific leaf area	0.117	0.084	-0.002	0.136	0.061	-0.351
Net photosynthetic rate ( $A$ )	0.082	-0.226	0.013	0.055	-0.119	0.006
Stomatal conductance ( $g_s$ )	0.171	-0.088	0.065	0.051	-0.048	0.035
Transpiration rate ( $E$ )	0.167	-0.085	0.082	0.070	-0.092	0.038
Ratio of intercellular $\text{CO}_2$ to ambient $\text{CO}_2$ concentration ( $C_i/C_a$ )	0.153	0.050	0.042	0.080	-0.092	0.026
Intrinsic water use efficiency ( $WUE_i$ )	-0.146	-0.015	-0.027	-0.102	0.167	-0.054
Instantaneous photosynthetic N use efficiency ( $PNUE_i$ )	0.085	-0.161	0.036	0.160	-0.087	-0.252
$\text{NH}_4^+$ fluxes	0.102	-0.213	0.063	-0.014	-0.032	0.044
$\text{NO}_3^-$ fluxes	0.098	-0.212	0.032	0.028	0.014	0.070
$\text{H}^+$ fluxes	0.064	-0.158	-0.223	-0.175	-0.086	0.122
PM $\text{H}^+$ -ATPase	0.096	-0.137	0.223	0.085	-0.030	-0.121
Root Ammonium	0.137	-0.139	-0.001	0.104	0.085	0.106
Root Nitrate	0.142	0.076	-0.104	0.073	0.141	0.020
Root Nitrite	0.042	-0.013	-0.015	-0.189	0.421	0.088
Leaf Ammonium	-0.116	-0.117	-0.024	-0.087	0.187	0.012
Leaf Nitrate	0.057	-0.149	0.014	0.011	0.327	-0.098
Leaf Nitrite	-0.063	0.199	0.209	0.107	-0.015	0.061
Root nitrate reductase (NR)	0.074	-0.174	-0.116	0.023	0.129	0.127
Root nitrite reductase (NiR)	-0.135	0.031	0.021	-0.014	0.129	-0.051
Root glutamine synthetase (GS)	0.053	0.136	-0.010	-0.309	-0.075	0.019
Root glutamate synthase (GOGAT)	-0.054	0.085	0.059	-0.026	0.149	-0.312
Root glutamate dehydrogenase (GDH)	-0.047	-0.038	0.082	0.170	0.086	-0.281
Leaf nitrate reductase (NR)	0.081	0.147	-0.007	-0.020	-0.134	0.132
Leaf nitrite reductase (NiR)	0.161	0.061	0.019	0.015	-0.130	-0.072
Leaf glutamine synthetase (GS)	0.140	0.144	-0.068	-0.033	-0.012	-0.107
Leaf glutamate synthase (GOGAT)	0.072	0.115	-0.103	-0.248	0.097	-0.022
Leaf glutamate dehydrogenase (GDH)	0.120	0.011	-0.004	-0.103	0.212	0.267
Root N concentrations	0.151	-0.117	-0.046	-0.001	-0.035	0.056

Continued:

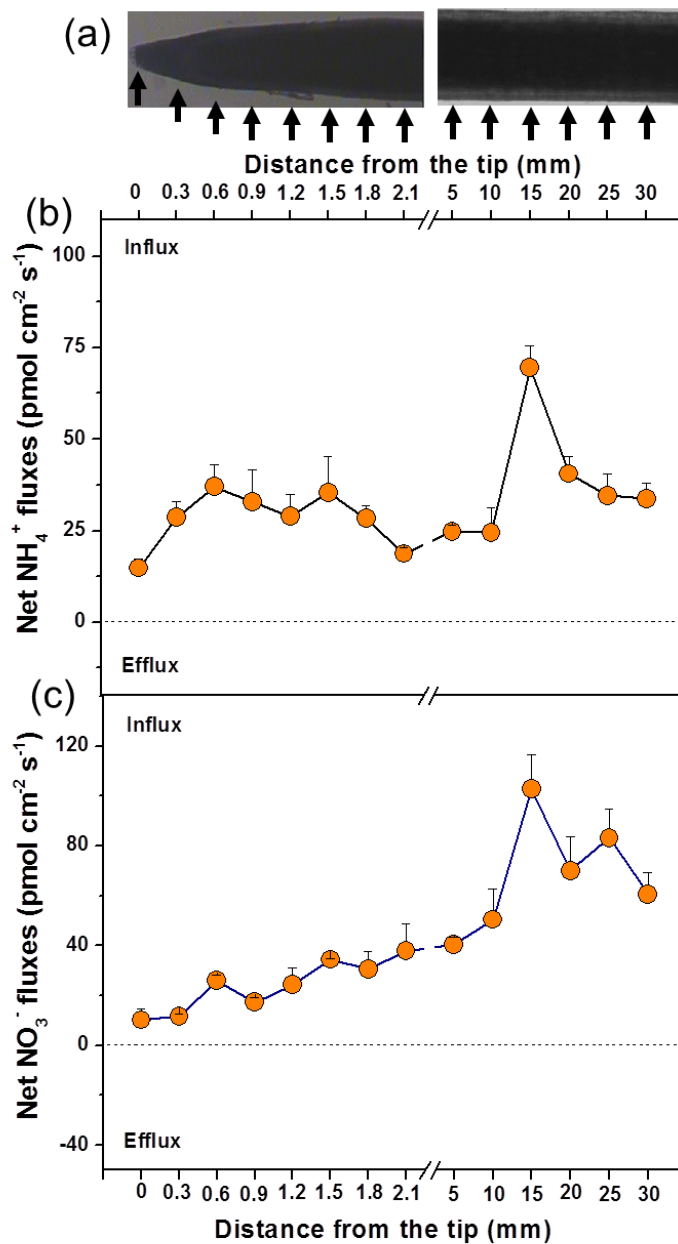
Root $\delta^{15}\text{N}$	-0.186	0.052	0.013	-0.058	0.010	-0.058
Root protein	0.136	0.029	-0.064	0.130	0.098	0.223
Leaf N concentrations	0.187	-0.003	-0.075	-0.003	0.001	-0.032
Leaf $\delta^{15}\text{N}$	-0.109	0.182	-0.007	0.012	0.002	-0.005
Leaf protein	-0.090	-0.192	-0.001	0.007	0.087	-0.127
Root P	0.066	0.021	-0.189	0.281	-0.043	-0.161
Root S	-0.121	0.091	0.169	-0.121	0.004	0.053
Root Na	0.079	0.063	0.093	0.014	-0.142	0.084
Root K	0.005	0.242	-0.011	-0.094	0.136	-0.045
Root Ca	-0.102	0.079	0.154	0.074	-0.175	0.050
Root Mg	-0.180	0.064	0.017	0.097	-0.041	0.003
Root Mn	-0.153	-0.084	-0.056	-0.004	-0.168	-0.003
Root Fe	-0.159	-0.052	-0.198	0.063	-0.053	0.034
Root Al	-0.146	-0.153	-0.074	-0.022	-0.058	0.045
Leaf P	0.109	-0.008	-0.158	-0.087	-0.202	-0.031
Leaf S	0.151	0.082	-0.155	-0.012	-0.055	-0.109
Leaf Na	0.056	-0.032	-0.130	-0.025	-0.071	-0.101
Leaf K	0.135	0.024	-0.191	-0.063	-0.083	-0.176
Leaf Ca	-0.067	0.218	-0.137	0.067	0.025	0.049
Leaf Mg	0.004	0.185	-0.207	0.021	-0.170	-0.019
Leaf Mn	-0.140	-0.067	-0.168	0.094	0.134	0.097
Leaf Fe	0.066	0.032	-0.036	0.326	0.024	0.160
Leaf Al	0.136	0.084	0.145	0.171	0.028	0.133
Root C concentrations	0.173	-0.079	-0.079	-0.021	0.040	0.026
Root $\delta^{13}\text{C}$	-0.138	-0.135	0.067	-0.111	-0.075	0.104
Root soluble sugar	0.116	0.079	-0.064	0.005	0.227	0.076
Root starch	-0.025	0.136	-0.220	0.200	0.043	0.029
Root phenolics	0.102	0.185	0.074	0.096	0.148	0.031
Leaf C concentrations	-0.096	0.035	0.251	0.133	0.056	0.143
Leaf $\delta^{13}\text{C}$	-0.088	-0.135	0.168	0.123	0.034	-0.002
Leaf soluble sugar	-0.129	-0.182	-0.028	-0.017	0.059	-0.041
Leaf starch	0.003	0.204	0.031	0.126	0.146	0.110
Leaf phenolics	-0.138	-0.129	0.118	0.032	0.094	-0.140
Proportion of variation (%)	37	20	7	6	5	4

**Supplementary Table S4.** PCA of transcriptional changes of representative genes involved in N uptake and assimilation in roots (A) and leaves (B) of *P. popularis* (Pp) and *P. alba* × *P. glandulosa* (Pg) exposed to 10, 100 or 1000  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . The PCA plot was also shown below. In PCA plot, data points are presented for 10 (yellow), 100 (orange) and 1000 (wine)  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ .

Variables	PC1	PC2	PC3
Root <i>AMT1;2</i>	-0.085	0.296	-0.095
Leaf <i>AMT1;2</i>	0.221	0.091	-0.075
Root <i>AMT1;6</i>	-0.169	-0.097	-0.272
Leaf <i>AMT1;6</i>	-0.227	-0.126	0.024
Root <i>AMT2;1</i>	-0.169	0.045	0.249
Leaf <i>AMT2;1</i>	0.154	-0.131	0.164
Root <i>NRT1;1</i>	-0.179	0.205	0.052
Leaf <i>NRT1;1</i>	-0.001	-0.175	-0.024
Root <i>NRT1;2</i>	0.057	0.285	0.218
Leaf <i>NRT1;2</i>	0.178	-0.215	0.076
Root <i>NRT2;4B</i>	0.098	0.230	0.272
Leaf <i>NRT2;4B</i>	0.216	-0.071	-0.057
Root <i>NRT2;4C</i>	-0.223	-0.096	-0.060
Leaf <i>NRT2;4C</i>	-0.227	-0.024	0.166
Root <i>NRT3;1B</i>	-0.130	0.224	-0.215
Leaf <i>NRT3;1B</i>	0.151	0.050	-0.355
Root <i>NRT3;1C</i>	-0.185	0.211	0.075
Leaf <i>NRT3;1C</i>	-0.196	-0.103	-0.251
Root <i>NR</i>	-0.219	-0.072	0.084
Leaf <i>NR</i>	-0.220	-0.006	0.194
Root <i>NiR</i>	-0.185	-0.194	-0.147
Leaf <i>NiR</i>	-0.094	0.257	-0.116
Root <i>GS1;3</i>	-0.154	0.226	-0.131
Leaf <i>GS1;3</i>	-0.121	-0.218	0.127
Root <i>GS2</i>	-0.194	0.117	-0.247
Leaf <i>GS2</i>	-0.190	-0.083	-0.129
Root <i>Fd-GOGAT</i>	-0.208	-0.021	0.146
Leaf <i>Fd-GOGAT</i>	-0.193	0.009	0.249
Root <i>NADH-GOGAT</i>	0.021	-0.305	0.156
Leaf <i>NADH-GOGAT</i>	-0.139	-0.231	0.159
Root <i>GDH</i>	0.173	0.166	0.115
Leaf <i>GDH</i>	0.202	-0.140	-0.002
Root <i>VHA1;1</i>	0.058	-0.225	-0.280
Root <i>VHA2;2</i>	-0.221	-0.106	-0.036
Proportion of variation (%)	48	26	16



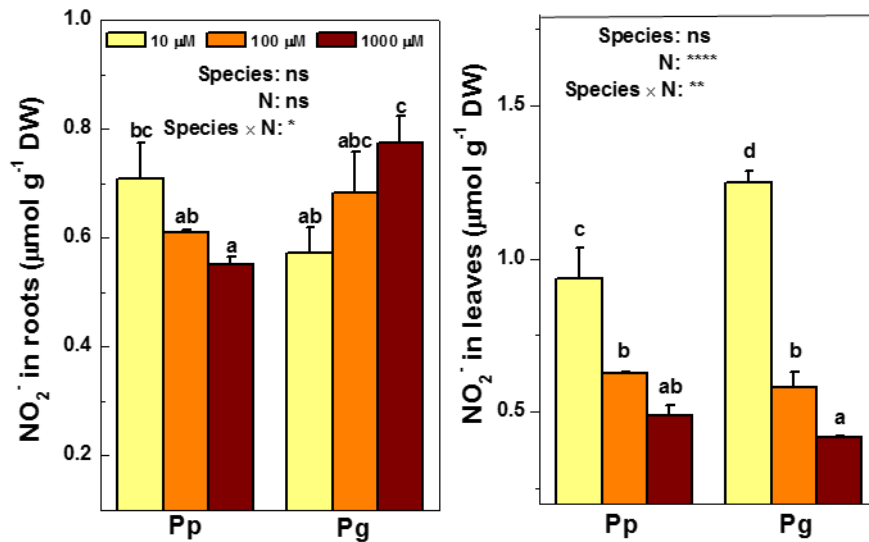
**Supplementary Fig. S1.** Root tip (a), net fluxes of  $\text{NH}_4^+$  (b) and  $\text{NO}_3^-$  (c) along the root tips of fine roots of *P. alba*  $\times$  *P. glandulosa* (Pg) exposed to 100  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . Data indicate means  $\pm$  SE (n = 6). The measuring solution contained 0.1 mM KCl and 0.1 mM  $\text{CaCl}_2$ , pH 5.5, besides either 0.1 mM  $\text{NH}_4\text{Cl}$  for  $\text{NH}_4^+$  or 0.1 mM  $\text{KNO}_3$  for  $\text{NO}_3^-$ . For net fluxes of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  along root tips of fine roots of *P. popularis* (Pp), please see Luo et al (2013) Net fluxes of ammonium and nitrate in association with  $\text{H}^+$  fluxes in fine roots of *Populus popularis*. *Planta* 237: 919-931.



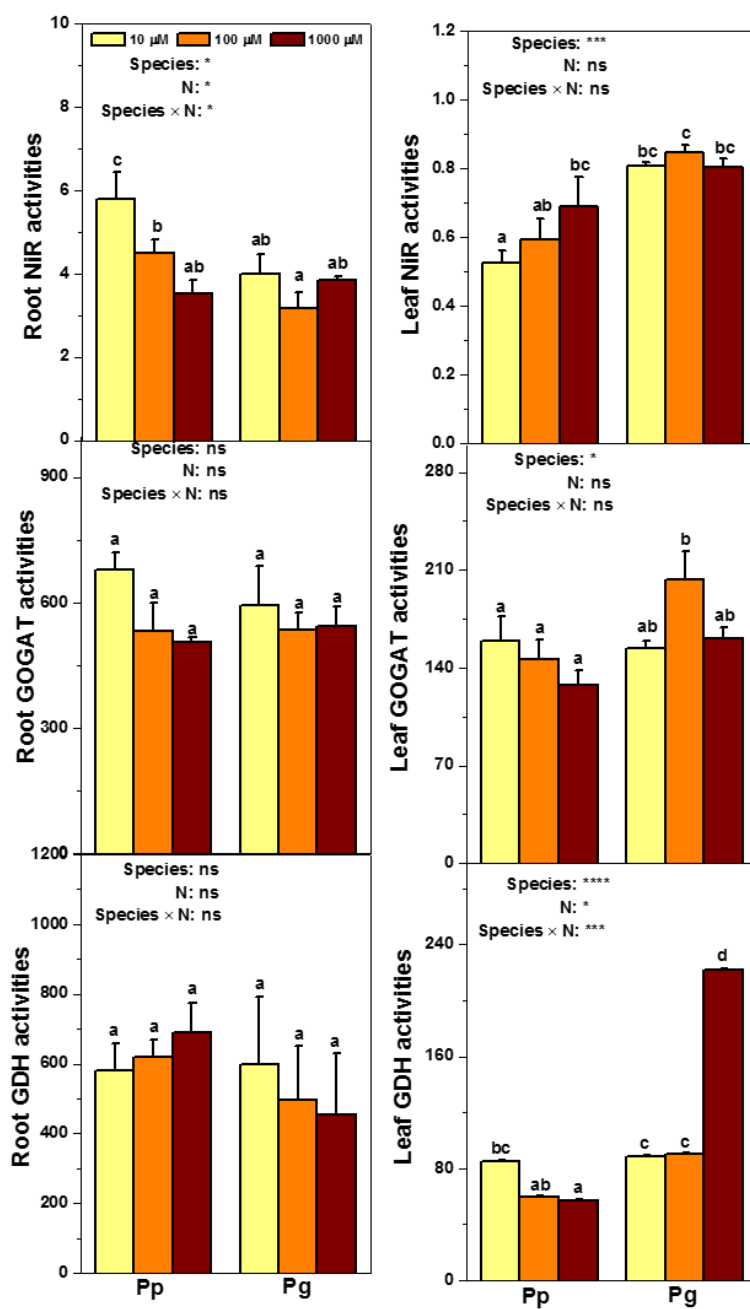


**Supplementary Fig. S2.** Alignments of representative genes. It is too large to be put here. For detailed information, please see the online version.

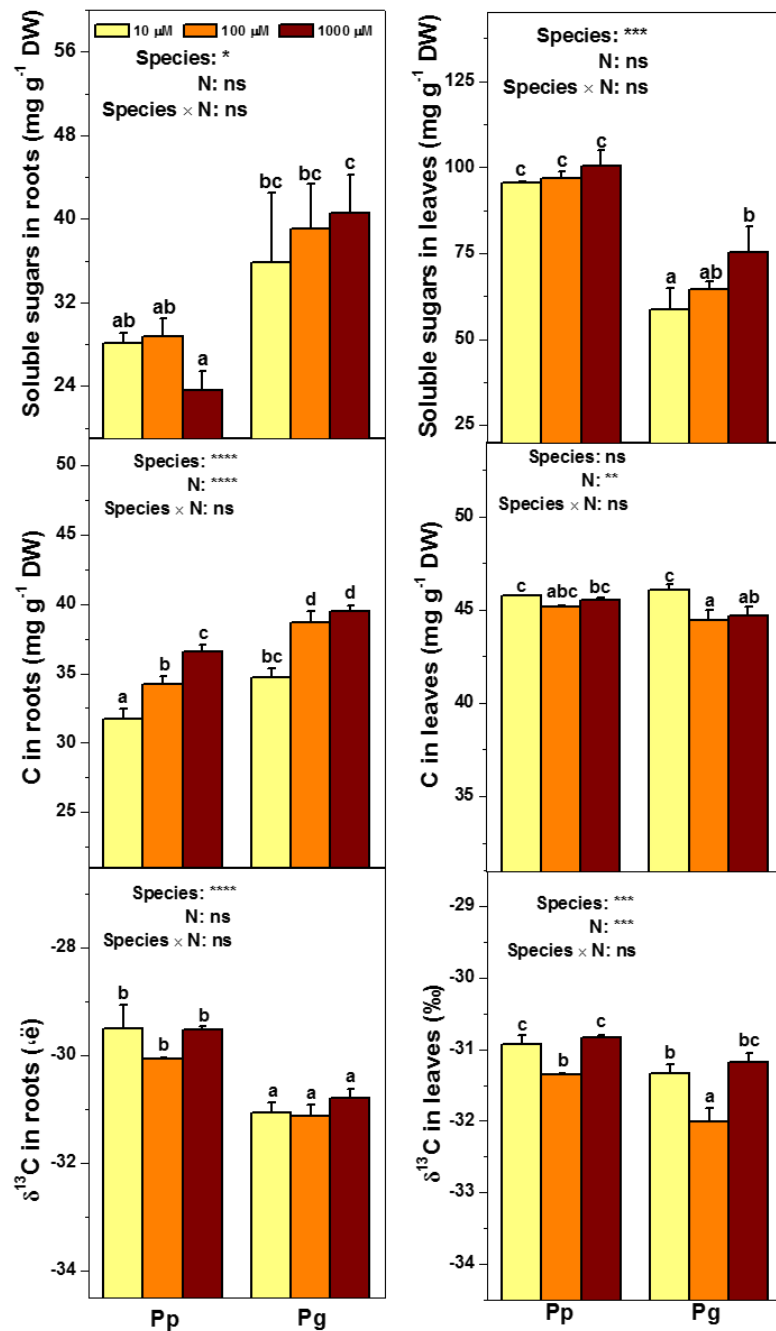
**Supplementary Fig. S3.**  $\text{NO}_2^-$  contents in roots and leaves of *P. popularis* (Pp) and *P. alba* × *P. glandulosa* (Pg) exposed to 10, 100 or 1000  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . Bars indicate means  $\pm$  SE (n = 6). Different letters on the bars indicate significant difference. P-values of the ANOVAs of species, N treatment (N) and their interaction are indicated. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; \*\*\*\*:  $P < 0.0001$ ; ns: not significant.



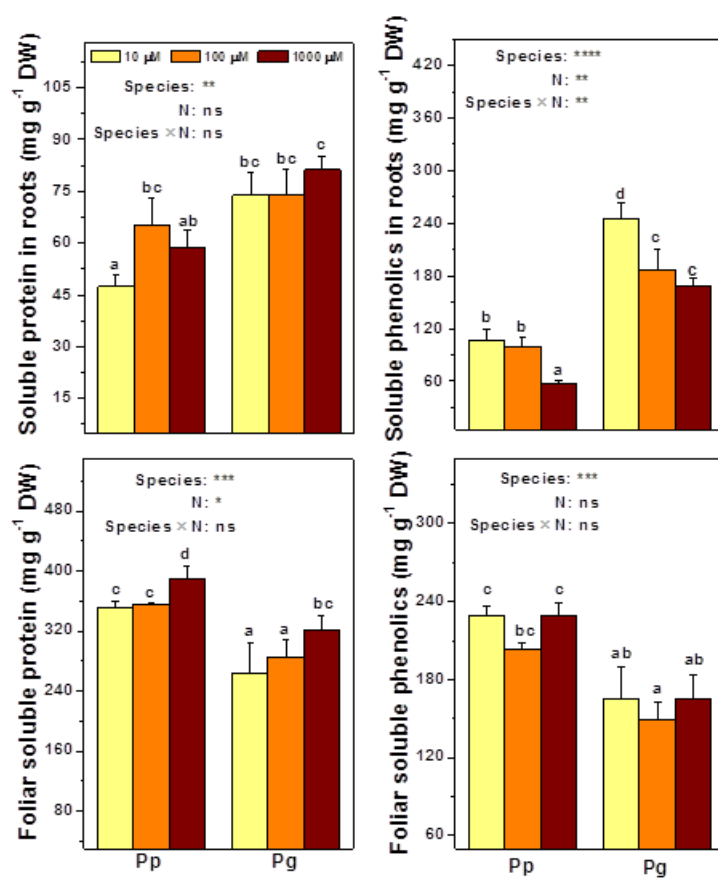
**Supplementary Fig. S4.** Activities of nitrite reductase (NiR,  $\text{mmol NO}_2^- \text{ h}^{-1} \text{ mg}^{-1}$  protein), glutamate synthase (GOGAT,  $\text{nkcat g}^{-1}$  protein) and glutamate dehydrogenase (GDH,  $\text{nkcat g}^{-1}$  protein) in roots and leaves of *P. popularis* (Pp) and *P. alba*  $\times$  *P. glandulosa* (Pg) exposed to 10, 100 or 1000  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . Bars indicate means  $\pm$  SE (n = 6). Different letters on the bars indicate significant difference. *P*-values of the ANOVAs of species, N treatment (N) and their interaction are indicated. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; \*\*\*\*:  $P < 0.0001$ ; ns: not significant.



**Supplementary Fig. S5.** Soluble sugars, C concentrations,  $\delta^{13}\text{C}$  in roots and leaves of *P. popularis* (Pp) and *P. alba*  $\times$  *P. glandulosa* (Pg) exposed to 10 (yellow), 100 (orange) or 1000 (wine)  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . Bars indicate means  $\pm$  SE ( $n = 6$ ). Different letters on the bars indicate significant difference. *P*-values of the ANOVAs of species, N treatment (N) and their interaction are indicated. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; \*\*\*\*:  $P < 0.0001$ ; ns: not significant.



**Supplementary Fig. S6.** Soluble protein and soluble phenolics in roots and leaves of *P. popularis* (Pp) and *P. alba* × *P. glandulosa* (Pg) exposed to 10, 100 or 1000  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ . Bars indicate means  $\pm$  SE (n = 6). Different letters on the bars indicate significant difference. *P*-values of the ANOVAs of species, N treatment (N) and their interaction are indicated. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; \*\*\*\*:  $P < 0.0001$ ; ns: not significant.



**Supplementary Fig. S7.** Correlations of related parameters. It is too large to be here.  
For detailed information, please see the online version.