Supplementary data

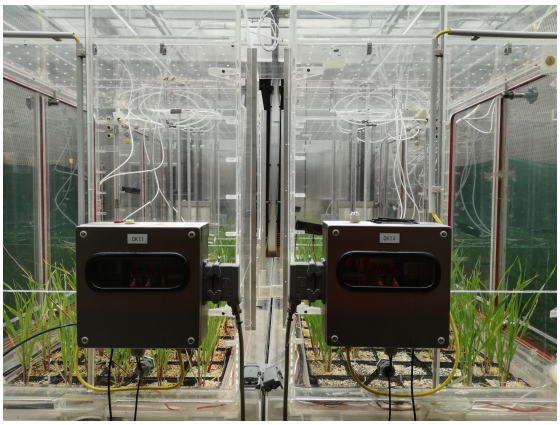
Table S1: Accession numbers of phytoglobin sequences employed in the multiple alignments and used to generate the phylogenetic tree

Protein	Species	Accession number
Class 1	*Arabidopsis thaliana* Phytoglobin 1	AAD26949.1
	Malus domestica Phytoglobin 1	AAP57676.1
	Pyrus communis Phytoglobin 1	AAP57677.1
	Gossypum hirsutum Phytoglobin 1	AAL09463.1
	Zea mays Phytoglobin 1	AAG01375.1
	Oryza sativa Phytoglobin 1.1	AAC49882.1
	Oryza sativa Phytoglobin 1.4	AAK72231.1
	Oryza sativa Phytoglobin 1.2	NM_001055972.1
	Oryza sativa Phytoglobin 1.3	NM_001056012.1
	Hordeum vulgare Phytoglobin 1.1	AAB70097.1
	Hordeum vulgare Phytoglobin 1.2	BAK07526.1
Class 2	*Arabidopsis thaliana* Phytoglobin 2	AAM65188.1
	Brassica napus Phytoglobin 2	AAK07741.1
	Grossypium hirsurtum Phytoglobin 2	AAK21604.1
	Beta vulgaris Phytoglobin 2	NP_001290022
Class3	*Arabidopsis thaliana* Phytoglobin 3	AEE86104.1
	Triticum aestivum Phytoglobin 3.1	ACH86231.1
	Triticum aestivum Phytoglobin 3.2	ACH86230.1
	Hordeum vulgare Phytoglobin 3	AAK55410.1

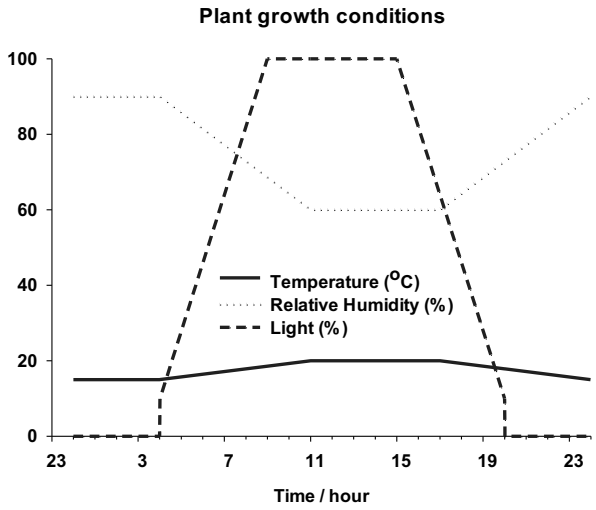
Table S2: Primers used for Real-Time PCR analysis.

Gene identifier	Name	Forward primer	Reverse primer
AY145451.1	*HvActin*	GCCGTGCTTTCCCTCTATGC	GCTTCTCCTTGATGTCCCTTAC
X60343.1	*HvGADPH*	GCTCAAGGGTATCATGGGTTACG	GCAATTCCACCCTTAGCATCAAAG
U94968.1	*HvPgb1.1*	TCGTCTTCAGCGAGGAGAAG	GATCTCGAAGATCTTGAGGAAG
AK376331.1	*HvPgb1.2*	ATGTGGACGCCGGAGATGAA	GCAGAGGCAGCGAGCTTCAT
AF376063.1	*HvPgb1.3*	CCTCTCCACCAACTTCTACACCA	TGGCCGATGTCGTCCTATCAAG
X57844.1	*HvNR*	GTCGACGCCGAGCTCGCCAA	GCGCACCTCGGACATGGT
LC097012.1	*HvNiR*	TCAAGTGGCTCGGCCTCTT	ACGCACACGTTCCACTTCCT
X53580.1	*HvGS2*	TGCTCGACATGGACACCA	CGTTTGTTAGTAGGGATGGGT
S58774.1	*HvFd-GOGAT*	TGCATGGAGCACCGTGGT	CCATCTAGGGCTTGTATTGGTACT

A



B



C

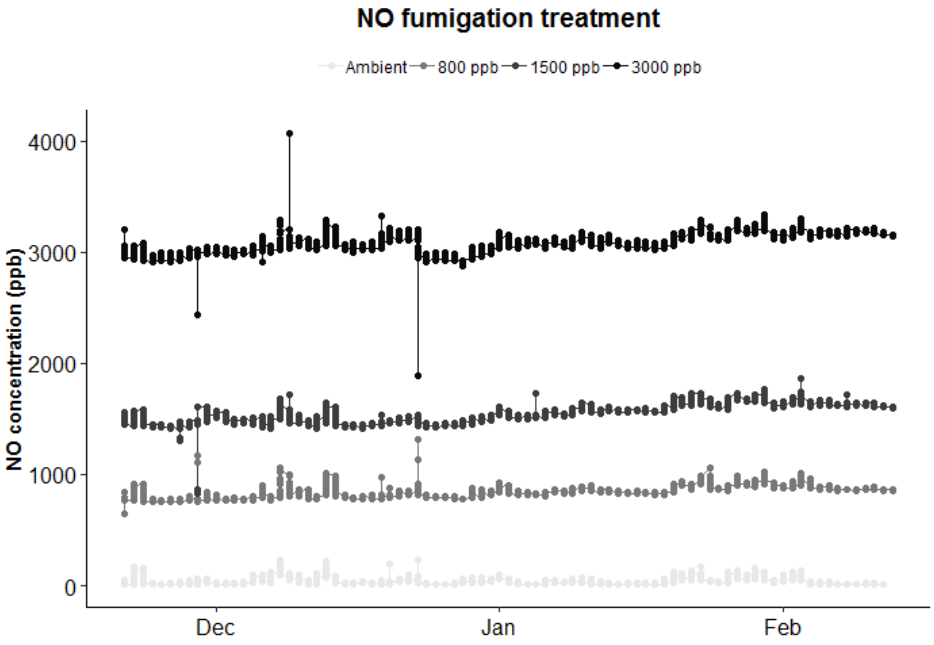


Fig. S1 Growth conditions for plants with long term NO fumigation treatment.

Barley plants were treated with various concentrations of NO in specially designed exposure chambers (A). The NO levels inside these chambers were continuously monitored using chemiluminescence detection method sensitive to as low as 1 ppb of NO. The plant growth conditions are showed in graph B, the photosynthetic photon flux density (PPFD) of light at 100% from 9:00-15:00 is 300 µmol m^{-2} s^{-1}, the photosynthetically active radiation (PAR) is 400 – 700 nm. The concentration monitored during the experiment is showed in graph C. All the chambers were supplied with ambient air that was directly drawn from the campus of Helmholtz Zentrum München, Germany. All the chambers were supplied with purified ambient air (see methods).

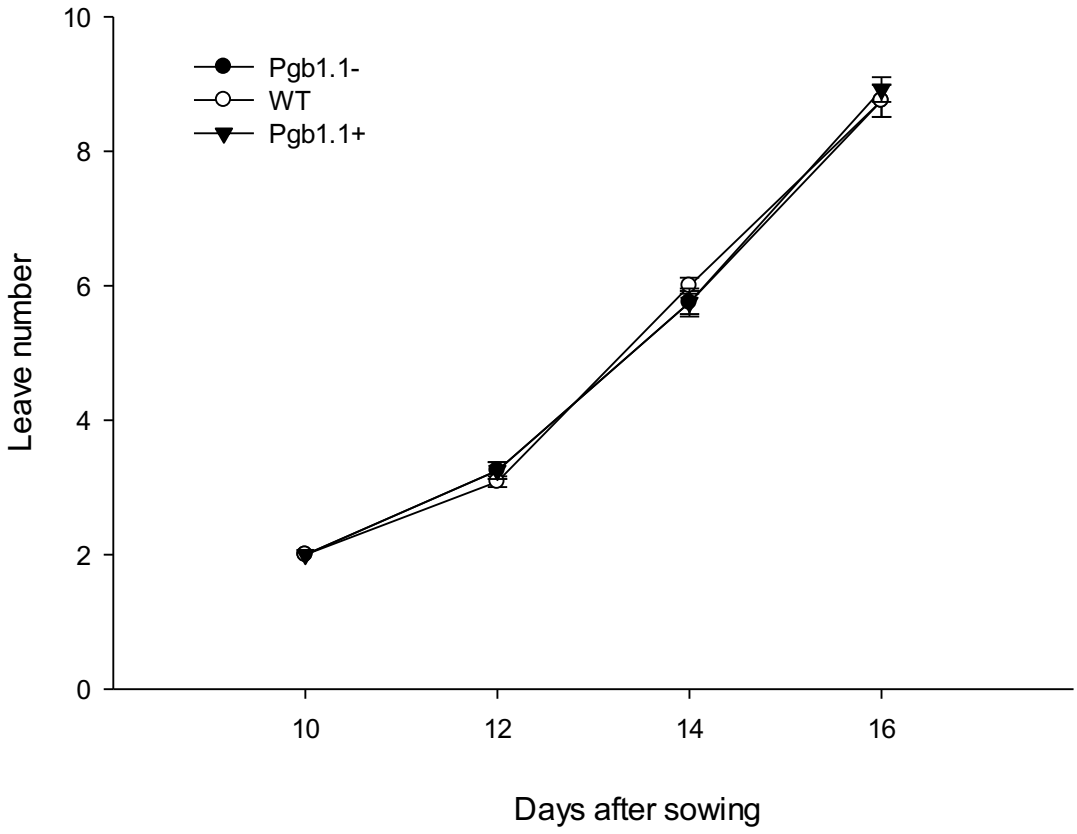


Fig. S2 Number of leaves during plant development.

To monitor plant development leaf numbers of WT, HvPgb1.1- and HvPgb1.1+ were determined at 10, 12, 14 and 16 days after sowing. 12 plants per line were analysed.

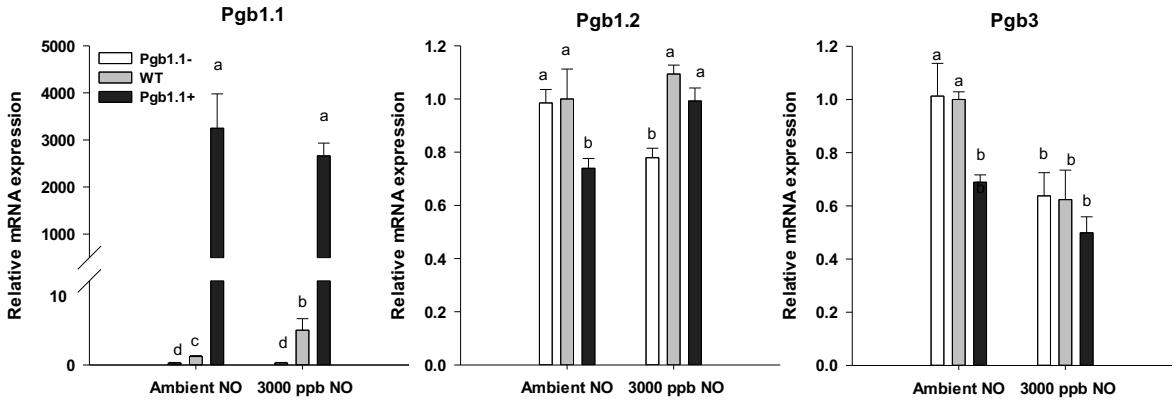


Fig. S3 Transcript levels of *HvPgb1.1*, *HvPgb1.2* and *HvPgb3* in barley leaves of Pgb1.1-, WT and Pgb1.1+ plants after NO fumigation.

Leaf samples were taken after 20 days of NO fumigation. *HvGADPH* and *HvACTIN* were used as housekeeping genes. Each data represents means ± SE (n=4). Different letters indicate significant differences among treatments at $P < 0.05$, according to Tukey's test.

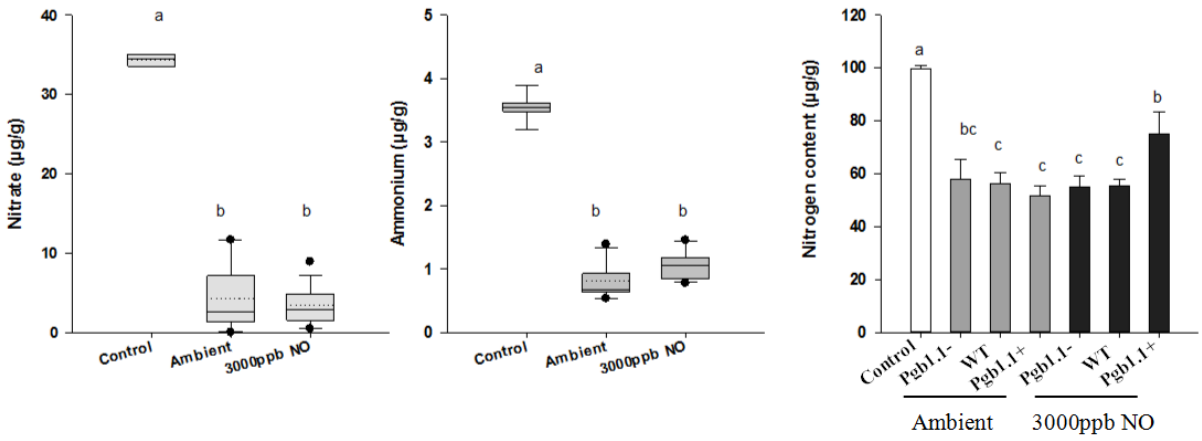


Fig. S4 Nitrate, ammonium and nitrogen content in soil after 30 days of NO fumigation. Nitrate, ammonium and nitrogen content in soil were measured after harvesting plants and totally removing of plant root. Control means the original soil. For nitrate and ammonium, 15 pots of soil (5 pots for each line) were measured. For nitrogen content, each data represents means ± SE (n=5). Different letters indicate significant differences among treatments at $P < 0.05$, according to Tukey's test.

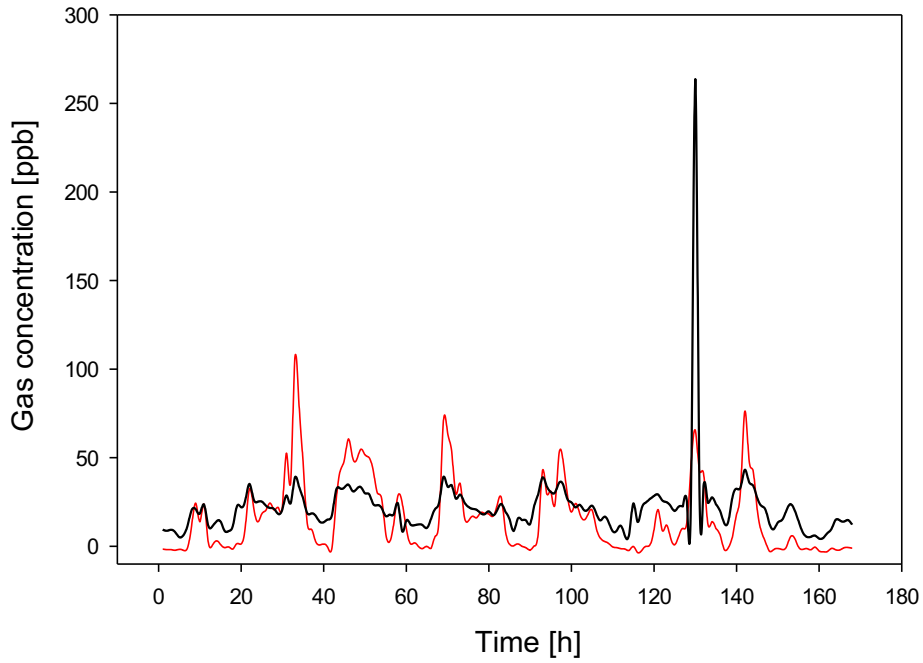
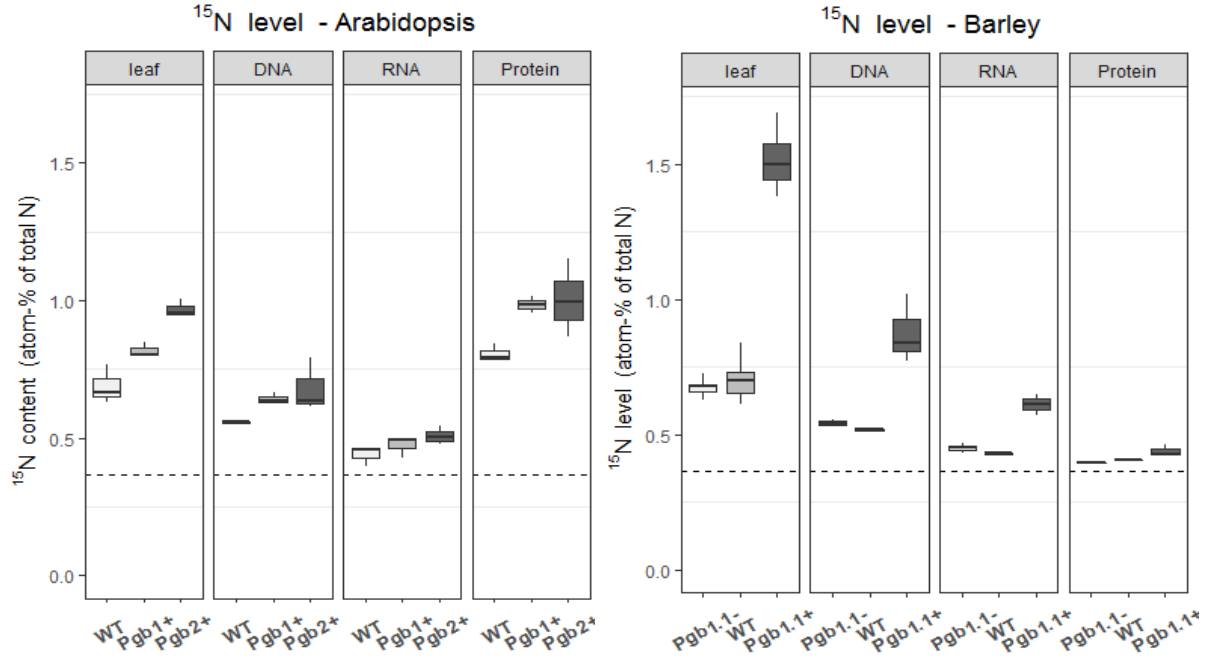


Fig. S5 NO and NO_2 measurements from 13.02.2019 – 19.02.2019 at the Helmholtz Zentrum München.

NO (red) and NO_2 (black) concentrations were monitored hourly using an Ecophysics chemiluminescence NOx Analyzer. Measurements started on 13.02.2019 at 0:00.

A



B

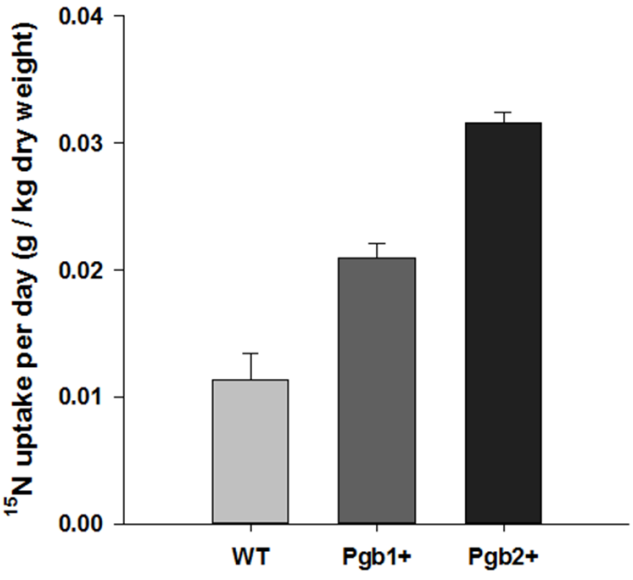


Fig. S6 ^{15}N level in barely and *Arabidopsis* leaves after ^{15}NO fumigation.

A) 20 days barley and 28 days *Arabidopsis* were fumigated with 90 ppb ^{15}NO during daytime (8:00-20:00). ^{15}N content was determined in barley and *Arabidopsis* leaves from at least 10 plants after 7 days. ^{15}N level in DNA, RNA, and protein were measured in the DNA, RNA, and protein extractions from leaves. The dashed line means ^{15}N level under control conditions is 0.37%. B) The ^{15}N uptake per day of *Arabidopsis* leaves were calculated based on the ^{15}N data of A.

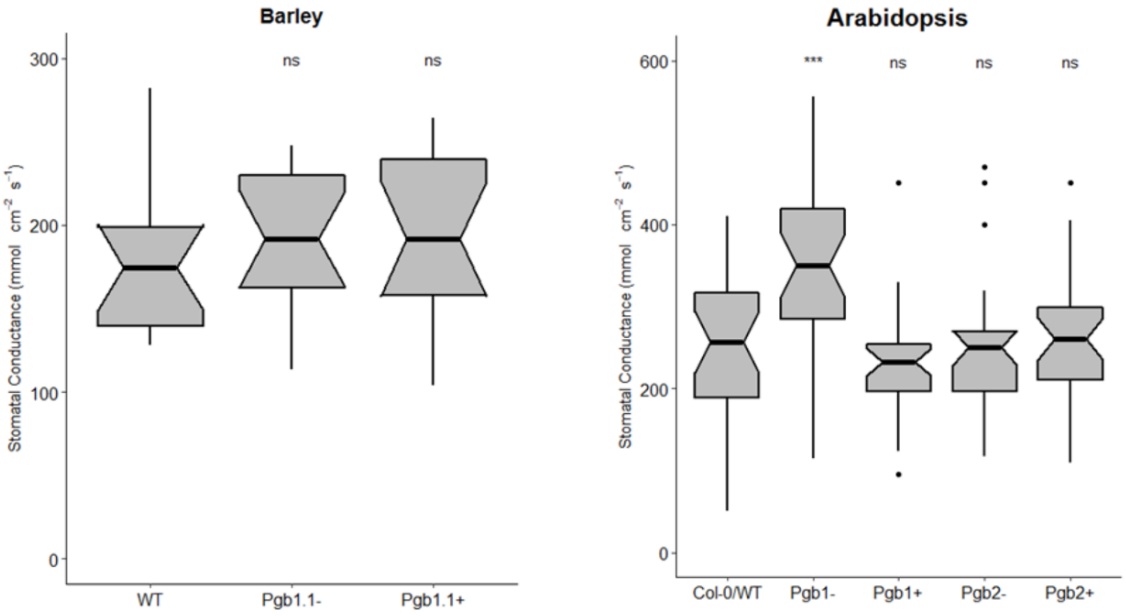


Fig. S7 Stomatal conductance of barley and *Arabidopsis* plants.

Stomatal conductance was measured during 10:00-12:00 from at least 14 plants per line. Asterisks indicate statistical significant differences from WT (Student's t-test; ***P<0.001)

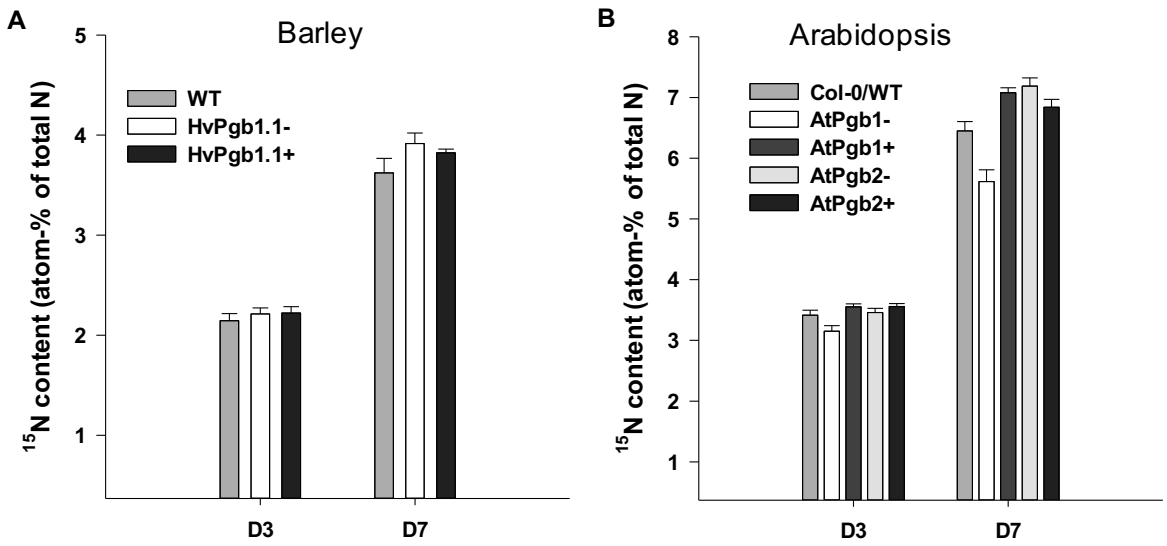


Fig. S8 ^{15}N level in barely and *Arabidopsis* leaves after $^{15}NO_2$ fumigation.

20 days barley and 28 days *Arabidopsis* were fumigated with 90 ppb $^{15}NO_2$ during daytime (8:00-20:00). ^{15}N content was determined in barley (A) and *Arabidopsis* (B) leaves from at least 10 plants after 3 and 7 days.