**Supplementary Information**

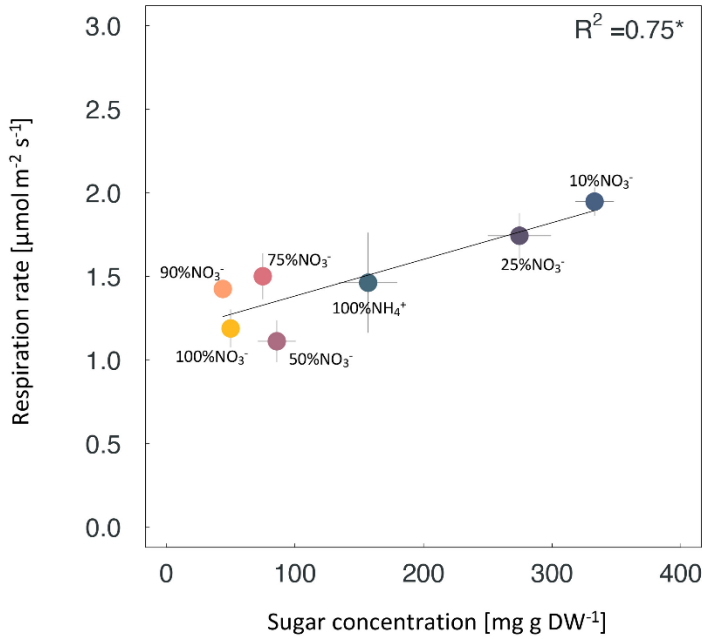


**Figure S1.** Linear regression between respiration rate (µmol $m^{-2}$ $s^{-1}$) and concentration of soluble sugars (mg g $DW^{-1}$) in tobacco leaves along the N species gradient from 100% $NO_3^-$ to 100% $NH_4^+$ (see Table 1). Different letters denote significant differences ($p$<0.05, n=5).

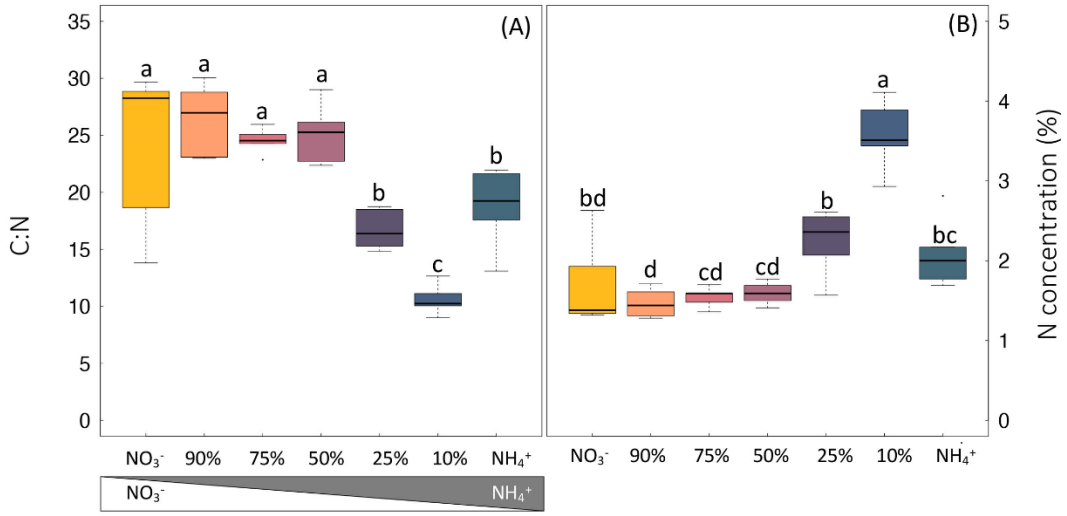


**Figure S2.** (A) Carbon to nitrogen ratio (C:N) and (B) nitrogen concentration (%) in of tobacco leaves along the N species gradient from 100% $NO_3^-$ to 100% $NH_4^+$ (see Table 1; percentages on the x axis represent the fraction of $NO_3^-$ in the nutrient solutions, decreasing from left to right). The bar underneath the figure reflects the gradient of N species changing from $NO_3^-$ to $NH_4^+$. Different letters denote significant differences ($p$<0.05, n=5).

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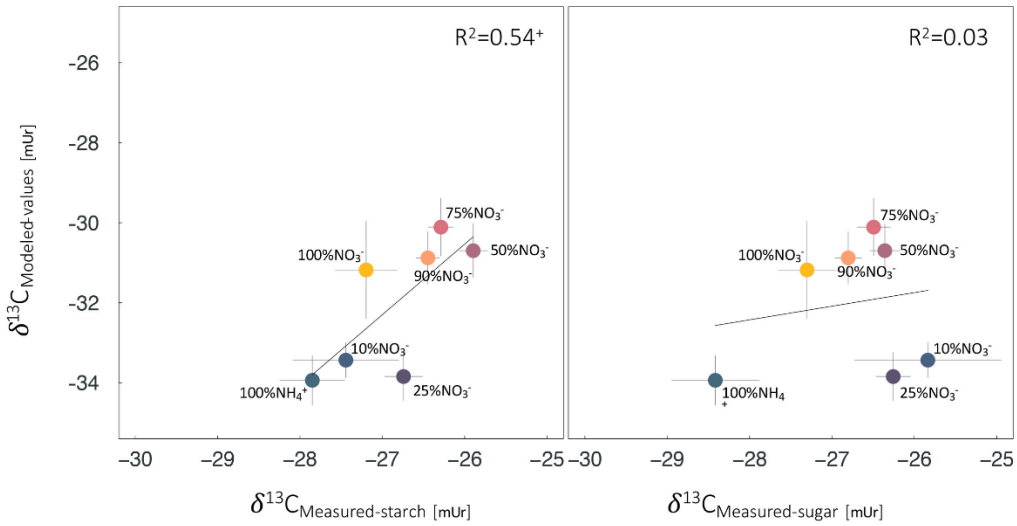


**Figure S3.** Linear regression between (a) measured $\delta^{13}C$ of starch and modeled $\delta^{13}C$ values using the simplified Farquhar model and between (b) measured $\delta^{13}C$ of sugar and modeled $\delta^{13}C$ values using the simplified Farquhar model along the N species gradient from 100% $NO_3^-$ to 100% $NH_4^+$ (see Table 1). Data represent average values ± SE (n=5). (+) denotes $p \leq 0.1$.

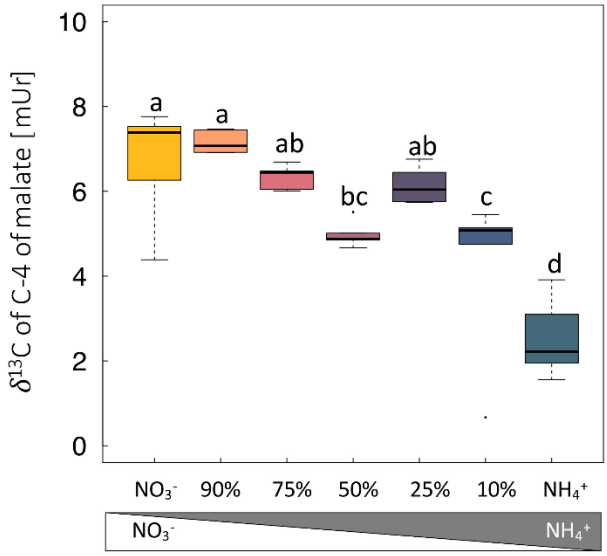


**Figure S4.** Modelled carbon isotopic composition of C-4 of malate in tobacco leaves according to Melzer and O'Leary (1991) along the N species gradient from 100% $NO_3^-$ to 100% $NH_4^+$ (see Table 1; percentages on the x axis represent the fraction of $NO_3^-$ in the nutrient solutions, decreasing from left to right). The bar underneath the figure reflects the gradient of N species changing from $NO_3^-$ to $NH_4^+$. Data represent average values ± SE (n=5).

***Compound-specific $\delta^{13}C$ and concentration analysis of amino acids and organic acids***

$\delta^{13}C$ and concentration analyses of individual organic acids in the organic acid fraction were performed with a high-performance liquid chromatograph (HPLC) coupled to a Delta V Advantage IRMS by an LC IsoLink (all ThermoFisher, Germany) as described by Lehmann *et al.* (2015). The organic acid fraction was filtered with a 0.45 µm PTFE syringe filter (Infochroma AG, Switzerland) into 1.5 mL glass vials (Macherey-Nagel GmbH and Co, Germany). The separation was performed on a 4.6 × 300 mm Allure Organic Acids HPLC column (Restek, USA) at 5-10 °C. The mobile phase was a 100 mM monopotassium phosphate buffer (pH=3.0) with a flow speed of 500 µL $min^{-1}$. A standard mixture of commercially available malate and citrate were analyzed at different concentrations (10-180 ng C $\mu L^{-1}$) between the samples to allow correction of the $\delta^{13}C$ offset, relative to measured $\delta^{13}C$ values with the EA coupling and used to determine the concentration of both organic acids (Rinne *et al.*, 2012; Lehmann *et al.*, 2015). The LC IsoLink measurement had a typical standard deviation of 0.2‰ for malate and 0.3‰ for citrate.

The amino acid fraction from the Dowex extraction was freeze-dried and re-dissolved in 400 µL deionized water. An aliquot of 200 µL was transferred to a tube, followed by an addition of 20 µL internal standard containing 0.25 µg $\mu L^{-1}$ norvaline and 0.25 µg $\mu L^{-1}$ norleucine in 0.1 M HCl. To derivatize the amino acids, 140 µL of ethanol:pyridine (4:1) and 20 µL ethyl-chloroformate were added to the solution. After 5 min incubation at room temperature, the samples were extracted by liquid-liquid extraction. 400 µL hexane:dichloromethane:ethyl-chloroformate (50:50:1, v/v/v) was added to the samples and an aliquot of 200 µL was transferred to a glass vial after a vortex mixing and centrifugation step (1 min, 2000 *g*). This step of extraction was repeated once, and 400 µL of the supernatant was combined with the first aliquot. The combined fractions were dried under a gentle $N_2$ flow and the residuals were re-dissolved in 50 µL ethylacetate according to Vlaardingerbroek *et al.* (2014) and Schierbeek (2017). Isotopic composition of amino acids and concentration measurements were performed with a Delta-XP isotope ratio mass spectrometer coupled online with a trace gas chromatograph and a combustion interface type 3 (GC-C-IRMS, ThermoFisher, Germany). Aliquots were injected into the gas chromatogram GC system by a PAL auto sampler (CTC, Switzerland). We corrected for the exogenous carbon added during the derivatization by analyzing a standard curve with known $^{13}C$ enrichments for each of the amino acids of interest. The slope and intercept of the curves are used to calculate the corrected $\delta^{13}C$ values of the samples. The GC-C-IRMS measurement had a typical standard deviation of about 1.1% for each amino acid.

***Enzymatic activities***

Nitrate reductase activity (NR, EC 1.7.1.1) was measured with an *in vitro* assay as described by Dier *et al.* (2017), with only minor modifications. Briefly, 100 mg of leaf material was mixed with four volumes of extraction buffer at 4 °C (100 mM HEPES-KOH (pH=7.5), 5 mM $Mg(Ac)_2$, 1 mM EDTA, 10% (v/v) glycerol, 0.5% (w/v) bovine serum albumin (BSA), 0.1% Triton X-100, 1% polyvinylpolypyrolidone (PVPP), 5 µM $NaMoO_4$, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM DTT, 20 µM FAD and 25 µM leupeptin). To start the enzymatic reaction for measuring the total NR activity, one volume of this leaf extract was added to five volumes of an assay buffer (100 mM HEPES-KOH (pH=7.5), 6 mM $KNO_3$, 6 mM EDTA, 0.6 mM NADH, 20 µM leupeptin, 12 µM FAD, 0.3 mM DTT and 6 µM $NaMoO_4$) preheated to 25.5 °C. After 5, 10 and 15 min, the reaction was stopped by adding 300 µL of this assay mixture to a new reaction tube containing 25 µL 600 mM $Zn(OAc)_2$. Unreacted NADH was removed by adding 75 µL of 0.25 mM phenazine methosulphate followed by a 15 min dark incubation. Produced $NO_2^-$ was then converted by diazotization with N-(1-naphthyl) ethylenediamine (NED) and sulfanilamide (SA) to a red dye that was determined colorimetrically as described by Scheible *et al.* (1997b) at 540 nm. Two technical replicates were carried out for each sample and afterwards averaged.

The activity of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) was determined as described by Gibon *et al.* (2004), with minor modifications. In brief, 150 mg of leaf plant material was shock frozen and ground in liquid nitrogen. This tissue was mixed with 1 mL of protein extraction buffer (50 mM HEPES, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA and 0.25% w/v BSA, pH=7.5). Additives containing 10% glycerol, 5 mM DTT and 1 tablet of protease inhibitor (Roche complete, Sigma Aldrich, CH) were added to this mixture, followed by a short vortex mixing and then samples were thawed on ice. After 10 min of centrifugation (10000 *g* at 4 °C), 20 µL protein extract was transferred to an assay volume containing 100 mM tricine (pH=8), 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 0.05% Triton X-100, 0.5 mM NADH, 0.2 U malate dehydrogenase (MDH), 1 mM PEP, pH 8.0. The reaction was assessed by monitoring the consumption of NADH spectrophotometrically at 340 nm over 2 hours with a plate reader (Infinite M1000, TECAN, Switzerland). Enzyme activity was calculated using the time window (approximately 30 min) when the reaction production rate of $NAD^+$ was constant.

NAD-malic enzyme (ME, EC 1.1.1.39) extraction was carried out by adding 200 µL of extraction buffer (50 mM 3-N-morpholino-propanesulfonic acid (MOPS)-NaOH, pH=7.0, 5 mM $MnCl_2$, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.05% (v/v) triton X-100, 20% (v/v) glycerol, and 1 mM PMSF, pH 7.0) to 100 mg shock frozen and ground plant material. After 15 min of centrifugation (10000 *g* at 4˚C), the supernatant was transferred to a new tube. 10 µL of the supernatant was directly pipetted into a 96 well plate containing 186 µL assay buffer (50 mM MOPS-NaOH, 4 mM $NAD^+$, 5 mM DTT, 10 mM $MnCl_2$ and 10 U MDH (pig heart, Roche, Switzerland, pH=7.0). The enzyme assay was started by adding 4 µL L-malate to a final concentration of 20 mM into each well. Production of NADH was then monitored spectrophotometrically at 340 nm for 20 min with a plate reader (Synergy HT Platereader, BioTek, USA).

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