$\delta^{15}\text{N}$ values in plants are determined by both nitrate assimilation and circulation

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Summary

- Nitrogen (N) assimilation is associated with $^{14}\text{N}/^{15}\text{N}$ fractionation such that plant tissues are generally ^{15}N -depleted compared to source nitrate. In addition to nitrate concentration, the $\delta^{15}\text{N}$ value in plants is also influenced by isotopic heterogeneity amongst organs and metabolites. However, our current understanding of $\delta^{15}\text{N}$ values in nitrate is limited by the relatively small number of compound-specific data.
- We extensively measured $\delta^{15}\text{N}$ in nitrate at different time points, in sunflower and oil palm grown at fixed nitrate concentration, with nitrate circulation being varied using potassium (K) conditions and waterlogging.
- There were strong interorgan $\delta^{15}\text{N}$ differences for contrasting situations between the two species, and a high ^{15}N -enrichment in root nitrate. Modelling shows that this ^{15}N -enrichment can be explained by nitrate circulation and compartmentalisation whereby despite a numerically small flux value, the backflow of nitrate to roots via the phloem can lead to a c. 30‰ difference between leaves and roots. Accordingly, waterlogging and low K conditions, which down-regulate sap circulation, cause a decrease in the leaf-to-root isotopic difference.
- Our study thus suggests that plant $\delta^{15}\text{N}$ can be used as a natural tracer of N fluxes between organs and highlights the potential importance of $\delta^{15}\text{N}$ of circulating phloem nitrate.

Introduction

Plant nitrogen (N) acquisition from nitrate fractionates between isotopes so that plant total organic matter is in general naturally ^{15}N -depleted compared to source N. When source nitrate is not limiting, plant matter can be depleted by several per mil (‰) (Högberg, 1997; Evans, 2001). Groundwater nitrate isotope composition (natural ^{15}N abundance, $\delta^{15}\text{N}$) is generally between -5 and $+5$ ‰ (Kendall & Aravena, 2000), and therefore the $\delta^{15}\text{N}$ value in plant matter is usually within -13 and $+5$ ‰, depending on species, plant organs and environmental conditions (for an example, see Handley *et al.*, 1997). The fractionation comes from the $^{14}\text{N}/^{15}\text{N}$ isotope effect associated with nitrate diffusion and transport via channels and transporters (presumably c. 1.003) and nitrate reductase (c. 1.016) (Ledgard *et al.*, 1985; Evans, 2001). The net fractionation associated with nitrate utilisation nevertheless depends on nitrate content, with low nitrate concentration in the soil solution leading to low isotope fractionation (Kolb & Evans, 2003; Stock & Evans, 2006). In addition, $\delta^{15}\text{N}$ values vary between plant organs, metabolites (Werner & Schmidt, 2002) and N-atom positions (Sacks & Brenna, 2005) due to nitrate redistribution within the plant, and isotope effects associated with enzymatic reactions in anabolism such as glutamine synthetase, glycine decarboxylase or transaminases

(Tcherkez, 2011). As a result, specific analysis of plant nitrogenous compounds can provide information on metabolic fluxes and nitrate circulation between plant organs. For example, it has been shown that isotope effects and metabolic fluxes in most important reactions (including photorespiratory NH_3 production and recycling) can explain the $\delta^{15}\text{N}$ values in individual amino acids of rapeseed leaves (Gauthier *et al.*, 2013).

It is believed that after having been absorbed, nonassimilated nitrate is ^{15}N -enriched due to nitrate reduction in roots, and therefore nitrate molecules transferred to shoots via the xylem are naturally ^{15}N -enriched (Evans, 2001; Tcherkez & Hodges, 2007). This should in principle lead to a ^{15}N -difference between plant organs, with leaves being ^{15}N -enriched compared to roots. Of course, partitioning of nitrate reduction between roots and shoots depends on species, environmental conditions, developmental stage and nitrate concentration and therefore the $\delta^{15}\text{N}$ value of plant matter can vary accordingly. In addition, the day/night cycle of transpiration and nitrate reduction activity in leaves leads to significant changes in $\delta^{15}\text{N}$ values not only in leaves but also in xylem and phloem sap. In fact, in *Ricinus communis* (where phloem sap can be relatively easily collected), it has been shown that there is a diel cycle of $\delta^{15}\text{N}$ values, with a ^{15}N -enrichment of several per mil in phloem at midday compared to the middle of the night, suggesting a progressive enrichment in

exported N-assimilates as leaves consume nitrate in the light (Peuke *et al.*, 2013).

Taken as a whole, N isotope composition of plant compounds is dictated by key mechanisms of N metabolism and allocation and, thus, $\delta^{15}\text{N}$ values could be exploited to obtain information on metabolic fluxes. However, this requires the use of models that account for nitrate redistribution, N partitioning and branching points at isotopically sensitive steps. This problem has been addressed previously with kinetic models (Yoneyama *et al.*, 1987; Robinson *et al.*, 1998; Comstock, 2001; Johnson & Berry, 2013). Although models can explain the $\delta^{15}\text{N}$ values with fitted flux parameters, they have three potential problems: first, the number of parameters required, up to nine in Robinson *et al.* (1998); second, they are implemented in the metabolic steady-state (except for pools formed by accumulation); and third, experimental data required for model validation and testing (in particular $\delta^{15}\text{N}$ values in nitrate) are very limited. As a result, the utilisation of $\delta^{15}\text{N}$ values in species relying on soil inorganic N in plant physiology remains limited. By contrast, $\delta^{15}\text{N}$ utilisation has been implemented in other species for a long time, typically to estimate the proportion of N derived from atmospheric N_2 in legumes (Mariotti *et al.*, 1983).

Here, we tackle this issue using, at the same time: experimental determination of nitrate isotope composition and content in several organs at different developmental stages; measurements of biomass and elemental N content; and a deliberately simple model of nitrate distribution and utilisation. To do so, we used two species (sunflower and oil palm) followed over an extended growth period, cultivated under low or high potassium (K) availability, with or without waterlogging, using a nitrate-based nutrient solution (we thus avoided complications associated with other N sources (ammonium or urea), such as isotope fractionation in ammonium acid/base protonation or in urea cleavage). We chose these two species because they usually grow in regions where K deficiency and waterlogging can occur simultaneously. In addition, their metabolic response to both K limitation and waterlogging (including N metabolism) has been described recently (Cui *et al.*, 2019a, b), and they are large enough to collect sufficient sample weight for nitrate purification. Manipulating potassium availability and waterlogging allowed us to change N assimilation and nitrate partitioning, while the availability and $\delta^{15}\text{N}$ of source nitrate were kept constant. Waterlogging affects ion (such as K^+ and NO_3^-) uptake efficiency in roots (Demidchik, 2014). In fact, oxygen shortage in roots leads to a deficiency in ATP generation by respiration as well as a change in transporter activity (such as an inhibition by reactive oxygen species) such that both nitrate and K^+ efflux from root cells increase (Sharma *et al.*, 2010; Barrett-Lennard & Shabala, 2013; Shabala & Pottosin, 2014; Zeng *et al.*, 2014). Under waterlogging, it is believed that the rate of nitrate reduction in roots does not decrease at least in the short- to mid-term so that there is a general decrease in nitrate concentration in tissues, as typically seen in sunflower (Agüera *et al.*, 1990). The simultaneous decrease in nitrate and water circulation from roots to shoots and the increase in root K^+ efflux tend to induce a potassium deficiency and thus a decline in sap circulation (Phukan *et al.*, 2016). K^+ is

by far the major cation in phloem sap and low K conditions are detrimental to phloem-mediated export by leaves (Hartt, 1969; Mengel & Haeder, 1977; Doman & Geiger, 1979; Cakmak *et al.*, 1994; Epron *et al.*, 2015). Low K not only affects phloem sap circulation but also leads to an increase in amino acid export from roots to shoots. Analyses of xylem composition have indeed shown an increase in glutamine and γ -aminobutyrate content, suggesting an increase in the root-to-shoot ratio of nitrate reduction (Sung *et al.*, 2015).

The present study represents a compilation of more than 2500 isotopic analyses including 600 analyses of $\delta^{15}\text{N}$ values in nitrate, using samples collected over one year (the time required to grow oil palm saplings in the glasshouse). Our objective was to address the following questions: Is leaf nitrate systematically ^{15}N -enriched compared to absorbed nitrate and root nitrate? Do K limitation and waterlogging effectively affect plant nitrate $\delta^{15}\text{N}$ values? Can we predict $\delta^{15}\text{N}$ values using a simple, nonsteady-state model and does modelling provide information on nitrate circulation? Our data show that plant nitrate is considerably enriched compared to source nitrate from the nutrient solution and that, surprisingly, root nitrate was more ^{15}N -enriched than leaf nitrate. Modelling suggests that this surprising result comes not only from root nitrate reduction preferentially consuming ^{14}N -nitrate and the ability of roots to accumulate nitrate, but also from the backflow of ^{15}N -enriched nitrate (although at a very small rate) via the phloem.

Materials and Methods

Plant material and sampling

Plant cultivation conditions were as in Cui *et al.* (2019a,b). Briefly, sunflower (*Helianthus annuus* L., var. XRQ) seed provided by the INRA Toulouse (France) and germinated oil palm seeds (*Elaeis guineensis* Jacq., *Dura* $\times$ *Pisifera*) obtained from Siam Elite Palm Co. Ltd (Krabi, Thailand) were sown directly in sand (washed with distilled water) in the glasshouse, using 7-l pots. Growth conditions were: 12 h : 12 h, 25°C : 18°C photoperiod and air temperature and 70/60% relative humidity, day : night for sunflower; and natural photoperiod (from 13.5 h : 10.5 h at emergence (February) to 14.5 h : 9.5 h at the end of the experiment (January), with a minimum of 9.5 h : 14.5 h in June), 30°C : 24°C air temperature and 70% : 60% relative humidity day : night for oil palm. Transpiration was measured using a portable open system Li-Cor 6400 XT device. Sampling time points are detailed in Supporting Information Fig. S1. Plants were subdivided into stems (+ apical bud), roots and leaves which were frozen in liquid N_2 and freeze-dried, weighed, and ground for further utilisation.

Nutrient conditions and waterlogging

The nutrient solution composition is given in Table S1. The amount of K^+ was varied by changing the amount of KCl. Two K availability conditions were used here: 'low K' (0.2 mM) and 'high K' (4 mM). The amount of nitrate was 12 mM in the

nutrient solution and was kept constant throughout the experiments. Under nonwaterlogging (control) conditions, 300 ml (sunflower) or 500 ml (oil palm) nutrient solution was provided to each pot every day. Excess liquid was allowed to drain freely from the pot, so that on average 250 ml was retained in pots each day. Waterlogging was performed by filling the pot with the nutrient solution. Some nutrient solution was provided every day to compensate for evaporation and transpiration (200 ml d⁻¹ pot). Thus, waterlogging led to a progressive hypoxia rather than abrupt anoxia. We used a relatively high nitrate concentration (12 mM) under our conditions to avoid changes in the $\delta^{15}\text{N}$ of source nitrate: in fact, renewal of nutrient solution (3.6 and 6 mmol nitrate per day per pot) compensated for the consumption by plants, so that source nitrate was kept isotopically constant ($\delta^{15}\text{N}$ variation during 24 h before the addition of nutrient solution of the next day would not have exceeded 0.05‰). The $\delta^{15}\text{N}$ value of source nitrate (nutrient solution) was +3.5‰.

Isotope analysis and %N

In total, 100 mg of powdered freeze-dried material was first extracted with hexane to obtain the total lipid fraction (supernatant that contained chlorophylls). The pellet was extracted with MilliQ water. After centrifugation, the supernatant was collected and heated at 100°C for 5 min. The heat-precipitated protein pellet was collected and the deproteinated supernatant was subdivided into two halves. One half was freeze-dried and used for total soluble N isotope analysis. The other half was used for nitrate extraction as barium nitrate with barium iodide, after Huber *et al.* (2011). Plant samples were weighed in tin capsules. $\delta^{15}\text{N}$ analysis was carried out using a Pyrocube Elemental Analyser (EA; Elementar France, Lyon France) connected on line (via an open split) in continuous flow mode to an Isoprime 100 isotope ratio mass spectrometer (IRMS; Elementar UK, Cheadle, UK), after Fourel *et al.* (2014). For combustion in the EA, the quartz tube was filled with tungsten oxide and the reduction tube was filled with copper. Combustion gases were separated through the 'purge and trap' module from the EA. The amount of N was measured via a thermal conductivity detector. $\delta^{15}\text{N}$ precision for replicate analyses was 0.1‰. Data were calibrated against international reference material IAEA-600, IAEA-CH6 and IAEA-N2. In addition, all sample batches included standards (glycine, +0.66‰; cysteine, +7.78‰; previously calibrated against IAEA standards glutamic acid USGS-40 and caffeine IAEA-600) every 10 samples. When the sample mass was occasionally low (typically < 0.08 µmol N per sample), the $\delta^{15}\text{N}$ value was corrected using the response curve of the $\delta^{15}\text{N}$ value to sample mass using standard glycine and cysteine. $\delta^{15}\text{N}$ values are expressed in δ notation, that is deviation in parts per thousand (‰) relative to atmospheric N₂ (air): $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1)$ where R denotes the $^{15}\text{N} : ^{14}\text{N}$ ratio.

Modelling and assumptions

The model considered here assumes that the plant can be subdivided into pools that exchange nitrate via xylem and phloem (Fig. 1; symbols are defined in Table 1). The model is

deliberately kept as simple as possible to minimise the number of variables, avoid lengthy parameterisation and therefore allow facile numerical resolution. In practice, the plant is considered to contain one mesophyll nitrate pool ('leaves') which is fed by the xylem, and can transfer some nitrate to the phloem for nitrate redistribution to (stem and) roots. Here, 'xylem' and 'phloem' are two terms used in a broad sense, that is, including tissues that inherit nitrate from vasculature (xylem vessels) and sieve tubes (parenchyma, sink organs), and therefore to avoid confusion with pure sap-conducting xylem and phloem cells (or saps themselves), they are thereafter referred to as xylem⁺ and phloem⁺. The model includes the possibility to have some transfer of nitrate from xylem to phloem (transfer rate B). Roots are assumed to comprise two nitrate pools (R_1 and R_2 , respectively): one of small size kept in the steady-state and used as a transfer compartment from soil to xylem sap, and another one that can be of varying size and used as the source of nitrate for root N assimilation. These two root pools can exchange nitrate (via back and forth fluxes, with rates w and $A + w$).

At this stage, at least three aspects deserve comments: first, we recognise that the transfer of nitrate from xylem to phloem (transfer rate B) has been found experimentally to be very small (see references in the Discussion section). As such, having a small value of B will be a way to check the relevance of our computations. Second, the model is not in the steady-state. That is, both nitrate $\delta^{15}\text{N}$ and pool sizes varied with time (during growth) and thus incorporating differential equations was necessary. Third, a backflow of nitrate from leaves to sink organs and ultimately to roots is considered. The reasons for including a phloem-driven flux of nitrate is further explained in the Discussion. As will become apparent below, this flux (although quantitatively small) is an important determinant of nitrate $\delta^{15}\text{N}$ because it can carry ^{15}N -enriched nitrate.

The equations used here are based on the general expression of derivatives of isotope ratios with respect to time. The derivative of the $^{15}\text{N}/^{14}\text{N}$ isotope ratio R in a given nitrate pool follows the relationship below that comes from ^{15}N mass balance:

$$\frac{d^{15}Q}{dt} = \frac{dQR}{dt} = Q \cdot \frac{dR}{dt} + R \frac{dQ}{dt} = \frac{F_{\text{in}}}{\alpha_{\text{in}}} R_{\text{in}} - \frac{F_{\text{out}}}{\alpha_{\text{out}}} R \quad \text{Eqn 1}$$

where ^{15}Q is the total ^{15}N content and Q is the total ^{14}N content, which is very close to the total pool size (because ^{15}N natural abundance is very small). F_{in} and F_{out} are influx and efflux, and R_{in} the isotope ratio of influx. $^{14}\text{N}/^{15}\text{N}$ isotope effects ($^{14}\nu/^{15}\nu$) are denoted as α . Note that F_{in} may be different from F_{out} if the nitrate pool of interest is not in the steady-state because by definition $dQ/dt = F_{\text{in}} - F_{\text{out}}$, rearranging Eqn 1 gives Eqn 2:

$$\frac{dR}{dt} = \frac{F_{\text{in}} \left(\frac{R_{\text{in}}}{\alpha_{\text{in}}} - R \right) - F_{\text{out}} R \cdot \left(\frac{1}{\alpha_{\text{out}}} - 1 \right)}{Q} \quad \text{Eqn 2}$$

The set of equations is given in Notes S1. When considered together as a matrix, the differential system can be simply written as $d\mathbf{Y}/dt = \mathbf{M}\mathbf{Y}$ where $\mathbf{Y}$ is the vector of isotope ratios and $\mathbf{M}$ the

Fig. 1 Model used to compute $\delta^{15}\text{N}$ values in nitrate. For simplicity, plants are assumed to comprise three compartments: leaf mesophyll (abbreviated 'leaves'), stem and roots. Pools that can vary in size (nonsteady-state) are indicated with dashed grey borders. The only pool that is of fixed size is the nitrate transfer pool (R_1) in the root. That pool is fed by external nitrate (influx i) and nitrate can also escape (efflux e). The stem is assumed to be composed of two pools: one associated with xylem tissues (including xylem parenchyma; referred to as xylem⁺) and one associated with phloem, phloem parenchyma and sink (developing) organs such as apical bud (referred to as phloem⁺). Rates of nitrate reduction (assimilation) are denoted as r while accumulation (build-up) rates are denoted as a . See also Table 1 for symbol definitions. Assimilates can circulate across the plant (amino acid or amide exchange between organs) and this is symbolised with blue arrows. This has no impact on nitrate in this model because it does not appear in equations. Note that this model is simple enough to rely on three main variables, once nitrate reduction capacities (r values) and accumulation rates (a values) are known: the rate of exchange between root pools (A), nitrate backflow (b_n) and xylem-to-phloem nitrate transfer (B).

matrix of (time-dependent) coefficients in equations. This equation cannot be solved directly because coefficients of $\mathbf{M}$ are not constant (pool sizes) and furthermore $\mathbf{M}$ could be nondiagonalisable (decomposed into singular values). Therefore, we solved numerically by decomposing time windows (referred to as 'steps' in Fig. S1) into n time increments. For any incremental time index k in $[0;n]$, the isotope ratio at $k+1$ is given by $R_{k+1} = R_k + \gamma \cdot (dR/dt)_k$ where γ is the time unit conversion factor between unit of fluxes F ($\mu\text{mol per plant d}^{-1}$) and time increments. n was chosen to be sufficiently large to avoid divergence (artefactual instability due to too large dR/dt values). In practice, time increments represented 3.6 and 3.36 min for sunflower and oil palm, respectively. Further details on parameterisation are provided in Notes S2 (as well as Figs S2, S3 and Table S2).

Verification of the isotope effect α_r

The fractionation associated with nitrate reduction (assimilation) is a critical component of the model and it is fixed at 16‰, which

is the value broadly accepted for the fractionation by nitrate reductase (for a review, see Tcherkez & Farquhar, 2006). The actual value of the fractionation was also estimated here using isotopic mass-balance in leaves. Leaves were chosen because they are an important site of nitrate reduction and redistribution of N assimilates (at the mature stage, they do not import reduced N to a large extent in both species) and thus the $\delta^{15}\text{N}$ of their organic compounds is minimally impacted by the contribution of other organs. Leaf samples were fractionated into their four main N-containing components (proteins, lipids, nitrate and total soluble fraction), the $\delta^{15}\text{N}$ of which was determined. Therefore, we have:

$$\delta_{\text{N-assimilates}} = \frac{\delta_{\text{nitrate}} - \Delta_r}{1 + \Delta_r} = \delta_{\text{lipids}}x_{\text{lipids}} + \delta_{\text{proteins}}x_{\text{proteins}} + \delta_{\text{TSF}}x_{\text{TSF}} \quad \text{Eqn 3}$$

where Δ_r is the fractionation associated with reduction ($= \alpha_r - 1$) and x are mole fractions of N represented by the

Table 1 Symbols used in the main text and equations.

Symbol	Definition	Units
Fluxes		
<i>A</i>	Net influx of nitrate from root pool R_1 to R_2	$\mu\text{mol N per plant d}^{-1}$
a_l	Variation in nitrate content in leaves	$\mu\text{mol N per plant d}^{-1}$
a_p	Variation in nitrate content in phloem ⁺	$\mu\text{mol N per plant d}^{-1}$
a_r	Variation in nitrate content in roots	$\mu\text{mol N per plant d}^{-1}$
a_x	Variation in nitrate content in xylem ⁺	$\mu\text{mol N per plant d}^{-1}$
<i>B</i>	Transfer of nitrate from xylem to phloem	$\mu\text{mol N per plant d}^{-1}$
b_n	Backflow of nitrate from leaves to phloem ⁺	$\mu\text{mol N per plant d}^{-1}$
<i>e</i>	Efflux of nitrate from root pool R_1 back to soil	$\mu\text{mol N per plant d}^{-1}$
<i>i</i>	Influx of nitrate from soil to root pool R_1	$\mu\text{mol N per plant d}^{-1}$
<i>Q</i>	Pool size	$\mu\text{mol per plant}$
r_l	Flux of nitrate reduction in leaves	$\mu\text{mol N per plant d}^{-1}$
r_p	Flux of nitrate reduction in phloem ⁺	$\mu\text{mol N per plant d}^{-1}$
r_r	Flux of nitrate reduction in roots	$\mu\text{mol N per plant d}^{-1}$
r_x	Flux of nitrate reduction in xylem ⁺	$\mu\text{mol N per plant d}^{-1}$
<i>T</i>	Flux of nitrate export from roots	$\mu\text{mol N per plant d}^{-1}$
<i>w</i>	Flux of nitrate exchange between R_1 and R_2	$\mu\text{mol N per plant d}^{-1}$
Isotopes		
α_d	$^{14}\text{V}/^{15}\text{V}$ isotope effect associated with nitrate transport through membranes (influx and efflux). Fixed at 1.003.	Dimensionless
α_r	$^{14}\text{V}/^{15}\text{V}$ isotope effect associated with nitrate reduction. Fixed at 1.016.	Dimensionless
$\delta^{15}\text{N}$	$^{14}\text{N}/^{15}\text{N}$ isotope composition with respect to air	‰
<i>R</i>	Isotope ratio ($^{15}\text{N}/^{14}\text{N}$)	Dimensionless

compounds of interest ($x_{\text{lipids}} + x_{\text{proteins}} + x_{\text{TSF}} = 1$). Eqn 3 neglects other components that have a small contribution to the total N budget (volatilisable ammonia etc.). In addition, 'TSF' refers to the nitrate-corrected total soluble fraction as the raw total soluble fraction also contains nitrate. Eqn 3 can thus be used to compute Δ_r .

Determination of total N assimilation and nitrate pool size change

Total N assimilation (denoted as r_{tot} in $\mu\text{mol N per plant d}^{-1}$) was calculated as a time average between two time points t and t' (two sampling dates) using dry weight (m , in mg), the ‰N in total plant matter and a correction for the quantity of nitrate (n in $\mu\text{mol mg}^{-1}$). That is:

$$r_{\text{tot}} = r_l + r_r + r_x + r_p$$

$$= \sum_{\text{leaf,stem,root}} \frac{\left(m \cdot \left(\frac{\%N}{14} \cdot 10^3 - n\right)\right)_{t'} - \left(m \cdot \left(\frac{\%N}{14} \cdot 10^3 - n\right)\right)_t}{t' - t}$$

Eqn 4

Similarly, the rate of change in nitrate pool size between time t and t' was calculated, for each organ, as:

$$a = \frac{(m \cdot n)_{t'} - (m \cdot n)_t}{t' - t}$$

Eqn 5

This allowed us to compute the total net nitrate demand ($i - e$):

$$i - e = \sum r + \sum a = r_{\text{tot}} + a_{\text{tot}}$$

Eqn 6

Therefore, gross nitrate influx was given by:

$$i = \frac{r_{\text{tot}} + a_{\text{tot}}}{1 - e/i}$$

Eqn 7

using the value of e/i chosen for parameterisation (Table S2). Note that the value of e/i had a very limited impact on calculations of $\delta^{15}\text{N}$ values simply because the isotopic contribution of efflux (isotope effect of 1.003) was very small compared to the range of ^{15}N -enrichment found here (up to 55‰).

Results

$\delta^{15}\text{N}$ in leaf organic matter

The N isotope composition of leaf raw material was within a rather narrow range, roughly between -8 and 0 ‰, that is, ^{15}N -depleted compared to source nitrate in the nutrient solution ($+3.5$ ‰) (Fig. S4). The isotope composition was then expressed relative to source nitrate ($\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{source nitrate}} - \delta^{15}\text{N}_{\text{leaf}}$) as the apparent N isotope fractionation, and plotted against ‰N expressed as the mean centred value (Fig. 2; see Fig. S5 for ‰N source data). Nearly all $\Delta^{15}\text{N}$ values were positive (only one value obtained at high K in sunflower was negative) showing that, overall, N acquisition in leaf tissues generally fractionated against ^{15}N . In both species, there was a significant effect of K availability: leaves were more ^{15}N -depleted (and less N-rich) at low K, suggesting that nitrate used for N assimilation in leaves was ^{15}N -depleted or more abundant (thereby allowing larger fractionation) than at high K. Waterlogging caused a ^{15}N -depletion, abolished the isotopic difference between high and low K, and led to a lower ‰N in leaf tissue. This suggests that waterlogging was detrimental to nitrate assimilation regardless of K availability, and changed nitrate circulation so that leaf nitrate was ^{15}N -depleted. Such a ^{15}N -depletion was also visible in raw material of sunflower stem and roots, and oil palm roots (Fig. S4).

There were differences between leaf fractions (proteins, lipids, total soluble fraction), the nitrate-corrected soluble fraction being

generally ^{15}N -depleted by a few per mil (Fig. S6). When calculated by mass-balance, the isotope fractionation associated with nitrate assimilation in leaves was 16.8‰ on average (Fig. 2, where fitting a Gaussian distribution gave a value of 17.8‰). This value is very close to the measured isotope fractionation by nitrate reductase (16‰) (Ledgard *et al.*, 1985; Tcherkez & Farquhar, 2006). While tissue fractions fell within a relatively narrow range in sunflower leaves, there were important differences in other organs of both species, with lipids being generally ^{15}N -depleted compared to proteins, by up to 30‰ (Fig. S6). In sunflower, the nitrate-corrected soluble fraction appeared to be ^{15}N -depleted in roots, suggesting that it contained organic compounds the synthesis of which was associated with a large isotope fractionation.

Nitrate content and $\delta^{15}\text{N}$ value

Nitrate was extracted and purified from all tissues and its $\delta^{15}\text{N}$ value was measured (Fig. 3). As expected, nitrate was found to be ^{15}N -enriched (up to 55‰ in sunflower stems) as a consequence of the isotope fractionation by N assimilation (so that consumed nitrate molecules were ^{15}N -depleted and nitrate left behind was ^{15}N -enriched). In sunflower, there was a ^{15}N -enrichment gradient from leaves to roots (Fig. 3a–c) regardless of K and waterlogging conditions. $\delta^{15}\text{N}$ values were not highly affected by K/waterlogging conditions except at high K where stem and root nitrate was very ^{15}N -enriched. In general, $\delta^{15}\text{N}$ values in oil palm were lower, except at high K where it reached nearly 30‰ 7 wk after the onset of our experiment (Fig. 3g–i). Taken as a whole, with the exception of the last sampling at high K in oil palm, root nitrate was always ^{15}N -enriched compared to other organs.

The $\delta^{15}\text{N}$ value in nitrate changed with time with a progressive ^{15}N -depletion, except at high K where it increased

considerably. Waterlogging generally caused a decrease in $\delta^{15}\text{N}$ values, and this was particularly visible at high K in sunflower. $\delta^{15}\text{N}$ changes were accompanied by changes in nitrate content. In fact, the nitrate content ($\mu\text{mol g}^{-1}$) showed a progressive decline (particularly pronounced in oil palm roots, Fig. 3l), except in sunflower roots where it remained fairly constant (Fig. 3f). It is worth noting that roots represented plant organs where nitrate was most concentrated (except at low K in sunflower, Fig. 3e).

$\delta^{15}\text{N}$ modelling

Biomass and nitrate content data were incorporated into a (nonsteady-state) model to assess whether observed $\delta^{15}\text{N}$ value can be reproduced satisfactorily, using observed N incorporation rates computed from biomass N increment and changes in nitrate pools in each organs (see the Materials and Methods section for details). Taken as a whole, the performance of modelling was good, with a R^2 between predicted and observed values of 0.95 (Fig. 4a). Modelling was not very sensitive to parameterisation and led to an average error in $\delta^{15}\text{N}$ of < 1‰ (Fig. S2). In addition, the predicted rate of nitrate transport via the xylem, T , was used to estimate leaf transpiration using leaf total surface area, and a fixed xylem concentration of 3 mM (although we recognise that nitrate transport may be disconnected from transpiration under certain circumstances (White, 2012)). The agreement between observed and predicted values was good under nonwaterlogged conditions (Fig. 4b). Unsurprisingly, there was no good agreement between observed and predicted values of transpiration under waterlogging conditions (Fig. 4b, grey symbols), demonstrating the disconnection between water flow and nitrate circulation when roots were waterlogged.

Fig. 2 $\Delta^{15}\text{N}$ values in leaf raw material in sunflower (a) and oil palm (b), with K and waterlogging conditions indicated with colours. In sunflower, additional measurements with medium K and low K + K resupply are also shown in green (squares and triangles, respectively). The net fractionation $\Delta^{15}\text{N}$ (calculated using source nitrate as the source material, $\delta^{15}\text{N} = +3.5\text{‰}$) is plotted against mean-centred nitrogen elemental content (%N), denoted as D . (c) Distribution of the isotope fractionation associated with nitrate assimilation, computed from purified protein, lipids and total soluble fraction from leaves. The average is shown with a blue arrow (16.8‰) and the Gaussian distribution with its average (17.8‰) are shown in red. $\delta^{15}\text{N}$ values in leaf components (lipids, proteins, soluble fraction) are shown in Supporting Information Fig. S5. Statistical significance ($P < 0.05$) is shown with asterisks: waterlogging effect at high K (*), waterlogging effect at both high and low K (**), and K effect under nonwaterlogged conditions (***).

Fig. 3 Nitrate content and isotope composition ($\delta^{15}\text{N}$) in leaves, stems and roots of sunflower (a–f) and oil palm (g–l). Same colour code as in Fig. 2. The $\delta^{15}\text{N}$ value is shown in per mil (‰) on the top row (a–c; g–i). The nitrate content ($\mu\text{mol NO}_3^- \text{g}^{-1} \text{DW}$) is shown on the bottom row (d–f; j–l). Values are mean $\pm$ SD ($n = 5$). Statistical significance ($P < 0.01$) is shown with upper-case letters (K, potassium effect; W, waterlogging effect; I, potassium $\times$ waterlogging effect) at each time point.

Nevertheless, modelled $\delta^{15}\text{N}$ values were not close to observed values (up to 10‰ difference) at high K in sunflower (Fig. 4a, red frames). This shows the limitation of the model used here, which was based on a minimal number of adjustable parameters. Possible explanations are further detailed in the Discussion section. Still, as expected, the model generated low values for all three exchange rates: nitrate transfer from xylem to phloem (B), nitrate backflow from leaves to roots (b_n) and exchange between rapid and slow nitrate pools in roots (w) (Fig. 4c). Also, waterlogging caused a decrease in modelled nitrate circulation (both T and b_n) in sunflower. In oil palm, the effect of waterlogging on T or b_n was more variable. In both species, modelling suggested that waterlogging caused a transient increased remobilisation of ^{15}N -enriched nitrate from the root storage pool (w) to feed nitrate circulation. As a result, the predicted $\delta^{15}\text{N}$ composition in xylem⁺ increased under waterlogging, and this effect was mostly visible in sunflower where stem nitrate content changed (Fig. S6).

It is worth noting that the modelled $\delta^{15}\text{N}$ value of nitrate in xylem⁺ and phloem⁺ differed substantially, with phloem⁺ nitrate being much more ^{15}N -enriched than xylem⁺ (for an example, see Fig. S7). This phenomenon is not surprising because nitrate reduction in leaves fractionates against ^{15}N and therefore nitrate exported by leaves is ^{15}N -enriched. As a result, nitrate carried by phloem⁺ back to roots is ^{15}N -enriched, thereby explaining the relatively high $\delta^{15}\text{N}$ values observed in root nitrate.

Discussion

^{15}N -enrichment in nitrate

The $\delta^{15}\text{N}$ in nitrate extracted from plant tissue is relatively poorly documented, but available data (e.g. data used for modelling in Robinson *et al.* (1998) or data in Yoneyama & Tanaka

(1999)) suggest a ^{15}N -enrichment. Such an enrichment can be large and, as a result, leaf nitrate content affects the $\delta^{15}\text{N}$ value in leaf total soluble fraction (Gauthier *et al.*, 2013). An important finding of the present study is the ^{15}N -enrichment, sometimes considerable, in root nitrate compared to other tissues, including leaves. This was visible in both sunflower and oil palm. This result is surprising because of the relatively higher $\delta^{15}\text{N}$ value in leaf organic matter (at least compared to roots, Fig. S3). This lower-than-expected isotope composition in leaf nitrate (and higher-than-expected in roots) could come from several potential mechanisms: (1) a Péclet effect whereby mesophyll ^{15}N -nitrate would behave as leaf water during transpiration, that is, would back-diffuse to xylem vessels against the convection flow (Barbour *et al.*, 2000, 2004); (2) the occurrence of two nitrate pools in roots, including a nitrate storage pool that can accumulate ^{15}N -enriched nitrate; (3) a flux of nitrate through the phloem from leaves back to roots; and (4) a metabolic process that consumes ^{15}N -depleted nitrate in roots, thereby enriching nitrate left behind. Amongst these, assumption (1) is unlikely because the diffusion coefficient of nitrate in water, $1.3 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$ (Yeh & Wills, 1970), is not large enough to compensate for the high water flux via the xylem. In other words, the Péclet number, which quantifies the diffusion-to-convection ratio would be too small to explain a significant change in the $\delta^{15}\text{N}$ value of leaf nitrate.

Assumptions (2), (3) and (4) are plausible. In fact, roots were a site of nitrate accumulation, with very high nitrate content up to $80 \mu\text{mol g}^{-1} \text{DW}$ in sunflower (Fig. 3), as observed previously (Alves *et al.*, 2016). The presence of two nitrate pools in roots has been demonstrated – although the biochemical nature of these two pools (cytosolic vs. vacuolar, apoplastic vs. symplastic, and so on) remains uncertain (Ferrari *et al.*, 1973; MacKown *et al.*, 1983; Siddiq *et al.*, 1991; Devienne *et al.*, 1994; Miller &

Fig. 4 Output of $\delta^{15}\text{N}$ modelling in nitrate. (a) Correlation between modelled and observed $\delta^{15}\text{N}$ values (in ‰). Red frames highlight points that deviate from the 1 : 1 line. Sunflower and oil palm symbols are discs and squares, respectively. With all points taken together, $R^2 = 0.95$. Each point is the average values of $n = 5$ replicates (all organs plotted together). (b) Relationship between predicted transpiration (assuming that xylem nitrate concentration is 3 mM) and observed transpiration across all K conditions, with (grey) and without (black) waterlogging. The determination coefficient (R^2) is 0.72 without waterlogging ($P < 0.01$) while there is no significant correlation under waterlogging. (c) Outputs of the model showing nitrate transport via xylem⁺ (T), xylem⁺-to-phloem⁺ transfer (B), nitrate backflow from leaves (b_n), and nitrate exchange between root pools (w). Fluxes are given in $\text{mmol nitrate per plant d}^{-1}$. Note the peak associated with w upon waterlogging onset.

Smith, 1996). It is generally believed that the flux of nitrate via the phloem is very small as nitrate is present at very low concentration if at all detectable in several species (especially legumes), and ^{15}N -nitrate labelling via the xylem suggests that shoot nitrate reduction is so efficient that only trace amounts of nitrate circulate via the phloem (Pate *et al.*, 1975). However, nitrate is detectable in phloem sap of other species (such as castor bean and yucca) and extensive measurements in castor bean (*R. communis*) have shown that it is *c.* 0.6 mM (Peuke, 2009). In palm trees, nitrate in palm wine obtained from phloem exudates has been found to be *c.* 20–30 $\mu\text{g ml}^{-1}$, that is 0.4 mM (Ojmelukwe, 2001; Chandrasekhar *et al.*, 2012). Interestingly, nitrate transporters that are present in phloem cells and essential to redistribute leaf nitrate to sink organs have been found in *Arabidopsis* (Fan *et al.*, 2009; Wang & Tsay, 2011). Thus, in principle, a

relatively small flux of nitrate via the phloem is possible. Here, $\delta^{15}\text{N}$ modelling suggests that it is indeed the case, with phloem⁺ carrying ^{15}N -enriched nitrate (by up to 30‰ compared to xylem⁺). The flux of nitrate export from leaves generated by the model (b_n) was rather small under normal conditions (no waterlogging), between 7% (sunflower) and 3% (oil palm) of the nitrate flux via the xylem (Fig. 4). This order of magnitude seems to match experimental data on palm trees: in adult palm trees, xylem water transport represents *c.* 600 l d^{-1} and thus *c.* 2 $\text{mol NO}_3^- \text{d}^{-1}$ (assuming a concentration of 3 mM) while phloem transport through the stem represents *c.* 50 l d^{-1} (Van Die & Tammes, 1975) and thus 0.02 $\text{mol NO}_3^- \text{d}^{-1}$ (at 0.4 mM), that is, 1% of the xylem flux. Interestingly, under waterlogging, b_n was found to decrease in sunflower, suggesting an inhibition of phloem circulation as in *Ricinus* (Peuke *et al.*, 2015) or forest

trees (Kreuzwieser & Rennenberg, 2014), but increased considerably in oil palm (suggesting an increase in leaf nitrate remobilisation).

Root nitrate is further ^{15}N -enriched by nitrate reduction in root metabolism: although small (shoots prevail in whole-plant nitrate reduction in the two species of interest), nitrate assimilation participates in enriching root nitrate by several per mil. It is worth noting that in sunflower roots, the $\delta^{15}\text{N}$ value of proteins was similar to that in leaves (Fig. S6a,c) and this shows that roots used nitrate of similar isotopic composition to that in leaves and/or inherited amino acids produced in leaves. In oil palm, the $\delta^{15}\text{N}$ value of root proteins was higher than that in leaves by several per mil (except at week 7 under low K + waterlogging) (Fig. S6d,f) showing that root metabolism did not only inherit amino acids from leaves but also assimilated nitrate that was more ^{15}N -enriched than leaf nitrate itself.

We recognise that our study did not allow the purification and isotopic analysis of nitrate from xylem and phloem sap. The volume of sap required would have been far too large (typically 0.1–1 ml xylem sap would have been necessary; even more for phloem sap because nitrate concentration is low) and thus this was technically not feasible. Nevertheless, our dataset includes isotopic analysis of stems. In sunflower, the $\delta^{15}\text{N}$ value of stem nitrate was generally intermediate between leaves and roots (Fig. 3b). This is unsurprising because stems should reflect a mixture of ^{15}N -depleted xylem nitrate (from root nitrate absorption) and ^{15}N -enriched phloem nitrate (inherited from leaf nitrate export), and such a mixture must be isotopically lighter than nitrate accumulated in roots. In oil palm, stem nitrate was generally less ^{15}N -enriched than in leaves (Fig. 3h). Nitrate concentration was also very low in oil palm stems (Fig. 3k), suggesting that there was much less opportunity for phloem nitrate to accumulate (meaning that, in mathematical terms, a_p was a small number) than in sunflower.

Interestingly, isotopic analyses in phloem and xylem sap of *Ricinus* are consistent with phloem carrying ^{15}N -enriched nitrate. In fact, during the day (when leaf nitrate reduction occurs), phloem $\delta^{15}\text{N}$ was quite close to that in xylem (within 0 and 4‰) while leaf soluble fraction was ^{15}N -depleted (c. -2‰ on average at midday) (Peuke *et al.*, 2013), suggesting that amino acids carried by phloem sap could not explain satisfactorily the isotopic signature of phloem sap and thus other N-containing compounds in phloem must cause a ^{15}N -enrichment. For example, if nitrate represented only 3% of phloem sap N, it could explain the results obtained by Peuke *et al.* (2013) with a $\delta^{15}\text{N}$ value of 100‰ . It is worth noting that the diurnal cycle of N transport via xylem and phloem is probably pronounced in oil palm because leaf %N (total N) varied by 0.05 units ($35\text{ }\mu\text{mol N g}^{-1}\text{ DW}$) between day and night (Scheidecker & Prevot, 1954).

Model validity

Key parameters associated with nitrate circulation could not be measured experimentally and thus we carried out modelling. Of course, our model was crude in that it could not capture all the subtleties of nitrate redistribution and utilisation. That is, we used three compartments, one of them (roots) having two pools.

In addition, our calculations do not take into account the contribution of veins in leaves, or the loss of organic matter in roots by exudation or fine root turnover. In that sense, our model is rather similar to the structure in basic elements of circulation described previously (Pate, 1975). In doing so, the model was deliberately kept simple to avoid the use of many parameters that are not accessible experimentally, but could reproduce observed $\delta^{15}\text{N}$ data (Fig. 4) by adjusting nitrate fluxes. As expected, the direct xylem-to-phloem nitrate transfer (B) was small (Van Bel, 1990) and was thus in agreement with experiments on legumes (Pate, 1975). Modelled values of nitrate flux in xylem⁺ decreased as expected under low K (see Mengel & Radomir (1973) in sunflower) and led to a consistent order of magnitude for leaf transpiration, using a fixed value of nitrate concentration in xylem sap of 3 mM. Of course, this value is debatable but 3 mM is realistic. In castor bean, xylem sap contains on average 7 mM nitrate (Peuke, 2009). In sunflower, measurements are scarce but samples of sap obtained with decapitation or a pressure chamber suggest a value of between 0.9 and 7.3 mM (Mengel & Radomir, 1973; Udayakumar *et al.*, 1981; Gollan *et al.*, 1992). To our knowledge, in oil palm, nitrate concentration in xylem sap has never been measured.

Of particular significance is the low performance of modelling in sunflower at high K (Fig. 4a, red frames): with the present model, it was not possible to reproduce perfectly the high ^{15}N -enrichment in roots (with a difference of up to 10‰). There are several potential causes for this discrepancy: root turnover and exudation, nitrate loss (efflux) from the storage pool or an underestimation of leaf nitrate $\delta^{15}\text{N}$. In fact, fine root turnover and exudation would represent a loss of organic matter that would have to be taken into account in r_r (nitrate reduction rate in roots). That is, not accounting for this loss would have led to an underestimation of r_r and thereby of the ^{15}N -enrichment in root nitrate. Similarly, not accounting for potential nitrate efflux back to soil from the storage pool would affect the $\delta^{15}\text{N}$ value because efflux fractionates between isotopes (by 3‰ , Table 1). Leaf nitrate could also be slightly underestimated because leaves contain xylem vessels in veins, and xylem nitrate is isotopically lighter. We have proposed previously that this effect might cause an underestimation of $\delta^{15}\text{N}$ in leaf nitrate by several per mil, and thus affects the isotope composition of nitrate going back to roots via the phloem (Cui *et al.*, 2019a).

We also recognise that our study used relatively high nitrate concentration (12 mM) and this allowed us to have isotopically constant conditions (the $\delta^{15}\text{N}$ value of source nitrate remained constant). However, our modelling would remain valid at lower nitrate concentration. Should nitrate concentration not be constant, it would only require the addition of a differential equation for medium nitrate to account for changes in content and $\delta^{15}\text{N}$.

Nitrate circulation under waterlogging and low K

There was an effect of waterlogging and K conditions on nitrate $\delta^{15}\text{N}$ and concentration. Waterlogging caused a general decrease in nitrate content in all tissues (Fig. 3), consistent with an inhibition of nitrate absorption (influx). It also led to a progressive

decline in $\delta^{15}\text{N}$ in nitrate in particular in roots, compared to control conditions, resulting from lower nitrate reduction rate (slower biomass N accumulation) and slower nitrate circulation coupled with transient remobilisation of root nitrate (Fig. 4). The former effect leads to a lower ^{15}N -enrichment in nonconsumed nitrate molecules and the latter leads to smaller flow of ^{15}N -enriched nitrate from phloem⁺ (lower b_n) and allows the consumption of nitrate from the root storage pool ($w > 0$). Such a situation was particularly visible in sunflower (Fig. 4) and is consistent with the general depressing effect of waterlogging on N metabolism in roots in both species but also on leaf-exported N assimilates such as asparagine (Cui *et al.*, 2019a,b).

Low K conditions also had a considerable effect on $\delta^{15}\text{N}$ values, with much less ^{15}N -enriched nitrate (except in sunflower leaves where leaf nitrate did not change much) (Fig. 3). K^+ is the cornerstone of phloem function and a common counter-cation for nitrate in xylem, so an effect on nitrate circulation was expected. In *R. communis*, potassium limitation increased nitrate concentration in both xylem and phloem (Peuke *et al.*, 2002). Here, in sunflower, both modelled xylem⁺ and phloem⁺ nitrate transport rates were much lower at low K. However, this was not reflected by the nitrate content, which was not lower but higher. This simply shows that plant nitrate absorption was less affected than nitrate circulation or utilisation by metabolism. That is, the N demand to form organic matter was proportionally lower, as demonstrated previously (Cui *et al.*, 2019a). In oil palm, nitrate concentration was lower under low K, suggesting an effect on nitrate absorption by roots and, furthermore, nitrate concentration correlated with the $\delta^{15}\text{N}$, in particular in stems (Fig. 3h). Thus, in contrast to sunflower, low K conditions inhibited not only nitrate transport but also nitrate acquisition and thus the ability to store (^{15}N -enriched) nitrate. The strong effect of potassium limitation on N acquisition is well known in this species, with many agronomical trials showing the strict dependence of N absorption on K (Chapman & Gray, 1949; Ollagnier & Ochs, 1973; Chan, 1981), and the strong decrease in elemental N content (%N) in all organs (data not shown).

Isotope fractionation in N assimilation

There were significant differences between tissue fractions, with nitrate being the most ^{15}N -enriched while lipids were ^{15}N -depleted, except in leaves. Accounting for the $\delta^{15}\text{N}$ of the different fractions allowed us to estimate the fractionation associated with nitrate reduction, which was, as expected, near 16‰ (Fig. 2) (Ledgard *et al.*, 1985; Tcherkez & Farquhar, 2006). In leaves, the lipid fraction, enriched in chlorophylls, is isotopically similar to proteins (Fig. S4), and this has been found previously in rapeseed leaves (Gauthier *et al.*, 2013). This simply comes from the fact that N atoms in chlorophylls come from glutamate (via δ -aminolevulinate) with little isotope fractionation. By contrast, N-containing lipids mostly originate from serine (e.g. ethanolamine and choline moieties of phospholipids), which is generally ^{15}N -depleted compared to other amino acids (Tcherkez, 2011; Gauthier *et al.*, 2013). In addition, there may be some isotopic fractionation during polar lipid synthesis (Werner & Schmidt,

2002). It is worth noting that the nitrate-corrected soluble fraction exhibited a rather variable $\delta^{15}\text{N}$ value, similar to that of proteins, intermediate, or as ^{15}N -depleted as in lipids. This reflects the fact that the composition of the soluble fraction in N-containing compounds (amino acids, polyamines, nucleotides, etc.) varied and these different compounds probably had distinct $\delta^{15}\text{N}$ values (not analysed here).

Perspectives

Our results provide an overview of nitrate $\delta^{15}\text{N}$ in plant organs and clearly show that nitrate is considerably ^{15}N -enriched in roots (Fig. 3) in the two species of interest, sunflower and oil palm. That is, root nitrate appeared to be much more enriched than would be expected if nitrate were simply transported to and accumulated in leaves, therefore suggesting that ^{15}N -enriched nitrate can be transported back to roots from leaves via the phloem ($\delta^{15}\text{N}$ values summarised in Fig. S8). It does *not* imply that the concentration or flux of nitrate in the phloem has to be high. Here, modelling shows that even small (a few per cent of xylem nitrate flux), the phloem nitrate flux has a considerable impact on root $\delta^{15}\text{N}$. Of course, this effect probably varies widely between species, which differ in their ability to carry nitrate via the phloem. For example, past measurements have shown that nitrate is usually undetectable in phloem sap of legumes. Still, our findings are highly significant because they suggest that the natural abundance in nitrate can be used as a tracer of plant sap circulation and support the view that nitrate remobilisation from leaves is an important aspect of plant N budget. We nevertheless recognise that more data would be required on the isotope composition of nitrate purified from saps but this is at present too challenging technically in particular for phloem, because of low nitrate concentrations and sap volumes that can be sampled (of the order of microlitres (Dinant & Kehr, 2013; Palmer *et al.*, 2014) and thus maximally 0.3 μmol nitrate per sample, while EA-IRMS would require minimally 1–2 μmol nitrate per sample). Conversely, N circulation in the plant also involves organic molecules highly concentrated in phloem sap, and some information could be drawn from compound-specific analysis. This will be addressed in a future study.

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Author contributions

GT and EL designed the experiments. JC performed palm cultivation, biomass measurements and isotopic analyses. FF

performed extra isotopic analyses. All authors contributed to writing the paper.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Scheme of the experimental design.

Fig. S2 Sensitivity of the model to parameterisation.

Fig. S3 Shoot : root ratio in both species over the course of the experiment.

Fig. S4 $\delta^{15}\text{N}$ values in total organic matter.

Fig. S5 N elemental content in total organic matter.

Fig. S6 $\delta^{15}\text{N}$ values in tissue fractions (proteins, lipids, soluble fraction).

Fig. S7 Example of $\delta^{15}\text{N}$ modelling under low K and waterlogging.

Fig. S8 Graphical summary of modelled $\delta^{15}\text{N}$ values.

Notes S1 Equations.

Notes S2 Model parameterisation.

Table S1 Nutrient solution composition.

Table S2 Model parameterisation.

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