

# Modelling N nutrition impact on plant functioning and root architecture in various genotypes of *Arabidopsis thaliana*

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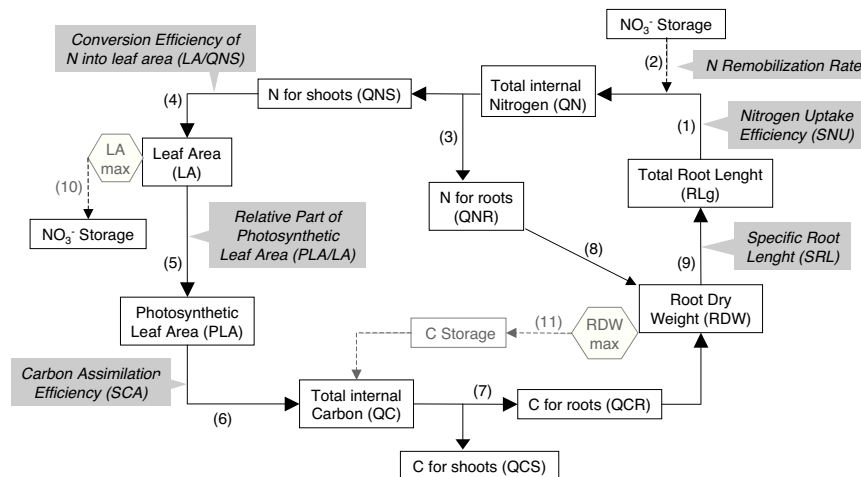
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**Keywords:** assimilate partitioning, root morphogenesis, genetic variability, nitrogen uptake efficiency, whole-plant modelling

## Introduction

Breeding new varieties adapted to low-input agricultural practices, mainly for nitrogen and pesticides, is of particular interest when considering economic and environmental concerns. However, screening pertinent traits for crop adaptation to low N supply remains difficult because plant response to N availability is a set of closely interacting processes, and because this response displays a wide genetic and environmental variability. Thus, Loudet et al. (2003) showed that most of the variables involved in N use efficiency exhibit wide variations in response to varying N supplies in a population of *Arabidopsis thaliana* recombinant inbred line (RILs). Therefore, a quantitative modelling of whole-plant C/N functioning should be a suitable tool to identify the key-parameters determining plant efficiency under low or high N nutrition, that will be pertinent for phenotype screening. Such an approach has been successfully employed to identify QTLs (Quilot et al., 2004; Yin et al., 2000) and to simulate phenotypes with allelic composition (Reymond et al., 2004).

A whole-plant structure-function model was developed for that purpose on one genotype (WS) of *A. thaliana*. The model is based on interactions between N and C fluxes and offers an explicit and dynamic description of root system and leaf area growths (Fig. 1). The model was then simplified (root system output was merely considered as biomass) to interpret the behaviour of several RILs of *Arabidopsis* and wheat, and of one *Arabidopsis* mutant impaired on high affinity nitrate uptake.

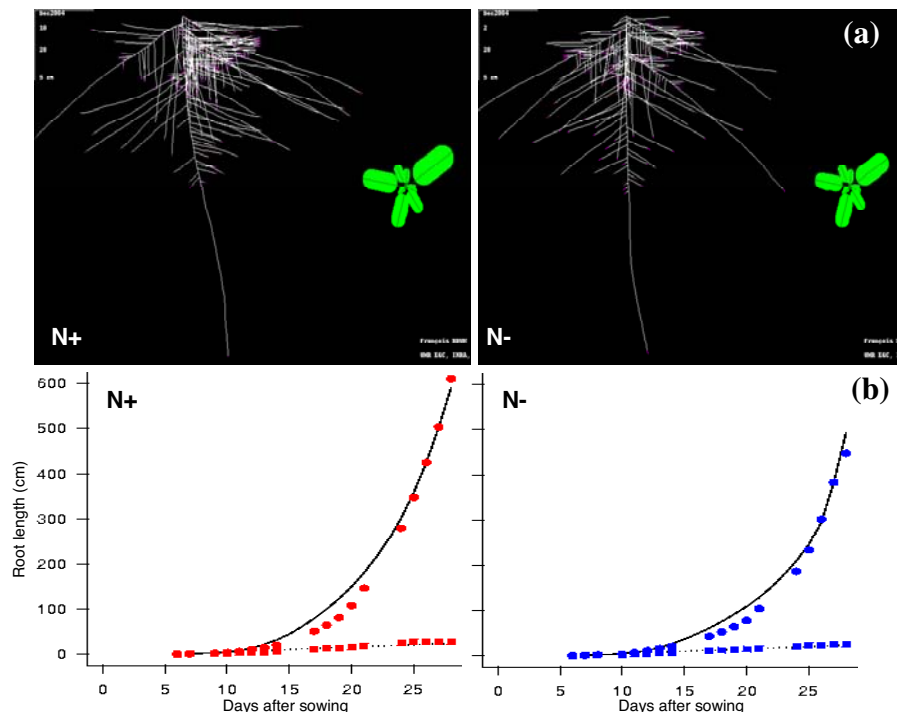


**Fig. 1:** Schematic diagram of the model. The model was constituted of a shoot and a root compartment, exchanging C and N fluxes. Priority was given to shoots for C and to roots for N. Leaf expansion and root growth were limited by relative maximum growth rates obtained in our culture conditions. Total internal N quantity resulted from root uptake (1) and reserve remobilization (2) and determined the increment of total leaf area (4), after satisfaction of root N demand (3). Total internal C quantity was produced by photosynthetic leaf area (6), obtained from total leaf area using the Beer-Lambert's law (5). Effective root growth resulted from the growth allowed by internal N quantity (8) and by the C quantity remaining after satisfaction of shoot C demand (7). Root dry mass was then partitioned in root length (9). N and C storage pools emerged (10, 11) when N and C internal quantities were not fully depleted by growth.

## Root architecture modelling for *Arabidopsis thaliana*

One of the aims of this work was to estimate to what extent the impact of an homogeneous N limiting nutrition on root architecture can be explained the decrease of endogenous carbon availability arising from leaf area reduction in response to low N supply. To answer this question, *A. thaliana* plants (WS) were grown in rhizotrons (Devienne-Barret et al., 2006) in growth chamber with two levels of N supply (2 and 10 mM NO<sub>3</sub><sup>-</sup>) and a combination of CO<sub>2</sub> or radiation levels (3 treatments). Root architecture was recorded daily and plants were harvested four times between 10 and 29 days after sowing to measure shoot and root dry weights and N metabolite contents.

A model was implemented in Python language, on the same basis and rules as other object-oriented models for root system architecture (Thaler and Pagès 1998, Drouet and Pagès 2003) and was designed to account for the effect of C availability on root system architecture. The model compares supply and demand for C within the root system described as a network of roots. The C supply was defined here from the flux of accumulated root dry weight. It was calculated from data as an input variable and corresponded to the flux of C provided by shoots (arrow (7) in Fig. 1). Thus, the effect of N supply was taken into account only through the modulation of this shoot-originating C flux, in accordance with our working hypothesis. Indeed, the quantity of nitrogen taken up by the roots was shown to be related to leaf area through a linear relationship (arrow (4) in Fig. 1). The "C demand" was calculated for each root according to its diameter (Farrar 1993) and summed for the whole root system. C availability was defined as the C Supply/Demand ratio and was used as a "satisfaction" coefficient of the demand. When C supply matched or exceeded C demand (*i.e.* C availability  $\geq$  1), effective elongation of each apex reached the potential elongation rate, calculated for each apex from its current diameter *via* a close relationship found between both variables. When C supply was lower than C demand (*i.e.* C availability  $<$  1), effective elongation was reduced, from the potential elongation rate, proportionally to the satisfaction coefficient. Apex size was also driven by C availability, the relative variation rate of apex diameter varying linearly with C availability, between a minimum (reached when C availability was null) and a maximum (reached when C availability was superior or equal to 1).



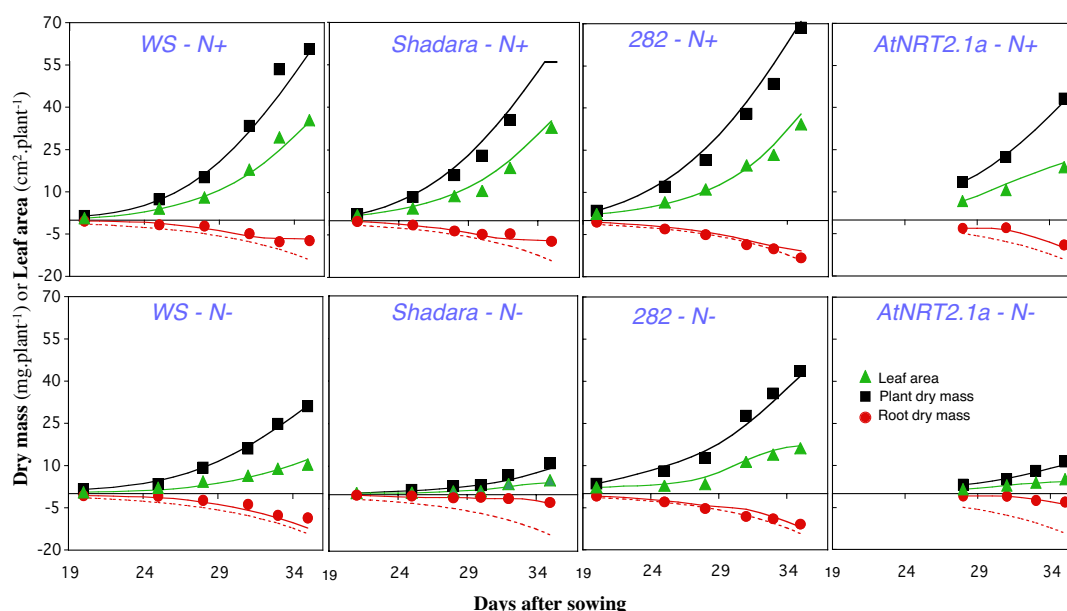
**Fig. 2:** Simulations of *Arabidopsis thaliana* root morphogenesis during vegetative growth under two levels of N nutrition. Plants (ecotype WS) were grown on soil with plethoric (left panel) or limited (right panel) N supply. (a) Graphical outputs generated by the model showing shoot and root architecture at 29 days after sowing. (b) Dynamics of root length during vegetative growth, measured (points) or simulated (lines) for taproot (squares, solid line) and for the whole root system (circles, dotted line).

Parameters were estimated under the high C x high N treatment and then the model was used with the same parameterization for the other CxN datasets. Only 12 model parameters were needed to simulated contrasted responses of root system architecture under our various C conditions when N supply was not limiting. Root length (Fig. 2) was well predicted as well as root branching, and root emergence in a lesser extent. Time of emergence of lateral root showed the expected distribution. Even if our model was not able to simulate local effect of nitrogen availability and presented limited conditions of validity, our modelling approach showed that major effect of global N availability on root system architecture was mediated by modifications of C fluxes. However, these modifications were not sufficient to fully predict root apical diameter or specific root length when N supply was low. A specific effect of N on root elongation had to be introduced to improved simulation in low N supply, and explained a part of root system adaptation to N limitation. C x N levels did not affect the other parameters. In particular, branching parameters were not affected even if branching adaptation was observed in our culture conditions.

### Toward a more functional model dedicated to genotype analysis and QTL detection

High-throughput phenotype analysis using simulation tools require the use of models with few and easily measurable parameters. With the aim to identify the key parameters determining genetic and environmental variability of plant response to N availability, we present a simple compartmental model of C and N absorption and partitioning for *A. thaliana* during the vegetative growth simplified from the above-presented one (Fig. 1). This model combines integrative variables (biomasses, N or nitrate accumulated), exchange surfaces for shoots (*i.e.* leaf area) but not for roots (in contrast with the above-model), and efficiencies (grey areas in Fig. 1) such as carbon assimilation efficiency, N uptake efficiency or N remobilization rate.

As the first step, the model was used to quantitatively interpret the behaviour of five contrasted genotypes of *A. thaliana* grown in soil (rhizotrons) with 3 levels of nitrate supply. Parameters were estimated on one genotype and one N level and then they were checked under the other genotype x nutrition conditions (Fig. 3). We found that nitrogen uptake efficiency (*snu*) and carbon assimilation efficiency (*sca*) were the key variables explaining the responses of the five genotypes to nitrogen nutrition under steady state conditions.



**Fig. 3:** Dynamics of leaf area (triangles), root dry mass (circles) and whole plant dry mass (squares) during vegetative growth in plethoric (upper part) or limited (lower part) N conditions for four genotypes of *A. thaliana*. Solid lines correspond to the simulations performed by the model. Values of root biomass were mirrored to increase legibility.

In the second step, the model was tested using the *atnrt2.1a* mutant. Due to the mutation of *AtNRT2.1* and *AtNRT2.2* genes, this mutant is impaired in high affinity nitrate uptake (Filleur et al. 2003). The model simulated satisfyingly the behaviour of the mutant (Fig. 3). This allowed us to validate the determining role of nitrogen uptake efficiency in plant response to N supply and to point out the main role of the *AtNRT2.1* gene in plant response efficiency to N limited nutrition.

In the last step, the model was used on a population of 120 doubled haploid lines of wheat, to characterize the genotypic variability of each parameter and to investigate QTLs involved in plant tolerance to a N deficiency. The lines were obtained from a cross between a N stress tolerant variety and a N stress sensitive one and were grown hydroponically under N limited conditions. We found that, as for *Arabidopsis*, the main source of variation relied on *snu*, indicating that this parameter was the major determinant of the variability of plant response to N supply. In addition, eight QTLs related to model parameters were detected in wheat.

## Conclusion

An *Arabidopsis* FSPM was built to study plant response to steady-state N nutrition with a focus to root system architecture. It was used as a reference to develop a simpler model, more suited to high-throughput analysis, which highlighted the determinant role of nitrogen uptake efficiency in plant response to N supply. The next step will be to study and model variations of nitrogen use efficiency within the root system, especially in case of heterogeneous N supply.

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