Technical Article

Totomatix: a novel automatic setup to control diurnal, diel and long-term plant nitrate nutrition

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Running title: An automatic setup to control nitrate nutrition

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ABSTRACT

• **Background:** Standalone nutritional setups are useful tools to grow plants at defined nutrient availabilities and to measure continuously nutrient uptake rates, in particular nitrate. Their use cannot be avoided when the measurements are meant to last for long time periods. These complex systems have, however, important drawbacks including a poor long-term reliability and low precision at high nitrate concentration. This explains why the information dealing with diel dynamics of nitrate uptake rate is scarce and concerns mainly young plants grown at low nitrate concentration.

• **Scope:** The novel system detailed in this paper has been developed so as to allow a versatile usage in growth rooms, greenhouses or open fields at nitrate concentration ranging from few micro- to several milli-mol L\(^{-1}\). The system controls at set frequencies, the solution nitrate concentration, pH and volumes. Nitrate concentration is measured by spectral deconvolution of UV spectra. The main qualities of the setup are its low maintenance (weekly basis), an ability to diagnose interferences or erroneous analyses and a high precision on nitrate concentration measurements (0.025% at 3 mM). The paper characterizes the precision on diurnal nitrate uptake rate measurements, which reveals sensitive to the solution volume at low nitrate concentration, whereas at high concentration, it is mostly sensitive to the precision on this volume.

• **Conclusions:** This novel setup allows one to measure and to characterise the dynamics of plant nitrate nutrition with a high temporal resolution (minutes-hours) over long term experiments, up to one year. It is reliable and also offers a novel method to regulate up to seven N treatments by adjusting the daily uptake of test plants relative to controls, in variable environments such as open fields and glasshouses.

**Key words:** Automatic setup; nitrogen; nitrate; nutrition; uptake rate; diel; diurnal; hydroponics; UV spectrophotometry; deconvolution.
INTRODUCTION

In hydroponic systems, nitrate concentration ([NO₃⁻]) of nutrient solutions is specified either by its theoretical initial value or by an actual in-line measurement. These data may largely diverge depending on the initial value and the plant N demand. For instance, plants demanding 100 µmol N per day decrease daily by 100% the nitrate stock of 1 L 0.1 mM solution but only by 1% in the case of 10 mM solution. Thus, in the low concentration range typical of physiological studies, maintaining concentration requires either frequent nitrate replenishments from stock solution and/or very large solution volumes. For example, Adamowicz and Le Bot (2008) fed 125 L of 50 µM [NO₃⁻] nutrient solution to each of their 1 g dry weight tomato (Solanum lycopersicum) plants (28 days old) and found necessary to correct manually the solution twice per day in order to limit concentration drifts below 15%. Thus, growing eight plants required 1000 L of solution posing important material and method problems (space occupation, size of containers, solution handling, number of repetitions etc.). These difficulties obviously worsen with lower [NO₃⁻] and/or bigger plants, thereby hindering long-term experiments based on manual methodologies.

In agronomic studies, most authors infer N uptake from plant intake between two harvests dates using plant analysis data. Over short periods of time (typically 1 hour), this destructive method proves to be insensitive in absence of isotopic tracing and must be replaced by non-destructive techniques. They rely almost exclusively on a balance sheet method of the nutrient solution, which is by far more sensitive in the low than in the large [NO₃⁻] range. This explains why most short-term measurements of uptake rates are made under low [NO₃⁻] and long-term measurements under high [NO₃⁻], denoting physiological and agronomical approaches.

A way to circumvent these difficulties in long-term experiments is to make programmed
nutrient additions (PNA) to water culture systems using an a priori knowledge of plant N demand (Asher and Blamey, 1987; Ingestad and Ågren, 1988, 1992). This is achievable under controlled environmental conditions (typically growth-rooms) when growth is regular and predictable from previous experiments. However, most ecophysiological studies request crops to be grown in the open field or the greenhouse so that PNA turns out to be unusable because the natural climatic variations provoke unpredictable plant N demand.

Growing plants at stable \([\text{NO}_3^-]\) requires frequent analyses of the nutrient solution. The knowledge of actual \([\text{NO}_3^-]\) and solution volume allows to calculate the amount of stock solution necessary to restore the initial concentration. This enables to calculate instantaneous uptake rates and to study their temporal variation. Automatic setups have been described to use various analytical techniques for concentration determination including HPLC (Goyal and Huffaker, 1986), UV spectrophotometry (Alloush and Sanders, 1990; Rockel, 1997), colorimetry (André et al., 1979; Lorenzen et al., 1998) and selective electrodes (Clement et al., 1974; Hansen, 1980; Glass et al., 1983; Blom-Zandstra and Lupijn, 1987; Koch et al., 1987; Bloom, 1989). They all control pH to compensate for net OH⁻ fluxes associated to \(\text{NO}_3^-\) uptake (Kirkby et al., 2009). The knowledge of solution volume is not compulsory to regulate \([\text{NO}_3^-]\) (Lorenzen et al., 1998), but it is essential for uptake calculation. Nevertheless, all papers cited above, but André et al. (1979) and Hansen (1980), ignored volume changes implying that uptake rates calculations may have carried errors.

Parker and Norvell (1999) have listed several drawbacks of these systems (expensive investments, few numbers worldwide, limited number of treatments, antagonism between constant concentration and sensitivity of uptake rate measurement), which also necessitate frequent manual maintenance. Most have also been described as a constitutive element of a specific cultural system, thus restricting their versatility.
(growth-room, greenhouse, open field, isolated plants versus stands). The current paper presents a novel automatic device, called Totomatix, overcoming some of the technical drawbacks of previous setups. It offers the reliable regulation of \([\text{NO}_3]\), pH, solution volume and the measurement of \(\text{NO}_3\) uptake rates over the long term (up to 1 year) with fine resolution (time scalable, down to 5 minutes), allowing for combined studies of seasonal and diel behaviours.

**MATERIALS AND METHODS**

The following description should suffice to understand Totomatix functioning, but upon simple request to the authors, the software and detailed schematics for the construction of this system can be made available to research projects for non-commercial use.

To ensure versatility, the automatic setup (Fig. 1) has been made independent of the cultural system considering that this latter must be hydroponic, typically a continuously flowing solution culture such as the nutrient film technique (NFT). Each nutritional treatment comprises a temperature-controlled tank of solution (Fig. 1a, 1 and 2), a pump (3) that injects (4a) continuously the solution into troughs or containers (not shown) holding the plants and return pipes (4b) to drain back the solution to the tank by gravity. The injection pipe (4a) comprises also a direct return pipe with a manual flow valve (5) setting the rate of delivery to the plants while mixing and aerating the tank. All materials in contact with the nutrient solution are made of rigid PVC, semi rigid black polyethylene and stainless steel.

A mere bypass (6a) branched on the main outlet (4a) redirects a continuous flow of solution through the automatic setup and returns it to the tank (6b). A manual valve (7) controls the flow rate, high enough to keep the lag between the tank and the analytical system below 1 minute. An optional filter (8) is placed at the inlet of the bypass.

**Automatic setup components**

Fig. 1b shows an overview of the setup components. At the inlet (6a), the solution
enters a flow-through cell equipped with a pH probe (9) and returns (6b) to the tank. Part of the nutrient solution is continuously filtered (10) and pumped (11) from the flow-through cell through a spectrophotometer cuvette (12) for nitrate measurement and returns (6b) to the tank. On analysis, a first group composed of a stock solution reservoir (13), a motorized syringe drive (14) and an 8-way motorized selection valve (15) injects acid into one nutrient solution through a manual 3-way valve (16) for pH correction. The 8-way valve (15) has one way connected to waste for priming and maintenance, and seven ways connected to the seven nutrient solutions. An identical second group (17-20) injects a nitrate stock solution to restore set [NO₃]. Deionized water is added (Fig. 1a, 21) to the tank (1) through a stainless-steel on/off valve (22) at a flow rate measured by a flowmeter (23) until the solution reaches an optical level sensor (24) positioned once in the tank. Unless mentioned, all other components in contact with the solution are in PTFE or glass. A computer (not shown) acquires signals from sensors and drives the actuators, except for solution temperature control, which has its own independent system. The setup allows the control of seven independent solutions implying that some components are present in seven units (1-12, 16, 20-22, 24).

Sensors
The dual-beam spectrophotometer (12, model UV=mc2, SAFAS, Monaco) with 2 nm spectral resolution is equipped with a motorized multi-cuvette holder fitted with up to seven quartz flow-through cells (series 170.700.QS and 176.700.QS depending on light path, Hellma GmbH & co, Müllheim, Germany). It communicates with the computer through RS232 interface.

The pH-meters (Aqua-Méditerranée, Aix-en-Provence, France) with 0.01 pH resolution and mV output are equipped with pH probes (9, model L 737, Schott, Clichy, France).

The flowmeter (23, model DPL-1V05 G4 L343, Kobold, Cergy-Pontoise, France) with analog 4-20 mA output measures flow rates in the range 0.025-0.5 L min⁻¹.
In each tank, an optical level detector (LLE 102000, Honeywell, Morristown, NJ, USA) with TTL output is placed upwards in a siphon (24) giving 1 mm hysteresis. In terms of solution volume, the resulting precision depends on the shape of the tank (80 mL and 540 mL in our 80 L and 300 L tanks, respectively). Level detectors are manually adjusted once at the beginning of the experiments.

**Actuators**

Two modular valve positioners (15 and 19, model MVP R0159699, Hamilton, Bonaduz, Switzerland) with RS232 interface drive two 8-way PTFE distribution valves (model HVXM R 36 766, Hamilton) that redirect the acid (13) and nitrate (17) stock solutions to the selected nutrient solution or to waste for priming and rinsing purposes. These stocks feed two syringe pumps (14 and 18, model PSD/2, Hamilton) with stepper motors (2000 steps) and RS232 interface, equipped with glass syringes (model TLL, Hamilton). The choice of their volumes (from 25 µL to 10 mL) is governed by experimental needs (i.e. low or large plant demands, fine or large temporal control) and can be changed during an experiment. Deionized water is added directly into each tank (1) by actuating the dedicated solenoid on/off valve (22, Parker, Cleveland, OH, USA).

**Service components**

The solution in the main bypass flows through an optional 25 µm charcoal filter (8, model CA10BX, Atlas Filtri, Padova, Italy) meant to trap optically active contaminants. In the analytical bypass, the spectrophotometer is permanently protected by in-line filtration (10, hand made using aquarium foam) to prevent cell clogging. The main solution is circulated by centrifugal pumps (3) made of inert material (plastic or stainless steel). Pumping in the analytical bypass is made upstream the spectrophotometer (12) by dedicated PTFE and PEEK micro pumps (11, model 7604, Bürkert, Triembach au Val, France). The bypass is also fitted with two T-flow path valves with 3 ports (16 and 20, model HV 4-3, Hamilton) that can be manually actuated...
in order to pump (11) a cleaning solution (see subsection Maintenance, below).

The solution temperature is regulated in each tank (1) using independent controllers (model EWDR 905/T, Elliwell, Villeneuve La Garenne, France), stainless steel coils (2) for solution cooling and aquarium glass coated heaters (not shown) for warming.

The computer and the spectrophotometer are protected by an online uninterrupted power supply (model Pulsar Extreme 1000 C, Merlin Gérin, Saint Ismier, France) that improves the stability of the spectrophotometer signal.

**Computer hard- and software**

The analog outputs from the flowmeter and pH-meters and the TTL outputs from the level detectors are acquired by a 24 bit multimeter board (model NI PCI-4351 with connector block TBX-68T, National Instruments, Austin, TX, USA). The on/off valves (22) are actuated through relays (model ER-16, National Instruments) driven by a digital I/O interface (model PCI 6503, National Instruments).

The main software was written in Visual Basic (Microsoft Corporation, USA) calling subroutines written in TK Solver (Universal Technical Systems Inc., Rockford, IL, USA) for the computation of [NO$_3$], acid additions and water flow-rates. From an appropriate graphical interface, the operator sets the number of regulated solutions (one to seven tanks), the time lapse between analyses, the concentrations of the stock NO$_3$ and acid solutions and the respective syringe volumes. For each solution, the operator sets also the tank volume, the [NO$_3$] and pH set points, and the light path of each analytical quartz cell. All settings can be modified during an experiment. The software allows also for pH, flowmeter and spectrophotometer calibrations.

After each analysis, the user interface displays the analytical results and the volumes of stock NO$_3$, acid and water added to restore set points in each tank. These values together with the raw data obtained from sensors are historicized on a hard disk and are
usable with usual spreadsheets.

**Regulation methods**

The regulation process follows a series of five steps performed sequentially on all nutrient solutions: (1) nitrate analysis (detailed in the next section); (2) pH measurement; (3) nitrate repletion; (4) pH correction and (5) volume correction.

A NO₃ stock solution of exact concentration Cₛₜ (typically 1 mol L⁻¹) is used to correct [NO₃]. It mixes (molar fractions) 0.408 KNO₃, 0.204 Ca(NO₃)₂ and 0.092 Mg(NO₃)₂.

From the knowledge of the measured [NO₃] (Cₘ), the concentration of the entire solution volume Vₛₚ is restored to the set point Cₛₚ by automatic injection of a volume Iₑ of this stock:

\[ I₁ = Vₛₚ \times \frac{Cₛₚ - Cₘ}{Cₛₜ} \]  

Eqn 1

Generally, plants fed with NO₃ alkalize the nutrient solution. Thus, pH regulation by acid injection was automated, but if needed, adding a syringe drive and a distribution valve for base injection can easily enhance the system. A stock H₂SO₄ solution (typically 1 N) is used to correct pH to its set value. The necessary volume Iₐ is calculated from the total nutrient solution volume, [H₂SO₄] and cubic spline interpolation of a titration curve previously made in the laboratory on a same nutrient solution. As pH regulation is a straightforward procedure, further details have been skipped in this paper.

In order to restore Vₛₚ, deionized water is added until the level sensor detects the upper limit. The exact addition volume Vₐ is calculated by integrating the flow rate during delivery, allowing the calculation of the solution volume (Vₘ) at the time of the analysis:

\[ Vₘ = Vₛₚ - Vₐ - Iₑ - Iₐ \]  

Eqn 2
Nitrate analysis

Vercambre and Adamowicz (1996) showed that Fe chelates are major interferers for UV nitrate determination in nutrient solutions. However, from the knowledge of NO₃⁻ and Fe-chelate specific absorbances, their respective concentrations in a solution mixture can be determined by spectral deconvolution analysis in the UV domain at two wavelengths or more. Their study compared Fe-EDTA to Fe-EDDHA and they found that the former had lower specific absorbance than the latter, which was also sensitive to pH. On this basis, they recommended using Fe-EDTA in nutrient solution in order to maximize sensitivity on nitrate determination. Since the measurement is independent of pH, the use of Fe-EDTA appears also safer because the UV method remains valid in case of pH-meter failure. It must be drawn to the reader’s attention that the use of Fe-EDTA in the present study does not preclude the use of other chelates if their spectra have been adequately characterized.

As a compromise between analytical precision and span, \( k = 5 \) wavelengths were selected (Table 1): 201 (NO₃⁻ peak), 206, 214, 257 (Fe-EDTA peak) and 280 nm. In order to improve the linearity of the spectrophotometer response at any wavelength (\( \lambda \)), the measured absorbance (\( A_{m,\lambda} \)) is corrected from the stray light fraction (\( \alpha_{\lambda} \), Table 1):

\[
A_{\lambda} = A_{m,\lambda} + \log_{10} \left( \frac{1 - \alpha_{\lambda}}{1 - \alpha_{\lambda} \times 10^{4\lambda_{m,\lambda}}} \right)
\]

Eqn 3

At any wavelength, the total absorbance of a mixture through a light path \( L \) is the sum of the individual absorbances of active species and of an error \( E_{\lambda} \) (\( C'_{m} \) being [Fe-EDTA] and \( \varepsilon \) being specific absorbances):

\[
A_{\lambda} = L \times \left( C_{m} \times \varepsilon_{N,\lambda} + C_{m} \times \varepsilon_{Fe,\lambda} \right) + E_{\lambda}
\]

Eqn 4

Spectral deconvolution uses a least squared error method, i.e. finds the values of \( C_{m} \) and \( C'_{m} \) that minimize the sum of squared errors at all \( k \) wavelengths:

\[
\sum E_{\lambda}^2 = \sum_{\lambda=1}^{k} \left( A_{\lambda} - L \times \left( C_{m} \times \varepsilon_{N,\lambda} + C'_{m} \times \varepsilon_{Fe,\lambda} \right) \right)^{2}
\]

Eqn 5
The mathematical solution, described in appropriate textbooks (see Clark et al., 1993), yields three major pieces of information, namely the nitrate and Fe-EDTA concentrations plus a mean error ($E$) between actual spectra and the restored one after deconvolution:

$$E = \sqrt{\frac{\sum E^2_k}{k-2}}$$  

Eqn 6

In order to detect transient $E$ outliers, the following transformed absorbance error $E_t$ is also computed from $E$ mean ($m_{10}$) and standard deviation ($sd_{10}$) of the ten previous analyses, by applying the following equation to the current $E$ value:

$$E_t = \frac{E - m_{10}}{sd_{10}}$$  

Eqn 7

This calculation shares the logics of reduced and centered variables, but it differs because $E$ is not included in $m_{10}$ and $sd_{10}$ computation.

The light path $L$ affects the sensitivity (proportional to $L$), and the stability and linearity of the spectrophotometer signal ($L$ should keep the peak absorbance $A_{201} \leq 2$).

Depending on experimental needs, the following light paths (in cm, followed by concentration range in mM between parentheses) are used to encompass nutrition studies and horticultural production: 1 ($\leq 0.2$), 0.5 (0.02-0.4), 0.2 (0.05-1), 0.1 (0.1-2) and 0.05 (0.2-4). Existing cells with $L = 0.01$ cm should allow measurements up to 20 mmol L$^{-1}$ NO$_3$.

**Nitrate uptake**

The balance between initial ($t_1$) and final ($t_2$) solution nitrate contents results from stock addition ($I_N$) at $t_1$ and plant uptake ($U$) between $t_1$ and $t_2$, which is thus calculated as:

$$U = V_{m,1} \times C_{m,1} - V_{m,2} \times C_{m,2} + I_N \times C_{st}$$  

Eqn 8

Thus, considering $n$ plants feeding on the same solution, their mean uptake rate ($F$) is:

$$F = \frac{U}{n \times (t_2 - t_1)}$$  

Eqn 9
The uncertainty on $U$ ($\Delta U$) can be computed from the uncertainties $\Delta V_m$, $\Delta C_m$ and $\Delta I_N$ on $V_m$, $C_m$ and $I_N$, respectively:

$$\Delta U = V_{m,1} \times \Delta C_{m,1} + V_{m,2} \times \Delta C_{m,2} + \Delta V_m \times C_m + \Delta I_N \times C_{st}$$  \hspace{1cm} \text{Eqn 10}

The formula omits the term $I_N \times \Delta C_{st}$ since a same stock solution provides for many injections, implying that $\Delta C_{st} = 0$. Because volume and $[\text{NO}_3]$ are regulated, they remain close to their respective set points $V_{sp}$ and $C_{sp}$, giving:

$$\Delta U \approx 2 \times (V_{sp} \times \Delta C_{sp} + \Delta V_m \times C_{sp}) + \Delta I_N \times C_{st}$$  \hspace{1cm} \text{Eqn 11}

The last term of this expression can also be written:

$$\Delta I_N \times C_{st} = \frac{\Delta I_N}{I_N} \times (I_N \times C_{st})$$  \hspace{1cm} \text{Eqn 12}

On average, the product $I_N \times C_{st}$ is the amount of nitrate restored to the solution that compensates for plant uptake, implying that:

$$\Delta I_N \times C_{st} \approx \frac{\Delta I_N}{I_N} \times U$$  \hspace{1cm} \text{Eqn 13}

By choosing the appropriate syringe volume, the relative uncertainty $\Delta I_N / I_N$ can be maintained $\leq 0.3\%$, making this term negligible, and thus:

$$\Delta U \approx 2 \times (V_{sp} \times \Delta C_{sp} + \Delta V_m \times C_{sp})$$  \hspace{1cm} \text{Eqn 14}

The uncertainty $\Delta F$ on $F$ follows from Eqns 9 and 14:

$$\Delta F \approx 2 \times \frac{V_{sp} \times \Delta C_{sp} + \Delta V_m \times C_{sp}}{n \times (t_2 - t_1)}$$  \hspace{1cm} \text{Eqn 15}

**Maintenance**

The setup has been devised in order to limit the frequency of maintenance operations. Routinely, the UV cuvettes (12, Fig. 1b) are cleaned only on a weekly basis (Hellmanex® II solution, Hellma). Indeed, at each pH correction, the acid injection upstream of the cuvettes (16, Fig. 1b) causes a self-cleaning operation. Every fortnight, the filters (10, Fig. 1b) are replaced, while the charcoal filters (8, Fig. 1a) may last for several months. The cleaning of level detectors (24, Fig. 1a) is made monthly. The
spectrophotometer Deuterium lamp (ref 71307445, SAFAS) lifetime is > 6000 hrs. 
Depending on the analytical frequency, the software performs extinction/ignition cycles 
in order to save its lifetime resulting, in practice, in more than one full year of use.

RESULTS

UV spectra and usage

Specificity

Usual nutrient solutions (for an example, Adamowicz and Le Bot, 2008) contain several 
chemicals that absorb light in the UV domain, namely nitrate, Fe-EDTA, molybdate and 
chloride (Fig. 2). Nitrate UV-print peaks at 201 nm and becomes negligible above 240 
nm. Fe-EDTA has two peaks, one at 208 nm interferes with NO₃ and a second at 257 
nm, higher than the former, rendering this situation favourable to spectral deconvolution 
of NO₃ and Fe-EDTA mixtures.

Molybdate shows also a peak at 208 nm followed by a smaller one around 227 nm. Its 
spectrum, however, is subject to pH changes (not shown) in particular below pH 5. 
Chloride UV absorption increases below 230 nm and its interference with nitrate 
increases sharply below 201 nm (for instance, 1 mM Cl absorbs like 3 and 19 µM NO₃ 
at 201 and 195 nm, respectively). In Fig. 2, the molybdate (0.28 µM) and Cl⁻ (23 µM as 
Mn counter-ion) concentrations correspond to those brought by a typical micronutrient 
mixture (Table 2). Under these conditions, molybdate and Cl⁻ interferences can be 
neglected. Indeed, restricting deconvolution to NO₃ and Fe-EDTA at the five selected 
wavelengths (Table 1), the analysis of the micronutrient mixture of Table 2 yields the 
right Fe-EDTA concentration and an erroneous 0.35 µM NO₃, which is the method bias.

Fig. 3a shows a monitoring of Cₐ made on 4 nutrient solutions feeding peach trees 
(Prunus persica) in an open field hydroponic orchard. The data represent a subset 
period (163-184 days after planting, DAP) during which tanks 1 and 2 contained high 
[NO₃] (Cₛₚ = 1 mM, L = 0.05 cm) while 3 and 4 contained low [NO₃] (Cₛₚ = 0.025 mM,
1 $L = 1.0 \text{ cm})$. It shows that $C_m$ was always lower than $C_{sp}$, which is normal as will be
2 explained below (see Fig. 4). Fig. 3b shows that for a given light path $L$, the $E$ values
3 are very similar. Conversely, they vary widely between cells of different light paths.
4 From around 174 DAP onwards, $E$ values drifted positively only in the low NO$_3$
5 treatments. We reckoned this was caused by a biological reaction (e.g. root exudation),
6 not an instrumental one. This was confirmed later because the replacement of charcoal
7 filters restored the original $E$ values (not shown).

Reliability
8 Fig. 3b shows the occurrence of an outlying $E$ value (tank 3, 183 DAP) that peaks above
9 the usual noise and reveals a dubious analysis. Indeed, we determined empirically that
10 such transient $E$ peaks occur when air bubbles flow through the spectrophotometer cells.
11 Outlier detection is easier on the transformed errors (Fig. 3c), because $E_t$ does not
12 depend on $L$ and is drift safe. The distribution of $E_t$ studied from a set of nearly 18000
13 analyses (not shown), is not gaussian ($p < 2 \times 10^{-16}$, d’Agostino test) but skewed
14 (skewness = 98) and leptokurtic (excess kurtosis = 9991) and thus is not suited for
15 ordinary statistics. However, the practical outcome is a rule of thumb associating
16 dubious analyses to outlying $E_t$. When $E_t > 10$, computation of $F$ values appear
17 doubtful, and when $E_t > 20$ nitrate regulation is also seriously impaired. Indeed, in this
18 case $C_m <<$ actual [NO$_3$] and the system attempts to inject a large volume of stock NO$_3$
19 unless it is blocked on the basis of $E_t$ by the software. Nevertheless, $E_t$ outliers proved
20 rare, their frequency in this dataset being 2 \(\%\) ($E_t > 10$) and 0.3 \(\%\) ($E_t > 20$).

Regulation process
21 In Fig. 4a are plotted the changes in [NO$_3$] at the time of analysis ($C_m$) and the
22 discontinuous injections of stock solution $I_N$ calculated from Eqn 1. It shows clearly that
23 the setup reacted to a drop in $C_m$ by an increased injection volume at time 7. But in any
24 case, due to plant uptake, $C_m$ is always lower than $C_{sp}$, the discrepancy being
proportional to the time interval between analyses. In the experiment involved in Fig. 4a, it was adapted to limit the discrepancy around 5%.

In diagrams, it is usual to connect data points to indicate that changes in concentration emerge from the continuous process of plant uptake, but this is misleading. Indeed, in Fig. 4b are plotted the intermediate $C_m$ changes every 20 minutes following injection at time 7. Although Fig. 4a suggests an increasing [NO$_3$] between times 7 and 10, Fig. 4b shows that in reality, the injection led to an initial steep increase that almost restored $C_{sp}$, followed by a gradual decrease by plant uptake until $C_m$ at time 10. It is thus clear that the analytical data point interpolation in Fig. 4a underestimates the mean [NO$_3$], which is intermediate between $C_{sp}$ and $C_m$.

**Precision**

**Nitrate concentration**

In order to test the setup precision in real growth conditions, [NO$_3$] was measured every 2 hours over a period of 14 hours following the final harvest of 92 tomato plants grown 23 days in a growth room at $C_{sp} = 3$ mM. During this period, all regulations ([NO$_3$], pH and volume) were cancelled and lights were off. Afterwards, the volume regulation was restored. Then, the amount of water added during a period of 48 hours represented the water losses due to evaporation. This data was used to estimate the mean hourly evaporation rate, which was 7.8 mL h$^{-1}$. During the first period, Fig. 5 shows that $C_m$ raised linearly along time from 2.9987 to 3.0084 mM, which is fully explained by the sole volume decrease resulting from a theoretical evaporation rate of 7.6 mL h$^{-1}$. From the good match between the theoretical and measured evaporation rates, we can infer that $C_m$ changes were due to evaporation and that the influence of microflora activity was negligible. Thus, the residual standard deviation between $C_m$ and the mean linear regression with time is a good estimate of the analytical precision. In this particular case, this figure was 0.00076 mM, i.e. 0.025% of $C_m$. 

Nitrate uptake

The above data were used to infer the precision on uptake rate measurements ($\Delta F$, Eqn 15). It increases 1) with increasing time intervals between successive analyses (Fig. 6a-d), 2) with increasing precision on volume ($\Delta V_{sp}$, Fig. 6a,c), 3) with decreasing total solution volume ($V_{sp}$, Fig. 6b,d) and 4) with decreasing [NO$_3$] (compare scales of Fig. 6a-b at $C_{sp} = 3$ mM to those of Fig. 6c-d at $C_{sp} = 0.1$ mM). For instance, in another experiment (not shown) we measured that 23 day old tomato plants absorbed on average $F = 90$ µM NO$_3$ h$^{-1}$. In order to measure this rate with 10% precision (i.e. $\Delta F = 9$), Fig. 6 shows that depletion must be measured over 1 h at $C_{sp} = 3$ mM and less than 5 minutes at $C_{sp} = 0.1$ mM. In the long term, the accuracy of nitrate uptake measurements (i.e. cumulative $U$) depends only on the accuracies of stock solution concentration ($C_{st}$) and of injected volumes ($I_N$). It is important to check the absence of significant biases by comparing $U$ to the intake inferred from plant growth and N content. Such a validation has been made on peach trees grown outdoors in a hydroponic orchard on high and low N nutritions (Jordan et al., 2011). After 65 days, $U$ differed only by 5% from the final tree N content measured from a destructive harvest.

There is an alternative method to improve $F$ calculation via a better knowledge of $V_m$ obtained through an appropriate modelling of water uptake. For instance, Fig. 7 shows nitrate uptake rates measured in unfavourable conditions, i.e. tomato plantlets grown at high [NO$_3$] in a growth room. Young plants having low water consumption, Fig. 7b shows that water additions ($V_a$) appear erratic in volume and frequency, particularly before 16 days after sowing (DAS). Each analysis did not provoke a water addition, but when it occurred it compensated for water uptake since the preceding water addition, not since the last analysis. In these conditions, Eqn 2 gives a biased $V_m$ because it assumes that following each analysis, $V_m$ is reset to $V_{sp}$. Accordingly, the computed $F$ (Fig. 7a, open symbols) followed jagged dynamics. Conversely, aging plantlets (16-17
DAS) took up more water forcing frequent water additions (Fig. 7b), which smoothed out the diel $F$ pattern (Fig. 7a, open symbols).

In order to free $V_m$ from such bias, $V_a$ can be distributed over larger time periods. For instance, in growth room conditions, Triboï-Blondel (1979) showed that water uptake rates remain fairly constant and high during the light period and low during the night. Thus, it is possible to infer \textit{a posteriori} the water uptake rate ($F_w$) from its mean value inside each period starting at $t_s$ and ending at $t_e$:

$$F_w = \frac{\sum_{t_s}^{t_e} (V_{a,t} + I_{N,t} + I_{H,t})}{t_e - t_s}$$

Eqn 16

Then, at any time, $V_{m,t}$ can be computed from the preceding volume instead of $V_{sp}$, and from water and stock solution additions:

$$V_{m,t_2} = V_{m,t_1} + V_{a,t_1} + I_{N,t_1} + I_{H,t_1} - (t_2 - t_1) \times F_w$$

Eqn 17

This last equation smoothed out the diel $F$ pattern (Fig. 7a, closed symbols), in particular before 16 DAS when water additions were erratic.

\textit{Other minerals}

This setup regulates only [NO$_3$], pH and volume, implying that the concentration of other ions may drift to some extent. For instance, in a peach orchard experiment, [P] required 5 restorations in a full 1-year growth. Opposite to phosphate, sulphate concentration increases with time (not shown) because the sulphuric acid added to regulate pH exceeds plant S needs. In all cases, K, Ca and Mg concentrations drifted upwards (not shown) avoiding any risk of growth limitation from these ions. The practical outcome of ion loads in solution is increased salinity diagnosed from conductivity measurements. Salinity is eventually remediable by total solution renewal.

Concerning micronutrients, manual Fe-EDTA additions are made according to the automatic analyses. Other micronutrients are not controlled, but we never observed any deficiency symptoms. The high initial micronutrient contents in solution, the initial
plant stocks and contaminations (air, water and fertilizers) are reasons explaining that deficiencies are difficult to obtain in hydroponics. This is reinforced by the strategy of tight pH control at acidic values.

DISCUSSION

Nitrate determination by the UV method appears adapted to nutrient solutions feeding plants. It requires the knowledge of spectral characteristics of solution components. Given the composition of our nutrient solutions (Fig. 2 and Table 2), only Fe-EDTA should be accounted for. For experiments using Cl\textsuperscript{−} in the millimolar range to increase salinity or as a macronutrient counter-ion, the method can be adapted by increasing the number of measured wavelengths and by accounting for Cl\textsuperscript{−} spectral characteristics. Nevertheless, plant roots and the associated microflora excrete optically active compounds (mucilage, acids, phenolics etc.) with unpredictable UV-prints that contribute to the absorbance error $E$ (Eqn 6). The Totomatix setup uses $E$ monitoring (Fig. 3b) to detect drifts due to their accumulation. They serve as a criterion for maintenance: quartz cell cleansing, filter replacement or full nutrient solution renewal. The setup uses also $E_i$ monitoring (Fig. 3c) to detect accidental false analyses that give erroneous $F$ measurements and may disturb $[\text{NO}_3]$ regulation. Indeed, when $C_m > \text{actual}[\text{NO}_3]$, $U$ and $F$ (Eqns 8 and 9) result underestimated and the calculated volume $I_N$ of stock $\text{NO}_3$ (Eqn 1) is inadequate to restore $C_{sp}$, which may lead to a drift towards low concentrations. Considering that the following analysis is correct, the next $U$ and $F$ values result overestimated, thus compensating for the previous errors, while the new $I_N$ value should restore $C_{sp}$. Thereby, the consequences of transient overestimates of actual $[\text{NO}_3]$ are transient $U$ and $F$ errors and transient regulation drifts, without any long-term effects. When $C_m < \text{actual}[\text{NO}_3]$, symmetrical transient effects occur on $U$ and $F$, but not necessarily on $[\text{NO}_3]$ regulation. In this case indeed, $I_N$ is overestimated and $[\text{NO}_3]$ overshoots $C_{sp}$. In this case, only plant uptake is able to restore $C_{sp}$, which may last for
long depending on $[\text{NO}_3]$ excess and on uptake rate. Thus, $[\text{NO}_3]$ underestimates put the regulation at risk, making the diagnosis of $E_i$ outliers valuable to improve the system reliability. In practice, this reliability combined with few maintenance operations (weekly basis) proved adapted to very long experiments such as an annual survey of peach N nutrition (partly published in Jordan et al., 2011).

Reliability and maintenance depend on the setup sophistication. Complex systems encompass the analysis of most major nutrients (André et al., 1979; Alloush and Sanders, 1990; Lorenzen et al., 1998), allowing uptake studies of individual ions and their interactions. Such systems replenish individual ions and thus they regulate wholly the solution composition. Thus, these setups allow theoretically long-term growth at set concentration in closed loops. In practice, however, their use has been reported in experiments lasting a couple of months at most, suggesting limited reliability and/or excessive maintenance operations in the long-term, such as the daily cleaning of selective electrodes (Glass et al., 1987) or the supervision of a flow injection analyser every 16 h (Lorenzen et al., 1998). Simpler systems are dedicated to the study and regulation of few ions (Goyal and Huffaker, 1986; Blom-Zandstra and Lupijn, 1987; Glass et al., 1987; Rockel, 1997) implying that other minerals are left to drift. In this case, the duration of experiments is limited unless frequent solution renewal is performed (for instance, twice a week for Blom-Zandstra and Lupijn, 1987). In the system described in this paper, we observed that phosphate is the only macronutrient whose concentration declines significantly over time. Since phosphate is the main pH buffer, it is advisable to keep $[\text{P}] > 0.5 \text{ mM}$ in order to facilitate pH regulation. Thus, it is a good practice to check periodically $[\text{P}]$ through manual sampling and analysis, followed by correction if necessary. In this context, the risk of growth limitation is low because $[\text{P}]$ remains well over the Michaelis constant for uptake (around 1 $\mu\text{M}$ according to Leitner et al., 2010). For all other major ions, concentration always
increased because of the chosen regulation methodologies. Hence, pH control by H₂SO₄
provokes sulphate buildup. SO₄ accumulation is common in recycled NFT but it does
not harm tomato growth and photosynthesis until at least 21 mM SO₄ (Lopez et al.,
1996). From the ionic charge viewpoint, nitrate-fed plants absorb more nitrate than
cations (Mengel and Kirkby, 1987) implying that NO₃ stock additions with
accompanying counterions, overcompensate for cationic charge uptake. Thus, if the
stock mixes K, Ca and Mg in a ratio close to plant uptake, their individual concentration
necessarily increases with time. Our NO₃ stock matches the mere cation composition of
tomato plantlets. It is thus optimal to experiment with these young plants, but it turned
out to be also suitable for fruiting tomatoes, Chinese cabbage (Brassica rapa) and peach
trees. According to our experience, concentration drifts are acceptable as long as there is
no salinity buildup in the rooting medium and solution renewals are rarely necessary.
Indeed, in the one year peach tree trial already cited, this occurred only once.

There is an antagonism between the quality of [NO₃] regulation and the precision on F
measurement. For instance, we observed that regulation was far tighter at high than low
Cₛₚ (Fig. 3a) while the calculation predicted the opposite for ΔF (Fig. 6). Improving
regulation at low Cₛₚ is easily achieved by increasing the analysis frequency and/or the
solution volume (Parker and Norvell, 1999) while improving ΔF remains a challenge at
high Cₛₚ. Indeed, the important outcome of comparing Figs 6a-d establishes that at high
Cₛₚ, ΔF is much more sensitive to volume precision ΔVₛₚ (Fig. 6a) than to Vₛₚ itself (Fig.
6b). Conversely, at low Cₛₚ ΔF is much more sensitive to volume (Fig. 6d) than to its
precision (Fig. 6c). For instance, at 6 min intervals, a threefold reduction in ΔVₛₚ
decreases ΔF by 2.7x and 1.3x at Cₛₚ = 3 and 0.1 mM, respectively, while in the same
conditions a threefold reduction in Vₛₚ decreases ΔF by 1.2x and 2.5x. ΔVₛₚ is set by the
design of solution tanks and by the level sensor characteristics. More precise sensors,
such as balances, have been used to infer volumes in systems holding few plants
but they seem unworkable in our NFT setups and in absence of real technological breakthrough, there is little hope for significant $\Delta V_{sp}$ reduction. This viewpoint is supported by the fact that most published $F$ data deal with $[NO_3] < 1$ mM. For instance, the Hurley system regulates $[NO_3]$ both in the micro- and millimolar ranges, but $F$ data were published only under 1 mM $[NO_3]$ (Clement et al., 1974; Clement et al., 1978b), while only cumulative $U$ was given above 1 mM (Clement et al., 1978a).

Horticultural productions are conducted at high $[NO_3]$ (Le Bot and Adamowicz, 2005) rendering necessary to assess $F$ in the domain of the low affinity nitrate transporters (Cárdenas-Navarro et al., 1999; Forde, 2000; Tsay et al., 2007). The setup presented in this paper is capable of such measurement provided that the plant water uptake rate provokes frequent water additions such as observed in Fig. 7, 16-17 DAS or that another model is applied to infer the solution volume (Fig. 7a, closed vs open symbols). Although both calculations yield identical long-term NO₃ uptakes, the short-term likeliness of this model is assessed by the similarity of both curves 16-17 DAS. It shows that $V_m$ calculation was little modified by the model when water additions became frequent. Models assuming constant evapotranspiration rates (e.g. Eqns 16-17) are straightforward for growth room conditions but their transposition to natural conditions relies on the existence of sound evapotranspiration models reacting to ambient climate, in order to distribute the measured daily water uptakes into hourly intervals.

Alternatively, the use of assimilation chambers or in-line transpiration devices could provide an objective and continuous means to infer instantaneous water uptakes.

We reckon that the system is suited to measure, especially in long-term experiments, uptake rates at unusually high $C_{sp}$ (3 mM) with reasonable time resolution (1h) and unusually high time resolution (5 minutes) at low $C_{sp}$ (0.1 mM) implying that it can bring invaluable insight to link short- and long-term processes (Adamowicz and Le Bot, 2005).
1 It is not attached to a specific growth system. It controlled a hydroponic orchard
2 in the open field (Jordan et al., 2011), young tomato plants in growth rooms (Huanost)
3 Magaña et al., 2009) and recently leafy and fruiting vegetables and young trees in the
4 greenhouse. Apart from classical experiments at fixed $C_{sp}$, it enables novel experiments
5 where N uptake rates are constrained relative to control by the means of adjusting $C_{sp}$
6 daily (Jordan et al., 2011). The latter share the logics of the “relative addition rate”
7 approach (Ingestad and Ågren, 1988, 1992) but extend to unpredictable environments
8 and non-exponential growth phases.
9 Finally, the setup provides a workable means to grow plants in closed nutrient systems
10 without drainage, which represents a sound environmentally friendly practice. Indeed, it
11 allows a high efficient use of N fertilizer, > 99% in practice.

ACKNOWLEDGMENTS

12 We wish to thank C Vigne for building the setup and P Orlando for writing the
13 software.
LITERATURE CITED


TABLES

1. **Table 1**: Stray light fraction and specific absorbances through 1 cm light path of 1 mmol NO$_3$ L$^{-1}$ and 1 mg Fe L$^{-1}$ as Fe-EDTA, as measured by a SAFAS UV=mc2 spectrophotometer with 2 nm spectral resolution. The parameters were determined by regression of absorbances against ranges of [NO$_3$] (nitrate standard 19811, Merck KGaA, Darmstadt, Germany) and [Fe-EDTA] (reference 03650, Fluka Chemie AG, Buchs, Switzerland).

<table>
<thead>
<tr>
<th>Wavelength ($\lambda$)</th>
<th>201 nm</th>
<th>206 nm</th>
<th>214 nm</th>
<th>257 nm</th>
<th>280 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$<em>3$ ($\varepsilon</em>{N,\lambda}$)</td>
<td>9.56240</td>
<td>9.21870</td>
<td>6.63190</td>
<td>0.00992</td>
<td>0.01068</td>
</tr>
<tr>
<td>Fe-EDTA ($\varepsilon_{Fe,\lambda}$)</td>
<td>0.12297</td>
<td>0.12429</td>
<td>0.12141</td>
<td>0.15042</td>
<td>0.11913</td>
</tr>
<tr>
<td>Stray light ($\alpha_{\lambda}$)</td>
<td>0.00169</td>
<td>0.00113</td>
<td>0.00047</td>
<td>0.00000</td>
<td>0.00000</td>
</tr>
</tbody>
</table>

2. **Table 2**: Typical micronutrient composition of nutrient solutions.

<table>
<thead>
<tr>
<th>micronutrient</th>
<th>Mo</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
<th>B</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>concentration (µM)</td>
<td>0.28</td>
<td>11.6</td>
<td>3.2</td>
<td>0.47</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>chemical</td>
<td>(NH$_4$)$_6$(Mo$<em>7$O$</em>{24}$)</td>
<td>MnCl$_2$</td>
<td>ZnSO$_4$</td>
<td>CuSO$_4$</td>
<td>H$_3$BO$_3$</td>
<td>Fe-EDTA</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Overview of the culture system showing (a) a solution tank with peripheral equipment and (b) the “Totomatix” analytical setup.

Key: arrows = flow direction; 1 = solution tank; 2 = cooling coil; 3 = pump; 4a = injection line to plants; 4b = drainage from plants; 5 = manual flow valve; 6a-b = bypass to analytical setup; 7 = manual flow valve; 8 = charcoal filter; 9 = flow-through cell with pH probe; 10 = filter; 11 = micro-pump; 12 = spectrophotometer with flow-through cuvettes; 13 = acid stock solution; 14 & 18 = motorized syringe drives; 15 & 19 = 8-way motorized selection valves; 16 & 20 = manual 3-way valves; 17 = nitrate stock solution; 21 = deionized water inlet; 22 = on/off solenoid valve; 23 = flowmeter; 24 = siphon with level sensor.

Figure 2. UV spectra of optically active nutrients (190 - 300 nm; 2 nm spectral resolution; 1 cm optical path). Left scale: NO$_3$ (25 µM, thick solid line), Fe-EDTA (29 µM, thin solid line); right scale: molybdate (0.28 µM, pH = 5, thick dashed line), Cl (23 µM, thin dashed line). Vertical dotted lines denote the wavelengths selected for spectral deconvolution.

Figure 3. Time course changes of (a) nitrate concentration $C_m$, (b) mean spectral error $E$ and (c) transformed error $E_t$ of 4 nutrient solutions. Notice the vertical axis breaks and uneven scales in (a) and (b). Conditions: four tanks of 500 L nutrient solution (pH = 5), each feeding 11 peach trees in a hydroponic orchard; nitrate analyses = 1 hour interval; nitrate set points = 1 mM (pink and blue) or 0.025 mM (red and black); optical paths = 1 mm (pink and blue) or 10 mm (red and black). Other major nutrients (mM): K = 3, Ca = 3.5, Mg = 1.5, H$_2$PO$_4$ = 1, SO$_4$ = 6 - NO$_3$ / 2. Micro nutrients as in Table 2.

Figure 4. Temporal changes in the measured nitrate concentration ($C_m$, in % of set point $C_{sp}$, closed symbols, left axis) and in the volume of injected stock nitrate ($I_N$, mL,
vertical bars, right axis). (a) Sequence of events recorded at 3 h time intervals over a 16
h period, (b) the same $C_m$ data plotted together with intermediate analyses (open
symbols) made in absence of regulation at 20 min intervals over the period 7-10 hrs.
Conditions: 27 plants feeding on 80 L of full solution ($C_{sp} = 0.5$ mM NO$_3$) in a
glasshouse, solution temperature = 23 °C, 32 days after sowing.

Figure 5. Drift in the measured nitrate concentration after the final harvest of 92 tomato
plants grown at $C_{sp} = 3.0$ mM and $V_{sp} = 33$ L. Conditions: growth room with lights off,
nitrate, pH and volume regulations off, air and solution temperature = 20 °C. Regression
line: [NO$_3$] = 2.99987 + 0.00069 x time ($R^2 = 0.952$).

Figure 6. Effect of the time interval between analyses (minutes) on the precision of
plant nitrate uptake rate ($\Delta F$, µmol h$^{-1}$) calculated from Eqn 15 with $n = 92$ plants, $C_{sp} =$
3 mM (a) and (b), and $C_{sp} = 0.1$ mM (c) and (d). In (a) and (c), effect of the volume
precision $\Delta V_{sp} = 0.04$ L (dotted line), 0.08 L (thin solid line) and 0.12 L (thick solid
line), with $V_{sp} = 66$ L. In (b) and (d), effect of total volume $V_{sp} = 33$ L (dotted line), 66 L
(thin solid line) and 99 L (thick solid line), with $\Delta V_{sp} = 0.08$ L.

Figure 7. Temporal variation of (a) individual plant nitrate uptake rate $F$ and (b) water
additions per tank. Conditions: growth room with 12 h photoperiod, 370 µmol m$^{-2}$ s$^{-1}$
PPFD during day, air and solution temperature = 20 °C, $n = 92$ plants, $V_{sp} = 30$ L, $C_{sp} =$
3.0 mM. Other major nutrients (mM): K = 3, Ca = 3.5, Mg = 1.5, H$_2$PO$_4$ = 1, SO$_4$ = 4.5.
Micro nutrients as in Table 2. In (a), $F$ was calculated either from Eqns 2 and 9 (open
symbols, dashed line) or from Eqns 9 and 17 (closed symbols, solid line). The shaded
areas denote the night periods.
**Figure 1**: Plot of nitrate concentration ($C_m$) in the nutrient solution over time for the diurnal and diel experiments. The graph shows a linear increase with time, indicating a consistent nitrate supply strategy.