# Soilless USDA-organic cultivation of tomato with 'Natural nitrogen'

A comparison study between 'Natural nitrogen' and organic nitrogen

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Rapport WPR-1089

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**Referaat**
In een USDA-organic teelt van tomaat op substraat wordt gebruik gemaakt van organische stikstof, die door micro-organismen rond de wortels moet worden omgezet in opneembaar stikstof voor het gewas. VitalFluid heeft een techniek ontwikkeld die op een natuurlijke manier in een reactor stikstofmeststoffen kan produceren met alleen lucht, water en elektriciteit, hierna genoemd "Natuurlijk stikstof" ($HNO_3$). In een teeltproef is onderzocht of deze meststof is in te passen in een USDA-organic bemestingsschema, en is een vergelijking gemaakt tussen een organic teelt met "Natuurlijk stikstof" en een standaard organic teelt (referentie). De proef heeft aangetoond dat "Natuurlijk stikstof" is in te passen in een voedingsoplossing en voordelen biedt ten opzichte van organisch gebonden stikstof. In de referentie kwam de mineralisatie van de organisch geboden stikstof moeizaam op gang, waardoor $NaNO_3$ ingezet moest worden om voldoende stikstof beschikbaar te hebben voor het gewas. Dit maakte recirculatie in de referentie onmogelijk (oplopend Na, daarmee minder K beschikbaar), terwijl in de behandeling met "Natuurlijk stikstof" al het drainwater is hergebruikt. Productkwaliteit en productiviteit van het gewas bleven in de behandeling met Natuurlijk Stikstof goed op peil, maar kon door de moeilijke start in de referentie niet goed worden vergeleken met deze referentiebehandeling. Er is ook aangetoond dat "Natuurlijk stikstof" als bron voor stikstof in organic teelt een emissieloze teelt mogelijk maakt en daardoor de Resource Use Efficiency in deze teeltmethode kan verbeteren.

**Abstract**
In a USDA-organic soilless cultivation of tomato organic nitrogen is used as N fertiliser, which needs to be converted to crop available nitrogen by microorganisms around the plant roots. VitalFluid developed a technology that produces nitrogen fertilisers in a natural way in a reactor, using only air, water and electricity ('Natural nitrogen'; $HNO_3$). Implementation of this nutrient source in a USDA organic soilless nutrient solution is investigated in a cultivation trial. A comparison is made between this 'natural nitrogen' nutrient solution and a standard USDA organic nutrient solution (reference). The trial has shown that 'Natural nitrogen' can be implemented in a nutrient solution and has advantages over organic nitrogen sources. In the reference treatment, the microbial conversion of organic N had a slow start, so that $NaNO_3$ needed to be applied to have enough readily available nitrogen for the crop to grow. This made it impossible to recirculate drain water in the reference treatment due to increased sodium levels (less K available), whereas the treatment with 'Natural nitrogen' could recirculate all drain water. Product quality and productivity of the crop were good in the 'Natural nitrogen' treatment, but due to the difficult start of the reference could not be compared to this reference treatment. It is also shown that 'Natural nitrogen' as source of N in USDA organic cultivation opens the way to adopting a closed-looped irrigation system. The technology can therefore enlarge the resource use efficiency (RUE) of such a system.

## Rapportgegevens

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## Disclaimer



## Adresgegevens

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# Summary

VitalFluid B.V developed a technology that produces plasma activated water (PAW) using air, water and electricity. This technique leads to the production of dissolved N in the plasma activated water (so called 'Natural nitrogen'). 'Natural nitrogen' is a liquid solution, containing dissolved nitrate ($NO_3^-$) in the form of nitric acid ($HNO_3$). Nitrate is an essential source of nitrogen for plants. The availability of $NO_3$ in organic soilless cultivation is often low due to a unpredictable mineralisation of organic nitrogen by rootzone microorganisms. In this trial a comparison is made between a standard USDA organic tomato (cv. Roterno) soilless cultivation (reference) and a treatment in which 'Natural nitrogen' was the main source of nitrogen. The crop was planted in a 120 $m^2$ greenhouse compartment on a peat substrate. The trial lasted from July 2021 until December 2021.

The 'Natural nitrogen' treatment maintained a good level of K, Ca, Mg and Na in the root zone. This treatment used a closed-looped irrigation system for the whole duration of the cultivation. $NO_3$ availability was never below critical levels, the input from 'Natural nitrogen' was 10 mmol/L which was determined by the required Ca input (5 mmol/L). The biggest challenge was to manage the micro-biological activity that was initiated by some of the organic fertiliser inputs in the B-stock solution. This caused strong pH rise and some changes in the nutrient composition.

The reference treatment showed an insufficient nitrification process in the rootzone of the crop. The most probable main reason for this is the inherently slow nitrification process in relation to the rapid increasing demand of nitrate by the crop. In addition to that, a high initial water content (despite 30% drain) in the growing media caused a lack of oxygen, which slowed down the nitrification process and could even have contributed to denitrification (causing loss of N by $N_2O$).

The lack of sufficient $NO_3$ reduced crop growth and yield in the reference treatment. To alleviate the shortage of $NO_3$, $NaNO_3$ was applied to the reference treatment, as this source is tolerated in the USDA approach, in form of mined Chilean Nitrate. As a consequence of drain water recirculation, Na accumulated rapidly at the expense of the other cations (K, Ca, Mg) in the nutrient solution. Drain water recirculation, Na accumulation and rising fruit load contributed to a rapid depletion of K between week 4 and 8 after transplanting. Due to K depletion, recirculation was reduced and additional $K_2SO_4$ was added.

The results showed a large difference in yield between treatments in favour of the 'Natural nitrogen' treatment. This was mainly due to the low nitrate availability in the reference due to a total lack of sufficient nitrification. The shortage of K during the first part of the cultivation might have also contributed to the low yield of the reference. Regardless the difficulties encountered in the reference, 'Natural nitrogen' treatment clearly showed an easier nutrients, pH and EC management. It is also shown that 'Natural nitrogen' as source of N in USDA organic cultivation opens the way to adopting a closed-looped irrigation system. The technology can therefore enlarge the resource use efficiency (RUE) of such a system.

# 1 Introduction

## 1.1 Background

In nature, most nitrogen is fixed by micro-organisms that live around the roots of plants. These microorganisms consume $N_2$ from air and transform it into ammonium ($NH_4$). Ammonium can be taken up by plants, but most of it is transformed into nitrate, in a so-called nitrification process. Nitrate is readily available for uptake by plants and is fixed in the biomass. Around 10% of the global nitrogen is fixed by lightning flashes, in addition to this microbial fixation.

In USDA organic cultivation, it is allowed to grow vegetables on substrate in soilless cultivation systems. There are restrictions on which fertilisers to use, mainly fertilisers with a complex organic composition are allowed. For nitrogen fertigation, organically fixed nitrogen is applied to the crop. In the rootzone of the plants this nitrogen is transformed by microorganisms to ammonium and nitrate for uptake by the crop. This process is continuous and requires the right circumstances for the microorganisms to perform this conversion (i.e. oxygen content, temperature). To react to an increased crop demand growers are allowed to use Chilean nitrate ($NaNO_3$). However, this brings in huge amounts of sodium that disrupt the possibility to recirculate drain water. All drain water (including nitrate) is discharged to the environment.

VitalFluid has designed a process that mimics lightning flashes in a reactor. As in nature, a lightning flash is produced by applying electricity to anodes. In the lightning flash the air is transformed from the gas phase to the plasma phase, thereby splitting $N_2$ and $O_2$ molecules into active radicals. These radicals are then lead through water, in which they finally dissolve as nitric acid ($HNO_3$, product name: 'Natural nitrogen'). In this form it can be used as a fertilizer, to apply directly available nitrate to the crop. The industrialized natural process is under consideration for certified application in USDA organic cultivation.

## 1.2 Goals and hypotheses

The demonstration project has two main goals:
- Implementation of 'Natural nitrogen' in the nutrient solution of a USDA organic cultivation of tomato.
- Evaluate the effect of the application of 'Natural nitrogen' as only source of nitrogen on crop productivity and product quality.

It is hypothesized that 'Natural nitrogen':
- opens opportunities for recirculation of drain water in USDA-organic cultivation systems.
- has a positive effect on crop productivity in USDA-organic cultivation, as it allows for application of directly available nitrate to the crop in times of rapid crop growth.
- increases the nitrogen use efficiency of cultivation systems.
- has no effect on product quality.

## 1.3 Approach

This trial compared a reference USDA organic soilless tomato cultivation (1) with a cultivation that used 'Natural nitrogen' as only source of $NO_3$, produced by VitalFluid (2). Both treatments use recirculation of drain water, as this can strongly improve the resource use efficiency of cultivation systems. The comparison study evaluated the yield, N-balance and the nutrient dynamic in the system for each treatment.

## 1.4 Organisation

VitalFluid B.V. commissioned the trial funded by the European Horizon 2020 SME-2 funding scheme, project Plan2fix (Grant agreement number 873862). For VitalFluid B.V., Mark van Boxtel (sales manager) and Erik Hertel (Commercial Director), were responsible for the input of VitalFluid. Rohit Chaudhary (R&D engineer) was responsible for the PAW production and consulting on HNO3 treatments.
Wageningen University & Research, Business Unit Greenhouse Horticulture was responsible for the implementation of the cultivation trial. Jim van Ruijven (scientific researcher in water treatment) was the project leader and responsible for decision making. Wim Voogt (scientific researcher in plant nutrition) was the scientific advisor on plant treatments and cultivation. Tommaso Barbagli (scientific researcher in plant nutrition) was responsible for calculation of the nutrient solutions, trial monitoring and results communication. Aat van Winkel (technical researcher in water and nutrition) was responsible for the execution of the trial.

# 2 Material and Methods

The trial was conducted by Wageningen University & Research, Business Unit Greenhouse Horticulture facilities at Violierenweg 1, Bleiswijk, the Netherlands. The trial lasted from July 2021 until December 2021.

## 2.1 Crop and management

One month old tomato (cv. Roterno) seedlings were transplanted at the beginning of July into BVB peat slabs. Up to this stage all the plants were fed by the nursery Plantise, with the same standard nutrient solution for tomato seedlings. The trial lasted from July 2021 until December 2021. The crop was managed according to a standard protocol: about 1.5 harvest events per week, fruits harvested as loose tomatoes; flower pruning to keep 5-7 tomatoes per truss; pruning of the last three leaves every 2 weeks; weekly stem twisting and bending, weekly pruning of lateral shoots.

## 2.2 Greenhouse layout and growth conditions

The trial was conducted in a 120 $m^2$ (net cultivation area) glasshouse Venlo-type compartment (12 m * 10 m) equipped with two independent irrigation compartments. The compartment hosted five double gutter rows and two side rows (Figure 1). Each row was 11 m long. Peat slabs with some perlite inside of 21 L were placed over the gutter resulting in 144 slabs in total (25 L/$m^2$). Each slab hosted two propagating cubes with one plant each, resulting in a plant density of 2.4 plants/$m^2$. A single stem per plant was adopted, resulting in an equal amount of plants and stems (2.4 stems/$m^2$). The average (24 h) temperature, $CO_2$ concentration and relative humidity during the experiment were respectively 18.5 °C, 520 ppm and 85%. Additional artificial light via HPS laps was used from September 15$^{th}$ to reach a sufficient daily radiation sum. The light intensity was 180 µmol/$m^2$/s. The irrigation was set to reach approximately 30% drainage. The drainage water of each treatment was collected separately with the possibility to recirculate into an individual mixing tank per treatment. Each treatment had an A and B stock tank connected to the respective mixing tank. The mixing tank could use drain water, fresh water and new stock solutions to reach a set EC. The pH correction in both treatments was operated by the mixing tank with HCl. Each mixing tank was connected to a daily supply tank where a daily stock of nutrient solution was stored. The nutrient solution was dispensed from the supply tanks to the greenhouse compartment. Drip irrigation with French capillaries was used. One dripper per plant was set, resulting in 2.4 drippers/$m^2$.


***Figure 1*** *Cultivation system with peat slabs, two tomato plants per slab. Double gutter configuration with one stem per plant scheme.*

## 2.3 Treatments

This trial compared a USDA organic tomato soilless cultivation (reference) with a cultivation that used $NO_3$ from plasma activated water ('Natural nitrogen') produced with VitalFluid's plasma equipment as main source of nitrogen, both applying recirculation of drain water. For both treatments the same standard nutrient solution was used as basis for the calculation of the fertiliser recipes. The reference treatment used organic sources of P, N, K, Ca and Fe, for which it was decided to use respectively Biota Phos 8% ($P_2O_5$), Biota Nitro (9.6% organic-N, 0.4% N-$NH^4$), Biota Kalium 15% ($K_2O$), Biota Calcium 8% (CaO), Biota Iron (5% Fe complexed by ligno-sulphonic acid). In addition, $K_2SO_4$ and $MgSO_4$ were applied, as these are mined fertilisers and also accepted within the USDA-organic regulation. The microelements were distributed as $MnSO_4$, Borax, $ZnSO_4$, $CuSO_4$, $NaMoO_4$. The 'Natural nitrogen' treatment used the same fertilisers, but substituted organic N and Ca with 'Natural nitrogen' ($HNO_3$) treated with lime ($CaCO_3$), for which the commercial product Dolokal (54% CaO, 5% MgO) was used. It resulted in a suspension with dissolved $Ca(NO_3)_2$ (Table 1).

The input of Mg and K (partly) was maximised up to the required $SO_4$ concentration. In addition for K, the organic source was used. For P, the organic source was used, which contained some organic-N (0.93 mol N/mol P). Except for this N-source and due to the mineral sources of Mg and K, the N in the 'Natural nitrogen' treatment could be supplied completely (90 %) from the $Ca(NO_3)_2$ source.

To realise the desired supply solution recipe, two independent mixing units were used and two separated A and B tanks per treatment were prepared as stock solution. In the reference treatment, organic Ca from Biota Calcium was stocked in tank A. Tank B stocked all the other fertilisers. Both A and B were 200 times concentrated. In the 'Natural nitrogen' treatment, tank A stocked a suspension of about 20 mmol/L of $Ca(NO_3)_2$. Tank B stocked all the rest of the fertilisers 4 times concentrated compared to the supply nutrient solution. The suspension of tank A was prepared in a third tank (Pre A) by mixing 'Natural nitrogen' (around 40 mmol/L $HNO_3$) and powdered Dolokal ($CaCO_3$) (Figure 2). 'Natural nitrogen' produced by VitalFluid's plasma equipment was stored into IBC tanks (1 $m^3$) with a consumption rate of approximately 1 IBC every two weeks. The Dolokal was added to the 'Natural nitrogen' as a suspension of concentration 1200 mmol/L. The preparation of the 'Natural nitrogen' solution with lime suspension was empirically determined and followed these steps: (I) Between 2% and 4% of the volume of 'Natural nitrogen' was added as Dolokal suspension in three steps, 2%, 1%, 1%. Between each step the pH was measured. The target was to reach a pH of 2.5. If pH 2.5 was reached before the next step, the addition of Dolokal suspension was stopped. (II) After a pH 2.5 was reached, the suspension was let to settle for at least 48 hours. (III) After at least 48 hours, a layer of sand precipitated on the bottom of the tank and a clear solution of $Ca(NO_3)_2$ was available on top of it with a pH around 4.5-6.5. (IV) After at least 48 hours, the clear solution was pumped to the A tank. In week 1 and 2 after transplanting, nitrifying microorganisms (Nite-Out II by Microbe-Lift) were added (1 L/60 $m^2$) in the supply tank of the reference treatment to facilitate the mineralisation of organic-nitrogen.

Table 1

*Nutrient recipes for reference and 'Natural nitrogen' treatment.*

| | Reference | $NH_4$ | K | Na | Ca | Mg | $NO_3$ | Cl | $SO_4$ | P | N-(organic) | N-total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| stock | mmol/L | 1.3 | 8.0 | 0.1 | 5.1 | 2.0 | 0.0 | 0.0 | 3.1 | 1.3 | 11.3 | 12.7 |
| A | Biota Calcium | | | | | | | | | | | |
| B | Biota kalium | | | | | | | | | | | |
| | Biota Phos | | | | | | | | | | | |
| | Biota Nutri | | | | | | | | | | | |
| | $K_2SO_4$ | | | | | | | | | | | |
| | $MgSO_4$ | | | | | | | | | | | |
| | Natural Nitrogen | $NH_4$ | K | Na | Ca | Mg | $NO_3$ | Cl | $SO_4$ | P | N-(organic) | N-total |
| stock | mmol/L | 1.2 | 8.0 | 0.0 | 5.1 | 2.0 | 10.3 | 0.0 | 3.0 | 1.3 | 1.2 | 12.6 |
| A | $Ca(NO_3)_2$ | | | | | | | | | | | |
| B | Biota kalium | | | | | | | | | | | |
| | Biota Phos | | | | | | | | | | | |
| | $K_2SO_4$ | | | | | | | | | | | |
| | $MgSO_4$ | | | | | | | | | | | |


***Figure 2*** *Layout of the irrigation system. From left to right: Reference treatment: (A1) A stock solution, (B1) B stock solution and (Mix 1) mixing unit; 'Natural nitrogen' treatment: (Mix 2) mixing unit, (B2) B stock solution, (A2) A stock solution, (Pre A2) mixing tank of $HNO_3$ + Dolokal.*

## 2.4 Experimental design and measurements

Each treatment occupied half greenhouse area (60 $m^2$). Every other pair of rows hosted one treatment for the full length of the rows. Six paths for crop management separated the rows. Each treatment had four blocks of plants in which crop parameters were measured. The replicate blocks were randomly distributed between rows (Figure 3). Each replicate comprised three peat slabs and therefore six plants (2.5 $m^2$). The number of fruits and the total weight was registered at each harvest event in every repetitive block. Old pruned leaves were collected and weighted for the two rows aside corridor 3 (Figure 3). At each pruning event, one subsample of fresh leaf material was collected and dried into an oven at 80 °C. The dry matter content was determined. At the end of the experiment, a pooled sample of dry material from each leaves pruning event was sent to the laboratory Groen Agro Control (GAC) to determine the nutrient content. Also a pooled fruit sample from two harvest events (October and November) was dried and the dry matter content as well as the nutrient content was determined. Weekly water analysis of supply solutions and drain water were sent to the laboratory Eurofins (EUR) to determine the nutrients concentration, pH and EC. Volumes of daily supply and drain water (L/$m^2$) were also measured in the greenhouse with graduated buckets. Substrate samples from the slabs were also collected during the first weeks of cultivation to monitor the nitrification process. Peat samples were sent to EUR to be analysed with 1:1.5 extraction method to determine the nutrient content. At the end of the experiment, one plant per replicate was harvested and the fresh weight (kg) was separately measured for green fruits, leaves and stem. Each organ was subsequently dried into an oven at 80 °C and the dry matter content was determined afterwards. The dry material from these organs was sent to EUR to determine the nutrient content. At the end of the experiment also peat samples were collected and send to EUR to determine nutrient concentration with 1:1.5 extraction method. To facilitate the conversion to absolute values, the water content (v/v %) of peat samples was measured in-situ by drying into an oven a known volume of the peat sample.

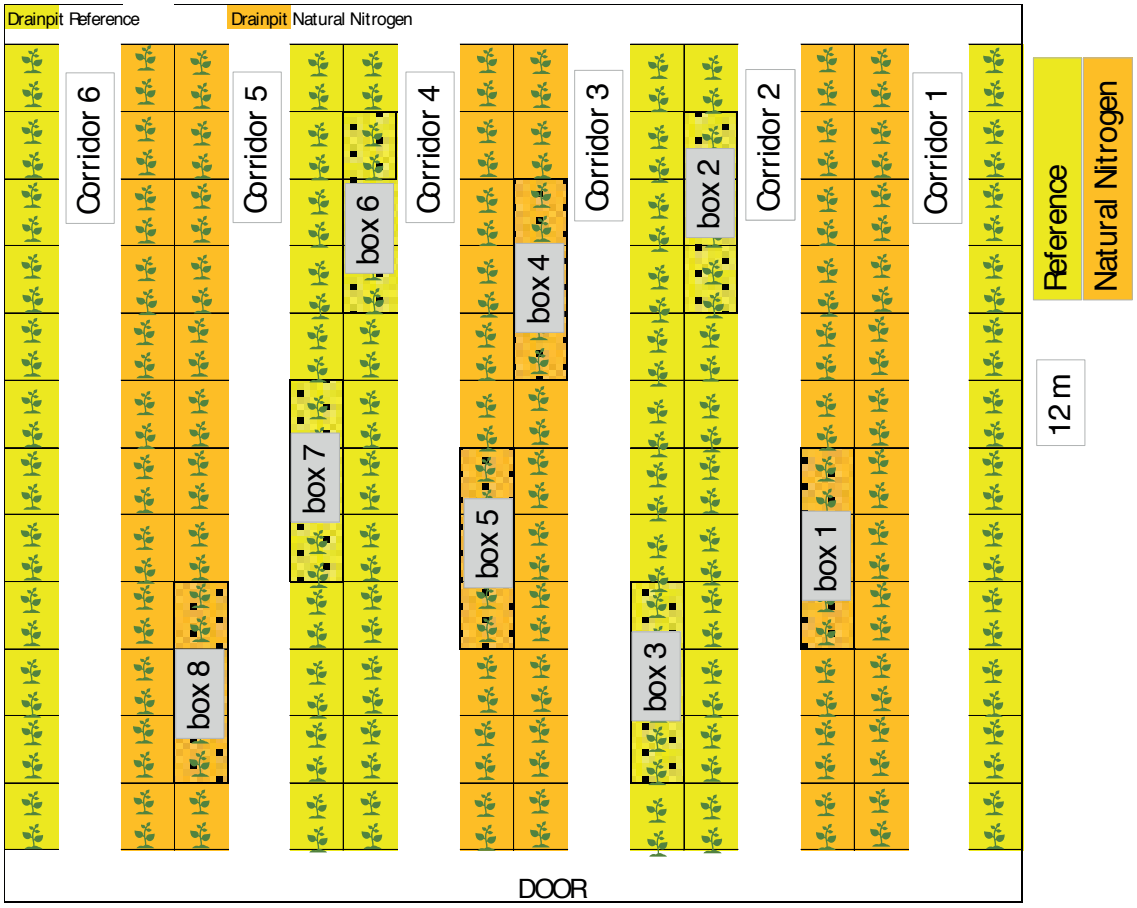


***Figure 3*** *Experimental layout. Five central double gutter rows plus two side rows. In yellow the reference USDA cultivation. In orange the 'Natural nitrogen' treatment. Four replicates per treatment, three peat slabs per replicate (6 plants/replicate).*

## 2.5 Evaluated parameters

An average cumulative yield per unit area (kg/$m^2$) and the average fruit size (g/fruit) were calculated with the harvesting observations from the replicates. With the measurements of the old leaves and the final observation on the fully grown plants (distinguishing between stem, leaves, green fruits), the average total plant fresh biomass (kg/$m^2$) was calculated. With the dry matter content of each organ and fruits, the average total dry biomass per unit area (kg dw/$m^2$) was calculated. With the nutrient concentration of each organ, the average total quantity of each nutrient per unit area (mmol/$m^2$) was calculated. With the daily measurement on supply and drain water, the daily water uptake was calculated (L/$m^2$). The nitrogen balance was calculated with the dry matter analysis. The absorbed fraction (mmol/$m^2$) was calculated multiplying the dry biomass (kg dw/$m^2$) for the nutrient concentration (mmol/kg dw) discriminating between yield (harvested fruits), stem, old leaves (pruned leaves), leaves (still on the plants at the end of the trial), green fruits (still on the plant at the end of the trial). A residual that comprises the volume left in the substrate (L/$m^2$) was also measured (mmol/L). An estimation of the total input in the system (mmol/$m^2$), was calculated as the sum of the product of the weekly supplied water concentrations (mmol/L) and the daily water supply registration (L/$m^2$). The nitrogen use efficiency (mol N/kg) expressed the quantity of N (mol/$m^2$) per cumulative yield (kg/$m^2$).

# 3 Results and Discussion

## 3.1 Nitrogen availability and water analysis

The standard USDA cultivation (reference) showed insufficient nitrification processes, as can be seen in Figure 4A. The peak in the $NO_3$ concentration is caused by manual addition of 5 mmol/L $NaNO_3$ at the end of August. During the rest of the test, the $NO_3$ content in the slabs as well as in the drain water was always close to zero. Several factors might have contributed to this:

- A high initial water content into the slabs despite an average of 30% drainage rate (Figure 5A).
- A very compact structure of the slabs was observed, which reduced the air content and air movement in the substrate. This was illustrated by little root growth in the central-lower part of the slab (Figure 5B).
- Consequently, a lack of oxygen might even have contributed to the denitrification processes and leaching of N as $N_2O$. A smell of $H_2S$ from the supply tank and from the slabs of the reference treatment was also noticed, suggesting that microbes reduced $SO_4$ to $S^-$ due to anoxic conditions.
- The high quantities of $NH_4$ analysed in both gift and drain water (Figure 4C and D) also indicates a high conversion of N-organic into $NH_4$ and in combination with a high pH it could have contributed to additional N-losses in form of $NH_3$.

Since the plants seriously started to show N-deficiency, it was decided to intervene with the application of Chilean Nitrate ($NaNO_3$) from the end of August. The USDA cultivation with 'Natural nitrogen' had a higher $NO_3$ availability (Figure 4 A and B) than the reference treatment. The $NO_3$ input was relatively lower (10 mmol/L) than in conventional cultivation (20-30 mmol/L; non-USDA-organic) to not exceed the required Ca input (5 mmol/L). The lime ($CaCO_3$) used to stabilise the 'Natural nitrogen' ($HNO_3$) produced by VitalFluid might represent a limiting factor for the large use of this $NO_3$ source. In fact, each mmol of $NO_3$ will bring 0.5 mmol of Ca, meaning that the overall desired or accepted concentration of Ca will define the maximum quantities of $NO_3$ to be applied from $HNO_3$. The limited organic-nitrogen as nitrogen source (only a little bit from the organic P fertiliser) avoided the presence of extreme $NH_4$ concentration in the supply and drain water (Figure 4C and D).

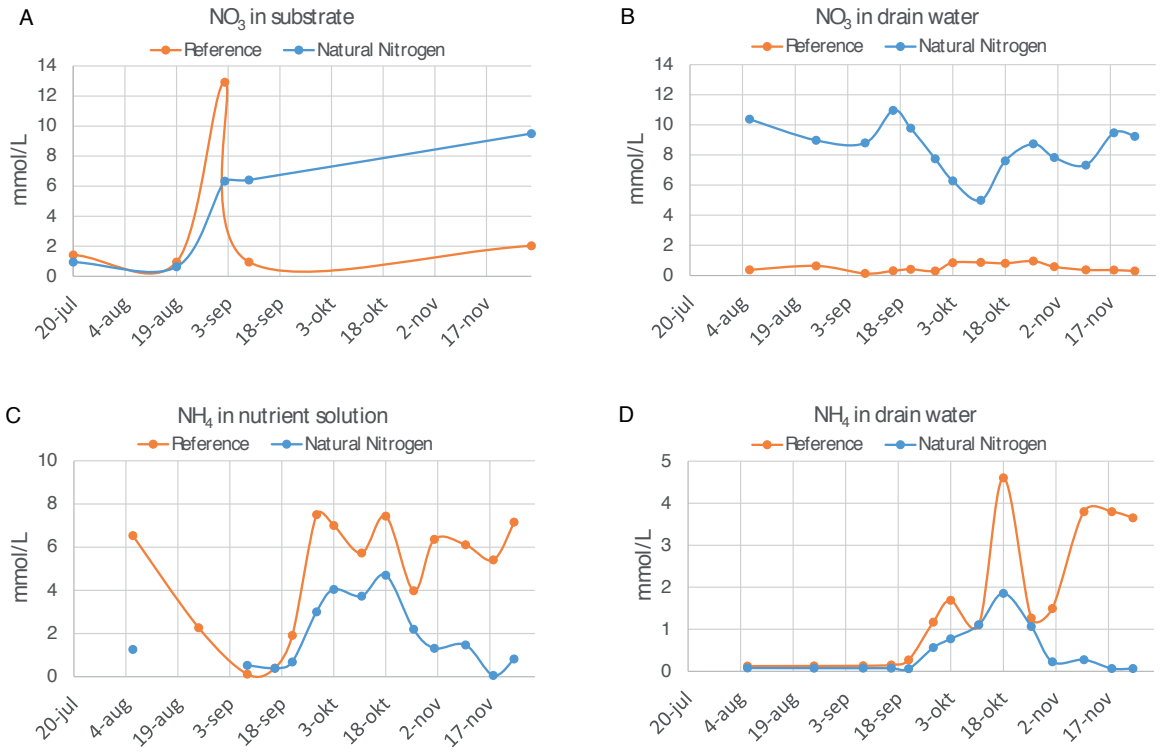


***Figure 4*** *(A) $NO_3$ concentration (mmol/L) in the substrate; (B) $NO_3$ concentration (mmol/L) in the drain water; (C) $NH_4$ concentration (mmol/L) in the supply water; (D) $NH_4$ concentration (mmol/L) in the drain water. One replicate sample per treatment.*

A B

***Figure 5* (A) H***igh water content in the substrate of the reference treatment at the start of the trial. (B) Limited root growth in the centre of the substrate, picture taken at the end of the cultivation from reference treatment.*

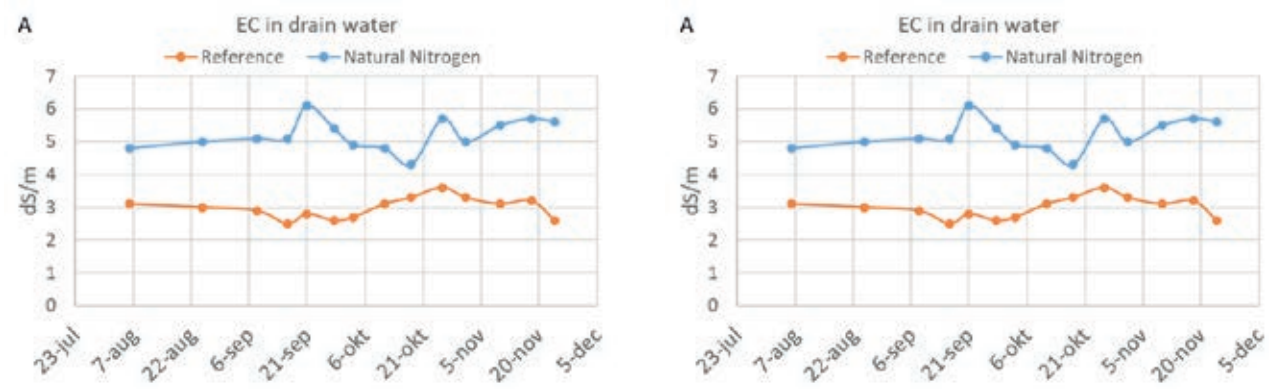


***Figure 6** (A) EC and (B) pH in drain water. One replicate sample per treatment.*

To alleviate the shortage of $NO_3$ in the reference treatment, $NaNO_3$ was added. As a result, Na accumulated rapidly in the drain in this treatment, due to the drain water recirculation strategy. This was at the expense of the other cations (Figure 7), as a consequence of keeping the drain water EC at the required stable level (Figure 6B). Drain water recirculation, Na accumulation and rising fruit load contributed to a fast depletion of K between week 4 and 8 after transplanting (20th August – 20th September) (Figure 7B). Due to the high Na accumulation and K depletion, drain water was partly discharged to the sewer and consequently the recirculation rate was reduced in the reference treatment from week 8 (September 15) onwards. In addition, extra $K_2SO_4$ was added. It was measured that 44% of the potentially recyclable drain water of the reference treatment was lost by this discharge. After week 8, K concentration raised, but not up to the drain target level (8 mmol/L).

Differently, the 'Natural nitrogen' treatment maintained a good level of Na and K (Figure 7A and B). The decreasing trend of K in drain in the last weeks of the cultivation was associated to the rising Ca and Mg, which was caused by pinching-off the growing point of the plant, to stimulate fruit development of the final fruit trusses, which is a standard crop management for tomato (end-of-crop strategy) (Figure 7C and D). In practice, the nutrient solution for tomato is adapted over time to follow the crop development. Relatively more K and less Ca is used in the period with the highest fruit load. This adaptation was deliberately not carried out in this trial to not interfere with the treatments. The fixed ratios of cations in the stock solution A and B and the end-of-crop-strategy contributed to this K decreasing trend, which was amplified by the full recirculation of drainage water. Ca and Mg were stably close to the drain target levels (respectively 10 and 4.5 mmol/L) except for the late rising mentioned above (Figure 7C and D). It is remarkable that the 'Natural nitrogen' treatment used a closed-loop irrigation system for the whole duration of the cultivation without showing any relevant nutrient unbalancing and no discharge.

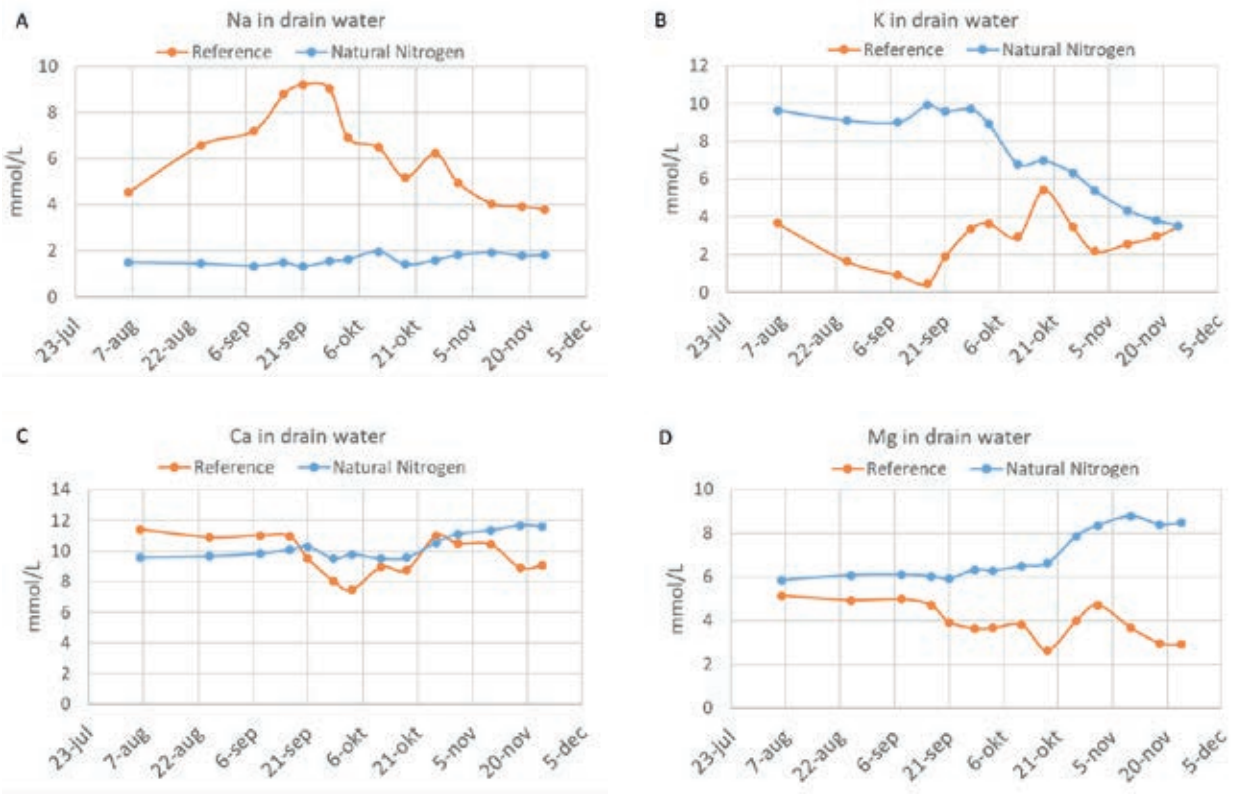


***Figure 7*** *Concentrations of Na (A), K (B), Ca (C) and Mg (D) in drain water. The elements concentrations are corrected to a target EC of 3.7 dS/m. The maximum concentration of Na accepted widely by grower is 8 mmol/L. The target concentration for K is 8 mmol/L, 10 mmol/L for Ca and 4.5 mmol/L for Mg at 3.7 EC. One replicate sample per treatment.*

High pH was often measured in the nutrient solution storage tanks of both treatments (Figure 8A). To restore it to the right levels, HCl (hydrochloric acid) was used in the mixing tanks to neutralise the pH before the nutrient solution passed to the nutrient solution storage tank. This pH increase was likely caused by microbial activity, which results in anoxic conditions and will cause pH to rise above 7. To counteract this, air pumps were placed in the nutrient solution storage tanks and the mixing tanks, to limit the rise of pH. However, the preventive effect of active oxygenation was limited. This was clearly observed by the smell of $H_2S$, often detected at the nutrient solution storage tank of the reference treatment. $H_2S$ will be produced by microorganisms by reduction of $SO_4$ under anaerobic conditions.

The microbial activity by rapid pH increase was also observed in the B stock solution of the 'Natural nitrogen' treatment. After a new B-stock was made, the pH raised from 5.5 to up to 9.0 in about one week. With the rising pH, a significant smell of $NH_3$ was observed and clearly released from the B stock with a peak of the smell at pH around 7.5-8.0. Aeration of the room was necessary. The presence of organic N, in form of some amino acids in the Biota P fertiliser was likely the source of the ammonification. The low $O_2$ rate in water easily led to anaerobic conditions causing the pH to rise. When pH is above 7, $NH_4^+$ is transferred to $NH_3$, which volatizes. The addition of air pumps into B did not help to avoid this process. It was believed to be not sufficient due to the large size of the tank (2000 L). This process was also prompted by the low concentration of the B-stock (4 times the supply recipe concentration). To avoid microbial activity (algae, bacteria, etc.,) a concentration of about 200 times the supply solution is often used in the stock solutions (i.e. for reference treatment). The low concentration of the B stock in the 'Natural nitrogen' treatment was aimed to equalise to the concentration of the $Ca(NO_3)_2$ solution from 'Natural nitrogen', which had a fixed concentration (40 mmol/L). An equal concentration of A and B was necessary to use the mixing unit properly. It is therefore recommended to use higher concentrations: 1 mol/L $HNO_3$ 'Natural nitrogen', resulting in 500 mmol/l $Ca(NO_3)_2$, which is 100 times the supply solution (assuming an aimed 5 mmol Ca/L). Then B will be 100 times concentrated as well. However a 100 times concentrated solution in B might be not sufficient to stop the microbial activity (not tested). VitalFluid can therefore consider to increase the 'Natural nitrogen' concentration to even 2 mol/L. If not possible, the use of a more sophisticated mixing unit which can dose stock solutions at different concentrations is advised. In this case, a 200 times concentrated B-stock is believed to be sufficient to stop the biological process. It is to be noted that there were differences in the supply EC between the two treatments. This was unavoidable, since the reference treatment consisted of a large portion of the nutrients (i.e all nitrogen and partly phosphorous) in organic form, which obviously do not contribute to the EC value as such, although the total quantity of the nutrients was kept equal.

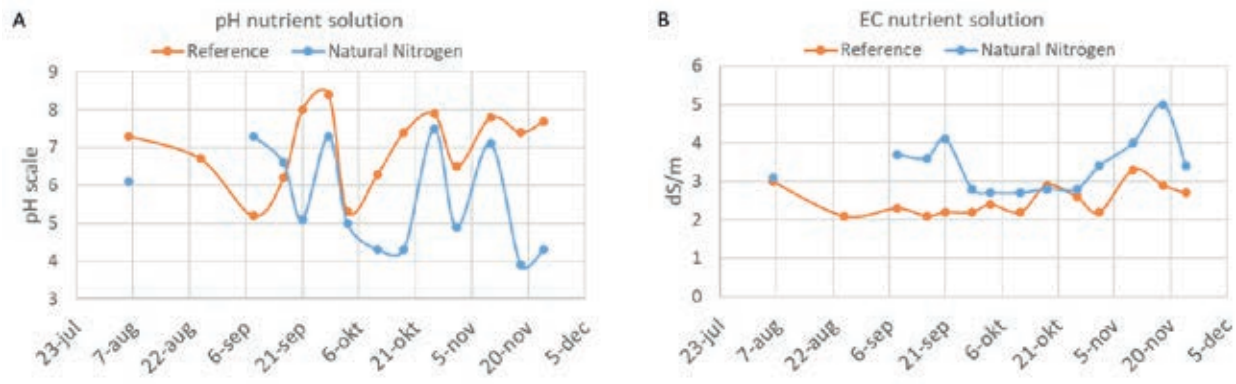


***Figure 8*** *(A) pH of the nutrient solutions; (B) EC in the nutrient solutions. One replicate sample per treatment.*

## 3.2 Yield and crop performance

The results showed that the yield of the standard USDA cultivation (reference) was significantly lower than cultivation with 'Natural nitrogen' (Figure 9A). This was merely correlated with the low availability of $NO_3$ in the reference. In addition, the shortage of K during the first part of the cultivation might have contributed as well. Also the average fruit size was significantly lower for the reference treatment during most of the cultivation (Figure 9B). The average fruit size can be used as a product quality parameter since the market has specific fruit size requirements. A large deviation from the market size means that the product loses value. The total plant dry biomass (kg/m$^2$) was lower in the reference treatment, in all plant organs (Figure 9C). The dry matter content was not significantly different between treatments, in all the organs (data not shown). Also the partitioning between organs did not differ much, except for a lower old leaves fraction in the reference treatment, due to the lower amount of pruning (Figure 9D). In fact, to reduce the difference in leaf area index (LAI) (leaf area/m$^2$) between treatments due to the lower growth in the reference treatment, less leaf pruning and stem bending was adopted in this treatment. This helped to keep a more equal light interception between treatments.

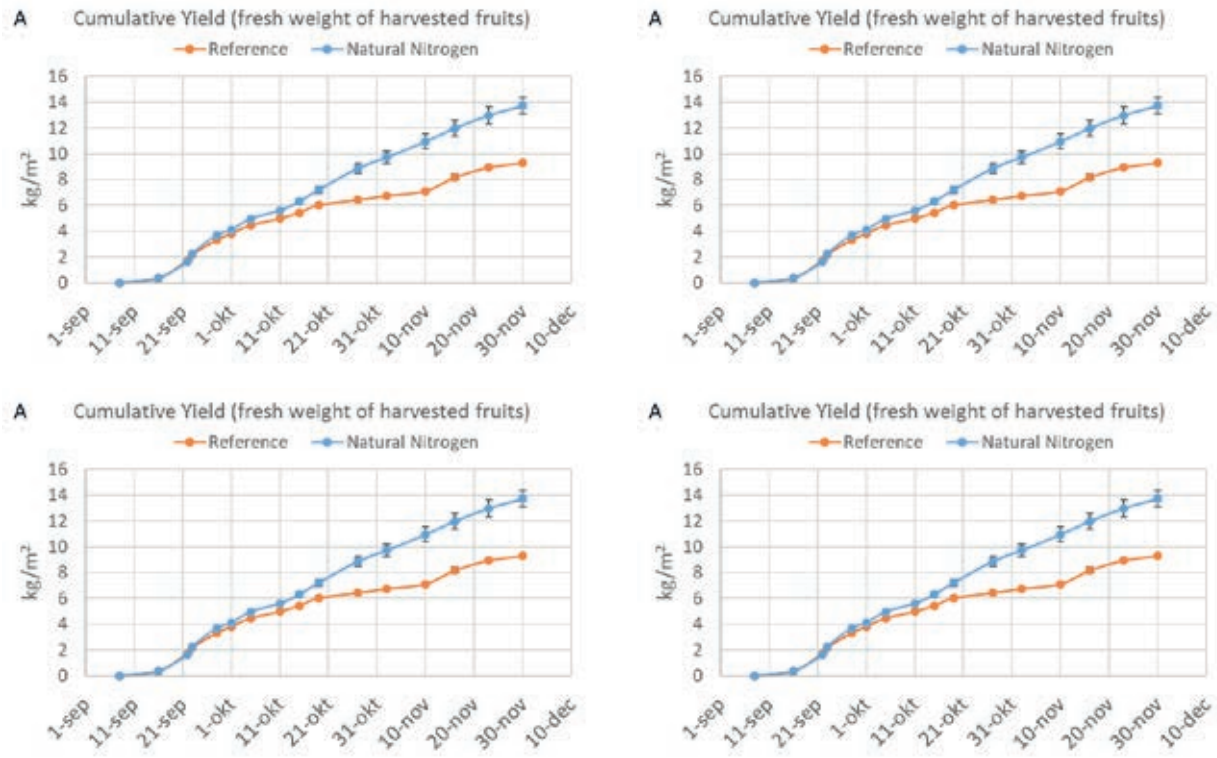


***Figure 9*** *(A) Cumulative yield; (B) Average fruit weight; (C) Average total plant dry biomass with the absolute contributions of each organ; (D) Relative contribution of each organ to the total plant dry biomass. Four replicates per treatment. Standard error of means are shown.*

A few weeks after the start of the trial, the difference in colour and growth between treatments was more clear (Figure 10A) than at the end (Figure 10B). The drain water recirculation reduction (44% drainage water discharged overall) and the manual additions of $K_2SO_4$ and $NaNO_3$ were believed to contribute to this reduction in difference. The average daily water uptake for reference and 'Natural nitrogen' treatment were respectively 2.2 and 2.5 L/m²/day, but it must be noted that the total fresh plant biomass was also respectively 15 and 20 Kg/m². The overall average drain rate was 35% and 27% for respectively the reference and the 'Natural nitrogen' treatment. This drain rate express the ratio between the drain flow (l/m²/day) and the supply flow (l/m²/day). The drain rate is different from the drain discharge percentage mentioned in 3.1 (44%) for the reference treatment. The latter expresses how much of the 35% was not reused over the all cultivation period.

A

B

***Figure 10*** *(A) At the start of the cultivation a visible nitrogen (N) deficiency in the reference treatment (right) with more light green leaves than 'Natural nitrogen' treatment (left). (B) At the end of the cultivation no difference in leaf colour between treatments thanks to the addition of* $NaNO_3$ *to reference treatment.*

## 3.3 Biomass analysis

The results of the dry biomass analysis showed similar concentrations of nutrients (mmol/kg) between the standard USDA cultivation (reference) and the 'Natural nitrogen' cultivation (Figure 11A and B; K and N showed).

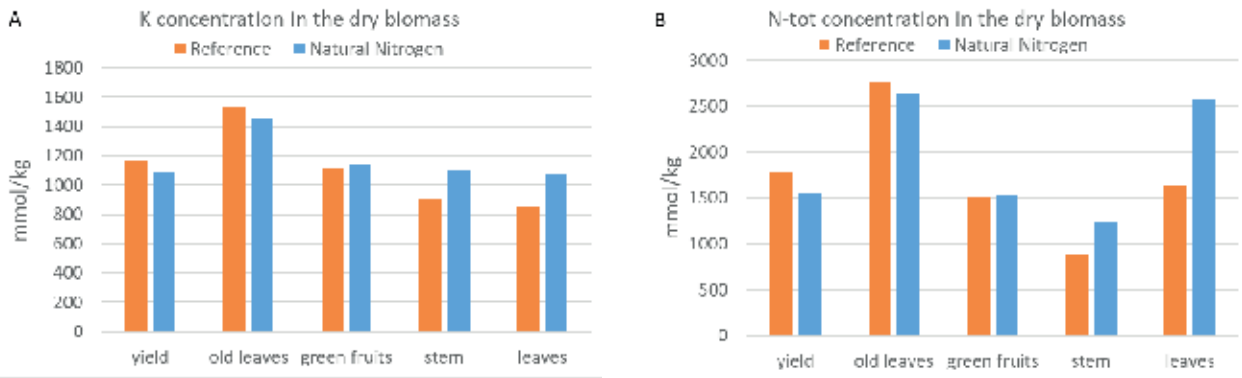


***Figure 11*** *K concentration (A) and Total-N concentration (B) in the dry biomass of different organs. One replicate sample per organ per treatment.*

However, the absolute quantities (mmol/$m^2$) were lower for the reference treatment for both cations and anions (K and N showed) (Figure 12A and B). This was the result of the lower total plant dry plant biomass of the reference treatment.

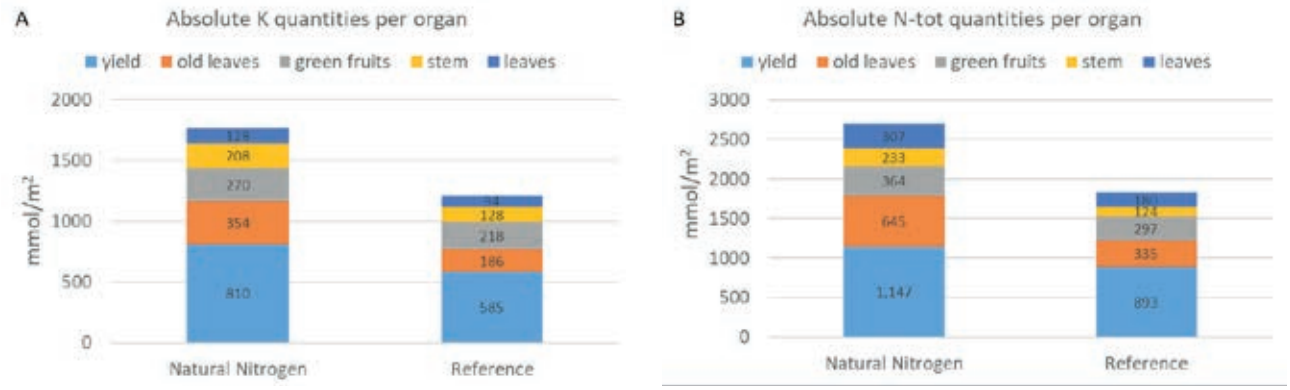


***Figure 12*** *Absolute quantity of K (A) and N (B) per organ. One replicate sample per organ per treatment.*

An estimation of the total average nutrients input showed how a large fraction of the distributed cations was occupied by Na and $NH_4$ in the reference treatment (Figure 13A). Consequently less Ca, K, and Mg were available for the crop. Similarly, Figure 13B shows that a large fraction of anions was occupied by Cl in the reference treatment. Hence less $NO_3$, S and P were available. The high Cl in the reference treatment derived mainly from the HCl used to correct the pH. In this trial, the rapid pH rise in reference and the consequent pH correction with HCl influenced the presence and therefore availability of the other anions. This issue can be eventually solved using $HNO_3$ if 'Natural nitrogen' is accepted in USDA organic cultivation.

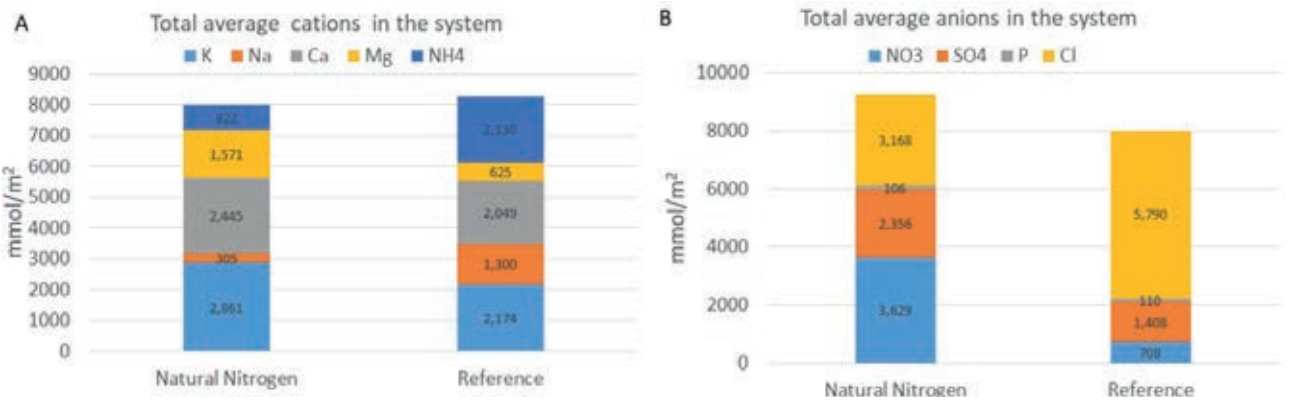


***Figure 13*** *Total average cations (A) and anions (B) input. One replicate sample per treatment.*

The total draft nitrogen balance shows how the total N was distributed within the plant and in which quantities (Figure 14A). Since part of the fertilisers for both treatments derived from organic sources, it was decided to express the "input" (mmol/m²) as the total average quantity of dissolved elements in the supply water (see 2.5). In addition, the reference treatment used an open system, therefore a large part of input has been lost via drain water discharge. Whereas the 'Natural nitrogen' treatment ran with a closed water system for the whole cultivation period. Hence, a comparison of the absolute input was considered not a fair way to express the "real" available quantities that the plants were exposed to. Figure 14A shows that the difference between "input" and quantities taken up by the crop is about 30%. A gap between input and dry biomass is often found in mass balance calculations. This is due to losses of elements by denitrification ($N_2O$), immobilisation (for N), volatilisation ($NH_3$ for N), sampling errors (more systematically and frequently is better), laboratory analysis errors (90-95% accuracy). Besides this gap, Figure 14A shows how the total absolute quantities in the crop (plus residual) were proportional to the "input" for both treatments. In Figure 14B it is possible to see the distribution of total N over the different components of the system. The lower N fraction in the residual of the reference treatment was also a confirmation of the N depletion condition. A higher fraction of N in the yield was found in the reference treatment. The nitrogen use efficiency (NUE) was very similar between treatments, supporting the assumption that N efficiency is correlated to plants genetics and is not influenced by the type of available N.

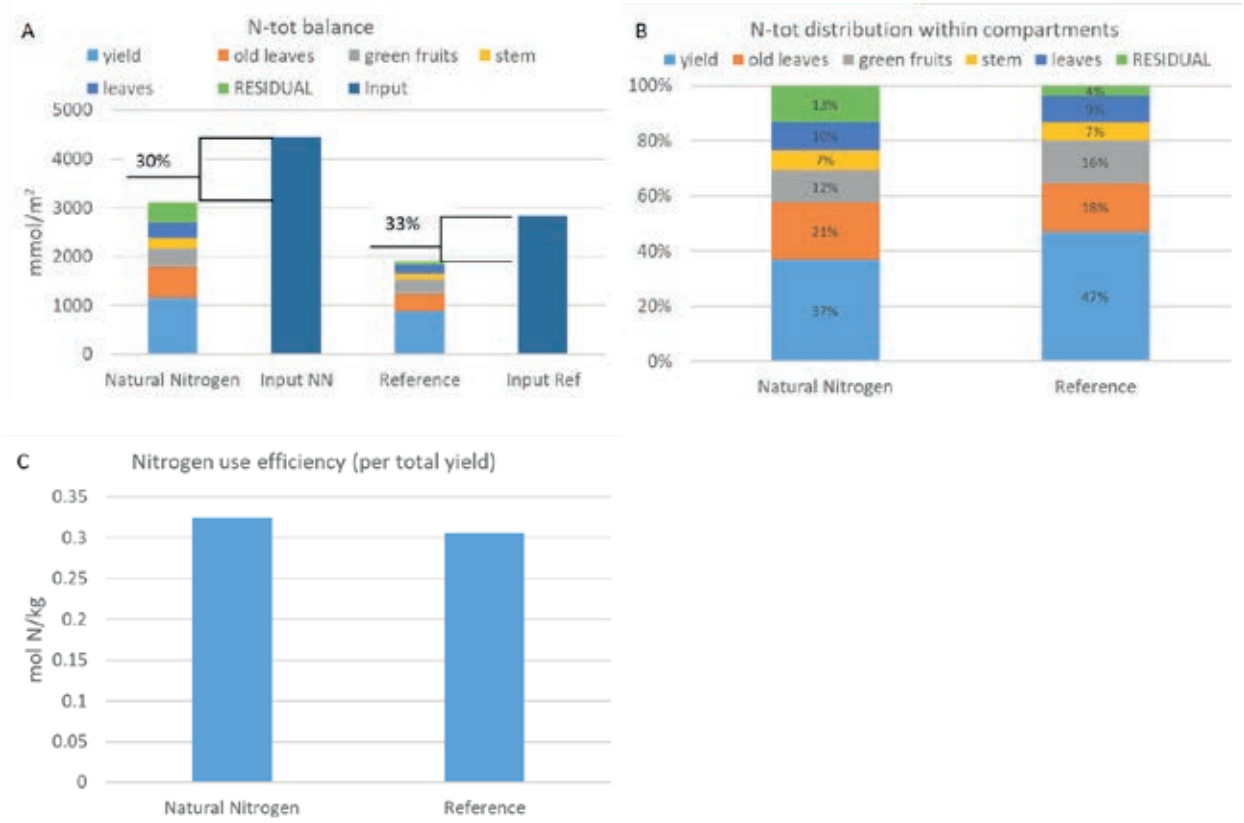


***Figure 14*** *(A) Total N balance and estimated total N input. (B) Total N distribution within different compartments. The residual is the quantity left in the substrate, detected via 1:1,5 extraction. (C) Nitrogen use efficiency expressed in terms of yield (harvested fruits) production. One replicate sample per treatment.*

# 4 Conclusions

The goal for this demonstration trial was to see if 'Natural nitrogen' could be used as N-source in a nutrient solution for USDA-organic cultivation of tomato. It was compared to a traditional USDA-organic cultivation (reference) to evaluate the effect on crop productivity and product quality of the directly available 'Natural nitrogen', compared to nitrogen from organic sources. Both treatments applied recirculation of drain water from the start.

It was shown that 'Natural nitrogen' ($HNO_3$) could be used as the major N-source in a nutrient solution. The only other nitrogen source was a small amount of N from an organic P fertiliser. $HNO_3$ was neutralised by pulverised limestone ($CaCO_3$), creating a solution of $Ca(NO_3)_2$, before adding it to the other elements. This form is readily available for the crop, so plant requirements can be followed closely. The biggest challenge was to manage the micro-biological activity that was initiated by some of the organic fertiliser inputs in the B-stock solution. This caused strong pH rise and some changes in the nutrient composition. This challenge can be counteracted by using higher concentrated stock solutions and a more sophisticated mixing unit, that can mix different concentrations from A and B stock. Restrictions on the concentration of $NO_3$ in the nutrient solution are set by the use of lime, which adds 0.5 mmol/L Ca for each mmol/L of $NO_3$, to ensure the ratio of K:Ca remains right.

The reference treatment with only organic nitrogen showed a reduced yield, due to several shortcomings:
- Difficulty to initiate and to manage mineralisation of organic-N;
- High pH rising in the mixing tank and the nutrient solution storage tank;
- High Na (due to unavoidably addition of $NaNO_3$) and Cl (due to HCl pH control) accumulation and consequently lower availability of other cations and anions. Consequently, recirculation of drain water had to be stopped;
- Loss of Sulphur and Ammonia due to microbial activity.

This resulted in big differences in crop growth between treatments at the start of the cultivation, which could not be corrected for during the remaining cultivation period. Therefore, a direct comparison between productivities of the two treatments is impossible. The demo showed at least that a USDA-organic cultivation is easier to control with directly available nitrogen, with less loss of nutrients due to complete recirculation of drain water. Productivity and product quality in the treatment with 'Natural nitrogen' seem to be good, but could not be related to a good reference treatment.

It was shown that the application of 'Natural nitrogen' as main source for nitrogen in USDA organic cultivation of tomato opens opportunities for an improved management and control of available nitrogen and thereby the other nutrients and will make complete recirculation of drain water possible. This approach will improve the resource use efficiency (RUE) of USDA-organic cultivation tremendously.

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