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Data Article

Responses of hydroponically grown maize to various urea to ammonium ratios: physiological and molecular data



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ABSTRACT

To date urea and ammonium are two nitrogen (N) forms widely used in agriculture. Due to a low production cost, urea is the N form most applied in agriculture. However, its stability in the soil depends on the activity of microbial ureases, that operate the hydrolysis of urea into ammonium. In the soil ammonium is subjected to fast volatilization in form of ammonia, an environmental N loss that contributes to the atmospheric pollution and impacts on farm economies. Based on these considerations, the optimization of N fertilization is useful in order to maximize N acquired by crops and at the same time limit N losses in the environment. The use of mixed nitrogen forms in cultivated soils allows to have urea and ammonium simultaneously available for the root acquisition after a fertilization event. A combination of different N-sources is known to lead to positive effects on the nutritional status of crops. It is plausible suppose that N acquisition mechanisms in plants might be responsive to N forms available in the root external solution, and therefore indicate a cross connection among different N forms, such as urea and ammonium.

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This DIB article provides details about the elemental composition and transcriptional changes occurring in maize seedlings when ammonium and urea mixture is applied to nutrient solution. An extensive and complete characterization of seedling response to urea and ammonium treatments is shown in the research article “Characterization of physiological and molecular responses of *Zea mays* seedlings to different urea-ammonium ratios” Buoso et al. [1].

Maize seedlings were grown under hydroponic system with N applied to nutrient solution in form of urea and or ammonium, hence five different urea (U) to ammonium (A) ratios were tested (100U, 75U:25A, 50U:50A, 25U:75A, 100A). As control maize were fed with nitrate as sole N source, or were maintained in N deficiency (-N). After 1 or 7 days of N-treatment, maize seedlings were collected, and physiological and transcriptional analyses were performed on maize roots. Depending on nutritional treatment, no significant changes in seedling biomass were observed comparing N treatments. At both sampling times, an overall higher N accumulation in shoots and roots were detected when the inorganic N sources were applied to nutrient solutions (as ammonium or nitrate). ¹⁵N experiments indicated that in comparison to -N seedlings, urea fed seedlings showed an increase of N accumulation and data showed that ureic-N was taken up by seedlings in lower amounts than inorganic N-forms. Through EA-IRMS, ICP-OES and ICP-MS a multielemental composition of maize tissues was performed as well as gene expression analyses by Real-time RT-PCR that allowed to monitor the expression profile of genes most involved in urea and ammonium nutritional pathways.

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Specifications Table

Subject	Biological sciences Plant Science: Plant Physiology
Specific subject area	Mineral nutrition: plant nitrogen nutrition in hydroponically grown plant
Type of data	Tables Figures
How data were acquired	N, C content and ¹⁵ N enrichment were determined by EA-IRMS (Vario Isotope Select and Isoprime 100, Elementar Analysensysteme GmbH, Hanau, Germany). Other element concentrations (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S and Zn) were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS NexION 300, Perkin Elmer Inc., Shelton, CT USA) or Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES 5800, Agilent Technologies, Santa Clara, USA). Real time RT-PCR analyses were performed using CFX96 Real Time RT-PCR Detection (Biorad).
Data format	Raw and Analyzed
Parameters for data collection	Five-day old maize seedlings were grown up to 7 days (13-d-old maize seedlings) to a combination of different N-forms in nutrient solution (urea, U; ammonium, A): 100U, 75U:25A, 50U:50A, 25U:75A, 100A treatments as described below; as controls maize seedlings were grown under N deficiency (-N treatment) or in presence of nitrate as only N source (Nitrate treatment).

(continued on next page)

Description of data collection	After 1 day and 7 days of N-treatment shoots and roots of maize seedlings were collected, and biomass data (fresh and dry weights) were measured. The multielemental composition of maize seedlings was analysed through EA-IRMS, ICP-OES and ICP-MS, moreover an isotopic analyses of maize tissues by EA-IRMS allowed to discriminate ¹⁵ N concentration deriving from ¹⁵ N-labelling N forms, when these latter were applied to nutrient solution. At molecular level, the expression profile of root genes was investigated by Real Time RT-PCR.
Data source location	Seedling growth and analyses were performed under controlled conditions at the Dipartimento di Scienze Agroalimentari, Ambientali e Animali (University of Udine, via delle Scienze 206, I-33100)
Data accessibility	Repository name: "Zea mays physiological data" Mendeley Data Data identification number, doi: http://dx.doi.org/10.17632/yjzs2kdwwy.2 Direct URL to data: http://dx.doi.org/10.17632/yjzs2kdwwy.2
Related research article	S. Buoso, N. Tomasi, D. Said-Pullicino, M. Arkoun, J. C. Yvin, R. Pinton, L. Zanin, Characterization of physiological and molecular responses of <i>Zea mays</i> seedlings to different urea-ammonium ratios, <i>Plant Physiol. Biochem.</i> 162 (2021): 613-623. https://doi.org/10.1016/j.plaphy.2021.03.037

Value of the Data

- Physiological and molecular characterization of maize seedlings in response to different ratios of urea and ammonium provides useful information to improve N nutrition in crops.
- The multielemental composition of maize seedlings is more influenced by ammonium in the external media than by urea.
- The expression profile of genes involved in N transport and assimilation was analysed in maize roots after 1 day and 7 days of treatment, highlighted a preferential induction by urea and ammonium of the cytosolic pathway (through GS, ASNS).
- This data can be used by researchers to further investigate on mechanisms involved in N acquisition in plants focusing on the occurrence of reciprocal interactions among different N-forms.
- This study contributes to pave the way for the optimization of N fertilization in agriculture. In particular, the information provided in this study gives useful indication about the proper ratio urea to ammonium useful to maximize the uptake efficiency of ammonium and therefore limit the economic and environmental impact of nitrogen fertilization.

1. Data Description

Physiological and transcriptional studies have been performed to identify plant response when maize seedlings were grown in hydroponic solution supplied with a mixture of urea and ammonium. Hence different N treatments were performed adding variable forms of N to a N-free nutrient solution (2 mM total N concentration): urea or ammonium were applied to nutrient solution as sole N forms (100U and 100A treatments, respectively), or urea and ammonium were applied in conjunction (treatments: 75U:25A, 50U:50A, 25U:75A urea:ammonium ratios). As controls maize seedlings were grown in presence of nitrate as sole N source (Nitrate treatment) or maintained in N deficiency (-N treatment). In Fig. 1 a schematic representation of the experimental set up is provided.

Up to 7 days of treatments, data indicate no differences on fresh and dry weights among N-treatments after 1 day and after 7 days (Fig. 2). The effect of each treatments on total N, C and C/N after 1 day and 7 days were presented in Figs. 3 and 4, respectively. Overall, higher N concentrations in shoots and roots were detected when the inorganic N sources were applied to nutrient solutions (as ammonium or nitrate; Fig. 3). After 1 day of treatment, seedlings fed with urea (100U) did not showed significant changes on total N concentration in comparison to -N fed

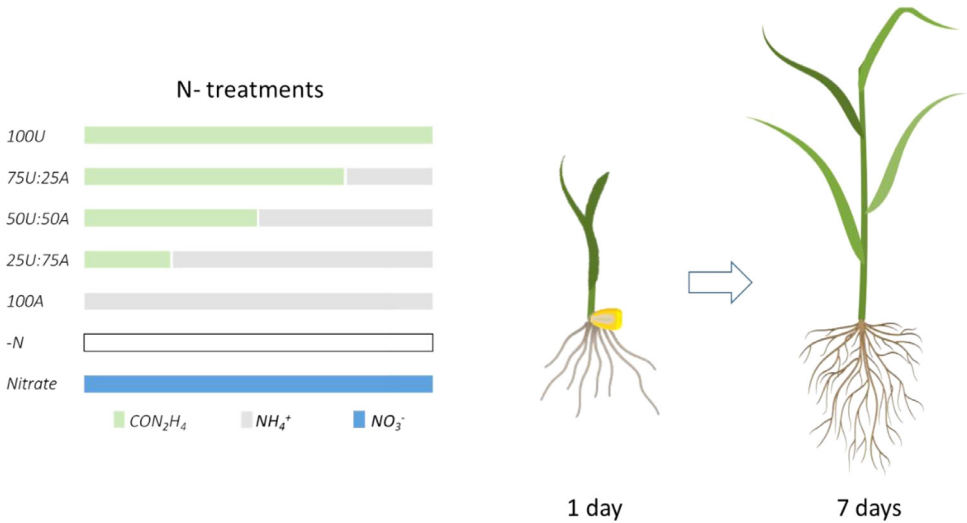


Fig. 1. Nitrogen treatments. Maize seedlings (5-day-old) were transferred to a N-free nutrient solution as described in material and methods containing N in form of urea (U), ammonium (A) or nitrate, the treatment was performed up to 7 days. Hence five nutritional treatments have been tested (2 mM total N): 1.00 mM $\text{CH}_4\text{N}_2\text{O}$ (100U), 0.75 mM $\text{CH}_4\text{N}_2\text{O}$ and 0.25 mM $(\text{NH}_4)_2\text{SO}_4$ (75U:25A), 0.50 mM $\text{CH}_4\text{N}_2\text{O}$ and 0.50 mM $(\text{NH}_4)_2\text{SO}_4$ (50U:50A), 0.25 mM $\text{CH}_4\text{N}_2\text{O}$ and 0.75 mM $(\text{NH}_4)_2\text{SO}_4$ (25U:75A), 1.00 mM $(\text{NH}_4)_2\text{SO}_4$ (100A). As controls, some seedlings were grown in N-free nutrient solution (-N) or in N-free nutrient solution containing 1 mM $\text{Ca}(\text{NO}_3)_2$ (Nitrate). Physiological and molecular analyses were performed on maize seedlings after 1 day and 7 days of treatment.

seedlings (Fig. 3), while significant higher values were recorded after 7 days (Fig. 4). Regarding N concentrations in seedlings treated with urea and ammonium mix, after 7 days similar values were measured among 75U:25A, 50U:50A, 25U:75A, 100A-fed seedlings, and especially in roots N concentration was significantly higher than that detected in 100U (Fig. 4).

The contribution of N treatments to nutritional level of maize seedlings was evaluated through ^{15}N -labelling experiments (Fig. 5). Seedlings were fed with ^{15}N -urea or ^{15}N -ammonium and the amount of ^{15}N taken up by seedlings was evaluated through EA-IRMS. The inorganic N forms (nitrate or ammonium) were preferentially taken up by seedlings. Over the time the capacity of maize seedlings to use urea as N source increased. Moreover, maize seedlings treated with urea and ammonium mixture absorbed similar amounts of ^{15}N regardless to urea and ammonium ratio in nutrient solution (Fig. 5).

Element concentrations in root and shoot of maize seedlings after 1 day and 7 days of treatment were presented in Figures 6, 7, 8 and 9. Table 1 reported the list of primers used for Real-time RT-PCR. The gene expression of maize root after 1 day or 7 days of treatment with the different N-sources was reported, respectively, in Tables 2 and 3. Interpretation of these data is provided in the research article “Characterization of physiological and molecular responses of *Zea mays* seedlings to different urea-ammonium ratios” Buoso et al. [1].

2. Experimental Design, Materials and Methods

Maize seeds (*Zea mays* L., P0423, Pioneer Hybrid Italia S.p.A.) were germinated over aerated 0.5 mM CaSO_4 solution as described in Zanin et al. (2018) [3]. Five-day old maize seedlings were grown in hydroponic system using the following N-free nutrient solution (μM : K_2SO_4 200; KH_2PO_4 175; MgSO_4 100; NaFe-EDTA 40; KCl 5; H_3BO_3 2.5; MnSO_4 0.2; ZnSO_4 0.2; CuSO_4

Table 1

List of primers used for Real-time RT-PCR (5'-3').

Gene	Gene ID	Gene ID	Primer For	Primer Rev
ZmNRT1.1	Zm00001d029932	GRMZM2G16145	CATCAGCGCCATCAACCTC	GACGGCAATAGACTCCTCTG
ZmNRT2.1	Zm00001d054057	GRMZM2G010280	CGACGATCACCTATACCTCT*	TCATGTCAACGGGACACACG*
ZmNRT2.2	Zm00001d054060	GRMZM2G010251	ATGTTACCTGCTACTACCC *	GAATATCGTTGGACATCTC *
ZmNAR2.1	Zm00001d017095	GRMZM2G179294	AGTGGCTGTCTGTTGCTGATT	GGTAATTTTGACGCACACAC
ZmNR	Zm00001d049995	GRMZM2G568636	TGCCAAITTTCTTCGTCGTG	TGGCAAGTCGGCTGTTTAT
ZmNIR2	Zm00001d052164	GRMZM2G079381	ACACCAGATTACTGCAACCCA	CTCACCGCTGAGGACTTGT
ZmGS2	Zm00001d026501	GRMZM2G098290	TGTGAAGCAGCTGAAGGATG	GAGCAGAGATCGCAAGACC
		GRMZM5G885867		
ZmFd-GOGAT	Zm00001d022388	GRMZM2G036609	GGTGAAGCGTTCCTCTGAAG	GCAACAGCTTGGACATCTCA
ZmAMT1;3	Zm00001d017249	GRMZM2G028736	TTCTTGGCGCTCAACAAGAT	CTCAAGCTCAAGTCGTCGTC
ZmAMT1;1a	Zm00001d025831	GRMZM2G175140	GTGGATCGTATCTGCCGGTC	GTAACACATGCGTCTCTGCT
ZmNOD26-like		GRMZM5G892338	GTACCCGTCATGATCTACCC	TCATTTGTCGGTACTCTGGAT
ZmUrease	Zm00001d007945	GRMZM2G461569	CTCTTTCGCTCCGCTTATCG	CCTCATGCTCTCTCTCTG
ZmGDH1	Zm00001d034420	GRMZM2G178415	CCAATCCACCACAGATCCA	ACCCACTCGAAGTGCCTCAC
ZmGS3	Zm00001d048050	GRMZM2G046601	CGATCAAGGGTGACTGGAAC	TCCTTGATCACCTGTAGCC
(ZmGLN1.5)				
ZmGLN1.2	Zm00001d033747	GRMZM2G024104	ACCGAGAAGGAAGGCAAGG	CTCCACAGCATGGTTGTCT
ZmASNS3	Zm00001d028750	GRMZM2G053669	GATCGCGTCTGACGAGAG	CCAGTTTATTGGCGCTGATCG
ZmASNS4	Zm00001d047736	GRMZM2G078472	CCTGCCGAGCATATTTCTGT	ACGACCATCAACTGCTGTT
ZmDUR3	Zm00001d037242	AC202439.3	CCTCAATCTGTGGGTGCT	ATTGGCCTTCTCCACAGC
ZmGLN1.4	Zm00001d051804	GRMZM2G036464	GTCCAACATGGACCCTACG	GAGCAAACCGACACGACAC
ZmAMT8	Zm00001d034782	GRMZM2G338809;	GCTGTGTATGTGAACGTCGC	TGCACAAGGACGAGGAAACA
		GRMZM5G883969		
ZmAMT9	Zm00001d016771	GRMZM2G043193	CATGCAGAGGATCGACGACA	CAGGCTCAGCAAAGAGTCCA
ZmGAPDH	Zm00001d049641	GRMZM2G046804	CCTGCTTCTCATGGATGGTT	TGGTAGCAGGAAGGAAACA
ZmTUA4	Zm00001d013367	GRMZM2G152466	AGGTCATCTCATCCCTGACG	TGAAGTGGATCCTCGGGTAG

* [2].

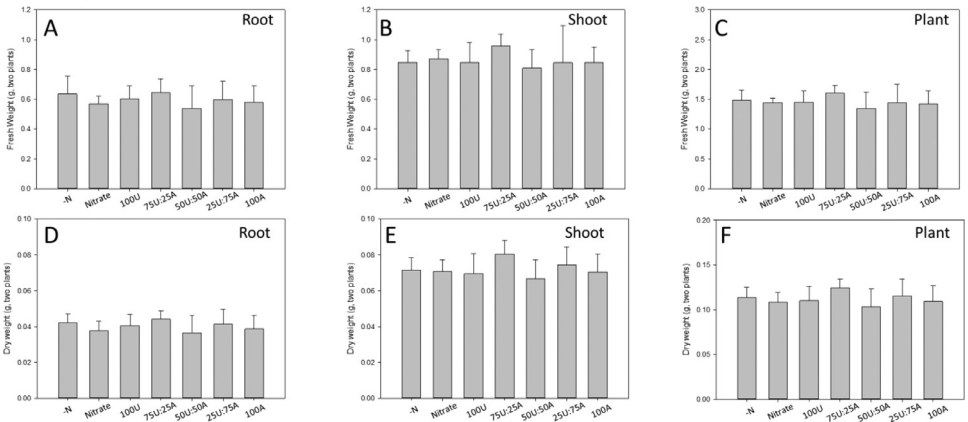
Table 2

Real-time RT-PCR gene expression of maize roots after 1 day of treatment with different N-sources.

1 day	Nitrate	100U	75U:25A	50U:50A	25U:75A	100A
<i>ZmNRT1.1</i>	1.51	1.10	1.26	1.29	1.43	1.19
<i>ZmNRT2.1</i>	0.16	0.27	-0.85	-0.57	-0.59	-0.18
<i>ZmNRT2.2</i>	0.88	1.13	0.21	0.22	0.40	0.89
<i>ZmNAR2.1</i>	1.21 ^a	0.66 ^{ab}	0.06 ^b	0.21 ^{ab}	0.08 ^b	0.04 ^b
<i>ZmAMT1;3</i>	0.26 ^e	2.99 ^a	1.66 ^d	2.14 ^c	2.35 ^{bc}	2.60 ^b
<i>ZmAMT1;1a</i>	-0.34 ^b	0.64 ^a	-0.17 ^b	0.38 ^a	0.66 ^a	0.52 ^a
<i>ZmAMT8</i>	-1.39	-1.20	-1.52	-0.96	-1.11	-1.39
<i>ZmAMT9</i>	0.16	0.16	-0.32	0.05	-0.04	0.00
<i>ZmDUR3</i>	-2.94	-2.08	-2.95	-3.09	-3.02	-2.43
<i>ZmNOD26-like</i>	0.01	0.35	0.63	0.47	1.00	0.61
<i>ZmNR</i>	3.37 ^a	-0.05 ^b	-0.51 ^b	-0.25 ^b	-0.16 ^b	-0.45 ^b
<i>ZmUrease</i>	-0.33	-0.23	-0.57	-0.34	0.13	-0.16
<i>ZmGS3</i>	0.91 ^d	3.37 ^a	1.53 ^{cd}	2.24 ^{bc}	2.72 ^{ab}	2.86 ^{ab}
<i>ZmGLN1.2</i>	1.47	0.47	0.30	0.58	-0.04	0.44
<i>ZmGLN1.4</i>	-0.71	-0.53	-0.86	-0.90	-0.82	-0.91
<i>ZmASNS3</i>	-0.28 ^b	3.00 ^a	3.07 ^a	3.56 ^a	3.24 ^a	3.69 ^a
<i>ZmASNS4</i>	1.81 ^b	3.60 ^a	3.38 ^a	3.90 ^a	3.70 ^a	3.91 ^a
<i>ZmNIR2</i>	3.24 ^a	0.48 ^b	0.14 ^b	0.38 ^b	0.09 ^b	0.08 ^b
<i>ZmGS2</i>	3.37 ^a	1.14 ^b	1.13 ^b	1.08 ^b	1.22 ^b	0.95 ^b
<i>ZmGOGAT</i>	0.82	-0.04	-0.43	-0.57	-0.15	-0.17
<i>ZmGDH1</i>	0.14	0.21	0.19	0.18	0.02	0.38

Values are expressed in comparison to N-deficient seedlings, as Log₂ FC. Letters refers to statistical significance (Holm-Sidak test ANOVA, N=3, p-value < 0.05).

After 1 day



After 7 days

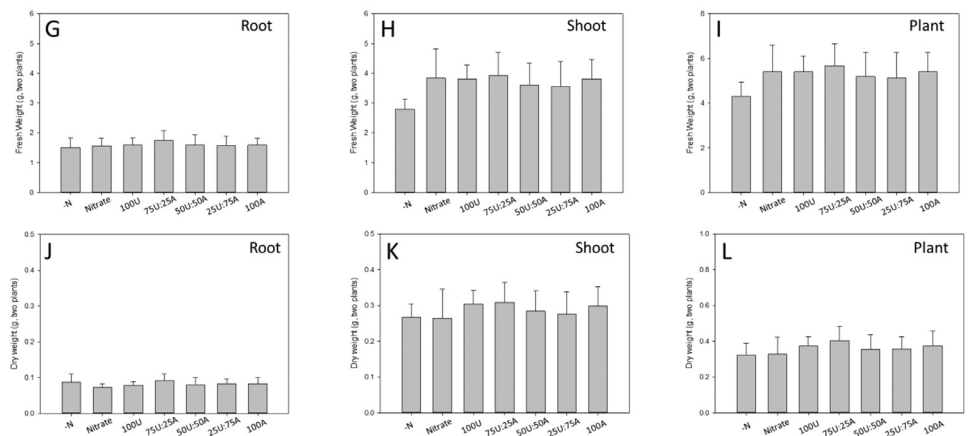


Fig. 2. Fresh (A-C, G-I) and dry (D-F, J-L) weights of maize seedlings after 1 day (A-F) or 7 days (G-L) of treatment with N-sources. Weights refer to roots (A, D, G, J), shoots (B, E, H, K), or total plant (C, F, I, L). No significant changes were observed among thesis (no significant differences: ANOVA, N=20, p-value < 0.05).

0.05; Na₂MoO₄ 0.05). Urea and/or ammonium were added to nutrient solution (2 mM total N): 1.00 mM CH₄N₂O (100U treatment); 0.75 mM CH₄N₂O and 0.25 mM (NH₄)₂SO₄ (75U:25A treatment); 0.50 mM CH₄N₂O and 0.50 mM (NH₄)₂SO₄ (50U:50A treatment); 0.25 mM CH₄N₂O and 0.75 mM (NH₄)₂SO₄ (25U:75A treatment); 1.00 mM (NH₄)₂SO₄ (100A treatment). As controls, maize seedlings were grown in presence of nitrate (1 mM Ca(NO₃)₂, Nitrate) or maintained in N-free nutrient solution (-N treatment; Fig. 1). The sulphate concentration was compensated among N-treatments. To evaluate the contribution of N-forms to N nutrition, [15N] labelling N-forms (10 atom% ¹⁵N) were added to nutrient solution. For further details on experimental set up and methods see the research article [1]. Briefly, after 1 day (24 hours) and 7 days of treatment, shoots and roots of maize seedlings were sampled to evaluate the elemental composition and, in roots, the gene expression profile. Carbon and N contents of roots and shoots of maize were analysed by EA-IRMS (Vario Isotope Select and Isoprime 100, Elementar Analysensysteme GmbH, Hanau, Germany) that allowed to discriminate also the isotopic composition ([15N]-tracer experiments). The tissue concentration of other nutrients (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S

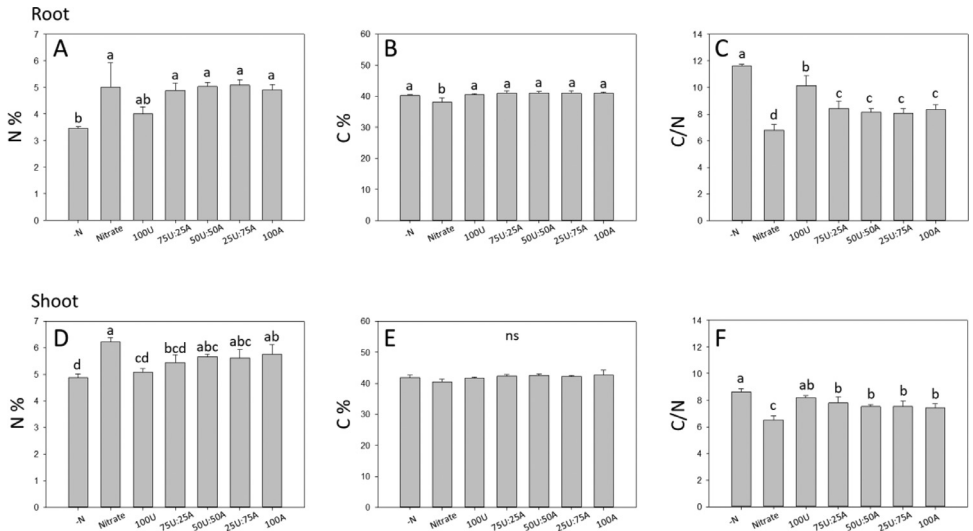


Fig. 3. N concentration (N%), C concentration (C%) and C to N ratio (C/N) in maize roots and shoots after 1 day of treatment (Holm-Sidak test ANOVA, N=3, p-value < 0.05).

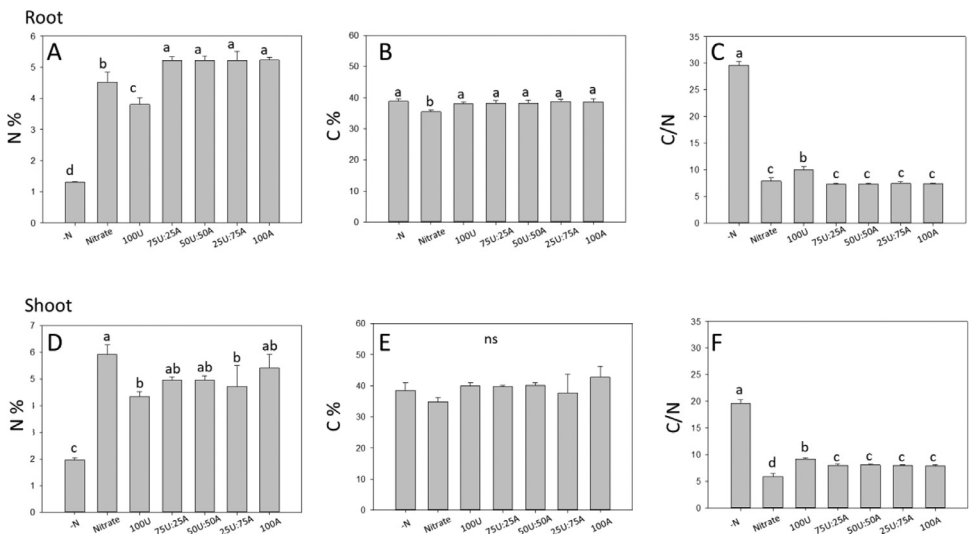


Fig. 4. N concentration (N%), C concentration (C%) and C to N ratio (C/N) in maize roots and shoots after 7 days of treatment (Holm-Sidak test ANOVA, N=3, p-value < 0.05).

and Zn) was investigated through multielemental analyses by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS NexION 300, Perkin Elmer Inc., Shelton, CT USA) or Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES 5800, Agilent Technologies, Santa Clara, USA). Data were referred to the dry weight of shoots and roots of maize. Gene expression profile of genes mainly involved in N acquisition pathway were analysed by Real-Time RT-PCR. The list of

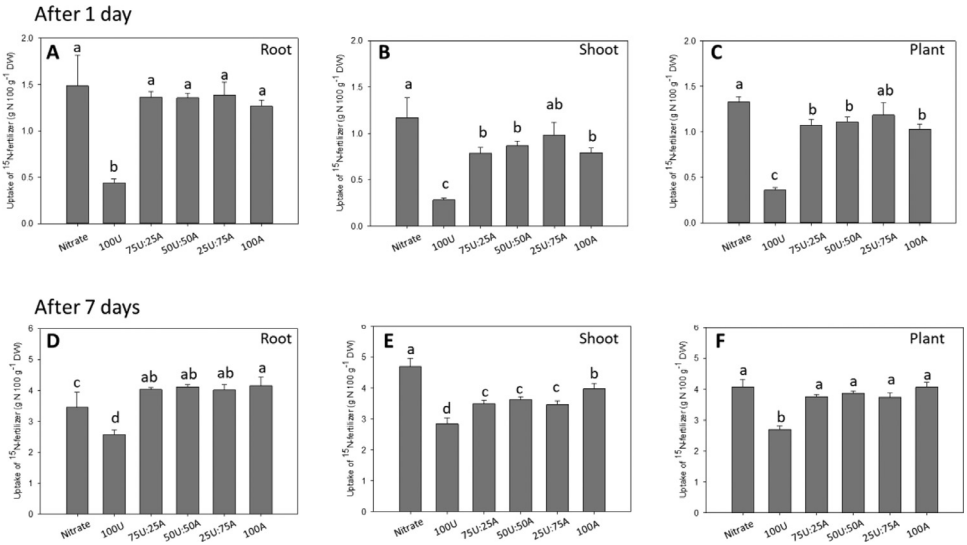


Fig. 5. ¹⁵N-concentration in roots (A, D), shoots (B, E) and whole seedlings (C, F) of maize after 1 day (A-C) or 7 days (D-F) of treatment with different N-sources. Letters refers to statistical significance (Holm-Sidak ANOVA, N=3, p-value < 0.05).

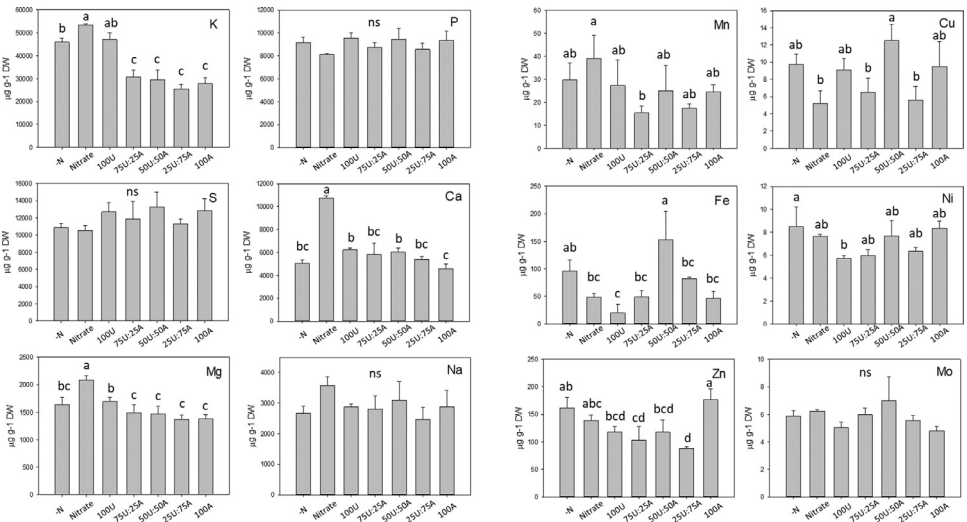


Fig. 6. Elemental composition in maize roots after 1 day of treatment (Holm-Sidak test ANOVA, N=3, p-value < 0.05).

primer is shown in Table 1. Data were normalized accordingly to the constitutive expression of two housekeeping genes *ZmGAPDH* and *ZmTUA* (Table 1) using the 2^{-ΔΔCT} method [4].

Significance differences among treatments were evaluated through statistical analysed performed by one-way analysis of variance (ANOVA Holm-Sidak test for multiple comparisons; p-value < 0.05, N = 3).

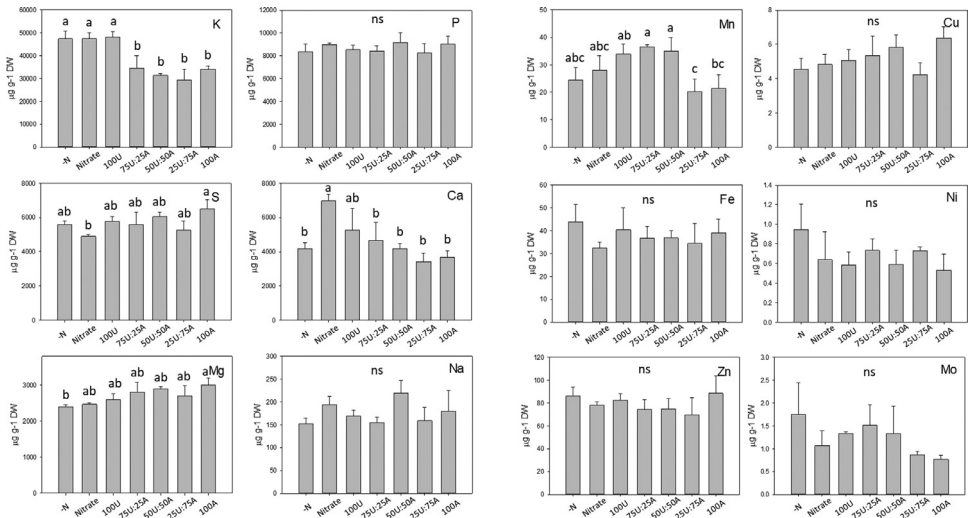


Fig. 7. Elemental composition in maize shoots after 1 day of treatment (Holm–Sidak test ANOVA, N=3, p-value < 0.05).

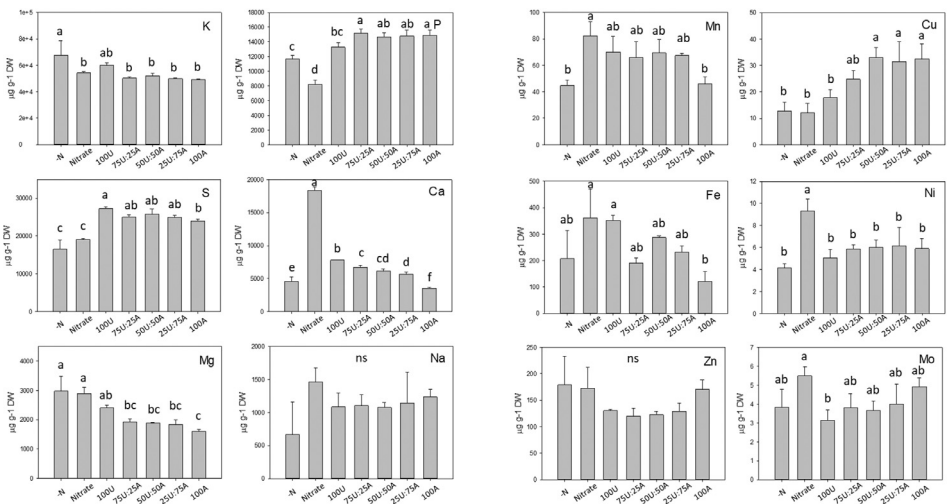


Fig. 8. Elemental composition in maize roots after 7 days of treatment (Holm–Sidak test ANOVA, N=3, p-value < 0.05).

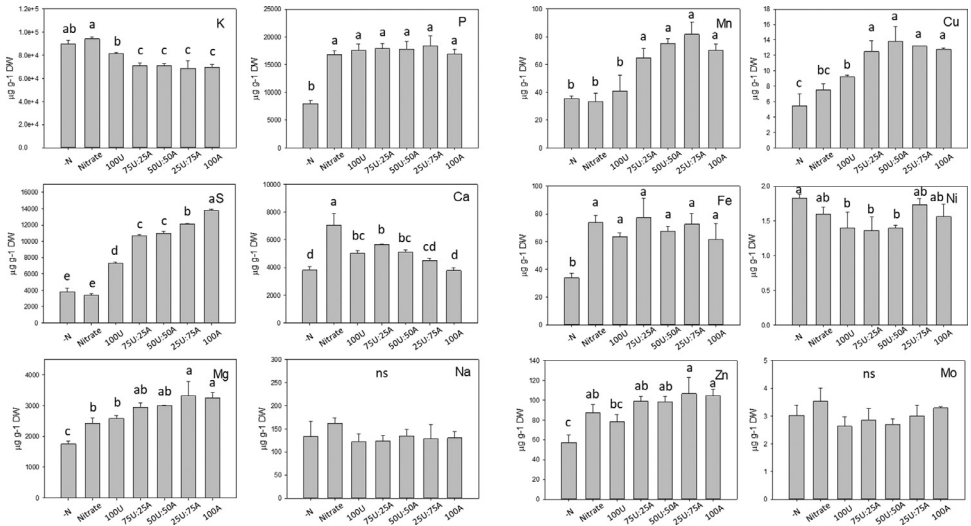


Fig. 9. Elemental composition in maize shoots after 7 days of treatment (Holm–Sidak test ANOVA, N=3, p-value < 0.05).

Table 3

Real-time RT-PCR gene expression of maize roots after 7 days of treatment with different N-sources.

7 days	Nitrate	100U	75U:25A	50U:50A	25U:75A	100A
<i>ZmNRT1.1</i>	4.16 ^a	3.01 ^c	3.66 ^{ab}	3.76 ^{ab}	3.90 ^{ab}	3.55 ^{bc}
<i>ZmNRT2.1</i>	2.20 ^a	1.21 ^b	0.29 ^d	1.02 ^b	0.66 ^{bcd}	0.44 ^{cd}
<i>ZmNRT2.2</i>	3.56 ^a	2.50 ^{ab}	2.04 ^b	2.25 ^b	1.88 ^b	1.67 ^b
<i>ZmNAR2.1</i>	2.16 ^a	0.41 ^b	0.32 ^b	0.65 ^b	0.16 ^b	-0.06 ^b
<i>ZmAMT1;3</i>	-0.30 ^b	1.83 ^a	2.20 ^a	2.25 ^a	2.55 ^a	2.33 ^a
<i>ZmAMT1;1a</i>	-0.29 ^{cb}	0.38 ^b	1.07 ^{ab}	1.26 ^a	1.10 ^{ab}	1.32 ^a
<i>ZmAMT8</i>	-3.72 ^a	-4.19 ^{ab}	-4.71 ^{bc}	-4.19 ^{ab}	-5.16 ^c	-4.72 ^{cb}
<i>ZmAMT9</i>	-1.25	-0.99	-1.22	-0.85	-1.06	-1.23
<i>ZmDUR3</i>	-2.64	-2.24	-2.34	-2.93	-3.79	-2.05
<i>ZmNOD26-like</i>	1.62 ^a	0.76 ^b	1.83 ^a	1.64 ^a	2.13 ^a	1.94 ^a
<i>ZmNR</i>	5.09 ^a	-0.48 ^b	-0.85 ^{bc}	-0.37 ^b	-0.96 ^{bc}	-1.18 ^c
<i>ZmUrease</i>	0.41	0.49	0.44	0.23	0.57	0.57
<i>ZmGS3</i>	1.15 ^c	2.18 ^b	2.84 ^{ab}	2.93 ^a	2.72 ^{ab}	2.91 ^a
<i>ZmGLN1.2</i>	1.07 ^b	3.12 ^a	2.79 ^a	2.70 ^a	2.92 ^a	3.10 ^a
<i>ZmGLN1.4</i>	-2.94	-3.02	-3.16	-2.79	-3.67	-3.31
<i>ZmASNS3</i>	3.01 ^b	6.16 ^a	7.00 ^a	6.96 ^a	6.90 ^a	7.11 ^a
<i>ZmASNS4</i>	6.17 ^b	8.42 ^a	9.10 ^a	9.05 ^a	9.33 ^a	9.19 ^a
<i>ZmNIR2</i>	6.29 ^a	1.17 ^b	0.88 ^b	1.63 ^b	1.11 ^b	1.08 ^b
<i>ZmGS2</i>	4.04 ^a	-0.42 ^b	-0.19 ^b	-0.22 ^b	-0.03 ^b	0.04 ^b
<i>ZmGOGAT</i>	1.08	-0.12	0.71	0.81	0.42	0.78
<i>ZmGDH1</i>	1.46 ^a	0.89 ^{ab}	0.88 ^{ab}	1.12 ^{ab}	0.58 ^b	0.96 ^{ab}

Values are expressed in comparison to N-deficient seedlings, as Log₂ FC. Letters refers to statistical significance (Holm–Sidak test ANOVA, N=3, p-value < 0.05).

Ethics Statement

The analysis did not involve the use of human subjects and animal experiments or data collected from social media platforms.

CRediT Author Statement

Sara Buoso: Data curation, Writing-Original draft preparation; **Nicola Tomasi:** Data analyses, Writing- Reviewing and Editing; **Daniel Said-Pullicino:** Elemental analyses; **Mustapha Arkoun:** Conceptualization; **Jean-Claude Yvin:** Conceptualization; **Roberto Pinton:** Conceptualization; **Laura Zanin:** Conceptualization, Methodology, Data analyses, Writing-Reviewing and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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