

This is the peer reviewed version of the following article:

Ammonium-charged zeolite effects on crop growth and nutrient leaching: Greenhouse experiments on maize (*Zea mays*) / Campisi, T.; Abbondanzi, F.; Faccini, B.; Di Giuseppe, D.; Malferrari, Daniele; Coltorti, M.; Laurora, A.; Passaglia, E.. - In: CATENA. - ISSN 0341-8162. - 140:(2016), pp. 66-76. [10.1016/j.catena.2016.01.019]

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

19/12/2025 03:02

Ammonium-charged zeolite effects on crop growth and nutrient leaching: greenhouse experiments on maize (*Zea mays*)

[T. Campisi](#), [F. Abbondanzi](#), [B. Faccini](#), [D. Di Giuseppe](#), [D. Malferrari](#), [M. Coltorti](#), [A. Laurora](#), [E. Passaglia](#)

Highlights

- We describe the selection of zeolite/soil ratio with two greenhouse experiments.
- The effect of NH₄-charged zeolite on nitrate leaching and plant characteristics was assessed in lysimeters.
- We carried out a ranking approach of all parameters analyzed for overall evaluation.
- The applications of 30 or 50 t ha⁻¹ NH₄-charged zeolite may be scaled-up in open field studies.
- The reduction of chemical fertilizer was feasible, allowing a limitation in groundwater pollution by nitrates.

Abstract

Nitrate leaching and the resulting groundwater contamination from intensive crop production has become a major concern for long-term farmland efficiency and environmental sustainability in Italy. The aim of this study was to evaluate a water-saving NH₄-charged zeolite (produced by a new prototype) for minimizing NO₃-leaching from soil and optimizing corn growth and yield. Forty-eight zeolite:soil lysimeters in two trials were installed in a greenhouse to study the growth and yield characteristics of maize (*Zea mays*) as well as the nitrate concentration in leachate under different fertilizing conditions (i.e., standard, high or 70%, medium or 50% and low or 30% of conventional fertilization rate) and NH₄-charged zeolite (control, 0; dose-1, 50 t ha⁻¹ and dose-2, 100 t ha⁻¹) treatments. The results implicitly suggest that plants may have a better response if NH₄-charged zeolite is used with a limited amount of conventional fertilizer, allowing a reduction of nitrate concentration in drainage.

Keywords

Zeolite chabazite, Nitrate leaching, Maize growth, Ammonium, Fertilizer,

1. Introduction

Agriculture remains one of the main sources of water pollution, and farmers need to adopt more sustainable practices, as huge efforts are still required to an optimal water quality across the European Union (EU) and abroad ([Bijay-Singh et al., 1995](#), [Thorburn et al., 2003](#), [Jalali, 2005](#), [Islam et al., 2011](#)). Generally, farming is responsible for the major N-compound discharges into surface waters and groundwater, and still nowadays farming practices in all Europe use a large amount of chemical fertilizers and animal manure, with large regional differences ([Velthof et al., 2014](#)). Of the total nitrogen input in the fields, indeed, a large amount is not absorbed by the crops and resides in the soil ([Mastrocicco et al., 2013](#), [Wang et al., 2013a](#), [Sebilo et al., 2013](#)), where it could be converted into highly soluble nitrates and flushed away into the water system ([Mastrocicco et al., 2009](#), [Arbat et al., 2012](#), [Aschonitis et al., 2012](#), [Wick et al., 2012](#), [Wang et al., 2013b](#)), triggering different degenerative processes and ultimately causing eutrophication phenomena ([Del Amo et al., 1997](#), [De Wit et al., 2005](#), [Statham, 2012](#)). Moreover, when denitrification processes occur in soils ([Rivett et al., 2008](#)), greenhouse gases are released into the

atmosphere ([Smith et al., 2007](#), [Benbi, 2013](#), [Ding et al., 2013](#), [Skinner et al., 2014](#)). Livestock effluents, whose NH_4 concentration may exceed 1000 mg l^{-1} , are also often used as fertilizers as they can also improve soil fertility for crop production ([Marinari et al., 2000](#), [Khan et al., 2007](#)); it is known that intensive livestock breeding is another major source of nitrogen pollution in water ([Goldberg, 1989](#), [Williams, 1995](#), [Widory et al., 2004](#)) and it heavily contributes to CO_2 and methane emissions worldwide ([FAO, 2006](#)). With the Nitrates Directive ([Directive 91/676/EEC, 1991](#)) and the Water Framework Directive ([Directive 2000/60/EC, 2000](#)) the EU aims at preventing nitrate pollution by promoting the use of good farming practices and established a protocol for protection and management of water; reporting measures that must be taken by each Member State, aim to favor the restoration of hydrological resources and reach a good chemical and ecological state of waters, by reducing dumping and toxic substance emissions.

Several previous investigations ([Lehmann et al., 2003](#), [Novak et al., 2009](#), [Ding et al., 2010](#), [Islam et al., 2011](#), [Nelson et al., 2011](#), [Sarkhot et al., 2012](#), [Hale et al., 2013](#)) have been focused on mixtures of soil and artificial high-CEC fertilizers (i.e. biochars or coating materials) showing that they can reduce the leaching of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, that therefore implies that these nutrients are bound to them when they are added to soil, and no further transformation reactions take place. For example, applying 20 g kg^{-1} biochar to an agricultural soil amended with swine manure had decreased the leaching of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ by 11% and 69% respectively ([Laird et al., 2010](#)). However, it is currently unclear how long-lasting these effects are ([Hale et al., 2013](#)) and if some of them may be toxic to soil ([Azeem et al., 2014](#)).

Zeolitites are rocks containing more than 50% of zeolites ([Galli and Passaglia, 2011](#)), minerals characterized by high and selective cation exchange capacity (CEC), molecular absorption and reversible dehydration ([Ming and Allen, 2001](#)). Natural zeolites have a remarkable selectivity for cations characterized by low ionic potential (i.e., NH_4^+ , K^+ , Pb^{2+} , and Ba^{2+}) and, in particular, are capable to uptake NH_4^+ from solutions in various environment and then to release it under proper conditions ([Ahmed et al., 2006](#), [Passaglia and Laurora, 2013](#)), such as slow release fertilizer (SRF). Moreover, a single application of water-saving zeolitites to the soil can increase soil quality for several growth seasons, producing long-term changes in physical properties and reducing yearly water and nutrient requirements for crop growth. When zeolitites are incorporated with soil, they should retain large quantities of water and nutrients, which are released as required by the plant. Thus, plant growth could be improved with limited water and nutrient supply. Zeolitite cost depends on type and source, and ranges approximately from 2.5 cent per kg (clinoptilolite in Iran, [Gholamhoseini et al. \(2013\)](#)) to 10 cent per kg (chabasite in Italy, in this study).

In this context, ZeoLIFE project (LIFE + 10 ENV/IT/000,321; [Coltorti et al., 2012](#)) has been conceived to assess an innovative integrated zeolitite cycle aiming at (i) reducing the amount of traditional (both chemical and organic) fertilizers, (ii) amending the agricultural soils for economization of fertilizers and water for irrigation and with improvement of the yield, (iii) and ultimately leading to a reduction of surface water and groundwater pollution and excessive exploitation of the water resource. [Colombani et al. \(2014\)](#) showed that NH_4 -charged zeolitite increase the water retention capacity even in silty-clay soils, thus limiting water and solute losses.

This study describes the selection of soil/zeolitite ratio to be applied in Maize (*Zea mays*) cultivation through greenhouse experiments, as an ex-situ trial to be subsequently reproduced at large scale in an agricultural field. The NH_4 -charged zeolitite (NH_4CZ , hereafter), produced by prototype (IT application MO2013A000354), was mixed to the agricultural soil of the ZeoLIFE experimental field (Codigoro, Ferrara, Italy; [Di Giuseppe et al., 2013](#) and [Di Giuseppe et al., 2014](#)) and to an artificial standard soil in two trials respectively, and in different ratios, in order to evaluate the reduction of NO_3 -concentration in leachate that could drain directly to groundwater and to optimize maize production in comparison to traditional practice (with the chemical fertilizer application).

87 2. Materials and methods

88 2.1. NH₄-charged zeolitite

89 The natural zeolitite used in this study is composed mainly of chabasite and comes from Sorano (Grosseto,
 90 Central Italy); chemical and mineralogical composition and physio-chemical properties of natural zeolitite
 91 are reported in [Malferrari et al. \(2013\)](#). To obtain NH₄CZ, NH₄-exchange experiments between natural
 92 zeolitite (fraction with particle size less than 3.0 mm) and swine manure were carried out in static mode
 93 ([Vassileva and Voikova, 2009](#)) in laboratory ([Faccini et al., 2015](#)), and the findings were reproduced in large-
 94 scale application, in a prototype located in Codigoro (Ferrara, Italy) near the experimental field arranged for
 95 ZeoLIFE project ([Coltorti et al., 2012](#), [Malferrari et al., 2013](#)). Briefly, the prototype (supplementary
 96 information SI-1) is composed by a 2.2 m (ø) × 5.3 m (h) tank for the swine manure storage (about 10 m³).
 97 The loading of swine manure is performed using a pump that takes manure directly from the manure pool;
 98 250 kg of natural zeolitite is introduced from the top into the vessel and mechanically stirred with swine
 99 manure for 45 min. After a resting time (4–16 h), NH₄CZ is discharged and recovered opening the ball valve
 100 at the bottom of the tank. A vibrating sieving system was inserted at the bottom of the vessel to separate
 101 the different particle size of NH₄CZ, with a total daily production of 500 kg. At the end of each production
 102 cycle, NH₄CZ was stored, air dried in controlled open-air conditions and then periodically characterized
 103 ([Faccini et al., 2015](#)).

104 2.2. Experiment set-up and general methodology

105 This study was conducted at CRSA Med Ingegneria facilities, north east of Italy (WGS84: 44°28'50"N
 106 12°16'21"E), in a 60 m³ greenhouse (3.3 m × 9 m x h 2 m) in 2012 (spring and summer).

107 The effect of zeolitite on leachate quality (EC and Cl, nitrate and ammonium concentrations) and plant
 108 characteristics was performed in lysimeters of 24 cm in diameter and 30 cm in depth, with a stone layer
 109 and a drain pipe at the bottom, for water sample collection. The soil used in the greenhouse trials was
 110 collected in the ZeoLIFE experimental field and sieved at 2 mm; it is a silty clay soil with 41.9, 38.9, and
 111 19.2% of silt, clay and sand, respectively and about 8% of organic matter ([Di Giuseppe et al., 2014](#)). Main
 112 characteristics of Codigoro soil at the beginning of the study are listed in [Table 1](#), and are consistent with
 113 the typical composition of an agricultural soil in Ferrara district, with a high content of organic matter, a
 114 medium-high nutrient content and low permeability ([Bortolami and Giandon, 2007](#)).

115 Table 1. Main chemical and physical characteristics of the Codigoro (FE, Italy) soil employed in the
 116 experiments ([Di Giuseppe et al., 2014](#)).

Property	u.m	Bulk soil
Cation exchange Capacity (CEC)	meq 100 g ⁻¹	33.6
Exchangeable Ca²⁺	mg kg ⁻¹	5660
Exchangeable K⁺	mg kg ⁻¹	582
Exchangeable K (as K₂O)	mg kg ⁻¹	701
Exchangeable Mg²⁺	mg kg ⁻¹	401
Exchangeable Na⁺	mg kg ⁻¹	368
Total nitrogen	mg kg ⁻¹	2.7–17.7

Property	u.m	Bulk soil
Soluble K	mg kg ⁻¹	76.5
Soluble P (as P ₂ O ₅)	mg kg ⁻¹	175.3
Soluble Fe	mg kg ⁻¹	62.4
Soluble Mg	mg kg ⁻¹	6.2
Soluble Zn	mg kg ⁻¹	1.9
Soluble B	mg kg ⁻¹	1.61
Copper	mg kg ⁻¹	42.8

117 The soil amendments were broadcast applied to the soil depth of the 7L lysimeters and incorporated to the
118 total depth prior to the planting of crops. In this study, maize was selected over other crops in view of its
119 rapid growth cycle, responsiveness to changes in nutrient availability, and represents a typical crop in the
120 farming system of the Region (also related to animal feeding). Three seeds of maize were planted 4 cm
121 deep in each lysimeter and at 26 days after sowing (DAS), maize in each lysimeter was thinned to two
122 plants. The lysimeters were surface irrigated and scheduled with 2-day intervals and, during each irrigation
123 event, 15% more water was applied to allow water drainage for sampling. In this study, the irrigation was
124 performed in the same way in all the treatments.

125 2.3. Greenhouse experiments

126 Two experiments were performed with a randomized complete block experimental design using a complete
127 factorial arrangement of treatments. The aim of the first trial was to find out the best zeolite/soil ratio to
128 be applied in the second trial and in the next 3-years open field experiments, evaluating the effect of
129 zeolite on nitrogen leaching and on seed germination and development. The second trial was mainly
130 devoted to select the best fertilizer reduction after zeolite application, assessing the effect of the
131 treatment on nitrates concentration in leachate, plant growth and physiology.

132 The treatments in both trials mainly consisted of (i) two soil amendment types with NH₄-charged (NH₄CZ)
133 and natural (nZ) zeolite, (ii) two soil amendment doses of 10 g kg⁻¹ (dose-1) and 20 g kg⁻¹ (dose-2), and
134 (iii) different applications of chemical fertilizer. The soil amendment doses were selected on the basis of soil
135 type ([Ming and Allen, 2001](#), [Leggo et al., 2006](#), [Malekian et al., 2011](#)) and the cost-effectiveness of the
136 treatment ([Islam et al., 2011](#)). Each treatment was performed in quadruplicate and four not amended soil
137 lysimeters were used as a positive control. The nitrogen source, applied once at the beginning of the trials,
138 was urea (46% N). The reductions of urea respect to each trial control (6 and 11% in the first trial, and 30,
139 50 and 70% in the second trial) were established considering the average nitrogen content in NH₄CZ
140 (5.81 mg N g⁻¹). In the first trial, 5 treatments per 4 replicates (20 lysimeters) were conducted for 89 days
141 of the experiment. Simulating a high nitrogen fertilization of full field for corn (about 370 kg N ha⁻¹) along
142 the soil profile in lysimeters (25 cm), 248 ± 2 mg kg⁻¹ urea have been added to the soil for traditional
143 farming practice (positive control); then two reductions of 6 and 11% in two different treatments were
144 carried out. In particular, for two treatments (10CZ_u and 20CZ_u) urea was added compensating for the
145 amount of nitrogen absorbed as ammonium in NH₄CZ by the prototype process ([Coltorti et al., 2012](#)). In
146 the last two treatments (20CZ_wo and 20nZ_wo), no urea was added in order to observe the effect of
147 zeolite (both natural and NH₄-charged) on plant growth. The application of nZ and NH₄CZ in each
148 lysimeter was calculated on the basis of the dry weight and the depth of plowing. More in detail, assuming
149 a depth of homogeneous distribution of zeolite along the soil profile equal to 40 cm (depth of plowing),

dose-1 (10 g kg^{-1}) and dose-2 (20 g kg^{-1}) correspond to 5 kg m^{-2} (or 50 t ha^{-1}) and of 10 kg m^{-2} (or 100 t ha^{-1}) of zeolite in the field, respectively. In order to evaluate the best approach and select the optimum zeolite application, the treatments were:

- Intensive (I): traditional farming practice with 370 kg N ha^{-1} (positive control)
- 10CZ_u: dose-1 of fine NH₄CZ, with 349 kg N ha^{-1} (– 6% urea-N application)
- 20CZ_u: dose-2 of fine NH₄CZ, with 329 kg N ha^{-1} (– 11% urea-N application)
- 20CZ_wo: dose-2 of fine NH₄CZ, without nitrogen application
- 20nZ_wo: dose-2 of fine natural zeolite (nZ), without nitrogen application (negative control)

The second trial (Table 2) was conducted using an artificial soil, except for one treatment performed with the already used zeolite/Codigoro soil, coming from the first trial (10CZ_u), with the aim to simulate the second year of production. The artificial soil (Std) was composed by 1:1 Po river sand and peat of northern European origin (46% organic carbon, 0.7% organic nitrogen, pH 4). This trial was carried out with 7 treatments per 4 replicates (total of 28 lysimeters), lasting 73 days.

Table 2. Treatment description of the second trial in summer 2012.

Treatments							
Type	Control (C)	T1	T2	T3	T4	T5	T6
Bulk soil ^a	Std	Std	Std	Std	Std	TdC	Std
NH ₄ CZ (g kg^{-1})	0	10	10	10 ^b	0	10 ^c	6
Natural zeolite (g kg^{-1})	0	0	0	0	10	0	0
Urea (mg kg^{-1})	161 ± 5	113 ± 5	78 ± 3	47 ± 2	111 ± 5	75 ± 5	3.8 ± 0.1

No new addition of NH₄CZ was performed at the beginning of the second trial; TdC: Codigoro soil.

a Std: artificial standard soil.

b 80% fine NH₄CZ and 20% ultra fine ($< 90 \mu\text{m}$) NH₄CZ, collected in prototype.

c Residual NH₄-charged zeolite from first trial (treatment 10CZ_u).

In order to simulate a full range of nitrogen fertilization on maize compatible with the Nitrates Action Program of Emilia Romagna Region (NAP, 2011), 240 kg ha^{-1} of nitrogen (equivalent to about 522 kg ha^{-1} of urea) were provided as the Maximum Application Standard (MAS).

The following treatments were chosen in order to evaluate the best approach and, thus, select the best nitrogen application:

- Control (C): traditional farming practice with 240 kg N ha^{-1} (positive control)
- T1: dose-1 of fine NH₄CZ with 168 kg N ha^{-1} (– 30% urea-N application)
- T2: dose-1 of fine NH₄CZ with 120 kg N ha^{-1} (– 50% urea-N application)
- T3: dose-1 of fine and ultrafine ($< 90 \mu\text{m}$) NH₄CZ, with 72 kg N ha^{-1} (– 70% urea-N application)
- T4: dose-1 of fine nZ with 168 kg N ha^{-1} (– 30% urea-N application)

178 • T5: dose-1 of fine NH₄CZ, residual from first trial with the residual Codigoro soil, and 120 kg N ha⁻¹ (– 50%
179 urea-N application) used as long-effect test.

180 • T6: minimum dose of fine NH₄CZ with 7.2 kg N ha⁻¹ (– 97% urea-N application) (MAS-complying test)

181 The treatment T1, T2 and T3, with the same content of zeolite (dose – 1, 10 g kg⁻¹), were supplied with a
182 reduction of 30, 50 and 70% Urea-N compared to the positive control. Moreover, in T3, the zeolite was
183 applied, adding 80% of the zeolite in coarse “fine” form (< 3.0 mm), like in the other treatments, and 20%
184 of an “ultrafine” form, obtained operating an additional sieving at < 90 µm using the *in-situ* sieving
185 apparatus. This fraction has a greater specific surface area and a higher content of both ammonium and
186 phosphorus than the coarser fraction. T4 was performed like T1 but using natural zeolite (nZ), in order to
187 observe the effect of zeolite type on soil and plant growth.

188 In the treatment T5, the soil of Codigoro was reused, sowing again the soil of the treatment 10CZ_u of the
189 first trial, in order to evaluate possible effects of residual nitrogen. Moreover, this test was performed in
190 order to assess the lasting effects of the use of zeolite; in particular, we want to check if the zeolite, once
191 the absorbed ammonium was consumed by the first crop cycle, could be recharged through the application
192 of chemical fertilizers to the soil.

193 An additional treatment (T6) was carried out providing a minimum amount of zeolite (6 g kg⁻¹) and
194 supplying it with a minimal Urea-N dose (3%) so that the N content in NH₄CZ plus Urea-N complied with
195 regulation of fertilizer distribution (240 kg N ha⁻¹, used in positive control). Indeed, the amount of NH₄CZ
196 to be added was calculated considering its N content and a urea-like behavior, adding a small amount of
197 Urea (3%) in order to lead to germinate the seeds.

198

199 2.4. Data collection

200 The leached solution from each lysimeter was sampled every 20 days in order to assess the NH₄-N and NO₃-
201 N concentration in leachate. The two trials were stopped at 97 and 73 DAS (growing stage R3 and VT,
202 respectively, described by [Abendroth et al., 2011](#)), before the influence of lysimeter volume on root
203 elongation and crop height. During the growth monitoring, measurements of the aerial biomass (height in
204 cm from the base of the plant to the top of the upper leaf) were performed approximately every 20 days.
205 At the end of each trial, all the plants were collected from each lysimeter, oven dried at 70 °C until constant
206 weight was attained, in order to assess the production in term of aerial biomass (dry weight).

207 Moreover, at the end of the second trial (day 73), the photosynthetic activity (PN) and leaf chlorophyll
208 content (soil–plant-analysis development, SPAD value) were measured in 5 leafs per plant, with an ADC-
209 LCPro + instrument (for determination of CO₂ per leaf area and time unit) and a portable SPAD meter
210 (Model SPAD-502, Minolta crop, Ramsey, NJ), respectively. The SPAD meter measures the transmission of
211 red light at 650 nm, in which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at
212 which no absorption occurs. On the basis of these two transmission values, the instrument calculates a
213 SPAD value that is well correlated with chlorophyll content and used as an indirect indicator of crop N
214 status. Joined to the evaluation of the aerial biomass, a quantitative and qualitative morphological study
215 (relative growth rate, density/appearance of the root) was conducted.

216 Then, several macronutrients in the corn leaves of the second trial were measured according to
217 international standards ([ISO 5378, 1978](#) for N determination; [EPA 3051 A, 2007](#) and [ISO 11885, 2007](#) for
218 other macronutrients determination). Briefly, after oven drying at 70 °C for 24 h and homogenizing, the leaf
219 samples were assayed for total N (Kjeldahl method, modified as described in [Cataldo et al., 1974](#)), and after
220 microwave-assisted mineralization (MLS 1200 Mega, Milestone), for P, S, Ca, Mg, K and Na (by inductively

coupled plasma mass spectrometry, Thermofischer). In particular, the leaf N content is an important physiological parameter that indicates the plant N status ([Lemaire et al., 2008](#)).

2.5. Data analysis

Treatment significant differences were calculated at Fisher's least-significant difference (LSD) at p-level < 0.05 in one-way ANOVA ([SAS, 2008](#)). Duncan's multiple range tests (DMRT) was performed for multiple significance between the treatments.

3. Results

3.1. First greenhouse trial

3.1.1. Nitrogen concentration in leachate

Results of the first trial are reported in [Table 3](#). The initial concentration of NO₃-N in the leachate was strictly related to the urea addition, and has been quickly reduced in all treatments after seed germination (at 36 DAS). Moreover, the treatment with natural zeolite and no urea-N application (20nZ_wo) showed a residual N content, probably deriving from previous agricultural practices on the agricultural soil used in the trial ([Table 1](#)). No significant differences were observed in NH₄-N concentrations between treatments and control, showing a decreasing trend during the monitoring period.

Table 3. First trial: Trend of NO₃-N and NH₄-N concentrations in leachate, for four treatments and control (intensive, I). Mean of four replicates was reported for 5 sampling times (every 15–20 days). A high variability in measurements was observed with a coefficient of variation (CV%) ranging from 11% to 57% and from 8% to 50% for NO₃-N and NH₄-N, respectively. No significant differences were observed in NH₄-N and NO₃-N trends between treatments and control.

	NO ₃ -N (mg l ⁻¹)					NH ₄ -N (mg l ⁻¹)					
Treatment	DAS	23	36	54	72	89	23	36	54	72	89
Intensive (I)		90.9	83.5	45.0	4.2	1.3	2.95	0.27	0.15	0.06	0.15
10CZ_u		124.0	93.7	20.7	9.8	4.2	2.32	0.21	0.08	0.06	0.16
20CZ_u		95.9	153.3	17.7	7.6	2.2	0.54	0.23	0.07	0.04	0.15
20CZ_wo		84.1	50.9	15.6	5.4	1.6	2.20	0.15	0.07	0.04	0.12
20nZ_wo		65.3	40.3	24.9	10.1	1.3	0.17	0.17	0.07	0.04	0.14

3.1.2. Biomass production

For the production of aerial biomass (dry weight) measured at the end of cycle, the treatment 20nZ_wo and treatment 20CZ_wo had a production lower than the control (I) and the other treatments with NH₄CZ (10CZ_u and 20CZ_u) (p-level: 0.02, [Fig. 1](#)).

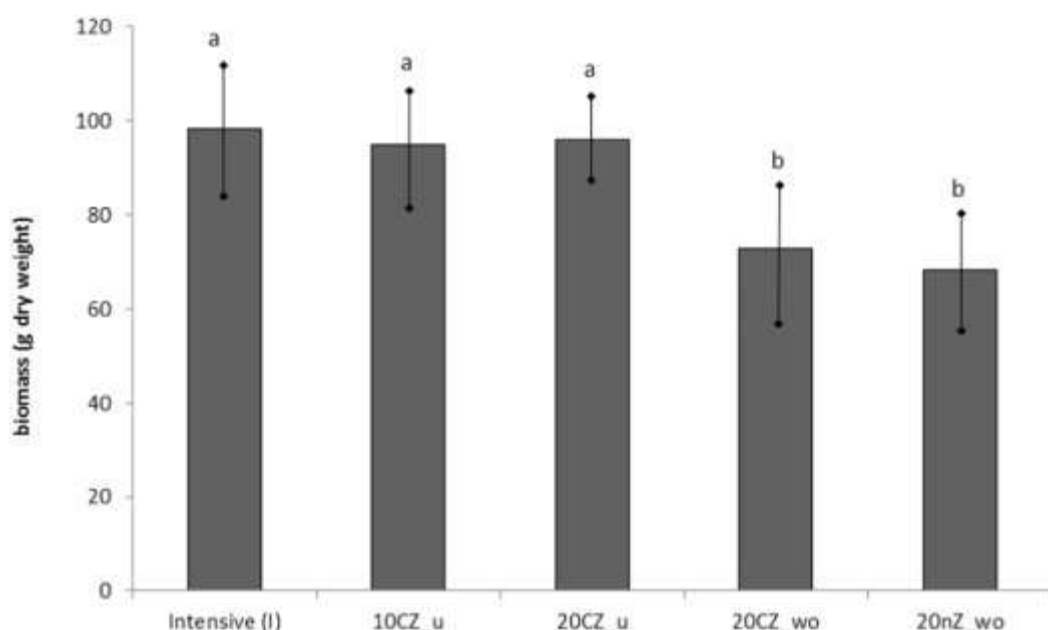


Fig. 1. Effect of treatments on the production of aerial biomass at the end of the first trial (dry weight). Values represent means \pm standard deviation ($n = 4$). Different letters indicate significant differences between treatments at the p-level < 0.05 .

3.2. Second greenhouse trial

3.2.1. Nitrogen concentration in leachate

In the second trial, the monitoring of leachate in the different treatments included the measurements of ammonium-N and nitrate-N concentrations, adding the measurements of conductivity and concentrations of chloride (Table 4).

Table 4. Water results for the second trial: trend of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ content, conductivity and chlorides in leachate for the six treatments and positive control. Mean \pm standard deviation of four replicates, except for (*) where three replicates were used.

Treatment	$\text{NO}_3\text{-N}$ (mg l^{-1})		$\text{NH}_4\text{-N}$ (mg l^{-1})		conductivity (mS cm^{-1})		Cl^- (mg l^{-1})	
	day 15	day 73	day 15	day 73	day 15	day 73	day 15	day 73
C	24.9 ± 5.4	4.6 ± 0.9	$1.2^* \pm 0.1^*$	$0.7^* \pm 0.1^*$	1.8 ± 0.3	1.1 ± 0.4	160.1 ± 95.5	154.1 ± 74.3
T1	35.6 ± 10.0	$17.5^* \pm 17.2^*$	3.1 ± 4.9	0.8 ± 0.2	2.2 ± 0.6	1.8 ± 0.5	175.7 ± 37.6	196.3 ± 76.8
T2	29.5 ± 7.6	$7.1^* \pm 2.6^*$	5.3 ± 4.4	1.3 ± 0.6	1.9 ± 0.7	1.4 ± 1.0	173.4 ± 13.8	151.9 ± 110.3
T3	32.3 ± 13.0	7.5 ± 2.9	0.5 ± 0.6	1.1 ± 0.7	1.5 ± 0.5	1.3 ± 0.2	164.5 ± 39.9	128.8 ± 46.7
T4	35.2 ± 13.6	4.2 ± 0.4	4.8 ± 8.3	0.9 ± 0.5	1.5 ± 0.3	1.5 ± 0.5	111.5 ± 25.2	153.8 ± 74.8
T5	20.0 ± 5.5	4.6 ± 0.4	0.02 ± 0.02	1.1 ± 0.7	1.4 ± 0.3	2.1 ± 0.5	120.8 ± 19.9	233.4 ± 75.7

	$\text{NO}_3\text{-N}$ (mg l^{-1})		$\text{NH}_4\text{-N}$ (mg l^{-1})		conductivity (mS cm^{-1})		Cl^- (mg l^{-1})	
Treatment	day 15	day 73	day 15	day 73	day 15	day 73	day 15	day 73
T6	28.8 ± 13.8	4.3 ± 0.4	0.11 ± 0.12	0.5 ± 0.1	1.4 ± 0.2	1.4 ± 0.5	155.1 ± 42.0	165.6 ± 119.9

As far as $\text{NO}_3\text{-N}$ concentration is concerned, no significant differences were found in 15 DAS between treatments and control. At the end of the experiment (73 DAS), in treatments T4, T5 and T6 a strong decrease occurred, reaching the value of the control; for the other treatments (T2 and T3), the decrease was moderate, while only treatment T1 was significantly higher. The nitrates were found lower than the regulatory limit in the majority of treatments (T2, T3, T4, T5 and T6) and in the control. In particular, considering treatments in order of decreasing nitrogen input, T6 (with low $\text{NH}_4\text{-charged}$ zeolite and N fertilization) had the significantly lowest nitrates content in water as expected.

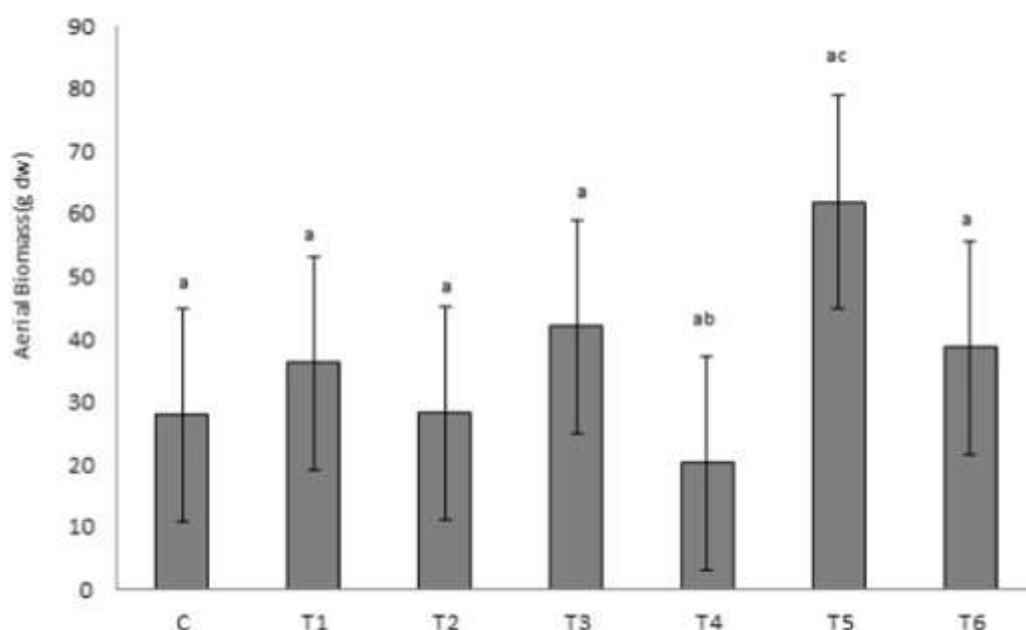
As regards the $\text{NH}_4\text{-N}$ content, in the first 15 days of the experiment, when the request of plant nutrients is not yet at the maximum, it can be observed a significantly lower concentration for the treatment with the highest urea reduction (T6) and in Codigoro soil (T5), compared to the positive control. Anyway, at 73 DAS, the amount of $\text{NH}_4\text{-N}$ (average $0.94 \pm 0.30 \text{ mg l}^{-1}$) leached from the lysimeters was unaffected by the amendment dose.

Conductivity remained stable in the leachate of all treatments with the only exception of T5, where an increase, probably linked to the leaching of the chloride present in the experimental field soil, had been observed. For the whole duration of the test, the pH was maintained at constant values for all treatments (7.5 ± 0.2).

276

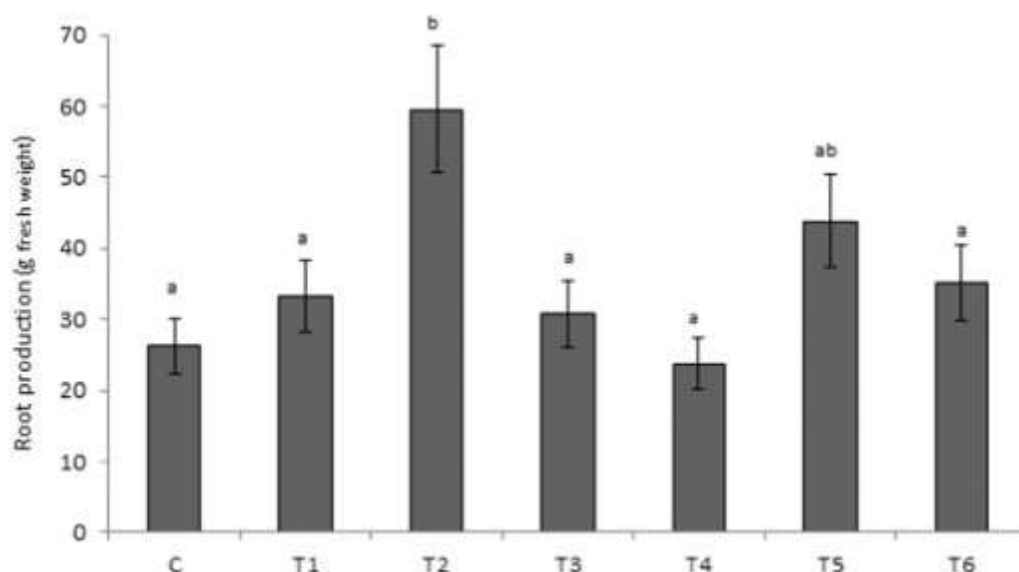
3.2.2. Biomass production

Final growth and root production of the corn grown under the different fertilization treatments are shown in [Fig. 2](#), [Fig. 3](#).



280

281 Fig. 2. Effect of treatments on the production of aerial biomass at the end of the second trial (dry weight).
 282 Values represent means \pm standard deviation ($n = 4$). Different letters indicate significant differences
 283 between treatments at the p -level < 0.05 .



284
 285 Fig. 3. Effect of treatments on the production of root biomass (fresh weight) in second trial. Different
 286 letters indicate significant differences between the treatments (p -level < 0.05).

287 At the end of the experiment, as far as the production of aerial biomass (dry weight) is concerned, the
 288 differences among treatments with the same artificial standard soil were not significant ([Fig. 2](#)). At the
 289 same time, there was no significant difference between artificial and Codigoro soil (T5), except for T4 with
 290 natural zeolite, which had the lowest production.

291 Moreover, the different fertilization treatments did not affect the root biomass (fresh weight) of the plants
 292 ([Fig. 3](#)), at either the normal or lower dose. This parameter was differed only for the treatments T5 and T2,
 293 both carried out with the 50% urea reduction and 10 NH₄CZ. Furthermore, T2 with artificial soil has yielded
 294 an even greater effect compared to T5 with agricultural soil, as expected.

295 In [Fig. 4](#), the assessment of the roots involved (i) the measurement of root biomass (dry weight) and (ii) the
 296 morphological analysis, considering the total length of roots, the number of primary roots and absorbent
 297 and the radical diameter. Considering these parameters, the treatment T5 showed the highest root
 298 biomass (dry matter), followed by T1 and T3. Other treatments induced significantly lower total production
 299 of roots.

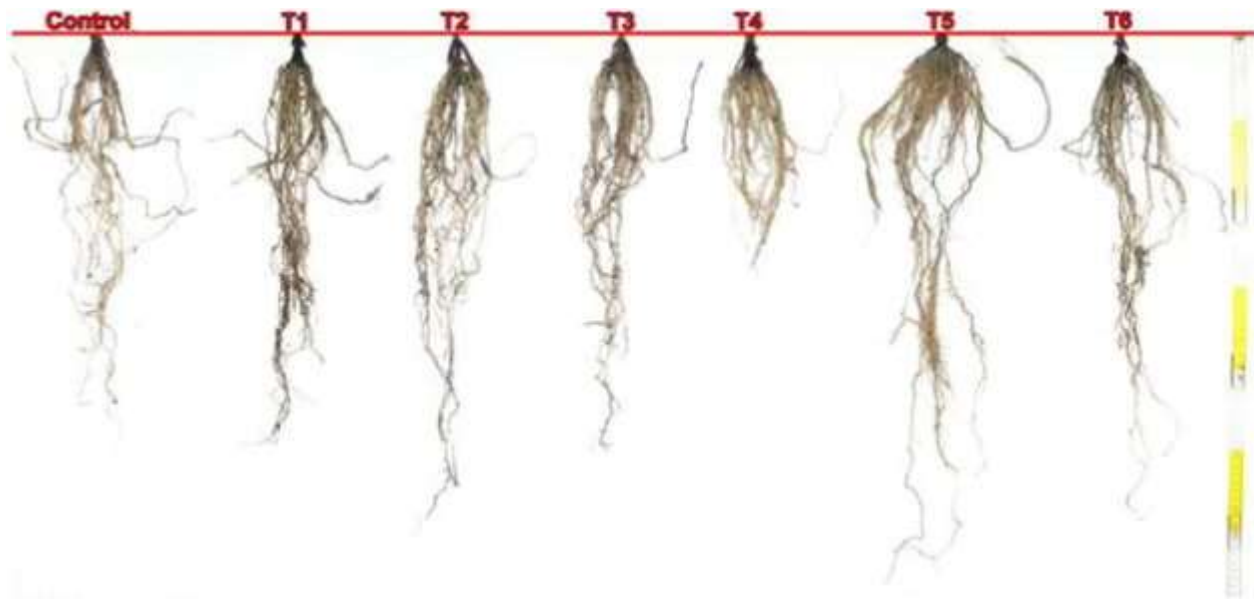


Fig. 4. Example of radical apparatuses of one plant in the 6 treatments and the control, at the end of the second trial. The roots have been cleaned, washed and air dried in order to observe type, elongation and structure.

The treatment T5 showed an impetus in the radical development already in the earliest stages of growth, when the volume of the primary structures was defined, that was maintained in the subsequent stage of production. As far as the architecture and hierarchical organization structures are concerned, T5 showed again features fully different from other treatments, developing a reduced amount of primary roots in the first crown, but having the greatest diameter. Furthermore, it is interesting to observe that the control (C) presented a reduced development in terms of accumulated biomass and minimum root diameter, with respect to the others.

Considering the treatments with artificial soil, T4 and T6 had the lowest number of roots in the first crown and the smallest average diameters, showing a behavior similar or lower than the control. Conversely, T1, T2 and T3 showed an overall increase of the primary structures and root biomass.

3.2.3. Measurements of the photosynthetic activity and chlorophyll content of plants

The leaves of the control C and T1 showed a greater net photosynthesis (PN), up to $30 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 5), while the treatments T2 and T3 recorded values around 25. The treatments T4, T5 and T6 showed PN values significantly lower than the other ones, in particular the treatment with Codigoro soil (T5) with the lowest values ever (just over 10).

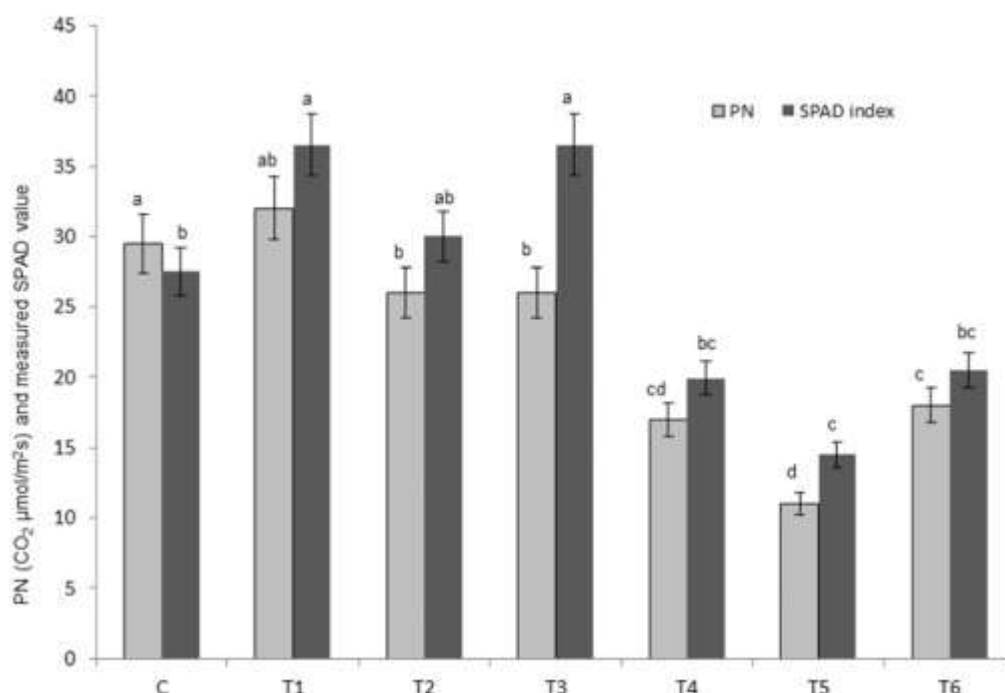


Fig. 5. Crop growth evaluation in the second trial on the basis of photosynthetic activity (PN) in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and SPAD index. Values represent means \pm standard deviation ($n = 4$). Different letters indicate significant differences between treatments at the p -level < 0.05 .

The SPAD index, which indicates the intensity of the green leaf area, is related to the presence of nitrogen and chlorophyll ([Yang et al., 2014](#)). Very low indices were found in T4, T5 and T6. In particular, the SPAD index in T5 was found close to 15, less than half compared to T1 and T3. Moreover, T1 and T3 showed a SPAD index significantly higher than the control, leading to suppose a positive effect of NH_4CZ on N availability. Effectively, during leaf senescence, the rapid drop in leaf SPAD readings is suppressed in plants subjected to the higher N application ([Yang et al., 2014](#)).

3.2.4. Macronutrients in leaves

Regarding the macronutrients in leaf at 73 DAS ([Fig. 6](#)), it can be observed that the concentrations of phosphorus, potassium, sulfur, calcium, magnesium and sodium were comparable in all treatments, favoring a good level of biomass growth, similar to the control. On the other hand, N leaf content was significantly higher in treatments T1, T2, T3, (containing NH_4CZ and a fertilizer reduction), than all the other treatments and the positive control. Moreover, across the fertilization regimes, 10 NH_4CZ increased N leaf from $23,100 \pm 2200$ up to $27,600 \pm 1700 \text{ mg kg}^{-1} \text{ dw}$, corresponding to a 1.2 and 1.8% for T1 (70% urea application) and T2 (50% urea application) respectively. Conversely, for T4, T5 and T6, the nitrogen content less than 1% suggests a suffering situation, with limitation on plant growth. Indeed, a typical growth maize stage presents 2.4% N leaf content at 75 DAS and 1.1% or more at 105 DAS, at the final stage ([Tajul et al., 2013](#), [Ahmed et al., 2008](#), [Jones et al., 2012](#), [Tejada and Benitez, 2011](#)).

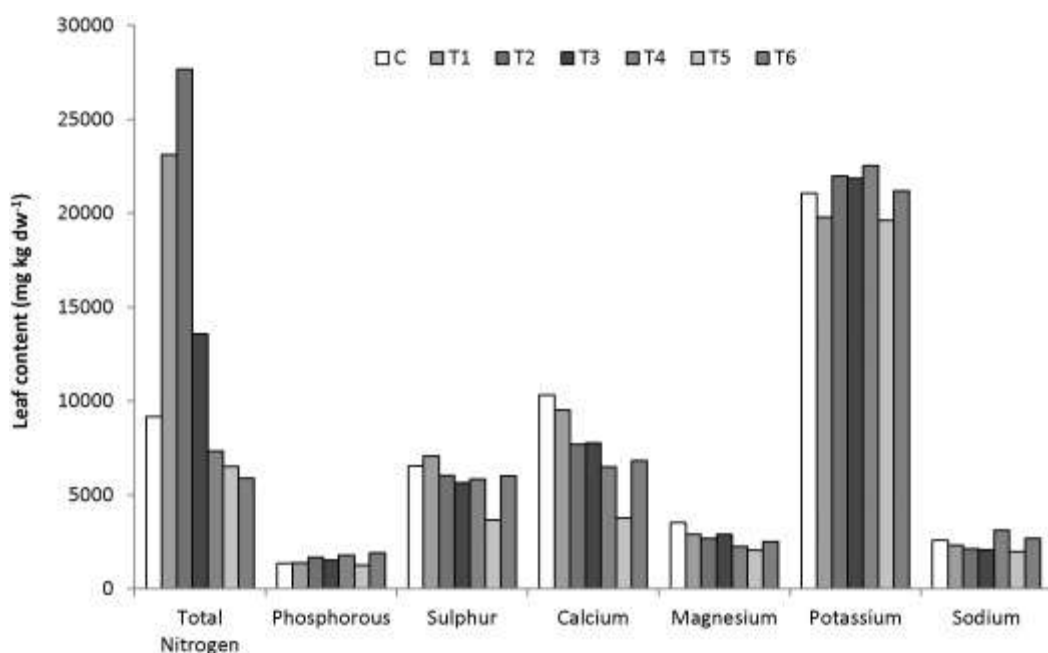


Fig. 6. Analysis of macronutrients in the corn leaves at 73 DAS, after harvest in the second trial. Optimal nitrogen content is set at 2% (20,000 mg kg⁻¹) while the sufficient level at 1% (Tajul et al., 2013, Ahmed et al., 2008, Jones et al., 2012). Calcium leaf content showed a significant difference between T5 and T1 (same urea application in two different bulk soil, p-level: 0.005) and between T5 and Control (p-level: 0.0004). All other compounds did not show significant difference among treatments (p-level > 0.05).

Phosphorous leaf content was not affected by the amount of fertilizer and there were no differences among the zeolite doses assayed, which showed a similar P leaf level (about 1550 mg kg⁻¹ dw), in line with other studies (about 1300 mg kg⁻¹ dw by Tejada and Benitez (2011) up to 2600 mg kg⁻¹ dw by Lazcano et al. (2011)). On the other hand, the K leaf content was higher in all the treatments of this study (average value of 21,100 mg kg⁻¹ dw) than those by Lazcano et al. (2011) and by Tejada and Benitez (2011), where a mean value of 13,500 mg kg⁻¹ dw was observed. Calcium leaf content was about 7.7% in all the treatments with artificial standard soil and low urea application (T2, T4 and T6), while the treatment with Codigoro soil and low urea application rate (T5) showed a significant lower Ca leaf content, more than half of treatment T1 with the same urea application on artificial soil (9.5%) or in the control C (10.3%). A similar trend was also observed for Mg leaf content; the Mg: Ca ratio was about 1:3 for the control and all treatments with artificial standard soil, and 1:1.8 for the treatment by Codigoro soil (T5). No marked changes were noted in sodium and sulfur content following the application of zeolites, corresponding to the standard leaf content at 73 DAS.

4. Discussion

4.1. First greenhouse trial

4.1.1. Nitrogen concentration in leachate

In this study, the nitrate concentration in drainage water in all treatments was over 60 mg l⁻¹, with a decreasing trend in 89 DAS for all treatments, without significant differences, reaching a low level (less than 5 mg l⁻¹) in 89 DAS. It is important to notice that, in the treatment 20nZ_wo, where any N source was supplemented, as occurred for NH₄-N, the nitrates were still present from 65 mg l⁻¹ to 1.3 mg l⁻¹. This could support the hypothesis of an effect of residual N fertilization coming from earlier crop years and supplied by the test soil: this could suggest an incomplete consumption of N by the crops previously seeded. This

371 residual N could have allowed the maize growth (1.22 cm day^{-1}) although lower than in the other
372 treatments (up to 1.34 cm day^{-1}).

373 The main result of the first trial was that applying the dose 10 g kg^{-1} of NH_4CZ and reducing urea
374 fertilization may offer a significant advantage by limiting the leaching of $\text{NO}_3\text{-N}$, and maintaining a good
375 crop growth rate. In this study, the phenomenon reported by [Ahmed et al. \(2006\)](#) where the inclusion of
376 1 g kg^{-1} zeolite have improved the soil retention of NH_4 as well as minimizing the conversion of NH_4 to
377 NO_3 was not observed, probably due to the tenfold lower urea addition (2 g kg^{-1} in [Ahmed et al. \(2006\)](#) and
378 about 0.2 g kg^{-1} in this study).

379 **4.1.2. Biomass production**

380 The fertilization regimes containing NH_4CZ and N fertilizer did not produce significant differences in plant
381 biomass with respect to the conventional fertilizer alone. However, the integrated fertilization regimes
382 (with urea application) produced differences in the plants, as the biomass of plants grown with integrated
383 organic fertilizer (20CZ_u) was significantly greater than this one grown with only NH_4CZ (20CZ_wo). This
384 suggested that an N integration with N fertilizer should be necessary even when NH_4CZ is used.

385 **4.2. Second greenhouse trial**

386 On the basis the outcomes from trial 1 and the economic viability, dose-1 was considered in the second
387 greenhouse trial, and then in the subsequent open-field experiments of ZeoLIFE project. Since Codigoro soil
388 contains minor amount of nitrogen in various chemical forms that can affect, though minimally, the
389 experimental results, in the second trial an artificial standard soil without any nitrogen residual source was
390 used, in order to observe the actual potential of zeolite.

391 **4.2.1. Nitrogen concentration in leachate**

392 The findings of the second trial showed that the nitrate concentration in water was significantly similar in
393 the treatments and in the control, except for the highest value at 73 days in T1 where 70% urea-N was
394 applied. Probably the high Urea-N content could contribute to maintaining a high level of nitrates in
395 leachate, also considering the low root production in the crop of this treatment. As regards the $\text{NH}_4\text{-N}$
396 content, after an initial difference in two treatments (T3 and T5) respect to the other treatments and the
397 positive control, the amount of $\text{NH}_4\text{-N}$ in drainage water was unaffected by the amendment dose and N
398 fertilization.

399 **4.2.2. Biomass production, photosynthetic activity and macronutrients in leaves**

400 Regarding crop production, for all fertilization treatments and zeolite doses assayed with artificial soil, no
401 significant changes in the production of aerial biomass were noted, while the treatment with Codigoro soil
402 showed the taller plants. The same results were found for root biomass, which only T2 determined a
403 significant difference compared to all other treatments, with the same artificial soil. Remarkably, T5 with
404 the same urea reduction of T2 (-50%) but with Codigoro soil is not significantly different to T2 and yielded
405 a good effect on root elongation.

406 As far as crop quality is concerned, the macronutrients content, except for nitrogen, in leaf was performed
407 at the end of the second trial, testify an overall good leaf health. Indeed, differences in N leaf content
408 subjected to varying NH_4CZ and urea application rates were evident during our observation: the 2.5% N
409 leaf content in T1 and T2 led to suppose the possibility to increase the production, while for the other
410 treatments it was less than 1%, suggesting a suffering situation, with limitation in plant growth. This
411 demonstrated that the unique mineral properties of chabazite zeolites, including high CEC and high affinity
412 for NH_4 + ([Malferrari et al., 2013](#)) significantly increased the N uptake by plants.

413 This was confirmed by the measurements of the photosynthetic activity and leaf chlorophyll content
414 (SPAD). In particular, SPAD index, related to the presence of nitrogen and chlorophyll in the leaf ([Yang et](#)
415 [al., 2014](#)), was very low in treatments with low amount of NH₄CZ or N-fertilizer (T4, T5 and T6). In
416 treatment T5, simulating the second year of sowing on used NH₄CZ, the SPAD index was close to 15 and the
417 N leaf content less than 10%, representing a typical situation of N lack ([Yang et al., 2014](#)). Moreover, the
418 color of the leaves in T5 was yellow indicating a chlorosis, process in which the leaves produce insufficient
419 chlorophyll, even if the plants were taller than those of the control and the other treatments. Even the
420 roots in T5 were the most developed, another reason could be attributed to stress in plants whose root
421 systems had already filled the volume of the container. At 52 DAS, the crop growth in T5 was higher than
422 19.57 cm at 40 DAS found in the field by [Singh et al. \(2014\)](#), and then drastically decreased probably due to
423 the effect of lysimeter volume. It can be supposed that plants in T5 had good availability of nitrogen at the
424 beginning of crop cycle (first 52 DAS) and the residual nitrogen of NH₄CZ was adequate for the
425 development of plants: in this case, it was difficult to discriminate between the role of the nitrogen
426 released by NH₄CZ (slow process) and that released by the Urea-N (ready-to-use). Anyway, in this study,
427 the Urea-N reduction of 50% in the second crop year could be a limitation for crop growth, even if the
428 NH₄CZ was present and could still support the crop development.

429 Focusing on the group of treatments based on artificial soil, T4 and T6 had produced a smaller radical
430 development and considerably more simplified from an architectural point of view (therefore less
431 efficient); measures of photosynthesis and SPAD index are in agreement with this behavior, also confirmed
432 by the reduced production of aerial biomass and root, at least for plants in T4.

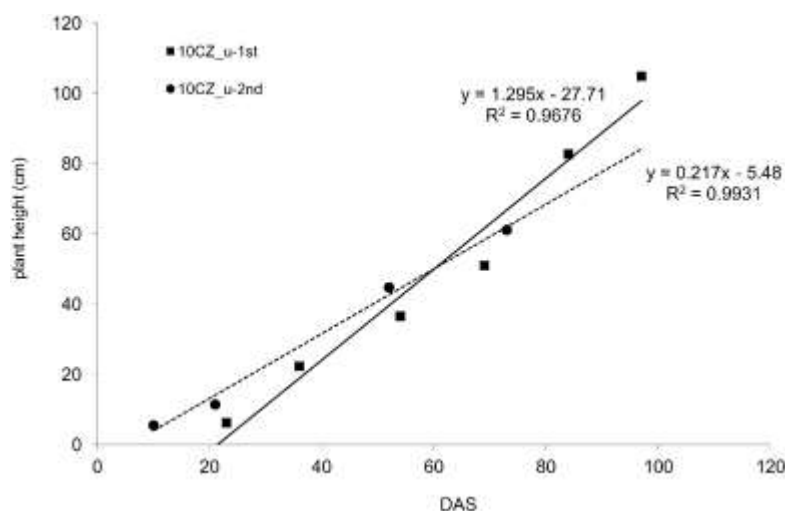
433 When natural zeolite was added (T4), some negative effects on plant physiology were observed and could
434 be partially explained by a “locking” of ammonia nitrogen by nZ, as reported by [Ahmed et al. \(2008\)](#). During
435 the initial step of crop cycle, NH₄-N probably was not ceased to plants in sufficient quantities, also as a
436 consequence of the limited Urea-N supply (– 30%). It has to be noticed that the nitrogen resulting from the
437 hydrolysis of urea was in the ammonia form and it represented the only source of this element in the
438 artificial soil for plants of maize (very demanding in nitrogen).

439 The reduced performance of T6 could be explained by the lower concentration of NH₄CZ and the minimal
440 application of Urea-N added to the substrate sand-peat (up to 10–20 times less compared to the other
441 treatments). Control, T1, T2 and T3 had maintained a good photosynthetic efficiency and chlorophyll
442 content, even in the last days of the crop cycle. However, the plants of the control C, despite the full supply
443 of urea, showed a significantly lower production of biomass and a more simplified radical organization with
444 respect to treatments T1, T2 and T3: this can be probably related to the presence of NH₄-charged zeolite
445 into the latter phase of crop cycle, and to its role in increasing water retention and nutrients in a naturally
446 poor substrate.

447 **4.3. Two crop years in Codigoro soil — trial 1 and 2**

448 One of the aims of ZeoLIFE project was to assess the long-term effect of zeolite, when only one
449 application of NH₄CZ in soil is enough for improving soil texture and maintaining its capability to exchange
450 cations with the plant roots over time. In order to simulate the effect of zeolite on plant growth for almost
451 2 crop years, the treatment 10CZ_u of first trial (hence called 10CZ_u-1st) was fertilized (reducing nitrogen
452 rate up to 50%) and sowed again in the second trial (T5, hence called 10CZ_u-2nd). The fertilization with
453 urea was required due to low content of residual nitrogen in the soil, after maize production in the first
454 trial. Moreover, it should be considered the contribution of the Codigoro soil, in relation to the nutrient
455 availability, as well as to an initial remarkable, content of macro-and micro-nutrients (as shown by chemical
456 analysis), compared to the artificial soil, constitutionally inert from the chemical point of view.

457 The comparison between 10CZ_u-1st and 10CZ_u-2nd (Fig. 7) showed a lower growth rate (0.92 cm day^{-1})
 458 in the second trial than in the first one (1.30 cm day^{-1}), probably due to a higher consumption of nitrogen
 459 (not present in leachate) by maize with respect to other plants of other studies. Indeed, [Gholamhoseini et](#)
 460 [al. \(2013\)](#) found an increase of sunflower yield during the two years experiment in open field. On the other
 461 hand, a study with natural zeolite and forage species demonstrate that the enhance forage yield was
 462 obtained by enhancing N fertilizer application ([Gholamhoseini et al., 2012](#)).



463

464 Fig. 7. Growth rate of two maize productions in Codigoro soil with 10 g kg^{-1} NH_4CZ and Urea-N progressive
 465 reduction (94.4% in 10CZ_u_1st and 50.0% in 10CZ_u_2nd). The comparison between first (square) and
 466 second trial (dot) allowed the assessment of the simulation of two crop years on the same NH_4CZ
 467 application, performed only in the first trial.

468 Furthermore, the comparison between first and second trial ([Table 5](#)) showed a downward trend of the
 469 final growth in terms of biomass and roots, in comparison to the control (I). Despite lower plant growth in
 470 the second trial, the N content in leachate reached the same value in both treatments, even with a
 471 significant reduction of urea (50% in 10CZ_u-2nd).

472 Table 5. Production assessment of Maize crop in Codigoro soil treatments and $\text{NO}_3\text{-N}$ concentration in
 473 leachate. Comparison of data collected in trial 1 and 2. The control treatment to be considered was the
 474 Intensive (I) in trial 1, with Codigoro soil and with 370 kg N ha^{-1} .

Treatment	Trial	DAS	Plant height (cm)	Aerial biomass ($\text{g}_{\text{fw}}^{\text{a}}$)	Roots ($\text{g}_{\text{fw}}^{\text{a}}$)	$\text{NO}_3\text{-N}$ in leachate (mg l^{-1})
Intensive (I)	1	89	106.4 ± 18.7	276.6 ± 25.5	533.7 ± 256.6	1.3 ± 0.3
10CZ_u-1st	1	89	104.9 ± 12.9	309.1 ± 63.0	430.0 ± 180.7	4.2 ± 2.8
10CZ_u-2nd	2	73	61.1 ± 13.9	128.4 ± 13.0	186.1 ± 43.8	4.6 ± 0.4

475 a fw: fresh weight.

476 4.4. Zeolite dose selection

477 In order to achieve an overall evaluation of all parameters analyzed in the second trial, a ranking approach
 478 was carried out ([Table 6](#)). As determined by [Liu et al. \(2014\)](#), using three parameters for amendment dose
 479 definition, three macro-groups of parameters were considered in order to evaluate the leaching process,
 480 the crop production and the crop quality before harvest. For each macro-group, three parameters were
 481 selected, respectively: (i) nitrates, ammonia and chloride content in drainage at 73 DAS for the leaching
 482 process, (ii) maize growth rate, aerial biomass and root elongation for crop production and (iii) N leaf

content, SPAD and PN activity for crop quality. Considering the positive control as a target for treatment evaluation, each parameter was compared to the control value by calculating the control/treatment ratio for leaching process parameters and treatment/control ratio for the other two macro-groups parameters, whereas the value greater than 1 as good result.

Table 6. Evaluation of the six treatments of the second trial (T1–T6, described in [Table 2](#)). The final score was obtained by the formula (1), where the single ratio of each parameter was weighted depending by type (weight 1 for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, Cl^- ; weight 0.5 for growth rate, aerial biomass and roots; weight 1.5 for N leaf content, SPAD and Chlorophyll-a content). The ratio versus control for each treatments was calculated considering the analytical results before harvest. When the ratio is > 1 , the treatment had a performance better than the control, when < 1 the worst. The ranking was “++++” for the best and “+” for the worst. T4 and T5 had very close final value so they obtained both the worst ranking (+).

Treatment	Leaching process			Crop production			Crop quality			Final score	Ranking
	NO ₃ -N	NH ₄ -N	Cl ⁻	Growth rate	Aerial biomass	Roots	N leaf content	SPAD	Chlorophyll <i>a</i> content		
C	1	1	1	1	1	1	1	1	1	9.0	/
T1	0.2	4.7	0.8	1.4	1.3	1.5	2.5	1.1	1.3	15.1	+++++
T2	0.1	2.6	1.0	1.2	1.0	1.4	3.0	0.9	1.1	13.1	+++
T3	0.6	3.1	1.2	1.5	1.5	1.7	1.5	0.9	1.3	12.8	++
T4	1.1	4.1	1.0	1.0	0.7	0.9	0.8	0.6	0.7	10.7	+
T5	1.0	3.3	0.7	1.7	2.2	2.9	0.7	0.4	0.5	10.8	+
T6	1.1	7.0	0.9	1.2	1.4	1.3	0.6	0.6	0.7	13.9	++++

The final score for each treatment was calculated using the formula:(1)

Where a_i the parameter weights, and y_i the ratio of parameters.

In this study, in order to assess the effect of NH_4 -exchange zeolite on the N process in soil and in the plant growth, the quality of the leachate and crop quality were considered very important, so the weight α was 1 and 1.5, respectively, while 0.5 weight was attributed to the crop production. Indeed, these experiments have been designed to observe any effects on crop development and the variables related to crop quality are of highest importance. Crop production is less important because the experiment does not comply with the conditions to simulate a real field experiment. In the variables related to leaching process, although Cl^- and nitrate concentration in drainage are of higher relevance than NH_4^+ , ammonium was assigned with the same weight because $\text{NH}_4\text{-N}$ reflected the behavior of the NH_4 -charged zeolite added to the soil. Indeed, considering that NH_4CZ exchange mainly NH_4^+ , nitrogen concentration is an indicator of NH_4CZ effect for the dose selection. In an open field experiment, due to the influence of many factors on these parameters, different weights should be defined and more importance should be given on crop production. Moreover, the water quality, coming from soil drainage, will be compare to regulation limits (i.e. 50 mg l^{-1} for nitrate concentration ([WHO, 1993](#))).

The ranking allowed a first selection of the best management practice compared to the traditional farming practice (positive control), to be performed in the field experiment. In particular, it was clear that the

511 application of NH₄CZ at 50 t ha⁻¹ (dose-1) plus 70% of standard fertilization or NH₄CZ at 30 t ha⁻¹ plus 3%
512 of standard fertilization (MAS-complying test) could both achieve higher results than conventional fertilizer
513 rate. This led to suppose that NH₄CZ gave a good contribution in N-availability during crop growth. Among
514 treatments with dose-1 of NH₄CZ, also the treatment T2 was a feasible solution, with 50% of conventional
515 fertilization. This was confirmed by the findings of T5, with the agricultural soil and two crop years (high
516 reduction in NO₃-N leaching and good crop production), although its score was low but even higher than
517 control. Also T4 was found with a lower score, but even higher than control, thanks to the good effect of
518 the natural zeolite on NO₃-N leaching and soil texture correction. Thus, considering the low content of
519 natural zeolite (50 t ha⁻¹) and the reduction of 30% fertilization, the treatment T4 could be also selected
520 for the open field activities of ZeoLIFE project. Similar results were obtained by [Liu et al. \(2014\)](#), using 30
521 and 40 t ha⁻¹ biochar amendment with about 6 g N kg⁻¹.

522 **5. Conclusions**

523 The study showed that the application of NH₄-charged zeolite to highly productive agricultural land had no
524 negative consequences in terms of crop growth and nutrition and may even provide high agronomic
525 benefits with lasting effect on soil properties. The lack of negative effects seen at application rates of either
526 30 or 50 t ha⁻¹ also suggested that the applications of NH₄-charged zeolite may be scaled-up in open field
527 studies with agricultural soils consisting of low permeability materials with naturally high content of organic
528 matter. Moreover, the reduction of chemical fertilizer was feasible, even at high degree, allowing a
529 reduction in groundwater pollution by nitrates. This demonstrated that the NH₄CZ behavior is different
530 with respect to chemical fertilizer one and the N content in NH₄CZ should not be considered an equivalent
531 of Urea-N. Thus, the maximum amount of NH₄CZ to be applied to soil could be selected on the basis of soil
532 type and not on the MAS regulation for fertilizer (for example, 240 kg N ha⁻¹ for maize). These results may
533 suggest that the employment of synthetic fertilizers foreseen for the different production regulations may
534 be revised downwards when they are associated with the use soil conditioners such as zeolite.

535 **Acknowledgments**

536 This work was undertaken using funds provided by the European Union under LIFE + “Environment Policy
537 and Governance” 2010, supporting the ZeoLIFE project (Project No. [ENV/IT/000321](#)). We want to thank
538 Prof. Davide Neri by University Politecnica delle Marche (Italy), for the measurements of the
539 photosynthetic activity and chlorophyll content, and PhD. Carlo Ponzio for his support and his work for the
540 experimental design.

541 **References**

542 [Abendroth et al., 2011](#)

543 L.J. Abendroth, R.W. Elmore, M.J. Boyer, S.K. Ma r l ay

544 **Corn growth and development**

545 PMR 1 009, Iowa State University Extension, Ames, Iowa (2011)

546 O.H. Ahmed, H. Aminuddin, M.H.A. Husni

547 **Reducing ammonia loss from urea and improving soil-exchangeable ammonium retention through mixing** 548 **triple superphosphate, humic acid and zeolites**

549 Soil Use Manag., 22 (2006), pp. 315-319

550 O.H. Ahmed, H. Aminuddin, M.H.A. Husni, A.A. Rahim, N.M.A. Majid

551 **Enhancing the Urea-N use efficiency in maize (*Zea mays*) cultivation on acid soils amended with zeolite**
552 **and TSP**

553 Sci. World J., 8 (2008), pp. 394-399

554 G. Arbat, A. Roselló, D. Olivé, F. Puig-Bargués, J.E. González Llinàs, M. Duran-Ros, J. Pujol, F. Ramírez de
555 Cartagena

556 **Soil water and nitrate distribution under drip irrigated corn receiving pig slurry**

557 Agric. Water Manag., 120 (2012), pp. 11-22

558 V.G. Aschonitis, M. Mastrocicco, N. Colombani, E. Salemi, N. Kazakis, K. Voudouris, G. Castaldelli

559 **Assessment of the intrinsic vulnerability of agricultural land to water and nitrogen losses via**
560 **deterministic approach and regression analysis**

561 Water Air Soil Pollut., 223 (2012), pp. 1605-1614

562 B. Azeem, K. KuShaari, Z.B. Man, A. Basit, H.T. Thanh

563 **Review on materials & methods to produce controlled release coated urea fertilizer**

564 J. Control. Release, 181 (2014), pp. 11-21

565 D.K. Benbi

566 **Greenhouse gas emissions from agricultural soils: sources and mitigation potential**

567 J. Crop Improv., 27 (2013), pp. 752-772

568 Bijay-Singh, Yadvinder-Singh, G.S. Sekhon

569 **Fertilizer-N use efficiency and nitrate pollution of groundwater in developing countries**

570 J. Contam. Hydrol., 20 (1995), pp. 167-184

571 P. Bortolami, P. Giandon

572 **L'interpretazione Delle Analisi del Terreno — Strumento per la Sostenibilità Ambientale**

573 ARPAV (2007)

574 (ISBN 88-7504-115-6)

575 D.A. Cataldo, L.E. Schrader, B.L. Youngs

576 **Analysis by digest and colorimetric assay of total nitrogen in plant tissue high in nitrate**

577 Crop Sci., 14 (6) (1974), pp. 854-856

578 N. Colombani, M. Mastrocicco, D. Di Giuseppe, B. Faccini, M. Coltorti

579 **Variation of the hydraulic properties and solute transport mechanisms in a silty-clay soil amended with**
580 **natural zeolites**

581 Catena, 123 (2014), pp. 195-204

582 M. Coltorti, D. Di Giuseppe, B. Faccini, E. Passaglia, D. Malferrari, M. Mastrocicco, N. Colombani

583 **ZeoLIFE, a project for water pollution reduction and water saving using a natural zeolite cycle**

584 Rend. Online Soc. Geol. Ital., 21 (2012), p. 853

585 R. De Wit, J. Leibreich, F. Vernier, F. Delmas, H. Beuffe, P. Maison, J.-C. Chossat, C. Laplace-Treytore, R.
586 Laplana, V. Clavé, M. Torre, I. Aubry, G. Trut, D. Maurer, P. Capdeville

587 **Relationship between land-use in the agro-forestry system of les Landes, nitrogen loading to and risk of**
588 **macro-algal blooming in the Bassin d'Arcachon coastal lagoon (SW France)**

589 Estuar. Coast. Shelf Sci., 62 (2005), pp. 453-465

590 Y. Del Amo, O. Le Pape, P. Tréguer, B. Quéguiner, A. Ménesguen, A. Aminot

591 **Impact of high-nitrate freshwater inputs on macrotidal ecosystems. I. Seasonal evolution of nutrient**
592 **limitation for the diatom-dominated phytoplankton of the Bay of Brest (France)**

593 Mar. Ecol. Prog. Ser., 161 (1997), pp. 213-224

594 D. Di Giuseppe, B. Faccini, M. Mastrocicco, N. Colombani, M. Coltorti, G. Ferretti

595 **Geochemical assessment of the unconfined aquifer in a recently reclaimed wetland area: a case study**
596 **from the Po river delta**

597 EQA Int. J. Environ. Qual., 10 (2013), pp. 37-49

598 D. Di Giuseppe, B. Faccini, M. Mastrocicco, N. Colombani, M. Coltorti

599 **Reclamation influence and background geochemistry of neutral saline soils in the Po River Delta Plain**
600 **(Northern Italy)**

601 Environ. Earth Sci., 72 (2014), pp. 2457-2473

602 Y. Ding, Y.X. Liu, W.X. Wu, D.Z. Shi, M. Yang, Z.K. Zhong

603 **Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns**

604 Water Air Soil Pollut., 213 (2010), pp. 47-55

605 W. Ding, J. Luo, J. Li, H. Yu, J. Fan, D. Liu

606 **Effect of long-term compost and inorganic fertilizer application on background N₂O and fertilizer-induced**
607 **N₂O emissions from an intensively cultivated soil**

608 Sci. Total Environ., 465 (2013), pp. 115-124

609 Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by
610 nitrates from agricultural sources.

611 Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a
612 framework for Community action in the field of water policy.

613 EPA 3051 A

614 **Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils**
615 (2007)

616 B. Faccini, D. Di Giuseppe, D. Malferrari, M. Coltorti, F. Abbondanzi, T. Campisi, A. Laurora, E. Passaglia

617 **Ammonium-exchanged zeolite preparation for agricultural uses: from laboratory tests to large-scale**
618 **application in Zeo-LIFE Project prototype**

619 Period. Mineral., 84 (2015), pp. 303-332

620 FAO

621 **Livestock Long Shadow**

622 FAO Eds, Rome (2006)

623 E. Galli, E. Passaglia

624 **Natural Zeolites in Environmental Engineering**

625 H. Holzapfel (Ed.), Zeolites in Chemical Engineering, Process Eng Engineering GmbH, Vienna (2011), pp. 392-
626 416

627 Gholamhoseini, M. AghaAlikhani, A. Dolatabadian, A. Khodaei-Joghan, H. Zakikhani

628 **Decreasing nitrogen leaching and increasing canola forage yield in a sandy soil by application of natural**
629 **zeolite**

630 Agron. J., 104 (2012), pp. 1467-1475

631 M. Gholamhoseini, A. Ghalavand, A. Khodaei-Joghan, A. Dolatabadian, H. Zakikhani, E. Farmanbar

632 **Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and**
633 **nutrient leaching**

634 Soil Tillage Res., 126 (2013), pp. 193-202

635 V.M. Goldberg

636 **Groundwater pollution by nitrates from livestock wastes**

637 Environ. Health Perspect., 83 (1989), pp. 25-29

638 S.E. Hale, V. Alling, V. Martinsen, J. Mulder, G.D. Breedveld, G. Cornelissen

639 **The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob**
640 **biochars**

641 Chemosphere, 91 (2013), pp. 1612-1619

642 M.R. Islam, S. Mao, X. Xue, A.E. Eneji, X. Zhao, Y. Hu

643 **A lysimeter study of nitrate leaching, optimum fertilisation rate and growth responses of corn (Zeamays**
644 **L.) following soil amendment with water-saving super-absorbent polymer**

645 J. Sci. Food Agric., 91 (2011), pp. 1990-1997

646 ISO 11885

647 **Water quality - Determination of selected elements by inductively coupled plasma optical emission**
648 **spectrometry (ICP-OES)**

649 (2007)

650 ISO 5378

651 **Starches and derived products — determination of nitrogen content by the Kjeldahl method —**
652 **spectrophotometric method**

653 (1978)

654 M. Jalali

655 **Nitrates leaching from agricultural land in Hamadan, western Iran**

656 Agric. Ecosyst. Environ., 110 (2005), pp. 210-218

657 D.L. Jones, J. Rousk, G. Edwards-Jones, T.H. DeLuca, D.V. Murphy

658 **Biochar-mediated changes in soil quality and plant growth in a three year field trial**

659 Soil Biol. Biochem., 45 (2012), pp. 113-124

660 A.U.H. Khan, M. Iqbal, K.R. Islam

661 **Dairy manure and tillage effects on soil fertility and corn yields**

662 Bioresour. Technol., 98 (2007), pp. 1972-1979

663 D. Laird, P. Fleming, B.Q. Wang, R. Horton, D. Karlen

664 **Biochar impact on nutrient leaching from a Midwestern agricultural soil**

665 Geoderma, 158 (2010), pp. 436-442

666 C. Lazcano, P. Revilla, R.A. Malvarb, J. Domingueza

667 **Yield and fruit quality of four sweet corn hybrids (Zeamays) under conventional and integrated**

668 **fertilization with vermicompost**

669 J. Sci. Food Agric., 91 (2011), pp. 1244-1253

670 P.J. Leggo, B. Ledèsert, G. Christie

671 **The role of clinoptilolite in organo-zeolitic**

672 Sci. Total Environ., 363 (2006), pp. 1-10

673 J. Lehmann, J.P. da Silva, C. Steiner, T. Nehls, W. Zech, B. Glaser

674 **Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon**

675 **basin: fertilizer, manure and charcoal amendments**

676 Plant Soil, 249 (2003), pp. 343-357

677 G. Lemaire, M.-H. Jeuffroy, F. Gastal

678 **Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N**

679 **management**

680 Eur. J. Agron., 28 (4) (2008), pp. 614-624

681 G. Liu, X. Chen, Y. Jing, Q. Li, J. Zhang, Q. Huang

682 **Effects of biochar amendment on rapeseed and sweet potato yields and water stable aggregate in upland**

683 **red soil**

684 Catena, 123 (2014), pp. 45-51

685 R. Malekian, J. Abedi-Koupai, S.S. Eslamian

686 **Influences of clinoptilolite and surfactant-modified clinoptilolite zeolite on nitrate leaching and plant**
687 **growth**

688 J. Hazard. Mater., 185 (2011), pp. 970-976

689 D. Malferrari, A. Laurora, M. Brigatti, M. Coltorti, D. Di Giuseppe, B. Faccini, E. Passaglia, M. Vezzadini

690 **Open-field Experimentation of an Innovative and Integrated Zeolite Cycle: Project Definition and**
691 **Material Characterization**

692 Rendiconti lincei. scienze fisiche e naturali - n. 24 (2013), pp. 141-150

693 (ISSN: 2037-4631)

694 S. Marinari, G. Masciandaro, B. Ceccanti, S. Grego

695 **Influence of organic and mineral fertilizers on soil biological and physical properties**

696 Bioresour. Technol., 72 (2000), pp. 9-17

697 M. Mastrocicco, N. Colombani, S. Palpacelli

698 **Fertilizers mobilization in alluvial aquifer: laboratory experiments**

699 Environ. Geol., 56 (2009), pp. 1371-1381

700 M. Mastrocicco, N. Colombani, D. Di Giuseppe, B. Faccini, M. Coltorti

701 **Contribution of the subsurface drainage system in changing the nitrogen speciation of an agricultural soil**
702 **located in a complex marsh environment (Ferrara, Italy)**

703 Agric. Water Manag., 119 (2013), pp. 144-153

704 D.W. Ming, E.R. Allen

705 **The Use of Natural Zeolites in Agronomy, Horticulture, and Environmental Soil Remediation**

706 D.L. Bish, D.W. Ming (Eds.), Natural Zeolites: Occurrence, Properties, Applications, Reviews in Mineralogy &
707 Geochemistry, 45, The Mineralogical Society of America, Washington (2001), pp. 619-654

708 NAP

709 **Nitrates Action Programme 2012-2015 "Regolamento regionale 28 ottobre 2011, n.1 ai sensi dell'articolo**
710 **8 della legge regionale 6 marzo 2007, n. 4**

711 Disposizioni in Materia di Utilizzazione Agronomica Degli Effluenti di Allevamento e Delle Acque Reflue
712 Derivanti da Aziende Agricole e Piccole Aziende Agro-alimentari" BURERT n. 161, October 28th 2011 (2011)

713 N.O. Nelson, S.C. Agudelo, W.Q. Yuan, J. Gan

714 **Nitrogen and phosphorus availability in biochar-amended soils**

715 Soil Sci., 176 (2011), pp. 218-226

716 J.M. Novak, W.J. Busscher, D.L. Laird, M. Ahmedna, D.W. Watts, M.A.S. Niandou

717 **Impact of biochar amendment on fertility of a southeastern coastal plain soil**

718 Soil Sci., 174 (2009), pp. 105-112

719 E. Passaglia, A. Laurora

720 **NH₄ exchange in chabazite, heulandite–clinoptilolite and phillipsite**
 721 Rend. Fis. Acc. Lincei, 24 (2013), pp. 369-376
 722 M.O. Rivett, S.R. Buss, P. Morgan, J.W.N. Smith, C.D. Bemment
 723 **Nitrate attenuation in groundwater: a review of biogeochemical controlling processes**
 724 Water Res., 42 (2008), pp. 4215-4232
 725 D.V. Sarkhot, A.A. Berhe, T.A. Ghezzehei
 726 **Impact of biochar enriched with dairy manure effluent on carbon and nitrogen dynamics**
 727 J. Environ. Qual., 41 (2012), pp. 1107-1114
 728 SAS
 729 **SAS/Stat User's Guide. Version 9.2**
 730 SAS institute inc., Cary, NC. USA (2008)
 731 M. Sebilio, B. Mayer, B. Nicolardot, G. Pinay, A. Mariotti
 732 **Long-term fate of nitrate fertilizer in agricultural soils**
 733 PNAS, 110 (2013), pp. 18185-18189
 734 S. Singh, L.S. Young, F.T. Shen, C.C. Young
 735 **Impacts of industrial waste resources on maize (*Zea mays* L.) growth, yield, nutrients uptake and soil**
 736 **properties**
 737 Waste Manag., 34 (2014), pp. 1877-1883
 738 C. Skinner, A. Gattinger, A. Muller, P. Mäder, A. Fließbach, M. Stolze, R. Ruser, U. Niggli
 739 **Greenhouse gas fluxes from agricultural soils under organic and non-organic management — a global**
 740 **meta-analysis**
 741 Sci. Total Environ., 468-469 (2014), pp. 553-563
 742 P. Smith, D. Martino, Z. Cai, D. Gwary, H.H. Janzen, P. Kumar, B.A. McCarl, S.M. Ogle, F. O'Mara, C. Rice, R.J.
 743 Scholes, O. Sirotenko, M. Howden, T. McAllister, G. Pan, V. Romanenkov, U.A. Schneider, S. Towprayoon
 744 **Policy and technological constraints to implementation of greenhouse gas mitigation options in**
 745 **agriculture**
 746 Agric. Ecosyst. Environ., 118 (2007), pp. 6-28
 747 P.J. Statham
 748 **Nutrients in estuaries — an overview and the potential impacts of climate change**
 749 Sci. Total Environ., 434 (2012), pp. 213-227
 750 M.I. Tajul, M.M. Alam, S.M.M. Hossain, K. Naher, M.Y. Rafii, M.A. Latif
 751 **Influence of plant population and nitrogen-fertilizer at various levels on growth and growth efficiency of**
 752 **maize**

753 Sci. World J., 2013 (2013), pp. 1-9

754 M. Tejada, C. Benitez

755 **Organic amendment based on vermicompost and compost: differences on soil properties and maize yield**

756 Waste Manag. Res., 29 (2011), pp. 1185-1196

757 P.J. Thorburn, J.S. Biggs, K.L. Weier, B.A. Keating

758 **Nitrate in groundwaters of intensive agricultural areas in coastal Northeastern Australia**

759 Agric. Ecosyst. Environ., 94 (2003), pp. 49-58

760 P. Vassileva, D. Voikova

761 **Investigation on natural and pretreated Bulgarian clinoptilolite for ammonium ions removal from**

762 **aqueous solutions**

763 J. Hazard. Mater., 170 (2009), pp. 948-953

764 G.L. Velthof, J.P. Lesschen, J. Webb, S. Pietrzak, Z. Miatkowski, M. Pinto, J. Kros, O. Oenema

765 **The impact of the nitrates directive on nitrogen emissions from agriculture in the EU-27 during 2000–**

766 **2008**

767 Sci. Total Environ., 468-469 (2014), pp. 1225-1233

768 L. Wang, A.S. Butcher, M.E. Stuart, D.C. Gooddy, J.P. Bloomfield

769 **The nitrate time bomb: a numerical way to investigate nitrate storage and lag time in the unsaturated**

770 **zone**

771 Environ. Geochem. Health, 35 (2013), pp. 667-681

772 Jiu Jiao, Y. Wang, J.A. Cherry, X. Kuang, K. Liu, C. Lee, Z. Gong

773 **Accumulation and transport of ammonium in aquitards in the Pearl River Delta (China) in the last**

774 **10,000 years: conceptual and numerical models**

775 Hydrogeol. J., 21 (2013), pp. 961-976

776 WHO

777 **Guidelines for Drinking Water Quality. 1. Recommendations**

778 (second ed.), World Health Organisation, Geneva (1993)

779 K. Wick, C. Heumesser, E. Schmid

780 **Groundwater nitrate contamination: factors and indicators**

781 J. Environ. Manag., 111 (2012), pp. 178-186

782 D. Widory, W. Kloppmann, L. Chery, J. Bonnin, H. Rochdi, J.-L. Guinamant

783 **Nitrate in groundwater: an isotopic multi-tracer approach**

784 J. Contam. Hydrol., 72 (2004), pp. 165-188

785 P.E.V. Williams

- 786 **Animal production and European pollution problems**
- 787 Anim. Feed Sci. Technol., 53 (1995), pp. 135-144
- 788 H. Yang, J. Li, J. Yang, H. Wang, J. Zou, *et al.*
- 789 **Effects of nitrogen application rate and leaf age on the distribution pattern of leaf SPAD readings in the**
- 790 **rice canopy**
- 791 PLoS One, 9 (2014), Article e88421, [10.1371/journal.pone.0088421](https://doi.org/10.1371/journal.pone.0088421)