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Ammonium-charged zeolitite 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 zeolitite/soil ratio with two greenhouse experiments.

• The effect of NH4-charged zeolitite 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} NH_4-charged zeolitite 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 NH_4-charged zeolitite (produced by a new prototype) for minimizing NO_3-leaching from soil and optimizing corn growth and yield. Forty-eight zeolitite: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 NH_4-charged zeolitite (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 NH_4-charged zeolitite is used with a limited amount of conventional fertilizer, allowing a reduction of nitrate concentration in drainage.

Keywords

Zeolitite 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 NO_3–N and NH_4–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 NO_3–N and PO_4–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 (NH4CZ, 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 NO3-concentration in leachate that could drain directly to groundwater and to optimize maize production in comparison to traditional practice (with the chemical fertilizer application).

2. Materials and methods

2.1. NH_4-charged zeolitite

The natural zeolitite used in this study is composed mainly of chabasite and comes from Sorano (Grosseto, Central Italy); chemical and mineralogical composition and physio-chemical properties of natural zeolitite are reported in Malferrari et al. (2013). To obtain NH4CZ, NH_4-exchange experiments between natural zeolitite (fraction with particle size less than 3.0 mm) and swine manure were carried out in static mode (Vassileva and Voikova, 2009) in laboratory (Faccini et al., 2015), and the findings were reproduced in large-scale application, in a prototype located in Codigoro (Ferrara, Italy) near the experimental field arranged for ZeoLIFE project (Coltorti et al., 2012, Malferrari et al., 2013). Briefly, the prototype (supplementary information SI-1) is composed by a 2.2 m (ø) × 5.3 m (h) tank for the swine manure storage (about 10 m^3). The loading of swine manure is performed using a pump that takes manure directly from the manure pool; 250 kg of natural zeolitite is introduced from the top into the vessel and mechanically stirred with swine manure for 45 min. After a resting time (4–16 h), NH4CZ is discharged and recovered opening the ball valve at the bottom of the tank. A vibrating sieving system was inserted at the bottom of the vessel to separate the different particle size of NH4CZ, with a total daily production of 500 kg. At the end of each production cycle, NH4CZ was stored, air dried in controlled open-air conditions and then periodically characterized (Faccini et al., 2015).

2.2. Experiment set-up and general methodology

This study was conducted at CRSA Med Ingegneria facilities, north east of Italy (WGS84: 44°28′50″N 12°16′21″E), in a 60 m^3 greenhouse (3.3 m × 9 m x h 2 m) in 2012 (spring and summer).

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

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

Property	u.m	Bulk soil
Cation exchange Capacity (CEC)	meq 100 g^{-1}	33.6
Exchangeable Ca^{2+}	mg kg^{-1}	5660
Exchangeable K^+	mg kg^{-1}	582
Exchangeable K (as K_2O)	mg kg^{-1}	701
Exchangeable Mg^{2+}	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_2O5)	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

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

2.3. Greenhouse experiments

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

The treatments in both trials mainly consisted of (i) two soil amendment types with NH_4-charged (NH4CZ) and natural (nZ) zeolitite, (ii) two soil amendment doses of 10 g kg^{-1} (dose-1) and 20 g kg^{-1} (dose-2), and (iii) different applications of chemical fertilizer. The soil amendment doses were selected on the basis of soil type (Ming and Allen, 2001, Leggo et al., 2006, Malekian et al., 2011) and the cost-effectiveness of the treatment (Islam et al., 2011). Each treatment was performed in quadruplicate and four not amended soil lysimeters were used as a positive control. The nitrogen source, applied once at the beginning of the trials, was urea (46% N). The reductions of urea respect to each trial control (6 and 11% in the first trial, and 30, 50 and 70% in the second trial) were established considering the average nitrogen content in NH4CZ (5.81 mg N g^{-1}). In the first trial, 5 treatments per 4 replicates (20 lysimeters) were conducted for 89 days of the experiment. Simulating a high nitrogen fertilization of full field for corn (about 370 kg N ha^{-1}) along the soil profile in lysimeters (25 cm), 248 ± 2 mg kg^{-1} urea have been added to the soil for traditional farming practice (positive control); then two reductions of 6 and 11% in two different treatments were carried out. In particular, for two treatments (10CZ_u and 20CZ_u) urea was added compensating for the amount of nitrogen absorbed as ammonium in NH4CZ by the prototype process (Coltorti et al., 2012). In the last two treatments (20CZ_wo and 20nZ_wo), no urea was added in order to observe the effect of zeolitite (both natural and NH_4-charged) on plant growth. The application of nZ and NH4CZ in each lysimeter was calculated on the basis of the dry weight and the depth of plowing. More in detail, assuming a depth of homogeneous distribution of zeolitite 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 zeolitite in the field, respectively. In order to evaluate the best approach and select the optimum zeolitite 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 zeolitite (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 zeolitite/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
NH4CZ (g kg^{-1})	0	10	10	10[b]	0	10[c]	6
Natural zeolitite (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 μm) NH4CZ, collected in prototype.

c Residual NH4-charged zeolitite 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 μm) 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)

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

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

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

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

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

2.4. Data collection

The leached solution from each lysimeter was sampled every 20 days in order to assess the NH_4-N and NO_3-N concentration in leachate. The two trials were stopped at 97 and 73 DAS (growing stage R3 and VT, respectively, described by Abendroth et al., 2011), before the influence of lysimeter volume on root elongation and crop height. During the growth monitoring, measurements of the aerial biomass (height in cm from the base of the plant to the top of the upper leaf) were performed approximately every 20 days. At the end of each trial, all the plants were collected from each lysimeter, oven dried at 70 °C until constant weight was attained, in order to assess the production in term of aerial biomass (dry weight).

Moreover, at the end of the second trial (day 73), the photosynthetic activity (PN) and leaf chlorophyll content (soil–plant-analysis development, SPAD value) were measured in 5 leafs per plant, with an ADC-LCPro + instrument (for determination of CO_2 per leaf area and time unit) and a portable SPAD meter (Model SPAD-502, Minolta crop, Ramsey, NJ), respectively. The SPAD meter measures the transmission of red light at 650 nm, in which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmission values, the instrument calculates a SPAD value that is well correlated with chlorophyll content and used as an indirect indicator of crop N status. Joined to the evaluation of the aerial biomass, a quantitative and qualitative morphological study (relative growth rate, density/appearance of the root) was conducted.

Then, several macronutrients in the corn leaves of the second trial were measured according to international standards (ISO 5378, 1978 for N determination; EPA 3051 A, 2007 and ISO 11885, 2007 for other macronutrients determination). Briefly, after oven drying at 70 °C for 24 h and homogenizing, the leaf samples were assayed for total N (Kjeldahl method, modified as described in Cataldo et al., 1974), and after 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 NO_3-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 zeolitite 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 NH_4–N concentrations between treatments and control, showing a decreasing trend during the monitoring period.

Table 3. First trial: Trend of NO_3–N and NH_4–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 NO_3–N and NH_4–N, respectively. No significant differences were observed in NH_4–N and NO_3–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).

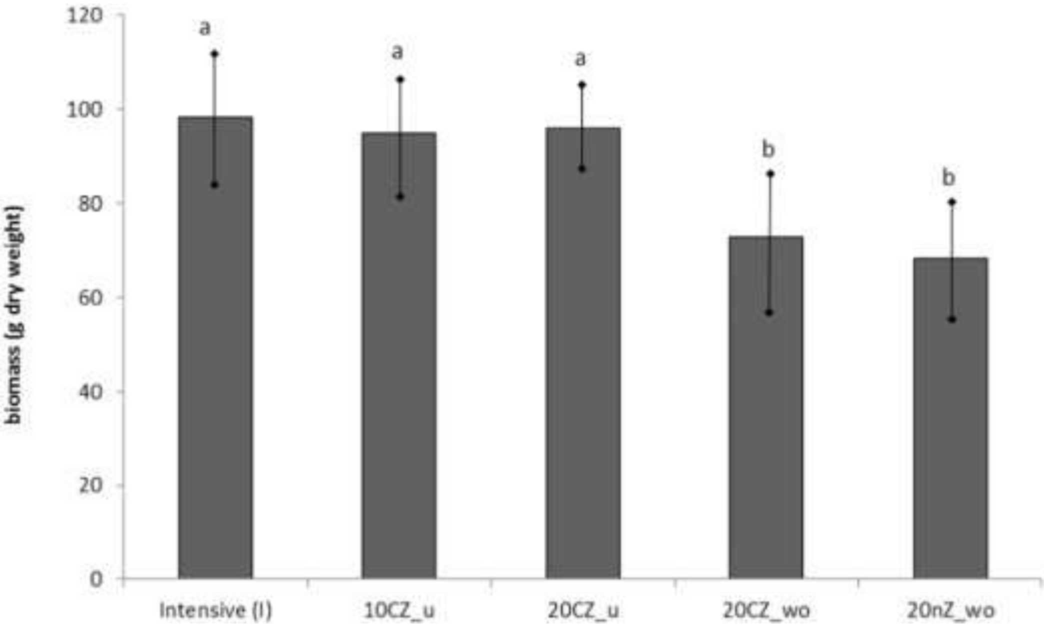

Fig. 1. Effect of treatments on the production of aerial biomass at the end of the first trial (dry weight). Values represent means ± standard deviation (n = 4). Different letters indicate significant differences between treatments at the p-level < 0.05.

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 NO_3-N and NH_4-N content, conductivity and chlorides in leachate for the six treatments and positive control. Mean ± standard deviation of four replicates, except for (*) where three replicates were used.

	NO_3-N (mg l^{-1})		NH_4-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**
C	24.9 ± 5.4	4.6 ± 0.9	1,2*±0,1*	0,7*±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*±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*±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

	NO_3-N (mg l^{-1})		NH_4-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 NO_3-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 NH_4-charged zeolitite and N fertilization) had the significantly lowest nitrates content in water as expected.

As regards the NH_4-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 NH_4-N (average 0.94 ± 0.30 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).

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.

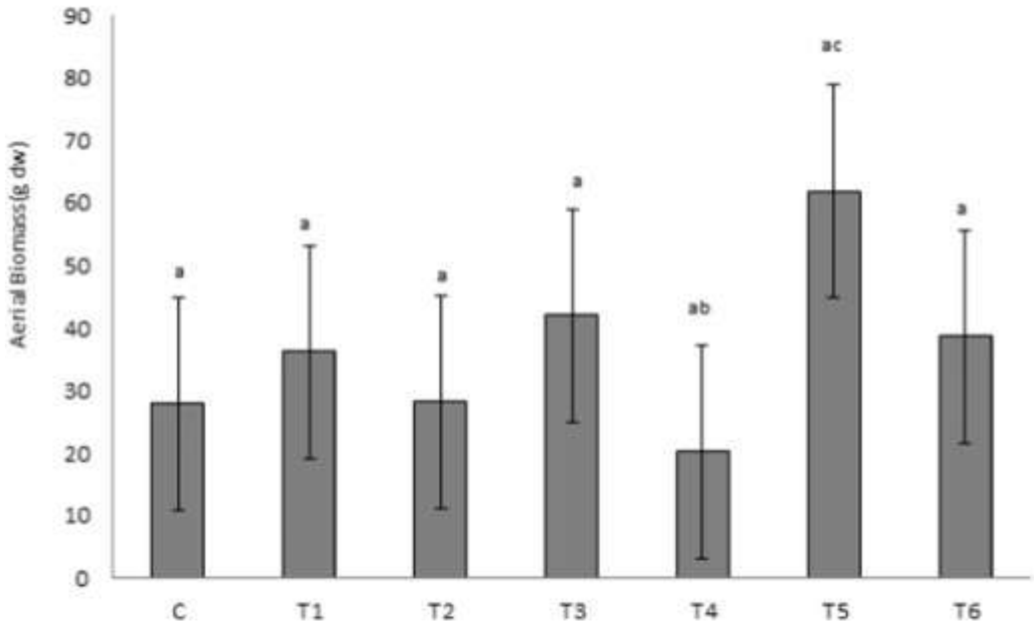

Fig. 2. Effect of treatments on the production of aerial biomass at the end of the second trial (dry weight). Values represent means ± standard deviation (n = 4). Different letters indicate significant differences between treatments at the p-level < 0.05.

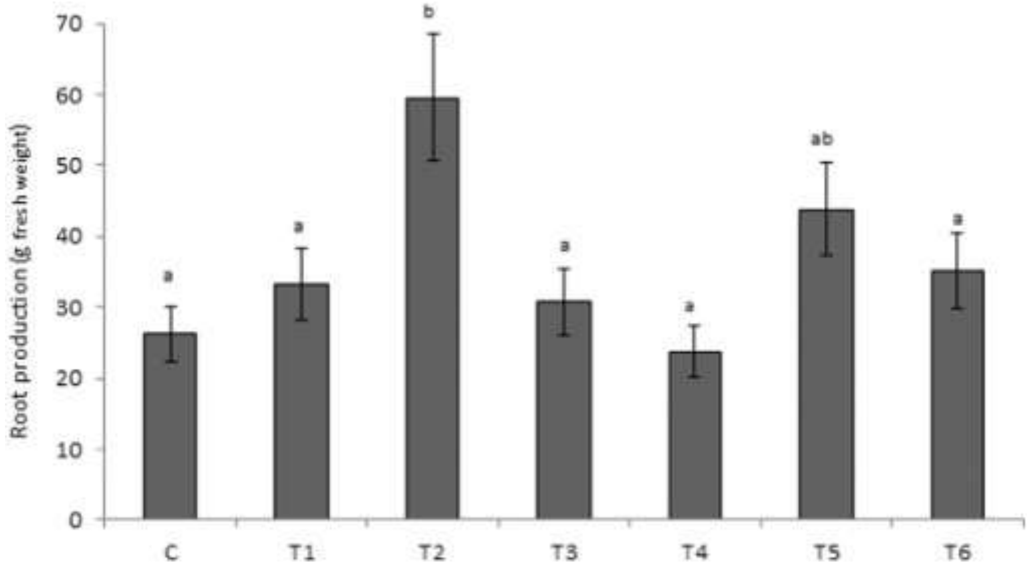

Fig. 3. Effect of treatments on the production of root biomass (fresh weight) in second trial. Different letters indicate significant differences between the treatments (p-level < 0.05).

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

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

In Fig. 4, the assessment of the roots involved (i) the measurement of root biomass (dry weight) and (ii) the morphological analysis, considering the total length of roots, the number of primary roots and absorbent and the radical diameter. Considering these parameters, the treatment T5 showed the highest root biomass (dry matter), followed by T1 and T3. Other treatments induced significantly lower total production 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 µmol CO_2 m^{-2} 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).

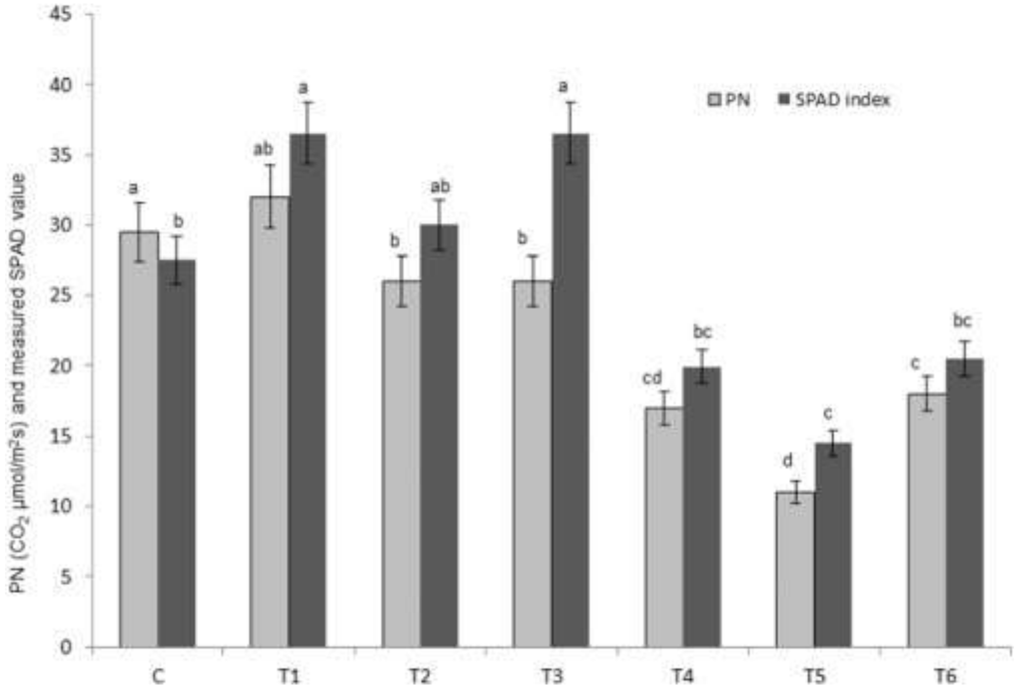

Fig. 5. Crop growth evaluation in the second trial on the basis of photosynthetic activity (PN) in µmol CO_2 m^{-2} s^{-1} and SPAD index. Values represent means ± standard deviation (n = 4). Different letters indicate significant differences between treatments at the p-level < 0.05.

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 NH4CZ 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 NH4CZ and a fertilizer reduction), than all the other treatments and the positive control. Moreover, across the fertilization regimes, 10 NH4CZ increased N leaf from 23,100 ± 2200 up to 27,600 ± 1700 mg $kg^{-1}{}_{dw}$, 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 Benıtez, 2011).

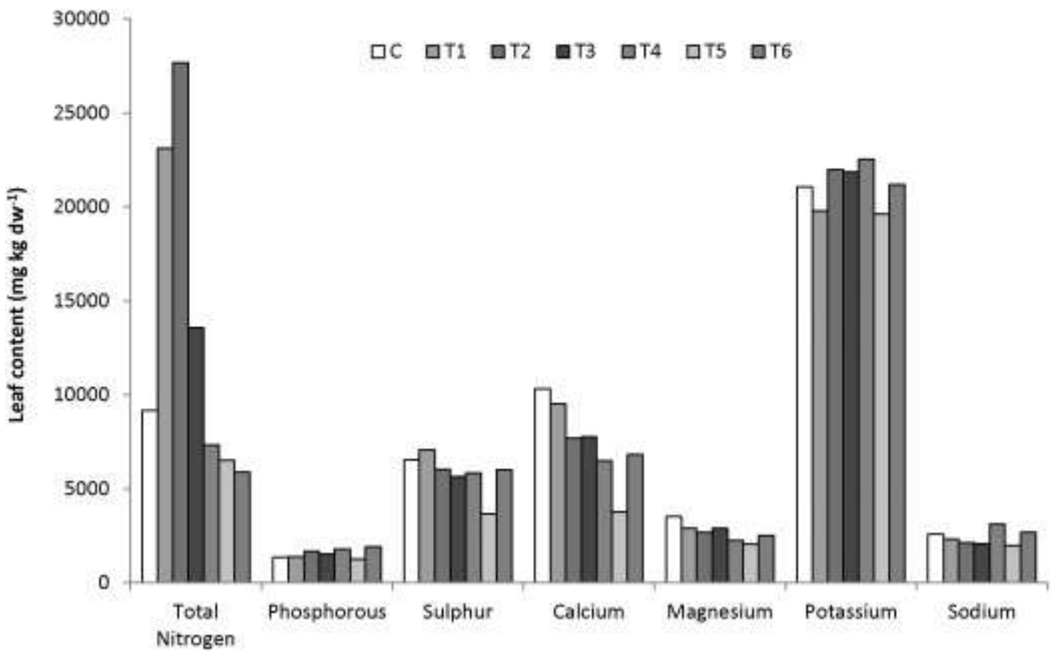

Fig. 6. Analysis of macronutrients in the corn leaves at 73 DAS, after harvest in the second trial. Optimal nitrogen content is set at 2% (20,000 mg kg^{-1}) while the sufficient level at 1% (Tajul et al., 2013, Ahmed et al., 2008, Jones et al., 2012). Calcium leaf content showed a significant difference 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 zeolitite 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 Benıtez (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 Benıtez (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 zeolitites, 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 NH_4-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 residual N could have allowed the maize growth (1.22 cm day^{-1}) although lower than in the other treatments (up to 1.34 cm day^{-1}).

The main result of the first trial was that applying the dose 10 g kg^{-1} of NH4CZ and reducing urea fertilization may offer a significant advantage by limiting the leaching of NO_3-N, and maintaining a good crop growth rate. In this study, the phenomenon reported by Ahmed et al. (2006) where the inclusion of 1 g kg^{-1} zeolitite have improved the soil retention of NH_4 as well as minimizing the conversion of NH_4 to NO_3 was not observed, probably due to the tenfold lower urea addition (2 g kg^{-1} in Ahmed et al. (2006) and about 0.2 g kg^{-1} in this study).

4.1.2. Biomass production

The fertilization regimes containing NH4CZ and N fertilizer did not produce significant differences in plant biomass with respect to the conventional fertilizer alone. However, the integrated fertilization regimes (with urea application) produced differences in the plants, as the biomass of plants grown with integrated organic fertilizer (20CZ_u) was significantly greater than this one grown with only NH4CZ (20CZ_wo). This suggested that an N integration with N fertilizer should be necessary even when NH4CZ is used.

4.2. Second greenhouse trial

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

4.2.1. Nitrogen concentration in leachate

The findings of the second trial showed that the nitrate concentration in water was significantly similar in the treatments and in the control, except for the highest value at 73 days in T1 where 70% urea-N was applied. Probably the high Urea-N content could contribute to maintaining a high level of nitrates in leachate, also considering the low root production in the crop of this treatment. As regards the NH_4-N content, after an initial difference in two treatments (T3 and T5) respect to the other treatments and the positive control, the amount of NH_4-N in drainage water was unaffected by the amendment dose and N fertilization.

4.2.2. Biomass production, photosynthetic activity and macronutrients in leaves

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

As far as crop quality is concerned, the macronutrients content, except for nitrogen, in leaf was performed at the end of the second trial, testify an overall good leaf health. Indeed, differences in N leaf content subjected to varying NH4CZ and urea application rates were evident during our observation: the 2.5% N leaf content in T1 and T2 led to suppose the possibility to increase the production, while for the other treatments it was less than 1%, suggesting a suffering situation, with limitation in plant growth. This demonstrated that the unique mineral properties of chabastite zeolites, including high CEC and high affinity for NH4 + (Malferrari et al., 2013) significantly increased the N uptake by plants.

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

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

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

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

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

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

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

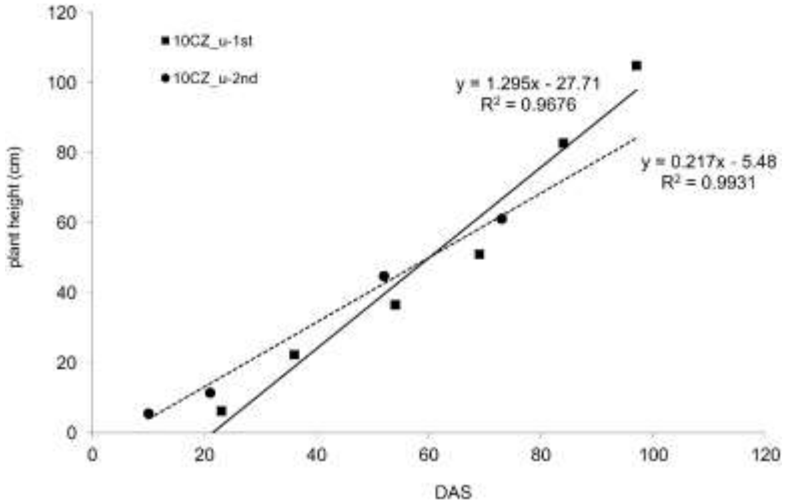

Fig. 7. Growth rate of two maize productions in Codigoro soil with 10 g kg^{-1} NH4CZ and Urea-N progressive reduction (94.4% in 10CZ_u_1st and 50.0% in 10CZ_u_2nd). The comparison between first (square) and second trial (dot) allowed the assessment of the simulation of two crop years on the same NH4CZ application, performed only in the first trial.

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

Table 5. Production assessment of Maize crop in Codigoro soil treatments and NO_3–N concentration in leachate. Comparison of data collected in trial 1 and 2. The control treatment to be considered was the Intensive (I) in trial 1, with Codigoro soil and with 370 kg N ha^{-1}.

Treatment	**Trial**	**DAS**	**Plant height (cm)**	**Aerial biomass (g $_{fw}$[a])**	**Roots (g $_{fw}$[a])**	**NO_3-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

a fw: fresh weight.

4.4. Zeolitite dose selection

In order to achieve an overall evaluation of all parameters analyzed in the second trial, a ranking approach was carried out (Table 6). As determined by Liu et al. (2014), using three parameters for amendment dose definition, three macro-groups of parameters were considered in order to evaluate the leaching process, the crop production and the crop quality before harvest. For each macro-group, three parameters were selected, respectively: (i) nitrates, ammonia and chloride content in drainage at 73 DAS for the leaching 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 NO_3–N, NH_4–N, Cl^{-1}; 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 (+).

	Leaching process			**Crop production**			**Crop quality**				
Treatment	**NO_3–N**	**NH_4–N**	**Cl^-**	**Growth rate**	**Aerial biomass**	**Roots**	**N leaf content**	**SPAD**	**Chlorophyll *a* content**	**Final score**	**Ranking**
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 NH4-exchange zeolitite 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 *a* 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 NH4 +, ammonium was assigned with the same weight because NH4-N reflected the behavior of the NH_4-charged zeolitite added to the soil. Indeed, considering that NH4CZ exchange mainly NH4 +, nitrogen concentration is an indicator of NH4CZ 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 application of NH4CZ at 50 t ha^{-1} (dose-1) plus 70% of standard fertilization or NH4CZ at 30 t ha^{-1} plus 3% of standard fertilization (MAS-complying test) could both achieve higher results than conventional fertilizer rate. This led to suppose that NH4CZ gave a good contribution in N-availability during crop growth. Among treatments with dose-1 of NH4CZ, also the treatment T2 was a feasible solution, with 50% of conventional fertilization. This was confirmed by the findings of T5, with the agricultural soil and two crop years (high reduction in NO_3-N leaching and good crop production), although its score was low but even higher than control. Also T4 was found with a lower score, but even higher than control, thanks to the good effect of the natural zeolitite on NO_3-N leaching and soil texture correction. Thus, considering the low content of natural zeolitite (50 t ha^{-1}) and the reduction of 30% fertilization, the treatment T4 could be also selected for the open field activities of ZeoLIFE project. Similar results were obtained by Liu et al. (2014), using 30 and 40 t ha^{-1} biochar amendment with about 6 g N kg^{-1}.

5. Conclusions

The study showed that the application of NH_4-charged zeolitite to highly productive agricultural land had no negative consequences in terms of crop growth and nutrition and may even provide high agronomic benefits with lasting effect on soil properties. The lack of negative effects seen at application rates of either 30 or 50 t ha^{-1} also suggested that the applications of NH_4-charged zeolitite may be scaled-up in open field studies with agricultural soils consisting of low permeability materials with naturally high content of organic matter. Moreover, the reduction of chemical fertilizer was feasible, even at high degree, allowing a reduction in groundwater pollution by nitrates. This demonstrated that the NH4CZ behavior is different with respect to chemical fertilizer one and the N content in NH4CZ should not be considered an equivalent of Urea-N. Thus, the maximum amount of NH4CZ to be applied to soil could be selected on the basis of soil type and not on the MAS regulation for fertilizer (for example, 240 kg N ha^{-1} for maize). These results may suggest that the employment of synthetic fertilizers foreseen for the different production regulations may be revised downwards when they are associated with the use soil conditioners such as zeolitite.

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