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C-N elemental and isotopic investigation in agricultural soils: insights on the effects of zeolite amendments

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Abstract

In this paper we present an elemental and isotopic investigation of carbon and nitrogen in the soil-plant system. Plants grown in an unamended soil were compared to plants grown in a soil amended with natural and NH_4^+ -enriched zeolites. The aim was to verify that zeolites at natural state increase the chemical fertilization efficiency and the nitrogen transfer from NH_4^+ -enriched zeolites to plants. Results showed that plants grown on plots amended with zeolites have generally a $\delta^{15}\text{N}$ approaching that of chemical fertilizers, suggesting an enhanced nitrogen uptake from this specific N source with respect to the unamended plot. The $\delta^{15}\text{N}$ of plants grown on NH_4^+ -enriched zeolites was strongly influenced by pig-slurry $\delta^{15}\text{N}$ (employed for the enrichment process), confirming the nitrogen transfer from zeolites to plants. The different agricultural practices are also reflected in the plant physiology as recorded by the carbon discrimination factor, which generally increases in plots amended with natural zeolites, indicating better water/nutrient conditions.

Keywords: *natural zeolites; NH_4 -enriched zeolites; $\delta^{15}\text{N}$; $\delta^{13}\text{C}$; amendments; soil-plant interactions; chabazite.*

1. Introduction

Intensive agricultural practices, which involve systematic use of chemical fertilizers, cause soil quality degradation, loss of soil organic matter, decrease of soil biological properties and widespread water pollution, particularly reflected by high concentration of dissolved nitrogen (N) compounds that contaminate the interacting surface and ground-water (Golchin et al., 1995; Sacchi et al., 2013; Marchina et al., 2015). Chemical fertilization implies significant N losses that may occur after their application (such as NO_3^- leaching/runoff, NO_x , N_2O and NH_3 volatilization), which have negative consequences from the economical, agronomical and

environmental point of view (Smil, 1999). In this framework, the application of organic and inorganic amendments has been recognized as a valuable technique for increasing soil physico-chemical properties as well as soil fertility (Waltz et al., 2003; Ferreras et al., 2006; Lima et al., 2009; Colombani et al., 2014). Among inorganic amendments, zeolitites are increasingly used. They are lithologies containing more than 50 % of zeolite minerals (Galli and Passaglia, 2011), which are crystalline hydrated aluminosilicates constituted by a three-dimensional framework of linked tetrahedra and by open cavities in the form of channels and cages (Gottardi and Galli, 1985; Coombs et al., 1997). They are known to be suitable in a wide range of agricultural applications (Reháková et al., 2004) in relation to their high Cation Exchange Capacity (CEC). Their structure allows in fact a controlled retention/release of water and nutrients (e.g. NH_4^+), thus reducing N leaching and NH_3 volatilization from agricultural soils. As a consequence, the use of these amendments generally increase crop yield, water and N use efficiency (Bigelow et al., 2001; Reháková et al., 2004; Passaglia, 2008; Sepaskhah and Barzegar, 2010; Ippolito et al., 2011; Latifah et al., 2011; Bernardi et al., 2013; Gholamhoseini et al., 2013; Li et al., 2013; Malferrari et al., 2013; Colombani et al., 2014; Colombani et al., 2015; Ozbahce et al., 2015; Di Giuseppe et al., 2016).

Among zeolitites, chabazite-bearing zeolitites (in which chabazite zeolite is prevalent) are recognized as one of the best zeolitites to be used for agronomical purposes, in relation to its very high CEC (2.0 - 2.1 meq g^{-1}), selective reversible NH_4^+ sorption and structure stability over long period (Baerlocher et al., 2001; Passaglia, 2008).

In this context, the ZeoLIFE project (LIFE10+ ENV/IT/00321) investigated the use of zeolitites amendments to minimize the nitrate pollution in agricultural soils and ground/superficial waters, increasing at the same time yield and fertilization efficiency in a nitrate vulnerable zone (Ferrara Province, Italy). Notwithstanding the high number of studies in which natural and NH_4^+ -enriched zeolitites were employed in agricultural applications (Barbarick and Pirela,

1984; Lewis et al., 1984; Dwairi, 1998; Reháková et al., 2004 and reference therein), the use of stable isotopes has never been applied for evaluating the effects of this practice on plant N uptake and for tracing the N transfer from zeolitites to plants.

In this framework, we used an isotopic approach to evaluate the nutrient transfer from zeolitites to plants as well as to verify the increase in fertilizers N uptake with respect to an unamended soil.

A very powerful tool that allows a better understanding of the plant-soil N dynamics is the measurement of the $^{15}\text{N}/^{14}\text{N}$ isotopic ratio (expressed as $\delta^{15}\text{N}$) in soils and associated plants (Högberg, 1997; Szpak, 2014). It is well known that variations in the N isotopic ratio can give robust information about N fluxes and plant N sources in ecosystems. Many studies consider that no variation of the isotopic ratio occurs after the absorption of N by the plant, thus, foliar or stem $\delta^{15}\text{N}$ can act as tracer, reflecting the isotopic ratio of the N source in the soil (Handley and Raven, 1992; Högberg, 1997; Evans, 2001). This assumption is however not completely corrected due to possible fractionations during N uptake, intra-plant N re-allocation and physiological factors due to mycorrhizal associations (Evans, 2001; Szpak, 2014).

Zootechnical effluents, such as pig-slurry that is commonly used as organic fertilizer, are strongly enriched in the heavier ^{15}N isotope ($> 20 \text{ ‰}$) due to NH_3 volatilization that causes depletion in the lighter ^{14}N (Högberg, 1997; Dittert et al., 1998; Schmidt and Ostle, 1999; Lim et al., 2007). This in turn implies that plants grown on organic fertilizers have higher $\delta^{15}\text{N}$ with respect to plants grown under unfertilized or under chemical N-fertilizers (Choi et al., 2002; Choi et al., 2003; Bateman et al., 2005; Yun et al., 2006; Szpak et al., 2012; Szpak, 2014). It is also known that site specific conditions such as water and nutrients availability, soil salinity and soil compaction can cause important variation in the carbon (C) discrimination factor ($\Delta^{13}\text{C}$), giving information on the physiological status of plants (Lasa et al., 2011; Maxwell et al., 2014).

The goal of this paper is to verify if soil amendments with zeolites at natural state can effectively increase chemical fertilization efficiency and to trace the N transfer from NH_4^+ -enriched zeolites to plants by taking into consideration the N and C elemental and isotopic distribution in the soil-plant system. The investigation has been carried out for two cultivation cycles (maize and wheat). During the experimentation, three different zeolite amendments, one employing NH_4^+ -enriched zeolites and two employing zeolites at natural state applied in various amount, were compared to an unamended soil.

Our hypothesis is that in soils amended with zeolites at natural state, plants are more influenced by the chemical fertilizers isotopic signature with respect to plants grown on an unamended soil, since zeolites should enhance the adsorption of NH_4^+ ions formed after the application of chemical fertilizers and allow a more efficient uptake of this specific N source. On the other hand, in soils amended with NH_4^+ -enriched zeolites (obtained by doping zeolites with pig-slurry characterized by a very high ^{15}N natural abundance), plants are expected to show a higher isotopic signature, as a consequence of the N transfer from the mineral to the plants.

2. Materials and methods

2.1 Zeolites used in the study

The natural zeolite (NZ) used in this study is a byproduct from a quarry located near Sorano village (central Italy) that is mainly exploited to obtain blocks and bricks for construction and gardening.

The quarried material is a zeolitized tuff composed of more than 68 % of K-chabazite constituting thus a “chabazite-bearing zeolite” (Malferrari et al., 2013). A grain size of 3-6 mm was selected and used in the experimentation. After sieving, part of the NZ was subjected

to an enrichment process which allowed the enrichment of the zeolitite with NH_4^+ ions, creating an NH_4^+ -enriched zeolitite (CZ). The enrichment process, based on a mixing procedure of pig-slurry and natural zeolitite in a specifically conceived prototype, has been described by Faccini et al. (2015).

2.2 Experimental field setting

This study has been carried out during the agronomic years 2014 and 2015 in a 6 ha agricultural field located 40 km eastward of Ferrara city ($45^\circ 50' 33''$ N and $12^\circ 05' 40''$ E, Italy) and 15 km from the Adriatic Sea. The average rainfall is between 500 and 700 mm per year with peaks in autumn and summer (sub-continental climate). Average daytime temperatures range from 5°C in January to 25°C in July. Marine thermoregulation helps to maintain minima temperatures over zero, reducing the number of night frosts (Mastrocicco et al., 2013). The experimental field lays at an average altitude of 3 m below sea level and consists of recently reclaimed (1860-1890) clayey-silt soils (Mastrocicco et al., 2013; Di Giuseppe et al., 2014a) defined as Calcaric Gleyic Cambisol, according to the World Reference Base for Soil Resources (IUSS Working Group, 2007) (see Table 1 for the main soil physico-chemical properties). These soils have been previously characterized from the geochemical point of view (Bianchini et al., 2012; Bianchini et al., 2013; Di Giuseppe et al., 2014b; Di Giuseppe et al., 2014c). The mineralogical composition is characterized by quartz, feldspar, calcite and clay minerals (illite, smectite, chlorite, serpentine and mixed-layer) (Malferrari et al., 2013).

The experimental field was parcelled at the begin of ZeoLIFE project (year 2012) in order to compare different zeolitite treatments with the traditional agricultural practices (Fig 1).

Distinct parcels were linear and continuous in order to facilitate the movements of farm machines. One parcel of 1.5 ha was cultivated without the use of zeolitites and thus left unamended (UA). Two parcels of 1 ha each were amended with 5 and 15 kg m^{-2} of zeolitites

(5NZ and 15NZ, respectively), and one parcel of 0.5 ha was amended with 10 kg m⁻² of NH₄⁺-enriched zeolite (10CZ). The 10CZ plot was relatively smaller with respect to other NZ plots because of the limited CZ availability. The experimental set-up is summarized in Table 2.

Maize (*Zea mays*) was sowed on March 28th, 2014 and harvested on September 7th, 2014, while wheat (*Triticum durum*) was sowed on November 11th, 2014 and harvested on June 30th, 2015. The project developed in a real agricultural context (the field was managed by a local farm company) in order to demonstrate the possibility of reducing N pollution from fertilization by increasing fertilization efficiency through the use of NZ and CZ as soil amendments. For this reason, since the begin of the project chemical fertilization was reduced in plots amended with zeolites, with respect to the fertilization rate applied in the UA plot (Table 2). Urea was applied in 2 steps on April 18th and May 13th, 2014, during maize cultivation, while NH₄NO₃ was applied in two steps on March 04th and April 29th, 2015, during wheat cultivation (see Table 2 for total fertilizers amounts in each plot). It is very important to specify that 10CZ plot was the only one subjected to a N input derived from pig-slurry in the agronomic years 2013 and 2014. The δ¹⁵N of each N input is listed in Table 3.

2.3 Plant and rhizosphere sampling

Six representative plants of maize and wheat from each treatment were sampled before the harvest (Fig 2). As representative of the above ground biomass, stems and grains were separated, dried at 50 °C for three days and then milled. Below ground biomass (roots) were gently shacked in order to obtain soil samples representative of the rhizosphere. The rhizosphere is a narrow zone in the vicinity of the roots characterized by the presence of mycorrhizal fungi and other rhizosphere microorganisms that depend on root exudates (Brüggemann et al., 2011).

2.4 C-N elemental and isotopic analyses

Measurements of elemental and isotopic C and N compositions have been carried out by the use of an Elementar Vario Micro Cube Elemental Analyzer (EA) in line with an ISOPRIME 100 Isotopic Ratio Mass Spectrometer (IRMS) operating in continuous-flow mode. Powdered samples are introduced in tin capsules that are wrapped and weighed; these capsules, that allow to load up to 40 mg of sample, are subsequently introduced in the Vario Micro Cube auto-sampler to be analysed. Flash combustion takes place in a sealed quartz tube kept at temperature of 950 °C filled with copper oxide grains (padded with corundum balls and quartz wool) which acts as catalyst, in excess of high purity (6 grade purity) O₂ gas. Freed gaseous species are transferred through a reduction quartz tube (at 550 °C) filled with metallic copper wires that reduce the N oxides (NO_x) to N₂. The formed analyte gases (N₂, H₂O and CO₂), carried by dry He (5 grade purity) gas, pass through a water-trap filled with sicapent ensuring complete removal of moisture, are sequentially separated by a temperature programmable desorption column (TPD) and quantitatively determined on a thermo-conductivity detector (TCD). Sample N₂ goes directly to the interfaced IRMS for the measurement of the isotopic ratio, while CO₂ is held by the TPD column, kept at room temperatures 20–25 °C. When N₂ isotopic analysis is over, CO₂ is desorbed from the TPD column raising the temperature to 210°C, and finally reaches the IRMS compartment for the determination of C isotopic ratios. The detection of the distinct isotopic masses of the sample are sandwiched between those of reference N₂ and CO₂ (5 grade purity) gases, which have been calibrated using a series of reference materials, in turn calibrated against IAEA international standards, such as the limestone JLs-1 (Kusaka and Nakano, 2014), the peach leaves NIST SRM1547 (Dutta et al., 2000), the Carrara Marble (calibrated at the Institute of Geoscience and Georesources of the National Council of Researches of Pisa), and the synthetic sulfanilamide provided by Isoprime Ltd. Mass peaks were recalculated as isotopic ratios by the Ion Vantage software package. Reference and carrier gases of certified purity were provided by SIAD Ltd.

The elemental precision estimated by repeated analyses of standards, and accuracy estimated by the comparison between reference and measured values, were in the order of 5% of the absolute measured value. Uncertainties increase for contents approaching the detection limit (0.001 wt%). C and N isotope ratios are expressed in the standard (δ) notation in per mil (‰) relative to the international Vienna Pee Dee Belemnite (V-PDB) and atmospheric air (AIR) isotope standard, respectively (Gonfiantini et al., 1995). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were characterized by an average standard deviation of ± 0.1 ‰ defined by repeated analyses of the above mentioned standards. The analyses have been repeated at least three times for each fraction in order to minimize the effect of inhomogeneity of the samples, to evaluate Standard Deviation (SD, 1σ) and to give consistency to the method.

2.5 Statistical analysis

For evaluating significant differences between the treatments, parametric and non-parametric statistics were applied to the dataset. In order to test ANOVA assumptions, results were subjected to Shapiro-Wilk normality test and Levene's Test (for testing homogeneity of variance). In case of both tests were passed, One Way ANOVA and Fisher (LSD) post-hoc pairwise multiple comparison tests were applied at $p=0.05$. In case ANOVA assumptions were not met, non-parametric Kruskal-Wallis One Way Analysis of Variance on Ranks and Student-Newman-Keuls pairwise multiple comparison tests were applied at $p=0.05$.

3. Results

3.1 Soil rhizosphere

The results of the elemental and isotopic C and N analyses of rhizosphere samples are reported in Table 4. All the parcels appeared very homogeneous in terms of total C-N contents and $\delta^{15}\text{N}$

in both agronomic years, apparently showing no differences between the distinct fertilization strategies. Average C content ranged from 3.31 to 3.43 wt% during maize cultivation and from 3.25 to 3.41 wt% during wheat cultivation while average N contents ranged from 0.24 to 0.25 wt% during maize cultivation and from 0.20 to 0.23 wt% during wheat cultivation. The $\delta^{15}\text{N}$ of the rhizosphere pool ranged between 4.20 to 4.91 ‰, showing comparable N isotopic fingerprint in all the parcels.

On the other hand, the $\delta^{13}\text{C}$ measured on maize and wheat rhizospheres showed systematic difference, and the wheat rhizosphere generally displays less negative (on average 1.8 ‰) $\delta^{13}\text{C}$ values.

3.2 Plant organs

The results of the elemental and isotopic C and N analyses with the associated statistical report are listed in Table 5.

3.2.1 C elemental and isotopic composition

Concerning maize (cropped in 2014), the C contents varied from 36.0 to 46.4 wt% in the stems and from 36.7 to 40.8 wt% in the grains; the relative isotopic compositions ranged from -15.4 (10CZ) to -13.8 (5NZ-15NZ) ‰ in the stems and from -13.9 (5NZ) to -12.6 (10CZ) ‰ in the grains. These values are conforming with those typical of C4 photosynthetic pathways worldwide, and particularly with those of maize plants from other Mediterranean occurrences; data coherently show physiological differences between stems and grains, with the latter systematically ^{13}C -enriched, leading to intra-plant isotopic fractionation in the order of $\delta^{13}\text{C}$ 1.0-1.5 ‰, comparable to what observed by Lasa et al. (2011).

Among the distinct parcels of the experimental field, the 10CZ treatment shows the most effective C intra-plant isotopic fractionation.

Concerning wheat (cropped in 2015), the C contents ranged from 38.0 to 43.8 wt% in stems and from 39.1 to 41.3 wt% in grains; the relative isotopic composition ($\delta^{13}\text{C}$) varied from -29.0 (5NZ) to -27.7 (15NZ) ‰ and from -27.7 (10CZ) to -25.4 (15NZ) ‰, respectively. These values highlight physiological differences between stems and grains, with the latter generally ^{13}C -enriched, leading to intra-plant isotopic fractionation in the order of $\delta^{13}\text{C}$ 1.3-2.4 ‰ comparable to what observed by Sanchez-Bragado et al. (2013). The obtained results are conforming with those typical of C3 photosynthetic pathways worldwide, and particularly with those of durum wheat plants from other occurrences in northern Italy (Brescia et al., 2002; Longobardi et al., 2015).

3.2.2 N elemental and isotopic composition

Maize N elemental contents ranged from 0.34 to 0.90 wt% in stems and from 0.93 to 2.23 wt% in grains. In spite of the relatively homogeneous elemental composition, the relative N isotopic fingerprint was highly variable in the distinct parcels of the experimental field. $\delta^{15}\text{N}$ varied between 7.60 (15NZ) and 28.5 (10CZ) ‰ in stems and between 7.99 (5NZ) and 23.5 (10CZ) ‰ in grains. On average, intra-plant comparison generally reveals $\delta^{15}\text{N}_{\text{stems}} > \delta^{15}\text{N}_{\text{grains}}$, while if we compare plants from different parcels we observe that $\delta^{15}\text{N}_{10\text{CZ}} > \delta^{15}\text{N}_{\text{UA}} > \delta^{15}\text{N}_{15\text{NZ}} \geq \delta^{15}\text{N}_{5\text{NZ}}$.

Regarding wheat, N elemental content varied in the ranges 0.19 to 0.43 and 1.63 to 2.48 wt% in stems and grains, respectively; the restricted elemental variation was associated with remarkable $\delta^{15}\text{N}$ heterogeneity. In particular, $\delta^{15}\text{N}$ varied between -0.13 (5NZ) and 16.3 (10CZ) ‰ in stems, and between 3.21 (5NZ) and 18.4 (10CZ) ‰ in grains. In this case, on average, the $\delta^{15}\text{N}_{\text{grains}} > \delta^{15}\text{N}_{\text{stems}}$. A comparison with the comprehensive dataset provided by Longobardi et al. (2015), highlights that the $\delta^{15}\text{N}$ values obtained in this study are generally high, thus

reflecting a significant signal of animal manure ($\delta^{15}\text{N}$ 21.7 ‰) which was preponderant in the 10CZ samples.

Summarizing, among the four investigated parcels, 10CZ showed the highest median N contents for all the analyzed plant parts, but for maize grains, whereas 15NZ generally showed the lowest. It is interesting to note that $\delta^{15}\text{N}$ is the parameter showing the highest correlation with the plant farming position. The median $\delta^{15}\text{N}$ values were higher in 10CZ for all the investigated plant parts, whereas the lowest median values were generally recorded in 5NZ. In particular, the highest median values were recorded in 10CZ parcel with $\delta^{15}\text{N}$ of 26.0 ‰ in the stems and 18.1 ‰ in grains of maize, 13.0 ‰ in the stems and 17.0 ‰ in the grains of wheat. Isotopic differences between distinct parcels ($\Delta^{15}\text{N}$) could be expressed as plant organs intraspecific variation respect to what observed in the UA assumed as the local baseline. In this light, the higher $\Delta^{15}\text{N}$ are recorded in 10CZ and account for 3.1 ‰ in grains and 13.01 ‰ in the stems of maize, 9.11 ‰ in grains and 10.2 ‰ in the stems of wheat.

3.3 Crop yield

In spite of the different agricultural managements, all zeolite amended plots obtained a similar or slightly higher yield with respect to UA (Table 2). During maize cultivation, the highest crop yield was obtained in 15NZ plot (118 q ha⁻¹) and the lowest was recorded in the UA plot (94.9 q ha⁻¹), whereas 5NZ and 10CZ yields were very similar (103 and 100 q ha⁻¹, respectively). During wheat cultivation, crop yield was more similar between the various plots. The highest yield was obtained in 5NZ (72 q ha⁻¹) and the lowest in the UA plot (63.7 q ha⁻¹), whereas 10CZ and 15NZ yields were 65.3 and 70.6 q ha⁻¹, respectively.

4. Discussions

While C and N contents do not appear to be correlated with the different agricultural managements, the isotopic composition efficiently reflects the distinct nutrient sources (Choi et al., 2003). These latter are mainly constituted by the two type of fertilizers used in this experiment, which are chemical fertilizers (urea and NH_4NO_3 having $\delta^{15}\text{N}$ ca. 0 ‰; Shearer et al., 1974; Freyer and Aly, 1974; Vitòria et al., 2004), variously applied in the distinct parcels, and zeolitite charged with pig-slurry ($\delta^{15}\text{N}$ ca. +20 ‰) applied only in 10CZ parcel (Table 2). In spite of a remarkable N-inputs reduction (approx. -30 % in 5NZ and 15NZ plots), yields were generally slightly higher in all zeolitite-amended plots with respect to UA, even if these results are not statistically supported by replicates of the different plots (Table 2). In this regard, it is also interesting to note that during wheat cultivation all the plants grown on zeolitite amended soils shown significantly higher total N content of grains with respect to UA plants. This may suggest that in this kind of soil common fertilizers application exceed the real plant N need or, alternatively, that the presence of zeolitite in the soil probably favour the preservation of plant nutrient budget and N use efficiency (Gholamhoseini et al., 2013).

The differences observed between maize and wheat $\delta^{13}\text{C}$ (Table 5) are due to the distinct fractionation attitude of the two crop species as a consequence of their photosynthetic pathways (C3 and C4). It is well known that wheat (C3 plant) tissues develop significantly more negative $\delta^{13}\text{C}$ values with respect to maize (C4 plant) (O'Leary, 1988; Staddon, 2004). In this respect, the slight differences in wheat and maize rhizospheres are noticeable (Table 4). These differences may be due to different isotopic compositions of plant associated rhizodeposits, since up to 40% of photosynthates are exudated by roots in the rhizosphere and immobilized/respired by rhizosphere microorganisms (Brüggemann et al., 2011).

In the two agronomic years, plants grown on 15NZ and on 5NZ plots showed significantly lower $\delta^{15}\text{N}$ with respect to UA plants in most of the organs. This behavior can be attributed to a stronger influence of the chemical fertilizers isotopic signature in the mentioned plots. It is

plausible that zeolites have captured part of NH_4^+ ions formed after urea and NH_4NO_3 hydrolysis due to their very high CEC and NH_4^+ affinity, avoiding part of the N losses that normally occur after the application of fertilizers (NO_3^- leaching/runoff, NO_x , N_2O and NH_3 volatilization). The retention of these ions probably allowed plants to uptake higher amounts of N from this specific source, resulting in a $\delta^{15}\text{N}$ significantly closer to that of chemical fertilizers (Fig 3). It is interesting to note that this effect was more visible in 5NZ plants instead of in 15NZ ones. The addition of high-doses of zeolites in 15NZ plot probably induced a relevant competition for the neo-formed ammonium ions from the fertilization between zeolites and plants, reflected by a relatively lower N uptake from this specific N pool.

Despite the different physiology and photosynthetic pathway of the investigated plants (C4 for maize, C3 for wheat), the $\delta^{15}\text{N}$ values recorded in the different organs reveal that pig-slurry signature has clearly influenced 10CZ plot (Fig 3). It has been demonstrated that generally plants $\delta^{15}\text{N}$ increases in parallel with fertilizer $\delta^{15}\text{N}$, and that plants grown in soils amended with pig-slurry can show a $\delta^{15}\text{N}$ up to +25 ‰ (Szpak, 2014). In this view, our results indicate that stem is the plant organ which mainly retains the $\delta^{15}\text{N}$ of the applied fertilizers. This evidence confirms that plants have mined the CZ pool, which in the considered experimental setup provides a source of nutrient for at least two cultivation cycles. However, according to Leggo (2000) soil microbial biomass can probably interact with NH_4^+ -enriched zeolites once added into the soil. Thus it is likely that the process does not involve a simple N transfer from zeolites to plants, but also complex interactions with soil microbial biomass that may have mediated the zeolite-plant N transfer in the investigated cultivation cycles.

Although the relationships between $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ in crops are difficult to be established and the literature provides contrasting evidences on their existence (Maxwell et al., 2014; Riehl et al., 2014), a significant trend in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation in maize and wheat grains can be observed in our case study (Table 5).

Distinct fertirrigation practices in fact influence the C discrimination factor ($\Delta^{13}\text{C}$) (Lasa et al., 2011; Bogaard et al., 2013; Maxwell et al., 2014) expressed as $[(\text{Ra}-\text{Rp})/(1+\text{Rp}/1000)]$ where Ra and Rp are the deviation of the C isotopic composition from the reference standard (Vienna Pee Dee Bee Belemnite) of atmospheric air and plant, respectively. The advantage to use “ Δ ” notation instead of “ δ ” is that the first can be used for directly express the consequences of biological processes, since it is independent of the isotopic composition of the standard (Farquhar et al., 1989). For this reason, the measurement of $\Delta^{13}\text{C}$ has been identified as a suitable indicator of the plant physiological status, especially in C3 plants where the variation in the $\Delta^{13}\text{C}$ is generally higher (Farquhar et al., 1989; Lasa et al., 2011; Maxwell et al., 2014). Fig 4 shows the $\Delta^{13}\text{C}$ - $\delta^{15}\text{N}$ relationship of maize and wheat grains. These trends seem to be coherent with those obtained in experimental studies where relationships between $\Delta^{13}\text{C}$ - $\delta^{15}\text{N}$ and manuring have been observed (Maxwell et al., 2014).

Contrary of what expected, a very clear trend was recorded in maize (C4 plant) instead of wheat (C3 plant). It is well visible that maize $\Delta^{13}\text{C}$ decreases not only when $\delta^{15}\text{N}$ of plant tissues (and thus of the N source) increases, but it seems also to be related with the amount of zeolite in the soil. On the other hand, the same trend was not maintained in wheat plants, where 15NZ and UA shown very similar $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. These differences in $\Delta^{13}\text{C}$ can be due to slight change in photosynthetic rates between the treatments as a consequence of the type of N source and amount of applied N (Maxwell et al., 2014). It cannot be excluded that also soil water availability played a role in these $\Delta^{13}\text{C}$ variations (Lasa et al., 2011; Bogaard et al., 2013; Maxwell et al., 2014). As described by Colombani et al. (2014), zeolite amendments influenced not only soil chemical properties but also physical and hydraulic conditions such as soil volumetric water content and surface dryness.

5. Conclusions

The obtained results demonstrate the reliability of isotope geochemistry in tracing the effects of zeolite applications to agricultural purposes.

We showed that zeolite amendment practices influenced N and C isotopic compositions in the soil-plant system. The observed $\delta^{15}\text{N}$ variations indicate that the addition of zeolites (especially in the low-dose plot, 5NZ) favored a more efficient uptake of N from chemical fertilizers notwithstanding a remarkable input reduction. The N transfer from NH_4^+ -enriched zeolites (CZ) to plants was confirmed for at least two cultivation cycles. The exact N transfer dynamics from zeolites to plants, which may have involved interactions with other soil N pools (like microbial biomass), will require a more in depth and specific investigation to be carried out. The C discrimination factor ($\Delta^{13}\text{C}$) is also influenced by the distinct practices; this parameter is generally interpreted as a proxy of the crop physiological status and seems to indicate that the best water/nutrient match is obtained for the 5NZ parcel, which coherently shows the highest agronomic yields.

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Figure Captions

Fig 1: Experimental field location and setting.

Fig 2: Sketch draw of the studied maize and wheat plants.

Fig 3: Scatter plot of plant organs $\delta^{15}\text{N}$ vs N for maize and wheat, respectively, together with compositions of the main N inputs (Chemical fertilizers and CZ). Rhombus, circles, reverse triangles and squares refers to UA, 5NZ, 15NZ and 10CZ treatments, respectively. Dark gray symbols refer to stems values, while white symbols refer to grains values.

Fig 4: $\Delta^{13}\text{C} - \delta^{15}\text{N}$ relationship found in maize and wheat grains, respectively, with the associated standard errors.