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# $^{15}\text{N}$ Natural Abundance, Nitrogen and Carbon Pools in Soil-Sorghum System Amended with Natural and $\text{NH}_4^+$ -Enriched Zeolitites

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**Abstract:** The use of rocks containing high amounts of natural zeolites (zeolitites) as soil amendment has been found as a valuable method for increasing agriculture sustainability. However, the potentialities and the effects of zeolitites on the biogeochemical cycles of nitrogen (N) and carbon (C) have still not been clearly addressed in the literature. The objective of this study was therefore to investigate the N and C pools and  $^{15}\text{N}$  distribution in an agricultural soil amended with both natural and  $\text{NH}_4^+$ -enriched zeolitites with the aim of understanding their effects on the soil-plant system, during sorghum cultivation, under fertilization reductions. Zeolitites were applied to an agricultural soil both at natural state (5 and 15  $\text{kg m}^{-2}$ ) and in an enriched state with  $\text{NH}_4^+$  ions from pig slurry (7  $\text{kg m}^{-2}$ ). Both zeolitites at natural and enriched state increased soil cation exchange capacity and affected microbial biomass, causing an initial decrease of microbial C and N and then a possible increase of fungal population.  $\text{N-NO}_3^-$  content was lower in natural zeolite treatments, that lead to a lower  $\text{NO}_3^-$  availability for denitrifying bacteria. Zeolitites slightly affected the fixed  $\text{N-NH}_4^+$  pool.  $\delta^{15}\text{N}$  turnover indicated that N from  $\text{NH}_4^+$ -enriched zeolitites remained in the soil until the growing season and that fertilizers partially substituted the fixed pool. Leaf  $\delta^{15}\text{N}$  content indicated that plants assimilated N from  $\text{NH}_4^+$ -enriched zeolitites and evidenced a higher fertilization recovery in natural zeolite treatments. Organic C tended to be higher in all zeolite treatment rhizospheres. In soils amended with zeolitites at natural state (at both application rates) sorghum yield was similar (+3.7%) to that obtained in the control while it was higher (+13.9%) in the plot amended with  $\text{NH}_4^+$ -enriched zeolitites.

**Keywords:** Zeolite; carbon and nitrogen pools;  $^{15}\text{N}$  natural abundance; zeolite agriculture; microbial biomass; nitrification and denitrification reduction

## 1. Introduction

The intensification of agricultural and zootechnical technology has brought food production to a high cost in terms of environmental quality [1–4]. The large use of fertilizers, as well as the need to apply manure, increase nutrients leaching and runoff risk to ground and surface water (e.g., eutrophication), soil desertification, biodiversity reduction and to an increase in greenhouse gases production (e.g.,  $\text{NH}_3$ ,  $\text{N}_2\text{O}$ ,  $\text{CO}_2$ ) [4,5].

In this respect, natural zeolites (NZ), particularly those containing clinoptilolite and chabazite, have been widely used in agriculture and horticulture. Zeolites are rocks containing more than 50% of zeolites [6], hydrated minerals capable of binding  $\text{NH}_4^+$  from solutions in their extra-framework sites, preventing  $\text{NH}_4^+$  leaching by rainfalls and/or irrigation. In this way, zeolites can increase nitrogen use efficiency (NUE) and behave as slow-releasing fertilizers, resulting in a more sustainable agriculture, where the amount of fertilizers can be decreased without lowering the production yield [6–12]. Due to their physic-chemical characteristics, zeolites can be used as soil conditioners, carriers of fertilizers, antibacterial agents, insecticides, pesticides, as enhancer of soil biological activity and additional reducer of the Sodium Adsorption Ratio (SAR) index [13–19]. A yield increases of up to 65% in several crops (sorghum, wheat, corn, rice, marrow, chard, basil, tomato, radish, watermelon, lettuce, sunflower) in soils amended with natural or  $\text{NH}_4^+$ -enriched zeolite (NEZ) were reported by several studies [6,20,21]. Analogously, Reháková et al. [14] found an increase of roots biomass in parsley (*Petroselinum sativum*), carrots (*Daucus carota*), and onions (*Allium cepa*). The same authors detected an increase in strawberry (*Senga sengana*) yield and green biomass of 70% and 49%; respectively, when natural zeolite was applied as fertilizer. The aforementioned studies provide very important and useful information regarding the beneficial effects obtained using NZ in terms of crop yield, environmental protection (reduction of nitrate leaching) and water use efficiency. Notwithstanding these promising results, a detailed study on the N and C pools dynamics in a zeolite-amended field is still lacking.

The implementation of the Water Framework Directive (2000/60/EC) and the Nitrate Directive (91/976/EEC) led to the designation of large areas vulnerable to nitrate pollution (Nitrate Vulnerable Zone, NVZ) such as the Ferrara Province (Emilia Romagna, Italy). It lays at the end of the Po Plain where the intensive agricultural practices of the entire Emilia-Romagna are conveyed. The high number of breeding farms located on this territory and the consolidate agricultural practice of animal slurry spreading (liquid mixture of urine, water and feces), increases  $\text{N}_2\text{O}$  emission by nitrification/denitrification and  $\text{NO}_3^-$  leaching, further emphasizing the vulnerability of this province [22].

In this framework, ZeoLIFE project (LIFE10+ ENV/IT/00321) was conceived to test different zeolite treatments (ZT) at the field scale aiming at reducing the input of N from fertilizers and the irrigation water in agriculture. This project exploits the high cation exchange capacity (CEC) of this material by up-taking  $\text{NH}_4^+$  from pig slurry using an especially designed prototype tank [23] or, alternatively, from chemical fertilizers directly in the field after addition to the soil [24].

The current scientific literature lacks field studies in which a detailed investigation of the dynamics of N and C pools in soil modified with natural zeolites was conducted. In this paper, we present a detailed study on the dynamics, of both organic and inorganic N and C pools in soils amended with NZ and NEZ (e.g., exchangeable and fixed  $\text{NH}_4^+$  in clay interlayers, nitrate, microbial biomass N and C, total organic carbon and carbon in humic substances).

Conventional agricultural practices (plowing, application of chemical fertilizers and monocropping) were compared with those related to the innovative zeolite methods. Non-amended soils (control) were compared with both NZ and NEZ amended soils (applied at different rates), over a cultivation cycle of sorghum (*Sorghum vulgare Pers.*). The main objectives of this work were therefore the following:

- (1) Evaluate how the addition of NZ and NEZ affects N and C pool dynamics in the soil-plant system over one agronomic year;
- (2) Determine if the use of NZ or NEZ can allow a reduction in the application of chemical fertilizers to soil;
- (3) Determine if the use of NZ or NEZ have beneficial effects on crop yield.

Considering the global need of improving agricultural sustainability and reducing environmental pollution, this work may help to unveil the potential of these methodologies.

## 2. Materials and Methods

### 2.1. Zeolitites

The natural K-rich, Na-poor zeolite used in this study is a byproduct from a quarry located near Sorano village (Italy, Grosseto province) (Figure 1, 42°41'20.65" N; 11°44'26.29" E) that is mainly exploited to obtain blocks and bricks for construction and gardening. It is a low-cost, natural and eco-friendly granular by-product of the quarrying activity, part of a large zeolitized pyroclastic deposit whose total zeolitic content is on average 70% (chabazite, 68.5%; Phillipsite, 1.8%; Analcime, 0.6%; [10]). The total CEC of the zeolite is 2170 mmol kg<sup>-1</sup>, of which 1460 are due to Ca<sup>2+</sup>, 600 to K<sup>+</sup>, 70 to Na<sup>+</sup> and 40 to Mg<sup>2+</sup>. The dry bulk density and water retention vary with grain size from 870 kg m<sup>-3</sup> and 48.4% for Ø <3 mm to 560 kg m<sup>-3</sup> and 34.2% for Ø 3–6 mm, respectively.

The grain size distribution of the employed zeolitites are reported in Figure 2. Part of the NZ was subjected to an enrichment process, which allowed the saturation of the zeolite with NH<sub>4</sub><sup>+</sup>, creating the NEZ. The enrichment process was carried out by static mode in a prototype tank where the zeolite is mechanically mixed with pig slurry (average NH<sub>4</sub><sup>+</sup> content of 2000 mg L<sup>-1</sup>) at specific solid/liquid ratio, stirring and resting times, gaining between 400 and 800 mmol of NH<sub>4</sub><sup>+</sup> per kg of material [23]. N and C pools of this material are reported in Table 1.



**Figure 1.** Location of the exploited zeolite quarry and ZeoLIFE experimental field.

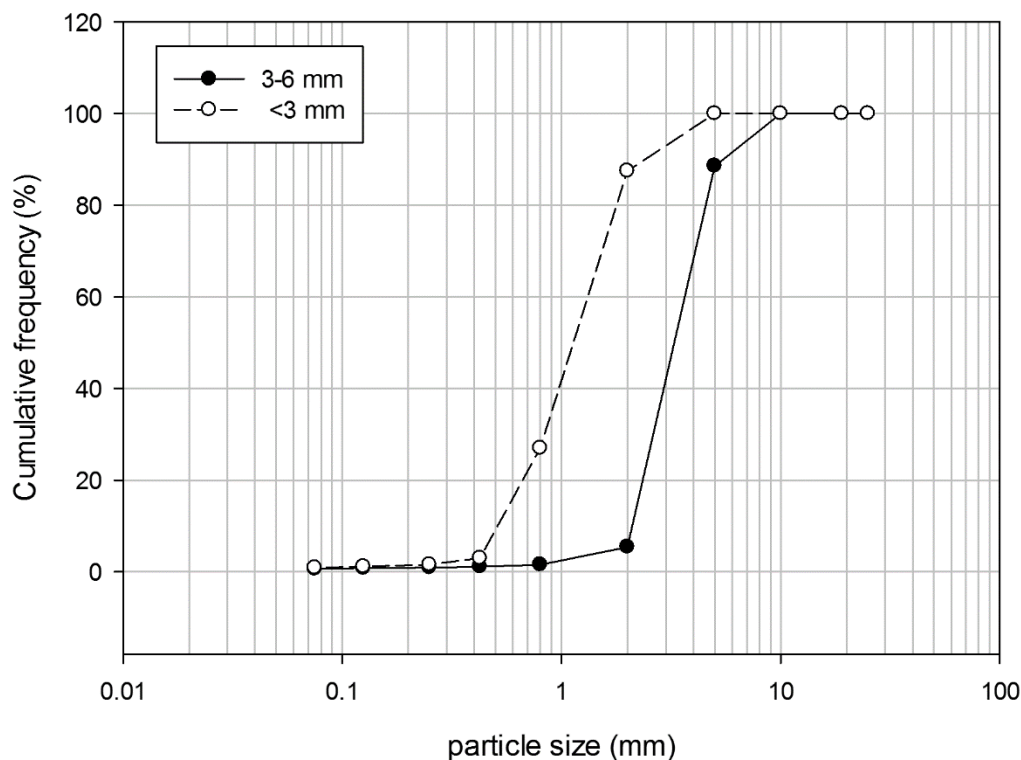


Figure 2. Particle size analysis of the employed zeolites.

## 2.2. Experimental Field Setting

The experimental field is located near Codigoro town (Italy), 40 km eastward of Ferrara (Figure 1, 44°50'33" N and 12°05'45" E) and 15 km from the Adriatic Sea in a reclaimed area at an average altitude of 3 m below sea level. The average daytime temperatures ranges from 3°C in January and 25 °C in July and the marine thermoregulation generally maintains the minima over zero, reducing the number of night frosts. The average rainfall is between 500 and 700 mm per year, representing the regional pluviometric minimum with peaks in autumn and summer (sub-continental climate).

The area belongs to the eastern Po River plain, where ancient interdistributary bays and brackish marshes were recently (1860–1960) reclaimed [25,26]. The soil of the experimental field belongs to the cartographic unit FOR1-LCO1 of the Emilia Romagna 1:50,000 Soil Map ([www.regione.emiliaromagna.it](http://www.regione.emiliaromagna.it)), and consisting of recent interfluvial silty-clay deposits classifiable as Calcaric Gleyic Cambisol [26,27]. The average grain size of the upper soil horizon is constituted by 0.6 ± 0.1% of medium sand (200–630 µm), 7.4 ± 0.3% of fine sand (63–200 µm), 49.2 ± 3.1 of silt (2–63 µm) and 42.0 ± 3.4% of clay (<2 µm) [28]. The average organic matter content is 8.1 ± 1.5%, hydraulic conductivity of the soil is 1.7 ± 2.4 m day<sup>-1</sup> while the average bulk density is 1.15 ± 0.05 kg m<sup>-3</sup> [28]. The upper soil horizon is also generally well drained due to the presence of a sub-surface drainage system at –1 m.b.g.l. Soil mineralogical composition is characterized by quartz, feldspar, calcite and clay minerals (illite, smectite, clorite, serpentine and mixed-layer) [10].

The experimental field was parceled in order to compare the different zeolite treatments with the traditional practices. The parcels were designed linear and continuous in order to facilitate the movements of farm machines. In each parcel, three different sub-parcels were considered to consider inter parcel variability and to have statistical significance. One parcel (0.5 ha) was amended with 7 kg m<sup>-2</sup> (7CZ) of NEZ (Ø < 3mm); two parcels (1 ha each) were amended with 5 (5NZ) and 15 (15NZ) kg m<sup>-2</sup> of coarse-grained (Ø 3–6mm) NZ, respectively, and 3.5 ha were used as control (CNTR). The smaller size of 7CZ parcel was due to the long time required to produce the NEZ using the “ZeoLIFE” prototype tank [23,24], which has a limited production rate of about 250 kg day<sup>-1</sup>. Addition and spreading into the field of NZ and NEZ were carried out between October 7th and

November 6th, 2012. NEZ supplied approximately 410 kg N ha<sup>-1</sup> in the 7CZ parcel. Immediately after spreading, the field was ploughed and the zeolite was located until an approximate depth of 30–40 cm. According to ZeoLIFE plan, in the first year of field experiment, sorghum (*Sorghum vulgare Pers.*) was sowed on May, 9<sup>th</sup>, 2013. Nitrogen fertilizers were distributed in two steps: di-ammonium phosphate during the sowing, with an application of 21.6 kg N ha<sup>-1</sup> in all parcels and urea on June 3<sup>rd</sup>, 2013, with different doses in the various parcels. In the CNTR parcel, an average of 170 (±16) kg N ha<sup>-1</sup> were supplied, while a reduction of approximately 30% and 50% of urea in NZ (both 5NZ and 15NZ for 122 and 127 kg N ha<sup>-1</sup>, respectively) and NEZ parcels (81 kg N ha<sup>-1</sup>) was applied, respectively. Harvest occurred in September, 23<sup>rd</sup>, and the yield was separately evaluated for each treatment.

### 2.3. Soil and Plant Sampling

A total of 60 soil samples (from 0 to 30 cm depth) were collected in the three sub-parcels for each treatment by manual drilling using an Eijelkamp Agrisearch auger. Soil samplings were carried out in four periods of the agronomical year: (1) November 2012 (Nov-2012), after the zeolite application; (2) May 2013 (May-2013), before sowing and the first fertilization; (3) June 2013 (Jun-2013), after the urea addition; (4) September 2013 (Sep-2013), at the harvest. Soil samples collected at the harvest were subdivided in bulk soil and rhizosphere. The rhizosphere was separated by gently shaking the plant roots. Three representative plants from each treatment and each sub-parcel were sampled during the harvest; each plant was subdivided in roots, stems, leaves and panicles that were subsequently dried at 50 °C and milled (total of 48 samples).

### 2.4. Analytical Techniques

CEC and exchangeable bases were determined using the Co(NH<sub>3</sub>)<sub>6</sub>Cl<sub>3</sub> method [29,30] on both bulk soil and rhizosphere samples collected in Sep-2013, pH was determined on 1:5 (w:v) extracts using a pH-meter, while carbonate content was determined using the volumetric method. Total nitrogen (TN) and organic C (TOC) of soil and plant samples were determined by an elemental CHNS-O EA 1110 Thermo Fisher Scientific analyzer coupled with mass spectrometry (Delta Plus, Finnegan, Thermo-Fisher). Soil samples were weighted in silver pots and treated with HCl to eliminate the inorganic C, while plant samples were weighted in tin pots. <sup>15</sup>N natural abundance was measured in total and fixed nitrogen pools (TN and Fix N-NH<sub>4</sub><sup>+</sup>) and in sorghum organs; the natural abundance was expressed in δ‰ (Equation (1); [31]), where δ represents the difference from the standard atmospheric N<sub>2</sub> (0.3663% <sup>15</sup>N):

$$\delta^{15}\text{N} (\text{‰}) = \left[ \frac{\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{sample}} - \left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{st}}}{\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{st}}} \right] \times 1000 \quad (1)$$

To evaluate the influence of the N source on the plant N isotopic signature, mixing lines were built between each end-member represented by soil, NEZ and urea.

NO<sub>3</sub><sup>-</sup> was extracted with Milli-Q (Millipore USA) water in a 1:5 (w/v) ratio, the solution was shaken for 1 hour and then filtered. NO<sub>3</sub><sup>-</sup> was determined by ion chromatography using an isocratic dual pump ion chromatography ICS-1000 Dionex. An AS-40 Dionex auto sampler was employed to run the analysis; Quality Control (QC) samples were run every 10 samples and the standard deviation for all QC samples was better than 4%.

Exchangeable N-NH<sub>4</sub><sup>+</sup> was extracted with 1M KCl in a 1:10 (w/v) ratio, the solution was shaken for 1 hour and then filtered. The solution was diluted and analyzed with an Ion Selective Electrode (ISE) Orion 95-12 connected to a Thermo Fisher Orion 4star pH-ISE benchtop. Interferences with Cl<sup>-</sup> and K<sup>+</sup> were verified and excluded by comparing measurement made with NH<sub>4</sub>Cl, CH<sub>3</sub>COONH<sub>4</sub> + 1M KCl and CH<sub>3</sub>COONH<sub>4</sub> + H<sub>2</sub>O standard solutions.

After the elimination of organic and exchangeable N, fixed N-NH<sub>4</sub><sup>+</sup> (Fix N-NH<sub>4</sub><sup>+</sup>) was determined by adding 20 mL of KBrO to 2 g of air-dried soil samples in two replicates. After 2 hours, 40 mL of

distilled water was added and a heating cycle of 10 min in a microwave oven was applied. Then, 20 mL of Milli-Q H<sub>2</sub>O was added and samples were left still for 24 hours. Finally, samples were centrifuged at 5000 rpm for 10 min and washed 2 times with 0.5 M KCl and analyzed by elemental analysis for the evaluation of TN and  $\delta^{15}\text{N}$  [32].

Total extractable carbon (TEC) was obtained adding 100 mL of 0.1 M NaOH + 0.1 M NaP<sub>2</sub>O<sub>7</sub>·10H<sub>2</sub>O to 10 g of air-dried soil. The solution was left in a Dubnoff bath at 60°C for 24 hours and then centrifuged at 6600 rpm for 15 min and filtered at 0.45 mm (Millipore). Part of this solution was acidified with HCl 1:1 to reach pH < 2 to allow the precipitation of Humic Acids (HA), which were separated after centrifugation at 7000 rpm for 25 min by the Non-Humic-Substances (NHS) and Fulvic Acids (FA). The separation of FA from NHS was performed by solid chromatography using a polyvinylpyrrolidone (PVP) polymer in acid ambient, which allows the retention of FA on the PVP and lets NHS pass through the column. The FA was then recovered by bringing the pH of the column to alkaline values with 0.5 M NaOH. The evaluation of C contained in TEC, HA and FA was obtained by reverse titration with 0.2 N Fe<sub>2</sub>SO<sub>4</sub> after oxidation of the C contained in the sample to CO<sub>2</sub> adding 5 mL of 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> + 20 mL of H<sub>2</sub>SO<sub>4</sub> and heating at 150 °C for 10 min [33]. The C extraction was carried out on one replicate.

Humification Index (HI) and Humification Degree (HD) were calculated as the ratio of C contained into NHS divided by the C contained into Humic Substances (HS) and as the ratio between HS and TEC expressed as percentage respectively (Equations (2) and (3), [33,34]).

$$HI = \frac{[TEC - (HA + FA)]}{HA + FA} \quad (2)$$

$$HD = \frac{[100 \times (HA + FA)]}{TEC} \quad (3)$$

where HI is the Humification Index, DH is the Humification Degree, TEC is Total Extractable C, HA is Humic Acids and FA are Fulvic Acids.

C and N immobilized by microbial biomass (C<sub>mic</sub> and N<sub>mic</sub>) were obtained by the chloroform fumigation-extraction method [35]. Following this, 10 g of soil samples in four replicates were humidified until 60% of water holding capacity (WHC) and incubated at room temperature for 1 week at constant WHC. After this period, two replicates were extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub>, representing the non-fumigate sample (NF). On the other two samples, CHCl<sub>3</sub> was added in a closed drier equipped with a void pump to allow CHCl<sub>3</sub> volatilization at room temperature. In these fumigate (F) samples the presence of CHCl<sub>3</sub> atmosphere guarantees the death of the microbial biomass and allows release of the immobilized C and N after the same extraction with 0.5 M K<sub>2</sub>SO<sub>4</sub> performed for NF. After the extraction, both NF and F samples were analyzed with a Shimadzu Total Organic Carbon Analyser TOC-V CPN coupled with a TN unit (TNM-1). Sorghum harvest was carried out with a harvester and the total yield of each parcel was weighted separately directly in the farm using the local vehicle scale. It was not possible to harvest separately the three sub parcels of each treatment, as this practice would have required an excessive time and precision not compatible with the host farm agricultural practices.

### 2.5. Statistical Analysis

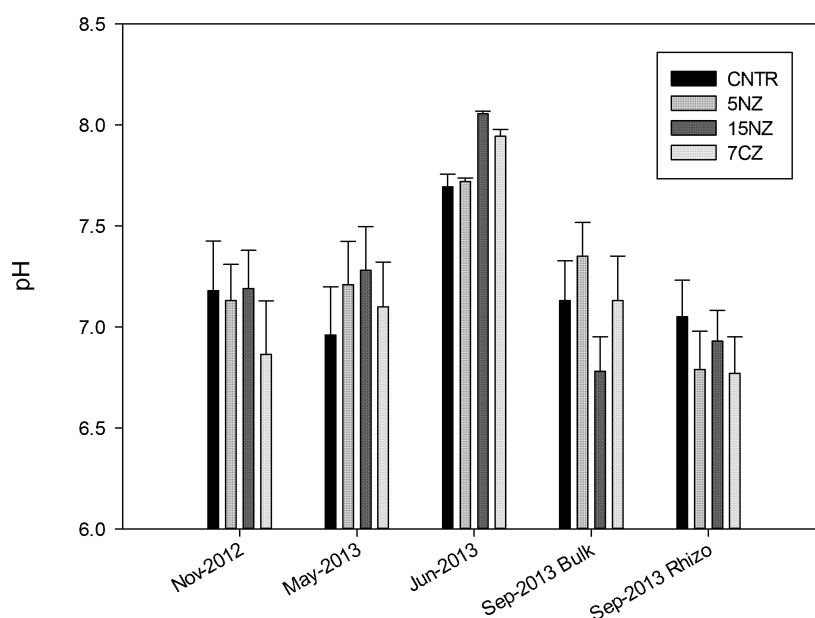
At least three replicates per treatments have been analyzed at each sampling point to have statistical significance. For evaluating significant differences between the treatments, parametric statistic was applied to the dataset. Two-Way ANOVA was employed (after verifying normality of data distribution with Shapiro-Wilk test) for testing significant differences within factors time and treatments and for testing interactions between factors (treatments\*time). Successively, a series of One-Way ANOVA and Tukey (HSD) post-hoc pairwise multiple comparison tests were applied at each sapling time at "p" level of 0.05 using Sigmaplot 12.0. Correlation analyses were also performed (using Pearson coefficient "ρ") in order to quantify linear associations between variables for each treatment.

### 3. Results

After the enrichment process with pig slurry, NEZ gains a total N load of  $5.84 (\pm 0.90) \text{ g kg}^{-1}$  (Table 1). Most of the N was present in the form of Exch  $\text{N-NH}_4^+$ , while Fix  $\text{N-NH}_4^+$  is considerably lower.  $\text{N-NO}_3^-$  load is on average  $148 \text{ mg kg}^{-1}$  (the material is not washed after the treatment before the introduction into the soil system). NEZ is characterized by a very high  $\text{N}_{\text{mic}}$  and a low  $\text{C}_{\text{mic}}$ , resulting in a  $\text{C}_{\text{mic}}/\text{N}_{\text{mic}}$  ratio lower than 1. TOC content is quite low ( $10.3 \text{ g kg}^{-1}$  on average) resulting in a low C/N. Soil pH was not affected by the addition of both NZ and NEZ ( $p > 0.05$ ), remaining close to neutral values in all treatments (with exception of Jun-2013 where after urea addition an increase toward sub-alkaline values ( $p < 0.05$ ), Figure 3). CEC increased significantly ( $p < 0.05$ ) in the bulk soil of all ZT.  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  were the major exchangeable bases, followed by  $\text{K}^+$  and  $\text{Na}^+$ , with the latter always higher in 7CZ than in the other treatments (Table 2). No significant differences were found between the various treatments regarding exchangeable  $\text{K}^+$  ( $p > 0.05$ ), while  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are generally higher in ZT, especially in the bulk soil with respect to the rhizosphere ( $p < 0.05$ ).

**Table 1.** Average N and C pools of  $\text{NH}_4^+$ -enriched zeolite (NEZ). Standard deviation within brackets.

TN	$\text{g}\cdot\text{kg}^{-1}$	5.84 (0.90)
Fix $\text{N-NH}_4^+$	$\text{mg}\cdot\text{kg}^{-1}$	343 (21)
Exch $\text{N-NH}_4^+$	$\text{mg}\cdot\text{kg}^{-1}$	4884 (330)
$\text{N-NO}_3^-$	$\text{mg}\cdot\text{kg}^{-1}$	148 (7.4)
$\text{N}_{\text{mic}}$	$\text{mg}\cdot\text{kg}^{-1}$	876 (152)
TOC	$\text{g}\cdot\text{kg}^{-1}$	10.3 (0.1)
$\text{C}_{\text{mic}}$	$\text{mg}\cdot\text{kg}^{-1}$	117 (1)
C/N		1.78
$\text{C}_{\text{mic}}/\text{N}_{\text{mic}}$		0.13



**Figure 3.** Mean soil pH for each treatment. Error bars represent standard deviation.

**Table 2.** Average of exchangeable bases and cation exchange capacity (CEC) of soil samples, both bulk soil and rhizosphere. Standard deviation within brackets. Significant ( $p < 0.05$ ) or non-significant ( $p > 0.05$ ) differences between treatments at each sampling time are indicated by capital letters.

Time	Treatment	Ca <sup>2+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	CEC
		cmol·kg <sup>-1</sup>	cmol·kg <sup>-1</sup>	cmol·kg <sup>-1</sup>	cmol·kg <sup>-1</sup>	cmol·kg <sup>-1</sup>
Sep-2013 Rhizo	CNTR	18.3 (0.7) A	8.3 (1.7) A	14.4 (1.2) A	1.8 (0.7) AB	42.8 (4.0) A
	5NZ	18.6 (0.6) A	9.9 (0.8) A	17.2 (0.9) B	1.3 (0.1) A	47.1 (1.9) A
	15NZ	17.7 (0.9) A	10.4 (0.8) A	17.9 (1.4) B	1.6 (0.6) A	47.5 (2.5) A
	7CZ	19.4 (1.1) A	7.7 (3.8) A	15.9 (0.6) AB	3.0 (0.4) B	46.0 (2.7) A
Sep-2013 Bulk	CNTR	19.9 (1.3) A	6.1 (3.3) A	16.8 (1.3) A	2.5 (1.1) AB	45.3 (2.3) A
	5NZ	20.3 (0.01) AB	4.9 (0.1) A	21.9 (0.1) B	2.0 (0.02) A	49.1 (0.04) AB
	15NZ	23.0 (0.02) B	5.9 (0.004) A	19.5 (0.1) AB	1.7 (0.1) A	50.2 (0.2) B
	7CZ	22.0 (0.2) AB	5.0 (0.1) A	19.9 (0.2) B	3.6 (0.04) B	50.6 (0.6) B

### 3.1. Nitrogen and Carbon Pools in Soil

Soil N pools significantly differed ( $p < 0.05$ ) between the treatments and among the sampling times, with the exception of TN that shows significant differences among sampling times only ( $p < 0.05$ ) (Table S1, Supplementary Materials). High TN were detected in Nov-2012 and Sep-2013, while a depletion was observed in May-2013 and Jun-2013.

Similar behavior is recorded for Fix N-NH<sub>4</sub><sup>+</sup> pool but in this case, all ZT shows slightly lower values ( $p < 0.05$ ) in Nov-2012 with respect to the CNTR, while in Jun-2013 only 15NZ treatment is significantly lower (Table S1, Supplementary Materials). At the beginning of the experiment, N-NO<sub>3</sub><sup>-</sup> content was significantly higher in the CNTR and 7CZ parcels than in those amended with NZ (5NZ and 15NZ, respectively) ( $p < 0.05$ ). In May-2013 a general depletion of N-NO<sub>3</sub><sup>-</sup> pool is noticed in all the treatments, increasing in Jun-2013 after urea addition especially in CNTR parcel. N-NO<sub>3</sub><sup>-</sup> values of ZT (especially 5NZ and 15NZ) were remarkably lower in this period with respect to the CNTR ( $p < 0.05$ ) (Table S1, Supplementary Materials). Exch N-NH<sub>4</sub><sup>+</sup> was significantly affected by NEZ addition in Nov-2012 ( $p < 0.05$ ) (138 mg kg<sup>-1</sup> vs 50 mg kg<sup>-1</sup> of 7CZ and CNTR, respectively) while starting from May-2013 these differences completely disappeared ( $p > 0.05$ ). In Jun-2013, there were no differences between the treatments ( $p > 0.05$ ) while a relative enrichment of the bulk soil Exch N-NH<sub>4</sub><sup>+</sup> with respect to the rhizosphere was apparent in Sep-2013.

Generally, at the beginning of the experiment, all treatments showed a low N<sub>mic</sub> content, following the order 7CZ > CNTR > 5NZ = 15NZ, suggesting that zeolitites differentially affected the N<sub>mic</sub> pool. N<sub>mic</sub> was increased by NEZ addition while it was decreased by NZ addition. The N<sub>mic</sub> pool increases in May, while it decreases in June after the chemical fertilization; as expected, N<sub>mic</sub> content in rhizospheric soil is higher than that determined in the bulk soil at the harvest (Table S1, Supplementary Materials). A remarkable increase of C<sub>mic</sub> and N<sub>mic</sub> and a contemporaneous decrease of N-NO<sub>3</sub><sup>-</sup> in all the treatments was observed in May-2013 (Figure 4).

During the monitored period, C<sub>mic</sub> ranged from 16 to 432 mg kg<sup>-1</sup>. At the beginning of the experiment large differences ( $p < 0.05$ ) were detected among the various treatments. In this period, C<sub>mic</sub> content decreases following the order CNTR (148 mg kg<sup>-1</sup>) > 15NZ (98.5 mg kg<sup>-1</sup>) > 7CZ (43.2 mg kg<sup>-1</sup>) > 5NZ (16.1 mg kg<sup>-1</sup>). In May-2013, C<sub>mic</sub> increased in all treatments, except for 15NZ, and decreased again in Jun-2013. As expected, C<sub>mic</sub> content in the rhizosphere is higher than that determined in the bulk soil. The average C<sub>mic</sub>/N<sub>mic</sub> ratio ranges from 3.3 to 94 (Figure 5). In Nov-2012, low values of C<sub>mic</sub>/N<sub>mic</sub> were observed in 5NZ and 7CZ treatments; concerning 5NZ, the low ratio is due to the lower C<sub>mic</sub> with respect to the other treatments, while for 7CZ the low ratio may have been due to the high N<sub>mic</sub>. All ZT have a higher C<sub>mic</sub>/N<sub>mic</sub> ratio than CNTR after May-2013 because of the lower N<sub>mic</sub>; on average, C<sub>mic</sub>/N<sub>mic</sub> ratio increased in order of 15NZ > 5NZ > 7CZ > CNTR. TOC showed significant differences between the treatments and among the sampling times ( $p < 0.05$ ). In particular, a depletion of TOC content was observed in 7CZ in Nov-2012 and in Jun-2013, after urea addition (Table S1,



Supplementary Materials). As expected, TOC content in Sep-2013 was higher in rhizosphere than in the bulk soil and there was a significant correlation between TN and TOC ( $\rho = 0.83, p < 0.05$ ). Soil C/N ratio showed significant differences among the treatments only at the beginning of the experiment because of the higher TN of 7CZ ( $p < 0.05$ ), while during the rest of the monitored period, soil C/N ratio remained constant around 10 (Table S1, Supplementary Materials). HS C content was reported in Table 3. TEC content ranged from 8.3 to 19.2 g kg<sup>-1</sup> (data not shown), whereas the extraction ratio ranged between 41 to 87%. Notably, the highest extraction ratio was observed in 15NZ parcel, followed by 5NZ. Generally, HA C content is higher than that determined in FA, with exception of 7CZ in Nov-2012, in which the FA/HA ratio is higher than 1. A low HI was generally recorded in all the treatments with the exception of 5NZ, where especially in May, June and Spet-2013 Bulk, HI increases through values above 0.5, highlighting a prevalence of NHS. The HD% generally varied between 30% and 50%, with the exception of 15NZ that showed large variations (from 27.8% in Nov-2012 to 68.2% in May-2013).

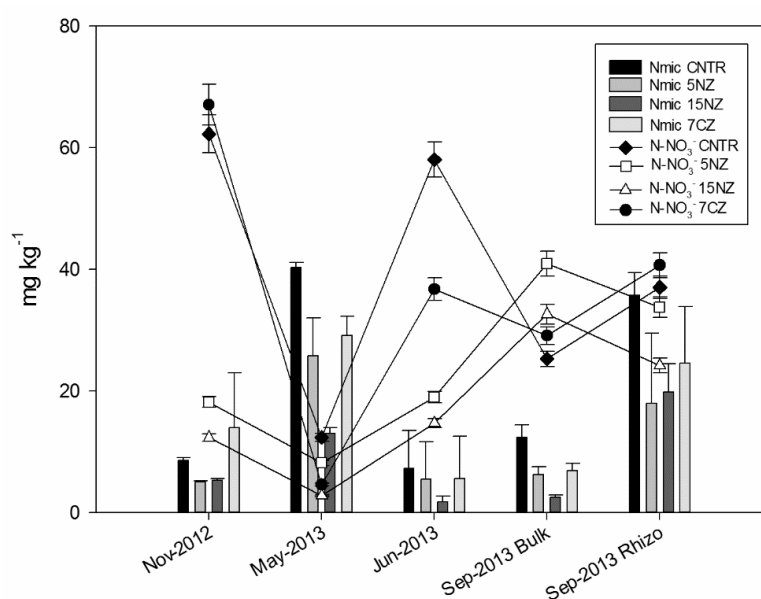


Figure 4.  $N_{mic}$  (columns) and  $N-NO_3^-$  (lines) content of soil samples across the monitoring period.

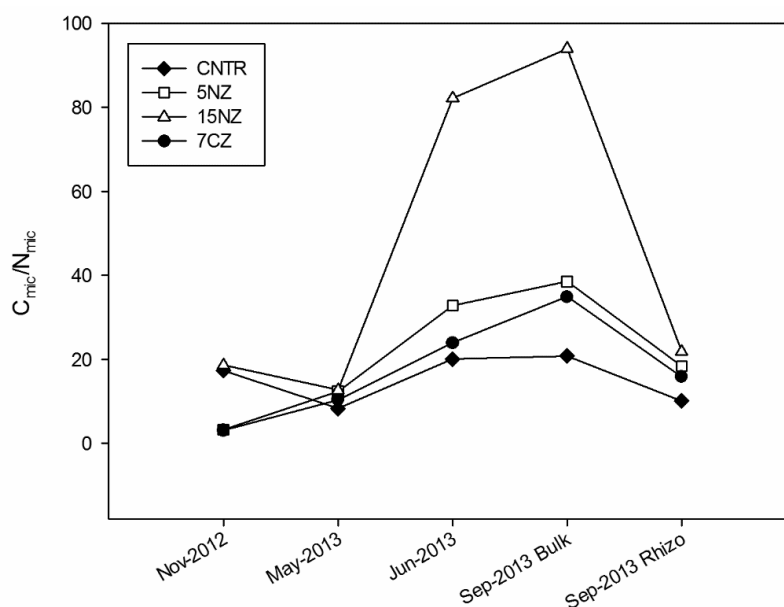


Figure 5. Microbial biomass C/N ratio over the monitoring period.

**Table 3.** C content of Humic Acids (HA) and Fulvic Acids (FA), percentage of Total Extractable and Total Organic Carbon (TEC/TOC) ratio, Humification Index (HI) and Humification Degree (HD) according to Ciavatta et al. [33] and Sequi et al. [34].

Time	Treatment	HA	FA	TEC/TOC	HI	HD
		g kg <sup>-1</sup>	g kg <sup>-1</sup>	%		%
Nov-2012	CNTR	5.3	3.4	47.4	0.4	34.4
	5NZ	4.3	5.0	46.2	0.2	37.7
	15NZ	3.9	3.7	41.4	0.5	27.8
	7CZ	2.7	3.9	40.1	0.3	31.9
May-2013	CNTR	7.4	3.2	63.0	0.5	43.1
	5NZ	8.6	3.5	74.4	0.6	46.9
	15NZ	11.4	3.6	87.3	0.3	68.2
	7CZ	5.9	2.5	48.6	0.1	43.8
Jun-2013	CNTR	4.5	3.1	52.3	0.4	38.6
	5NZ	4.3	2.9	65.0	0.8	35.5
	15NZ	3.8	3.1	50.6	0.3	39.2
	7CZ	3.4	3.0	43.6	0.6	27.4
Sep-2013 Bulk	CNTR	5.9	3.2	52.9	0.2	44.2
	5NZ	3.9	2.9	54.5	0.9	29.4
	15NZ	8.6	3.3	70.7	0.4	51.3
	7CZ	5.9	3.5	57.9	0.4	41.2
Sep-2013 Rhizo	CNTR	8.1	3.1	58.1	0.3	43.4
	5NZ	6.9	3.2	48.5	0.4	34.7
	15NZ	9.7	3.2	49.2	0.2	41.0
	7CZ	7.1	3.3	48.8	0.3	37.0

### 3.2. Total N and C Content in Sorghum Plant Components

No significant differences in TN and TOC content were detected in the sorghum organs (e.g., roots, stems, leaves and panicles) among the treatments ( $p > 0.05$ ) (Table S2, Supplementary Materials). Generally, leaves and panicles had a higher TN content than roots and stems, while TOC was similar in all the plant organs, resulting in decline of the C/N ratio following the order stems > roots > panicles > leaves.

### 3.3. $\delta^{15}\text{N}$ Natural Abundance in Soil and Sorghum Organs

TN and Fix N-NH<sub>4</sub><sup>+</sup>  $\delta^{15}\text{N}$  differs significantly ( $p < 0.05$ ) among treatments and time (Table 4). At the beginning of the experiment (Nov-2012), 7CZ shows a remarkably higher isotopic signature ( $\delta^{15}\text{N}$  of 7.46‰ and 8.27‰ for TN and Fix N-NH<sub>4</sub><sup>+</sup>, respectively) with respect to the other treatments ( $p < 0.05$ ). Furthermore, without considering 7CZ treatment, Fix N-NH<sub>4</sub><sup>+</sup> pool showed a high  $\delta^{15}\text{N}$  variability (from 4.55‰ to 6.00‰) with respect to the TN pool (from 3.38‰ to 3.86‰). In May-2013, TN  $\delta^{15}\text{N}$  is still significantly higher ( $p < 0.05$ ) in 7CZ treatment, while no significant differences were observed regarding Fix N-NH<sub>4</sub><sup>+</sup> pool. In Jun-2013 (after the chemical fertilization), all treatments showed a homogeneous  $\delta^{15}\text{N}$  in both TN and Fix N-NH<sub>4</sub><sup>+</sup> pools ( $p > 0.05$ ). In Sep-2013, rhizosphere samples show higher  $\delta^{15}\text{N}$  with respect to the bulk soils in both TN and Fix N-NH<sub>4</sub><sup>+</sup> pools (especially in the latter). The isotopic signatures of NEZ, urea and di-ammonium phosphate were: +18.4 (± 0.5) ‰, −1.18 (± 0.06) ‰ and −0.89 (± 0.09) ‰, respectively (Table 5). Isotopic analysis on the harvested plants indicated that the  $\delta^{15}\text{N}$  of plant tissues was influenced by the different agricultural managements employed in the experimentation (Table 5).  $\delta^{15}\text{N}$  was significantly higher in 7CZ plants leaves and significantly lower in all 5NZ plants organs with respect to the CNTR plants ( $p < 0.05$ ). In Figure 6, the TN vs  $\delta^{15}\text{N}$  of the different plants' organs are plotted together with urea, NEZ and bulk soil. It is evident that the 7CZ leaves are strongly affected by NEZ contribution, while 5NZ plants are more influenced by a low  $\delta^{15}\text{N}$  N-source.

### 3.4. Sorghum Yield

The CNTR parcel returned a yield of 5818 kg ha<sup>-1</sup>. The two NZ returned 6032 kg ha<sup>-1</sup> each, while 7CZ parcel gave 6627 kg ha<sup>-1</sup>. Compared with the CNTR, the three zeolite-treated parcels gained from 3.7 to 13.9%, although the yield results are not statistically significant.

**Table 4.** Average <sup>15</sup>N natural abundance in soil N pools (TN and Fix N-NH<sub>4</sub><sup>+</sup>). Standard deviation within brackets. Significant ( $p < 0.05$ ) or non-significant ( $p > 0.05$ ) differences between treatments at each sampling time are indicated by capital letters. “\*” and “x” symbols indicates the presence or the absence, respectively, of a significant difference among the variables for Treatments and Time in the experiment period and correlating treatments with time (Treatment\*Time).

Time	Treatment	TN $\delta^{15}\text{N}$ vs air	Fix $\delta^{15}\text{N}$ vs air
		‰	‰
Nov-2012	CNTR	3.86 (0.15) A	6.00 (0.17) B
	5NZ	3.83 (0.22) A	5.28 (0.45) AB
	15NZ	3.38 (0.19) A	4.55 (0.31) A
	7CZ	7.46 (0.53) B	8.27 (0.36) C
May-2013	CNTR	3.75 (0.20) AB	3.34 (0.15) A
	5NZ	3.34 (0.06) A	3.38 (0.30) A
	15NZ	3.31 (0.62) A	3.84 (0.04) A
	7CZ	4.87 (0.17) B	3.11 (0.12) A
Jun-2013	CNTR	3.11 (0.28) A	3.65 (0.43) A
	5NZ	3.37 (0.10) A	3.38 (0.17) A
	15NZ	3.32 (0.05) A	3.46 (0.13) A
	7CZ	3.27 (0.17) A	3.73 (0.19) A
Sep-2013 Bulk	CNTR	2.68 (0.11) A	1.37 (0.11) A
	5NZ	2.39 (0.09) A	1.83 (0.23) AB
	15NZ	2.73 (0.14) A	1.79 (0.03) AB
	7CZ	2.55 (0.29) A	2.09 (0.16) B
Sep-2013 Rhizo	CNTR	4.22 (0.38) A	2.47 (0.26) A
	5NZ	4.17 (0.31) A	2.81 (0.75) A
	15NZ	4.55 (1.11) A	2.40 (0.46) A
	7CZ	4.45 (0.19) A	2.52 (0.31) A
NEZ		18.4 (0.5)	17.3 (0.4)
Urea		-1.18 (0.06)	-
Diammonium Phosphate		-0.89 (0.09)	-
	Treatment	*	*
	Time	*	*
	Treatment*Time	*	*

**Table 5.** Average <sup>15</sup>N natural abundance in sorghum organs at the harvest. SD within brackets. Significant ( $p < 0.05$ ) or non-significant ( $p > 0.05$ ) differences between treatments for each organ are indicated by capital letters.

Plant Organ	Treatment	$\delta^{15}\text{N}$ vs Air
		‰
Roots	CNTR	10.2 (1.4) AB
	5NZ	6.33 (1.69) A
	15NZ	10.3 (2.8) AB
	7CZ	11.6 (2.7) B

Table 5. Cont.

Plant Organ	Treatment	$\delta^{15}\text{N vs Air}$
		‰
Stems	CNTR	9.95 (0.81) B
	5NZ	6.08 (1.56) A
	15NZ	11.1 (1.1) B
	7CZ	11.0 (1.5) B
Leaves	CNTR	12.3 (1.6) B
	5NZ	8.88 (0.94) A
	15NZ	13.8 (2.2) BC
	7CZ	18.1 (1.7) C
Panicles	CNTR	10.3 (0.4) B
	5NZ	6.82 (0.97) A
	15NZ	10.0 (1.8) B
	7CZ	11.7 (2.0) B

#### 4. Discussion

As expected, CEC was generally higher in ZT with respect to the CNTR, especially in the bulk soil. Ozbahce et al. [36] and Gholamhoseini et al. [20] found a similar tendency after mixing zeolite in the first 30 cm of soil or after the spreading in combination with cattle manure. Both bulk and rhizosphere soils in the 7CZ parcel showed higher exchangeable  $\text{Na}^+$  with respect to the other treatments, likely because of the NEZ enrichment process with pig slurry.

##### 4.1. NEZ Influence on Soil N and C Pools.

###### 4.1.1. NEZ Effects on N Pool Size and Isotopic Signature

The addition of NEZ into the soil (7CZ treatment) did not lead to significant differences in TN or Fix  $\text{N-NH}_4^+$  content regardless the N input of about 410 kg of  $\text{N ha}^{-1}$  brought by NEZ introduction. On the other hand, this N input has clearly influenced Exch  $\text{N-NH}_4^+$ ,  $\text{N-NO}_3^-$  and  $\text{N}_{\text{mic}}$  pools, increasing their size. Through the application of a simple mass balance equation, it was estimated that the increment in 7CZ Exch  $\text{N-NH}_4^+$  pool corresponded to the amount of N introduced with NEZ in the first 30 cm of soil.

As it is known,  $\text{NH}_4^+$ -enriched zeolites can act as slow-released fertilizer [37–39] and in our experiment this behavior was clarified by the isotopic signature of the TN pool during the agricultural management. The  $\delta^{15}\text{N}$  increase of TN pool in 7CZ with respect to that found in the other treatments is in fact due to the N isotopic signature of the pig slurry contained into the NEZ. Dittert et al. [40] found a  $\delta^{15}\text{N}$  of 5.90‰ in agricultural soil treated with slurry while a  $\delta^{15}\text{N}$  of 3.90‰ was found in soils treated with inorganic fertilizers. Pig slurry usually has high  $\delta^{15}\text{N}$  values, as a result of the fractionation processes occurring during volatilization of  $\text{NH}_3$  after the excretion, which causes an enrichment in the heavier  $^{15}\text{N}$  atoms in the substrate (slurry) with respect to the product (ammonia gas) [40–44]. The significantly higher  $\delta^{15}\text{N}$  of TN in May-2013 suggested that a significant amount of N introduced with NEZ was still present in the soil before the sowing. At the beginning of the experimentation, the organic C cycle of 7CZ treatment was affected by the high N input provided by NEZ, resulting in a decrease of TOC content and C/N ratio; furthermore, an increase of FA fraction can be interpreted as an increase in depolymerization processes.

###### 4.1.2. NEZ Specific Effects on Soil Microbial Biomass

On the other hand,  $\text{N}_{\text{mic}}$  increment in 7CZ parcel was not counterbalanced by a parallel  $\text{C}_{\text{mic}}$  increment, resulting in a strong decrease of the  $\text{C}_{\text{mic}}/\text{N}_{\text{mic}}$  and likely in an increase of organic matter (OM) mineralization processes [44]. No evidence of enhanced nitrification was observed in 7CZ parcel

after the zeolite spreading as  $\text{N-NO}_3^-$  content was very similar to the CNTR notwithstanding the large N input provided by NEZ spreading (with residual  $\text{N-NO}_3^-$  from the pig slurry treatment). The agronomic year before the experimental cultivation was low in precipitation and the drought caused low yield and accumulation of high amounts of N from fertilizers in the upper soil horizon, thus no differences were expected between CNTR and ZT. A possible explanation of this behavior is that controlled retention and release capacity of  $\text{NH}_4^+$  contained in the microsite of the open-ring structure of the mineral. These ions are physically protected and likely less available to nitrifying bacteria (leading consequently to a lesser  $\text{N-NO}_3^-$  content in the pore-waters) [45–48]. This hypothesis is plausible but not supported by a direct measurement of gross nitrification rates (e.g., by the mean of  $^{15}\text{N}$  pool dilution technique). In this light, further studies are urgently needed where gross N transformation rates are measured in soil amended with NEZ and NZ to efficiently quantify gross N production and consumption through mineralization, nitrification and denitrification processes.

In May-2013,  $C_{\text{mic}}$  and  $N_{\text{mic}}$  increased significantly in all treatments, while  $\text{N-NO}_3^-$  pool significantly decreased (but not Exch  $\text{N-NH}_4^+$  pool with the exception of 7CZ) (Figure 4), suggesting that microbial immobilization prevailed over mineralization [49]. In addition to  $\text{NO}_3^-$  decrease, 7CZ treatment showed a reduction of Exch  $\text{N-NH}_4^+$  pool, where the N surplus caused by the NEZ introduction completely disappeared. A fraction of this mineral N depletion can be attributable to  $\text{NO}_3^-$  leaching and/or to  $\text{N}_2\text{-N}_2\text{O}$  losses through denitrification process (probably enhanced by waterlogged conditions; [25]). It is important to consider that NEZ introduction affected mainly the Exch  $\text{N-NH}_4^+$  and not the Fix  $\text{N-NH}_4^+$  pool and that 7CZ still had high TN  $\delta^{15}\text{N}$  in May-2013, confirming that an important fraction of NEZ-N was still present in the soil system. It is thus possible that a large fraction of the exchangeable N contained into NEZ has been immobilized by the microbial biomass [50,51].

#### 4.1.3. NEZ Specific Effects on Fixed $\text{N-NH}_4^+$ Pool

A possible hypothesis is that Fix  $\text{N-NH}_4^+$  pool is in dynamic equilibrium with exchangeable and soluble  $\text{N-NH}_4^+$  pools [52,53]. In our case, this hypothesis is supported by the  $\delta^{15}\text{N}$  turnover of fixed pool that suggested a continuous N replacement during the monitoring period. At the start of the experimentation, Fix  $\delta^{15}\text{N}$  of CNTR and NZ treatments was probably still affected by previous slurry treatments as shown by the higher isotopic signature. Generally, microbial activity increases with the increase of soil temperature, affecting the other N pools, in particular, a decrease in  $\text{N-NO}_3^-$  and Exch  $\text{N-NH}_4^+$  pools (as in May-2013 sampling) can alter the equilibrium with fixed interlayer ammonium. This decrease can cause a release of N from Fix  $\text{N-NH}_4^+$  pool in order to maintain equilibrium, with a continuous N turnover and a consequent change in  $\delta^{15}\text{N}$ . No  $\delta^{15}\text{N}$  turnover of fixed ammonium was observed immediately after the chemical fertilization applied in Jun-2013, while the addition of urea was marked by a pH increase [54]. On the contrary, in Sep-2013, a probable storage of N from chemical fertilization was visible in Fix  $\text{N-NH}_4^+$  pool (and consequently in TN pool), as demonstrated by the significantly lower  $\delta^{15}\text{N}$  (very close to the  $\delta^{15}\text{N}$  of urea and di-ammonium phosphate). This behavior is recorded only in the bulk soil, where the exploitation by the plant roots is less important than in the rhizosphere.

#### 4.2. NZ Influence on Soil N and C Pools

The introduction of natural zeolites (5NZ and 15NZ, respectively) affected the soil C and N pools. The decrease in the total reserve of Fix  $\text{N-NH}_4^+$  in 15NZ in Nov-2012 was probably due to a dilution effect operated by the high amount of NZ (devoid of N) introduced. Instead, the decrease of Fix  $\text{N-NH}_4^+$  during the growing season suggest an exploitation by plants, confirming the active role of this pool in crop nutrition [55,56]. Nevertheless, no significant differences in the amount of Fix  $\text{N-NH}_4^+$  pool were found between the various treatments, suggesting that the N subjected to CEC processes by zeolites belongs to the exchangeable pool. The generally lower  $\text{N-NO}_3^-$  content in the NZ treatments may be attributed both to the aforementioned controlled retention of  $\text{NH}_4^+$  by the zeolite, which can

reduce the availability of N to nitrifying bacteria and to the lower amount of N fertilizer (from 30 to 50%) with respect to CNTR (where  $\text{N-NO}_3^-$  pool increased significantly). The introduction of NZ also affected microbial biomass N and C immobilization. In Nov-2012, lower  $C_{\text{mic}}$  was observed in all ZT, especially in 5NZ, suggesting that NZ introduction cause an initial disequilibrium in microbial biomass. During the sorghum-growing season (Jun-2013), microbial biomass was probably influenced by the competition with the plants, resulting in a consistent decrease in  $C_{\text{mic}}$  and  $N_{\text{mic}}$  in all the treatments. Apparently, 15NZ treatment had a stronger influence on microbial biomass N, resulting in a lower N immobilization that was already visible in May-2013 (in this case even  $C_{\text{mic}}$  pool was affected) and lasted until Sep-2013, suggesting more zeolite was in the soil the less N was easily available to soil microorganisms. The C/N ratio of microbial biomass may give information regarding bacterial or fungal prevalence in the microbial community; usually bacteria have on average a lower C/N than fungi because of a lower N requirement by the latter [57,58]. Agricultural management (tillage or no-tillage), nutrient availability, soil pH, temperature and moisture may influence fungal/bacterial ratio [15,59,60]. In this respect,  $C_{\text{mic}}/N_{\text{mic}}$  ratio suggested that the introduction of natural zeolites caused a shift in microbial population through a greater fungal prevalence [15,59,60] especially in 15NZ. A greater fungal/bacteria biomass ratio can be often interpreted as marker of a more sustainable agricultural system, where the nutrients required for the plant growth are supplied by OM decomposition and N mineralization with probably lesser N loss in the environment [15,59,60]. It is thus likely that the presence of zeolites caused a lower N accessibility into the N soil pools, making it less available to soil microorganisms and favoring the development of fungal biomass. These implications on microbial biomass affect the soil C cycle, where a lower HI and a greater FA fraction content can contribute to lower C immobilization.

#### 4.3. Zeolite Influence on Rhizosphere

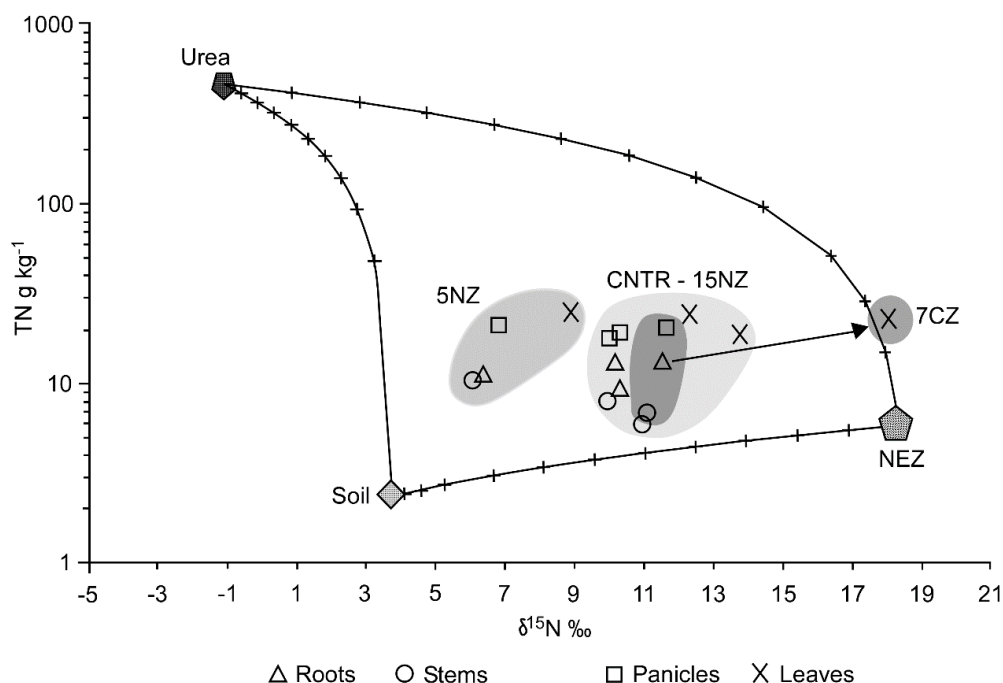
At the harvest, rhizosphere was depleted in Exch  $\text{N-NH}_4^+$  with respect to bulk soils but significantly enriched in microbial biomass C-N, TOC, HA and TN. It is reasonable that the decrease in the exchangeable pool was due to an exploitation by plant roots while bulk soil results relatively enriched. Rhizosphere is known to be a zone with higher metabolic activity, thus the release of organic compounds from roots may explain the increase in microbial biomass, TOC, HA and TN [61,62] and also the slight fractionation observed in TN and Fix  $\text{N-NH}_4^+$  pools  $\delta^{15}\text{N}$ . It is interesting to note that at the end of the growing season, rhizosphere TOC was probably influenced by zeolite addition (especially in 15NZ), showing an increase with respect to the CNTR. This evidence suggests a higher efficiency of microbial biomass, but this is not supported by a parallel increment in  $C_{\text{mic}}$  and  $N_{\text{mic}}$  with respect to CNTR. On the other hand, Ozbahce et al. [36] found that the application of NZ mixed in the first 30cm soil layer increased OM content, although a clear explanation of this phenomenon was not given. Italian zeolitic tuffs can adsorb a significant amount of HS from solutions, especially when divalent cations (such as  $\text{Ca}^{2+}$ ) act as bridge between the negatively charged mineral surface and the organic phase [63]. A plausible hypothesis is that zeolite can adsorb HS reducing in this way organic C losses and increasing thus TOC in the soil [64].  $\text{Ca}^{2+}$  is indeed the major exchangeable base of the studied NZ and it can act as bridge for the sorption of HS in zeolitic tuff. Further studies are required to confirm this very important aspect.

#### 4.4. Plant-Zeolite Interactions

N is preferentially allocated in leaves and panicles and the lack of significant differences in N content with respect to the distinct treatments suggests that, notwithstanding the fertilization reductions, plants uptake similar N amounts, reflecting a higher fertilizer recovery or N use efficiency. As reported in literature [65,66]  $\delta^{15}\text{N}$  of leaves is determined by the physiological mechanism within the plants and by the isotopic ratio of external N sources. Moreover, intra-plant N isotopic variations, between roots and leaves can be due to different patterns of N assimilation or reallocation. These intra-plant  $\delta^{15}\text{N}$  variations were recognizable in the sorghum plants, where leaves  $\delta^{15}\text{N}$  was generally

greater (from 2 to 3‰) than that determined in roots, except for 7CZ where differences were even greater (about 6‰). Many authors used  $^{15}\text{N}$  natural abundance of leaves in order to trace the N source in the soil [65–69] mostly because leaves represent the primary sink of N and the largest plant N pool [41]. Average  $\delta^{15}\text{N}$  of 7CZ leaves was very close to NEZ  $\delta^{15}\text{N}$  and significantly higher than the leaves from the other parcels (Figure 6). The higher isotopic signature of 7CZ leaves indicated that during the growing season, an uptake from a significantly higher  $\delta^{15}\text{N}$  occurred. Assuming negligible fractionation effects during uptake, the main N source was probably represented by NEZ-N. Indeed, 7CZ is the only parcel where a high  $\delta^{15}\text{N}$  input (represented by pig slurry) was added during the experimentation. It has been demonstrated that crops grown under chemical fertilizers (such as urea) have lower  $\delta^{15}\text{N}$  with respect to plants grown under organic fertilizers (which have usually higher  $\delta^{15}\text{N}$ ) [70–72]. It is important to consider that the other N inputs were urea and di-ammonium phosphate, which have a negative  $\delta^{15}\text{N}$  and were applied to all treatments, supporting the hypothesis that NEZ was an efficient slow release fertilizer and retained N even after several months and with no important N losses occurring (from leaching or denitrification). Most N in soil is bound in forms not immediately available to plants [41,71], so usually the  $\delta^{15}\text{N}$  of TN is not a good approximation of the isotopic signature of the N source preferentially used by plants. Anyhow,  $^{15}\text{N}$  natural abundance in leaves of CNTR, 15NZ and especially 5NZ reflect the use of N characterized by a lower  $\delta^{15}\text{N}$  and related to chemical fertilizers signature. It is important to note that notwithstanding the lower urea application (–30% with respect to the CNTR), in 5NZ and 15NZ treatments, leaves  $\delta^{15}\text{N}$  is similar or even lower with respect to CNTR. In particular, as visible from the mixing model (Figure 6), 5NZ plants showed the lowest  $\delta^{15}\text{N}$  not only in leaves but also in roots, stems and panicles, suggesting a higher efficiency in the uptake of N from a source with lower  $\delta^{15}\text{N}$ , i.e., chemical fertilizers.

The positive yield results of all ZT must be attributed also to the soil physical properties amelioration induced by zeolite amendments. In our case, zeolites can in fact increase soil water retention and, thanks to their coarser size with respect to the fine-grained soil (particle-size analysis performed within ZeoLIFE project are not shown in this work) they can also increase soil permeability and aeration [28,36].



**Figure 6.**  $\delta^{15}\text{N}$  vs total nitrogen (TN) mixing diagram for sorghum plant organs. Chemical fertilizers, NEZ and Soil have been chosen as end-members.

## 5. Conclusions

In this work, a detailed investigation of the N-C pools and  $\delta^{15}\text{N}$  dynamics in the soil-sorghum system were studied in a field amended with natural and  $\text{NH}_4^+$ -enriched zeolitites, under fertilization reductions. ZT generally had a weak influence on the total reserve of Fix N- $\text{NH}_4^+$  pool. However, its reserve decreased during the growing season and a  $\delta^{15}\text{N}$  turnover occurs, confirming both its active role in plant nutrition and its dynamic equilibrium with the other soil N pools. Moreover, Fix  $\delta^{15}\text{N}$  turnover indicate that part of N from chemical fertilizers was probably stored in this pool at the end of the agronomic year. The N introduced in the soil system with NEZ increased the Exch N- $\text{NH}_4^+$  reserve, suggesting that N adsorbed by zeolitites preferentially affected this pool. ZT (especially NZ) influenced the N- $\text{NO}_3^-$  pool, resulting in lower nitrate contents suggesting a possible nitrification and denitrification rate reduction. Microbial activity was strongly affected by NEZ and NZ introduction which seems to cause at first a disorder, then a possible change in microbial population towards a fungal prevalence, usually indicative of a system with lower N accessible to microorganisms.  $^{15}\text{N}$  natural abundance in soils and plants, together with the positive yield results, suggested that plants benefited from N NEZ. However, in all likelihood, plants did not directly exploit N from NEZ, but rather the transfer involves complex interactions among soil microbial biomass. Further investigations focused on this biotic mediation are needed. Most of the N-C pools dynamics were not affected by NZ amendments but, in all likelihood, an increase in fertilizers recovery by plants occurred. The spreading of zeolitites may improve soil physical-chemical properties, enhance nutrient use efficiency as well as augment economical and an environmental saving.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2076-3417/9/21/4524/s1>, Table S1: Average of N and C pools. Table S2: Average of TN, TOC and C/N of sorghum organs.

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## References

1. Conway, G. *The Double Green Revolution: Food for All in the 21st Century*; Penguin: London, UK, 1997; p. 334.
2. Smil, V. Nitrogen in crop production: An account of global flows. *Glob. Biogeochem. Cycles* **1999**, *13*, 647–662. [[CrossRef](#)]
3. Tilman, D.; Fargione, J.; Wolff, B.; D'Antonio, C.; Dobson, A.; Howarth, R.; Schindler, D.; Schlesinger, W.H.; Simberloff, D.; Swackhamer, D. Forecasting agriculturally driven global environmental change. *Science* **2001**, *292*, 281–284. [[CrossRef](#)] [[PubMed](#)]
4. Oenema, O.; Kros, H.; Vries, W. Approaches and uncertainties in nutrient budgets: Implications for nutrient management and environmental policies. *Eur. J. Agron.* **2003**, *20*, 3–16. [[CrossRef](#)]
5. Kroeze, C.; Aerts, R.; Breemen, N.; Dam, D.; Hoek, K.; Hofschreuder, P.; Hoosbeek, M.; Klein, J.; Kros, H.; Oene, H.; et al. Uncertainties in the fate of nitrogen I: An overview of sources of uncertainty illustrated with a Dutch case study. *Nutr. Cycl. Agroecosyst.* **2003**, *66*, 43–69. [[CrossRef](#)]
6. Passaglia, E. *Zeoliti Naturali, Zeolititi e Loro Applicazioni*; Arvan: Padova, Italy, 2008; p. 104.
7. Wu, Q.; Chi, D.; Xia, G.; Chen, T.; Sun, Y.; Song, Y. Effects of Zeolite on Drought Resistance and Water—Nitrogen Use Efficiency in Paddy Rice. *J. Irrig. Drain. Eng.* **2019**, *145*, 4019024. [[CrossRef](#)]
8. Wu, Q.; Chen, T.; Chi, D.; Xia, G.; Sun, Y.; Song, Y. Increasing nitrogen use efficiency with lower nitrogen application frequencies using zeolite in rice paddy fields. *Int. Agrophys.* **2019**, *33*, 263–269. [[CrossRef](#)]
9. Li, Z.; Zhang, Y.; Li, Y. Zeolite as slow release fertilizer on spinach yields and quality in a greenhouse test. *J. Plant Nutr.* **2013**, *36*, 1496–1505. [[CrossRef](#)]



10. Malferrari, D.; Laurora, A.; Brigatti, F.; Coltorti, M.; Di Giuseppe, D.; Faccini, B.; Vezzalini, M. Open-field experimentation of an innovative and integrated zeolite cycle: Project definition and material characterization. *Rend. Lincei-Sci. Fis.* **2013**, *24*, 141–150. [[CrossRef](#)]
11. Colombani, N.; Di Giuseppe, D.; Faccini, B.; Ferretti, G.; Mastrocicco, M.; Coltorti, M. Estimated water saving in an agricultural field amended with natural zeolites. *Environ. Process.* **2016**, *3*, 617–628. [[CrossRef](#)]
12. Di Giuseppe, D.; Ibáñez Insa, J.; Melchiorre, M.; Coltorti, M. On the potential effect of micronized zeolites on seed germination: A prospective study. *Period. Mineral.* **2018**, *85*, 179–183.
13. Ferretti, G.; Di Giuseppe, D.; Faccini, B.; Coltorti, M. Mitigation of sodium risk in a sandy agricultural soil by the use of natural zeolites. *Environ. Monit. Assess.* **2018**, *190*, 646. [[CrossRef](#)] [[PubMed](#)]
14. Reháková, M.; Čuvanová, S.; Dživák, M.; Rimár, J.; Gaval'ová, Z. Agricultural and agrochemical uses of natural zeolite of the clinoptilolite type. *Curr. Opin. Solid State Mater. Sci.* **2004**, *8*, 397–404. [[CrossRef](#)]
15. Ferretti, G.; Keiblinger, K.M.; Di Giuseppe, D.; Faccini, B.; Colombani, N.; Zechmeister-Boltenstern, S.; Coltorti, M.; Mastrocicco, M. Short-term response of soil microbial biomass to different chabazite zeolite amendments. *Pedosphere* **2018**, *28*, 277–287.
16. Bernardi, A.C.C.; Oliviera, P.P.A.; Monte, M.B.M.; Souza-Barros, F. Brazilian sedimentary zeolite use in agriculture. *Microporous Mesoporous Mater.* **2013**, *167*, 16–21. [[CrossRef](#)]
17. Colombani, N.; Mastrocicco, M.; Di Giuseppe, D.; Faccini, B.; Coltorti, M. Variation of the hydraulic properties and solute transport mechanisms in a silty-clay soil amended with natural zeolites. *Catena* **2014**, *123*, 195–204. [[CrossRef](#)]
18. Ferretti, G.; Keiblinger, K.M.; Zimmermann, M.; Di Giuseppe, D.; Faccini, B.; Colombani, N.; Mastrocicco, M.; Zechmeister-Boltenstern, S.; Mentler, A. High resolution short-term investigation of soil CO<sub>2</sub>, N<sub>2</sub>O, NO<sub>x</sub> and NH<sub>3</sub> emissions after different chabazite zeolite amendments. *Appl. Soil Ecol.* **2017**, *119*, 138–144. [[CrossRef](#)]
19. Ferretti, G.; Di Giuseppe, D.; Natali, C.; Faccini, B.; Bianchini, G.; Coltorti, M. CN elemental and isotopic investigation in agricultural soils: Insights on the effects of zeolite amendments. *Chem. Der Erde* **2017**, *77*, 45–52. [[CrossRef](#)]
20. Gholamhoseini, M.; Ghalavand, A.; Khodaei-Joghan, A.; Dolatabadian, A.; Zakikhani, H.; Farmanbar, E. Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and nutrient leaching. *Soil Tillage Res.* **2013**, *126*, 193–202. [[CrossRef](#)]
21. Di Giuseppe, D.; Ferretti, G.; Faccini, B.; Blasi, E.; Passeri, N.; Bianchini, G.; Coltorti, M. Is it possible to cultivate corn in a sustainable way using a quarry waste? *Period. Mineral.* **2016**, *85*, 179–183.
22. Arcara, P.G.; Gamba, C.; Bidini, D.; Marchetti, R. The effect of urea and pig slurry fertilization on denitrification, direct nitrous oxide emission, volatile fatty acids, water-soluble carbon and anthrone-reactive carbon in maize-cropped soil from the Po plain (Modena, Italy). *Biol. Fertil. Soils* **1999**, *29*, 270–276. [[CrossRef](#)]
23. Faccini, B.; Di Giuseppe, D.; Malferrari, D.; Coltorti, M.; Abbondanzi, F.; Campisi, T.; Laurora, A.; Passaglia, E. Ammonium-exchanged zeolite preparation for agricultural uses: From laboratory tests to large-scale application in ZeoLIFE project prototype. *Period. Mineral.* **2015**, *84*, 303–321.
24. Faccini, B.; Di Giuseppe, D.; Ferretti, G.; Coltorti, M.; Colombani, N.; Mastrocicco, M. Natural and NH<sub>4</sub><sup>+</sup>-enriched zeolite amendment effects on nitrate leaching from a reclaimed agricultural soil (Ferrara Province, Italy). *Nutr. Cycl. Agroecosyst.* **2018**, *110*, 327–341. [[CrossRef](#)]
25. Mastrocicco, M.; Colombani, N.; Di Giuseppe, D.; Faccini, B.; Coltorti, M. Contribution of the subsurface drainage system in changing the nitrogen speciation of an agricultural soil located in a complex marsh environment (Ferrara, Italy). *Agric. Water Manag.* **2013**, *119*, 144–153. [[CrossRef](#)]
26. Di Giuseppe, D.; Faccini, B.; Mastrocicco, M.; Colombani, N.; Coltorti, M. Reclamation influence and background geochemistry of neutral saline soils in the Po River Delta Plain (Northern Italy). *Environ. Earth Sci.* **2014**, *72*, 2457–2473. [[CrossRef](#)]
27. IUSS Working Group. *World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports, No. 106; FAO: Rome, Italy, 2007.
28. Colombani, N.; Di Giuseppe, D.; Faccini, B.; Ferretti, G.; Mastrocicco, M.; Coltorti, M. Inferring the interconnections between surface water bodies, tile-drains and an unconfined aquifer-aquitard system: A case study. *J. Hydrol.* **2016**, *537*, 86–95. [[CrossRef](#)]
29. Orsini, L.; Remy, J.C. Utilization du chlorure de cobaltihexammine pour la détermination simultanée de la capacité d'échange et des bases échangeables des sols. *Sci. Sol.* **1976**, *4*, 269–275.

30. Ciesielsky, H.; Sterckeman, T. Determination of cation exchange capacity and exchangeable cations in soils by means of cobalt heamine trichloride. *Effects of experimental conditions. Agronomie* **1997**, *17*, 1–7.
31. Mariotti, A. Natural N-15 abundance measurements and atmospheric nitrogen standards. *Nature* **1984**, *311*, 251–252. [[CrossRef](#)]
32. Silva, J.A.; Bremner, J.M. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 5. Fixed ammonium. *Soil Sci. Soc. Am. J.* **1966**, *30*, 587–594. [[CrossRef](#)]
33. Ciavatta, C.; Govi, M.; Vittori Antisari, L.; Sequi, P. Characterization of humified compounds by extraction and fractionation on solid polyvinylpyrrolidone. *J. Chromatogr.* **1990**, *509*, 41–146. [[CrossRef](#)]
34. Sequi, P.; De Nobili, M.; Leita, L.; Cercignani, G. A new index of humification. *Agrochimica* **1986**, *30*, 175–179.
35. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [[CrossRef](#)]
36. Ozbahce, A.; Tari, A.F.; Gönülal, E.; Simsekli, N.; Padem, H. The effect of zeolite applications on yield components and nutrient uptake of common bean under water stress. *Arch. Agron. Soil Sci.* **2015**, *61*, 615–626. [[CrossRef](#)]
37. Barbarick, K.A.; Pirela, H.J. Agronomic and horticultural uses of zeolites: A review. In *Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture*; Pond, W.G., Mumpton, F.A., Eds.; Westview Press: Boulder, CO, USA, 1984; pp. 93–103.
38. Eslami, M.; Khorassani, R.; Coltorti, M.; Malferrari, D.; Faccini, B.; Ferretti, G.; Di Giuseppe, D.; Fotovat, A.; Halajnia, A. Leaching behaviour of a sandy soil amended with natural and  $\text{NH}_4^+$  and  $\text{K}^+$  saturated clinoptilolite and chabazite. *Arch. Agron. Soil Sci.* **2018**, *64*, 1142–1151. [[CrossRef](#)]
39. Dwairi, I.M. Evaluation of Jordanian zeolite tuff as a controlled slow-release fertilizer for  $\text{NH}_4^+$ . *Environ. Geol.* **1998**, *34*, 1–4. [[CrossRef](#)]
40. Dittert, K.; Georges, T.; Sattelmacher, B. Nitrogen turnover in soil after application of animal manure and slurry as studied by the stable isotope  $^{15}\text{N}$ : A review. *Z. Pflanzenernähr. Bodenkd.* **1998**, *161*, 453–463. [[CrossRef](#)]
41. Högberg, P. Tansley review No. 95  $^{15}\text{N}$  natural abundance in soil-plant systems. *N. Phytol.* **1997**, *137*, 179–203.
42. Schmidt, O.; Ostle, N.J. Tracing nitrogen derived from slurry in earthworms using  $^{15}\text{N}/^{14}\text{N}$  stable isotope ratios at natural abundances. *Appl. Soil Ecol.* **1999**, *12*, 7–13. [[CrossRef](#)]
43. Sang-Sun, L.; Woo-Jung, C.; Jyn-Hyeob, K.; Jae-Woon, J.; Scott, X.C.; Han-Yong, K.; Kwang-Sik, Y.; Soo-Myung, C. Nitrogen and carbon isotope responses of Chinese cabbage and chrysanthemum to the application of liquid pig manure. *Plant Soil* **2007**, *295*, 67–77.
44. Kitayama, K.; Iwamoto, K. Patterns of natural  $^{15}\text{N}$  abundance in the leaf-to-soil continuum of tropical rain forests differing in N availability on Mount Kinabalu, Borneo. *Plant Soil* **2001**, *229*, 203–212. [[CrossRef](#)]
45. Gualtieri, A.F.; Passaglia, E. Rietveld structure refinement of  $\text{NH}_4$ -exchanged natural chabazite. *Eur. J. Mineral.* **2006**, *18*, 351–359. [[CrossRef](#)]
46. Hedström, A. Ion exchange of ammonium in zeolites: A literature review. *J. Environ. Eng.* **2001**, *127*, 673–681. [[CrossRef](#)]
47. Widiastuti, N.; Wu, H.; Ang, H.M.; Zhang, D. Removal of ammonium from greywater using natural zeolite. *Desalination* **2011**, *277*, 15–23. [[CrossRef](#)]
48. Malekian, R.; Abedi-Koupai, J.; Eslamian, S.S. Influences of clinoptilolite and surfactant-modified clinoptilolite zeolite on nitrate leaching and plant growth. *J. Hazard. Mater.* **2011**, *185*, 970–976. [[CrossRef](#)]
49. Azam, F.; Malik, K.A.; Hussain, F. Microbial biomass and mineralization-immobilization of nitrogen in some agricultural soils. *Biol. Fertil. Soils* **1986**, *2*, 157–163. [[CrossRef](#)]
50. Ito, O.; Watanabe, I. Immobilization, mineralization and availability to rice plants of nitrogen derived from heterotrophic nitrogen fixation in flooded soil. *Soil Sci. Plant Nutr.* **1981**, *27*, 169–176. [[CrossRef](#)]
51. Schulten, H.R.; Schnitzer, M. The chemistry of soil organic nitrogen: A review. *Biol. Fertil. Soils* **1998**, *26*, 1–15. [[CrossRef](#)]
52. Meurant, G. *Soil Organic Matter and Its Role in Crop Production*; Elsevier Science: Amsterdam, The Netherlands, 1973; p. 634.
53. Scherer, H.W.; Werner, W. Significance of soil microorganisms for the mobilization of nonexchangeable ammonium. *Biol. Fertil. Soils* **1996**, *22*, 248–251. [[CrossRef](#)]

54. Martens, D.A.; Bremner, J.M. Urea hydrolysis in soils: Factors influencing the effectiveness of phenylphosphorodiamidate as a retardant. *Soil Biol. Biochem.* **1984**, *16*, 515–519. [[CrossRef](#)]
55. Li, C.; Fan, X.; Mengel, K. Turnover of interlayer ammonium in loess-derived soil grown with winter wheat in the Shaanxi Province of China. *Biol. Fertil. Soils* **1990**, *9*, 211–214. [[CrossRef](#)]
56. Marzadori, C.; Vittori Antisari, L.; Gioacchini, P.; Sequi, P. Turnover of interlayer ammonium in soil cropped with sugar beet. *Biol. Fertil. Soils* **1994**, *18*, 27–31. [[CrossRef](#)]
57. Hodge, A.; Robinson, D.; Fitter, A. Are microorganisms more effective than plants at competing for Nitrogen? *Trends Plant Sci.* **2000**, *5*, 304–308. [[CrossRef](#)]
58. Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G.; Renella, G. Microbial diversity and soil functions. *Eur. J. Soil Sci.* **2003**, *54*, 655–670. [[CrossRef](#)]
59. Vries, F.T.; Hoffland, E.; Eekeren, N.; Brussaard, L.; Bloem, J. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biol. Biochem.* **2006**, *38*, 2092–2103. [[CrossRef](#)]
60. Strickland, M.S.; Rousk, J. Considering fungal:bacterial dominance in soils—Methods, controls, and ecosystem implications. *Soil Biol. Biochem.* **2010**, *42*, 1385–1395. [[CrossRef](#)]
61. Merckx, R.; Van Ginkel, J.H.; Sinnaeve, J.; Cremers, A. Plant induced changes in the rhizosphere of maize and wheat. I. Production and turnover of root-derived material in the rhizosphere of maize and wheat. *Plant Soil* **1986**, *96*, 85–93. [[CrossRef](#)]
62. Liljeroth, E.; Van Veen, J.A.; Miller, H.J. Assimilate translocation to the rhizosphere of two wheat cultivars and subsequent utilization by rhizosphere microorganisms at two soil nitrogen levels. *Soil Biol. Biochem.* **1990**, *22*, 1015–1021. [[CrossRef](#)]
63. Capasso, S.; Salvestrini, S.; Coppola, E.; Buondonno, A.; Colella, C. Sorption of humic acid on zeolitic tuff: A preliminary investigation. *Appl. Clay Sci.* **2005**, *28*, 159–165. [[CrossRef](#)]
64. Söderström, B.; Hedlund, K.; Jackson, L.E.; Kätterer, T.; Lugato, E.; Thomensen, I.K.; Jørgensen, H.B. What are the effects of agricultural management on soil organic carbon (SOC) stocks? *Environ. Evid.* **2014**, *3*, 2. [[CrossRef](#)]
65. Evans, D.R. Physiological mechanism influencing plant nitrogen isotope composition. *Trends Plant Sci.* **2001**, *6*, 121–126. [[CrossRef](#)]
66. Evans, D.R.; Bloom, A.J.; Sukrapanna, S.S.; Ehleringer, J.R. Nitrogen isotope composition of tomato (*Lycopersicon Esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. *Plant Cell Environ.* **1996**, *19*, 1317–1323. [[CrossRef](#)]
67. Erskine, P.D.; Bergstrom, D.M.; Schmidt, S.; Stewart, G.R.; Tweedie, C.E.; Shaw, J.D. Subantarctic Macquarie Island ± a model ecosystem for studying animal-derived nitrogen sources using <sup>15</sup>N natural abundance. *Oecologia* **1998**, *117*, 187–193. [[CrossRef](#)] [[PubMed](#)]
68. Hulton, B.Z.; Sigman, D.M.; Schuur, E.A.G.; Edin, L.O. A climate-driven switch in plant nitrogen acquisition within tropical forest communities. *Proc. Natl. Acad. Sci. USA* **2007**, *114*, 8902–8906. [[CrossRef](#)] [[PubMed](#)]
69. Kahmen, A.; Wanek, W.; Buchmann, N. Foliar δ<sup>15</sup>N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia* **2008**, *156*, 861–870. [[CrossRef](#)] [[PubMed](#)]
70. Choi, W.J.; Ro, H.M.; Hobbie, E.A. Patterns of natural <sup>15</sup>N in soils and plants from chemically and organically fertilized uplands. *Soil Biol. Biochem.* **2003**, *35*, 1493–1500. [[CrossRef](#)]
71. Bateman, A.S.; Kelly, S.D.; Jickells, T.D. Nitrogen Isotope Relationships between Crops and Fertilizer: Implications for Using Nitrogen Isotope Analysis as an Indicator of Agricultural Regime. *J. Agric. Food Chem.* **2005**, *53*, 5760–5765. [[CrossRef](#)]
72. Yun, S.I.; Ro, H.M.; Choi, W.J.; Chang, S.X. Interactive effects of N fertilizer source and timing of fertilization leave specific N isotopic signatures in Chinese cabbage and soil. *Soil Biol. Biochem.* **2006**, *38*, 1682–1689. [[CrossRef](#)]

