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The application of natural and NH_4^+-enriched chabazite zeolites as soil amendment: a bio-geochemical exploration

Settore Scientifico Disciplinare: GEO/07

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Summary

1.0 Introduction

1.1 Nitrogen and agriculture

The great majority of Nitrogen (N) on Earth is present in the form of molecular N_2 and the atmosphere represents the main pool of this unreactive form of N. Only the 0.002 % of the global N is stocked in living tissues and organic matter (Schlesinger, 1997; Howarth, 2008) while the reactive N species (rN), represented by molecules like nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+), are orders of magnitude less present (Howarth, 2008).

N is an essential element for all living organisms, but the amount of biologically available N forms is a very small proportion of the global N on Earth. For this reason, it limits productivity in many of world's ecosystems (Howarth, 2008).

Before the 20th century, the only mechanisms for converting unreactive N_2 into rN compounds were bacterial N fixation and chemical reactions with oxygen associated with lightning and volcanoes (Howarth, 2008). N biological fixation is estimated to produce from 90 to 195 Tg N year^{-1}, while lightning and volcanoes less than 10 Tg N year^{-1}(Howarth, 2008).

Before the dawn of agriculture, the hunter–gatherer lifestyle supported about 4 million people globally (Tilman et al., 2002). Modern agriculture now feeds 6,000 million people. The discovery that triggered this drastic increase of agricultural productivity occurred at the begin of the 20th century, when the "artificial N fixation" process, that allows the industrial synthesis of ammonia (the so called Haber-Bosch process), was invented. This discovery led to an enormous increase in the global rN (Galloway et al., 2004) because of the introduction of "anthropic rN". From that moment, it was possible the artificial production of synthetic N-based fertilizers and thus to increase the N inputs in agricultural lands and consequently crop yields. This discovery fueled the "green revolution" and allowed a massive expansion of global agriculture, with concomitant decrease in hunger and malnutrition (Smil, 2001).

Half of all of the synthetic N fertilizers ever used on Earth has been produced since 1985 (Howarth et al., 2002, 2005) and at the begin of 21th century, human-controlled rN creation occurred at a rate of approximately 165 Tg N year^{-1}, increasing thus the global creation of rN by 33-55 % (Howarth, 2008). These inputs helped to keep world crop productivity ahead of human population growth and to enhance rural economic development.

Notwithstanding the new technologies, high N inputs in modern agriculture are still fundamental for a good crop production. Unfortunately, it is well known that an important fraction, often over 50 %, of the N applied is not efficiently exploited by crops but can be lost from agricultural systems as N_2, trace gases, or leached nitrate (Aulakh and Singh, 1997; Vitousek et al., 1997; Tilman, 1998; Adesemoye and Kloepper, 2009). N losses from the root zone (e.g by leaching or volatilization), are known to have a high cost in terms of environmental quality degradation. For this reason, the large use of N-based fertilizers, as well as N rich zoo-technical effluents, are diminishing environmental quality worldwide, leading to the degradation of downstream and groundwater quality, eutrophication of coastal marine ecosystems, soil desertification, biodiversity reduction, increase of photochemical smog and the rising of global concentration of powerful greenhouse and harmful gasses like N_2O, NO_x ($NO + NO_2$) and NH_3 (Oenema et al., 2003; Kroeze et al., 2003; Vitousek et al., 2009 and references therein).

By 2050, global population is projected to be 50 % larger than at present and global food demand is projected to double (Tilman et al., 2002). In this optic, the development of land use strategies and ecologically oriented agricultural production systems in combination with a good and efficient water management are necessary for decreasing N losses from the root zone and for increasing N use efficiency.

1.2 The use of natural zeolites in agriculture

The application of organic and inorganic amendments to soil is recognized as a valuable technique for reducing compaction and leaching, while increasing available water content and nutrient holding

capacity (Waltz et al., 2003; Ferreras et al., 2006; Lima et al., 2009; Colombani et al., 2014). Within natural inorganic amendments, zeolites are considered a well suitable material for agronomical purposes because of their very peculiar properties.

According to the recommended definition of zeolites proposed by Coombs et al. (1997), "a zeolite mineral is a crystalline substance with a structure characterized by a framework of linked tetrahedra, each consisting of four O atoms surrounding a cation. This framework contains open cavities in the form of channels and cages. These are usually occupied by H_2O molecules and extra-framework cations that are commonly exchangeable. The channels are large enough to allow the passage of guest species. In the hydrated phases, dehydration occurs at temperatures mostly below about 400°C and is largely reversible."

Thanks to these properties, zeolites are capable of sorbing into their cavities or channels many polar/non-polar inorganic/organic molecules and also bio and agro-chemically effective compounds and odoriferous compounds (Rehákova et al., 2004).

Zeolites can be natural or synthetic and within natural zeolites, the most occurring worldwide is clinoptilolite (Kennedy, 1990). However, one of the first studied natural zeolite as cation exchanger was the chabazite (CHA) zeolite (Dyer and Zubair, 1998). CHA zeolites are commonly found in volcanoclastic deposits, especially in the Italian peninsula, where many quarries are actually exploiting zeolite-rich tuffs for the production of construction bricks (Passaglia, 2008). If the zeolite content of these tuffs is above 50 %, they can be defined as "zeolitites" (Galli and Passaglia, 2011). From the cutting process of these construction bricks, high amount of zeolitites remains generally unused, constituting a waste for the quarry, but an interesting granular by-product which can be used for many purposes, including the use as soil amendments.

Among all the properties of this peculiar mineral, from the agronomic point of view, the most important characteristic is the very high cation exchange capacity (CEC), i.e the capacity of adsorbing positively charged ions or molecules from a solution by replacing the extra-framework cations actually present into the mineral cavities or channels (Fig 1).

The ability of exchanging cations is crucial for potential reduction of N losses from agricultural fields, since one of the principal N-molecules formed after fertilizer hydrolysis in the soil is the positively charged ammonium ion (NH_4^+). Retarding or preventing the biotical or abiotical transformation of NH_4^+ into more mobile anionic molecules like NO_2^- and NO_3^- (generally subjected to anionic repulsion from soil clay minerals) or into harmful gaseous species (NH_3, NO_x and N_2O) is essential for reducing N losses and increasing fertilizer N-use efficiency.

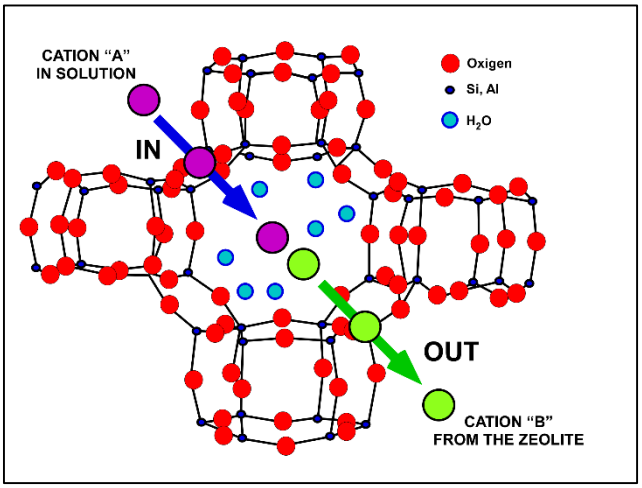

Fig 1: Cation exchange reaction in a zeolite (modified from Passaglia, (2008)).

Natural zeolites can be easily modified from their natural state by enrichment processes which cause the adsorption of specific cations (i.e. NH_4^+, Na^+) as guest species (Dwairi, 1998; Leggo, 2000; Faccini et al., 2015). In agricultural context, these kind of enriched-zeolites, especially the NH_4^+-enriched forms, are used as a slow-release source of nutrients for plants. The theory behind the use of this kind of zeolites is that the NH_4^+ adsorbed by the mineral during the enrichment process is slowly released by cation exchange processes in the soil system, reducing the high N losses that normally occurs immediately after fertilizer application and increasing thus fertilization efficiency.

The use of different kinds of natural and enriched-zeolites as soil amendments has been studied extensively in terms of soil physicochemical characteristics modification (Passaglia, 2008; Colombani et al., 2014, 2015, 2016), reduced N leaching, increased N use efficiency (NUE), water

use efficiency and crop yield (Reháková et al., 2004; Sepaskhah and Barzegar, 2010; De Campos Bernardi et al., 2013; Gholamhoseini et al., 2013; Di Giuseppe et al., 2015).

Colombani et al. (2014) found that the application of Italian K-CHA to agricultural soils reduced surface dryness and increased volumetric water content, water storage and permeability.

Malekian et al. (2011) found that the application of natural clinoptilolite reduced significantly NO_3^- leaching and increased maize N uptake and yield with respect to an unamended soil. Gholamhoseini et al. (2013) found that the application of natural zeolite together with fresh manure reduced N leaching, increased plant-available N and consequently NUE. Sepaskhah and Barzegar (2010) showed that zeolite addition to soil increased NUE even after years later from the first application. Haruna Ahmed et al. (2008a) and De Campos Bernardi et al. (2013) indicated that zeolite addition to soil increased Urea-N use efficiency, while Eberl et al. (1995), Passaglia (2008), Sepaskhah and Barzegar (2010) and Gholamhoseini et al. (2013) reported yield increments of up to 65 % in several cultures (sorghum, wheat, rice, marrow, chard, basil, tomato, radish, watermelon, lettuce, sunflower) in soil treated with natural or NH_4^+-enriched zeolites. Analogously, in the review of Reháková et al. (2004) increments of roots biomass in parsley (*Petroselinum satilvum*), carrots (*Daucus carota*), and onions (*Allium cepa*) attributable to the use of natural zeolites are reported.

Campisi et al. (2016) indicated that the application of NH_4^+-enriched Italian CHA zeolites to agricultural soils had no negative consequences in terms of crop (*Zea Mays*) growth and nutrition and may even provide high agronomic benefits with lasting effect on soil properties. They also indicated that reducing chemical fertilizers after the application of NH_4^+-enriched CHA to soil was feasible, even at high degree.

These studies are only some of the many present actually in the scientific literature. Moreover, some studies were also conducted on the effects of zeolite application to soil CO_2, NH_3 and N_2O emissions as potential option for reducing harmful and greenhouse gasses.

Natural clinoptilolite zeolite as amendment to soils have been described to reduce respiratory activity probably because of the adsorption capacity of zeolites for CO_2 (Mühlbachová and Šimon, 2003).

Kučić et al. (2013) showed that natural clinoptilolite zeolite adsorption potential for CO_2 and NH_3 evolved during co-composting of grape and tobacco waste was 31 % and 100 %, respectively. In addition, pasture and wetland soils amended with clinoptilolite zeolite showed a reduction in daily and total N_2O emissions during a 90-day incubation compared to unamended soils (Zaman et al., 2007). These reductions of N_2O emissions have been attributed to the sorption of NH_4^+ by zeolites that should have lowered the available substrate for microbial nitrification and hence potential losses of N_2O. The mitigation potential of zeolites for soil NH_3 emissions is known to be very high since NH_3 volatilization is a physical process that can be reduced if NH_4^+ ions are "physically protected" (e.g. within a mineral lattice). The scientific literature about NH_3 emission reduction is in fact more consistent (He et al., 2002; Haruna Ahmed et al., 2008a,b; Bundan et al., 2011; De Campos Bernardi et al., 2013; Kučić et al., 2013). However, the very majority of these studies were conducted on surface applied zeolites in combination with chemical fertilizers and not as soil amendment. On the other hand, the effects of zeolite application on soil NO_x emissions are actually largely unexplored.

1.3 What is missing in the scientific literature?

As shown in the previous chapter, the scientific literature is very rich of studies concerning the application of zeolites in agricultural context. These works focused mainly on evidences of crop yield increase, reduced N leaching, increased NUE, soil physical properties changes and few of them on the reduction of gaseous emissions.

It is clear that zeolites are recognized as a valuable material for reducing the environmental impact of agricultural activity and for increasing its sustainability.

However, an in deep investigation on how this mineral component is influencing the N cycle after its insertion in the soil system is missing. Another aspect that was never investigated before is the effective transfer of nutrients from zeolites to plants. In all of the studies mentioned above, scientists

have always only observed yield increments, but they never traced and certified any nutrient transfer from the mineral to the plants, especially for the studies in which NH_4^+-enriched zeolites were used. Lastly, almost no studies were conducted about the interaction with the "living" community of the soil system. The soil is in fact a complex microhabitat colonized by billions of different types of microorganisms (i.e bacteria, fungi, actinomycetes, algae, protozoa, nematodes). Considering that 80-90 % of the processes in the soil are microbes-mediated reactions (Nannipieri et al., 2003), the nutrients cycling in soil is strictly related to microbial activity and interactions with soil mineral components.

If we think about the N cycle (Fig 2) it is evident that the strong majority of the involved reactions are mediated by soil microbial biomass (MB) (e.g N fixation, ammonification, nitrification, denitrification etc.).

This in turns implies that if we are going to insert a "new" mineral component in the soil system, with the important characteristics that zeolites have, it will probably influence **microbial community and activity**, **the N cycle** and the **production of gaseous N species**.

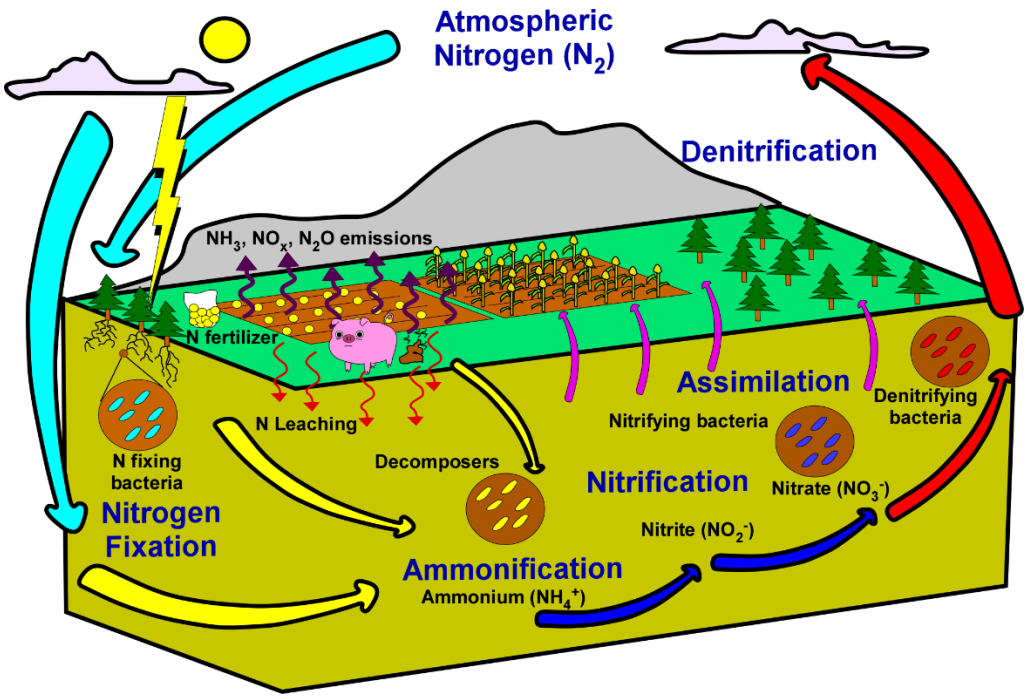

Fig 2: Scheme of the N cycle.

Lastly, the very majority of the studies in the scientific literature have been conducted employing clinoptilolite zeolites as soil amendment, thus the specific effects of CHA zeolites are largely less investigated.

1.4 Aim and structure of the thesis

The aim of this thesis was therefore to investigate and gain information on how soil amendments with natural and NH_4^+-enriched CHA zeolites influence soil N cycling with particular attention to the interaction with soil microbial biomass.

This thesis is structured in 6 main chapters. Each chapter (with the exception of chapter 1, the introduction and chapter 2, in which materials are described), represent a different separate investigation performed during my PhD project. In the chapter 3, a general investigation of the soil C-N pools dynamics over one year of monitoring in the experimental field of the ZeoLIFE project[1] is presented. In the chapter 4, the attention is focused on verifying the nutrient transfer from zeolites to plants by the mean of isotopic approaches at natural abundance. In the chapter 5, the results of a short term incubation experiment about the effects of natural and NH_4^+-enriched CHA amendments on soil gaseous emissions (CO_2, N_2O, NO_x and NH_3) are presented. Lastly, in chapter 6, an in deep investigation of the effects of natural and NH_4^+-enriched CHA amendments on soil microbial biomass is presented.

[1] The ZeoLIFE project ("Water pollution reduction and water saving using a natural zeolitite cycle", LIFE+10 ENV/IT/000321) is a European LIFE project that is testing the application of natural and NH_4^+-enriched CHA zeolites to agricultural soils at the open field scale since 2012. The experimental field is located in the Ferrara province (Emilia Romagna, Italy).

2.0 Materials: employed natural and NH_4^+-enriched CHA zeolitites and ZeoLIFE experimental field description

2.1 Employed natural and NH_4^+-enriched CHA zeolitites

The natural K rich - Na poor CHA zeolitite **(NZ)** (Fig 3) used in this study is a byproduct from a quarry located near Sorano village (central Italy, 42°41'20.65"N, 11°44'26.29"E) that is mainly exploited to obtain blocks and bricks for construction and gardening.

The selected NZ comes from a zeolitized pyroclastic deposit quarried in the Lithic-Yellow Tuff body of the Sorano formation, where CHA is the overall prevalent zeolite specie (Malferrari et al., 2013). The Sorano Formation is part of phonolitic-tephritic ignimbrite with black pumices deposits, erupted during Quaternary by the volcanic complexes of Bolsena, Vico and Bracciano lakes (Passaglia et al., 1990). These rocks underwent extensive zeolitization thanks to the activity of pore fluids heated by the thermal energy of the pyroclastic deposit itself, resulting in a sort of "geoautoclave" (Passaglia and Vezzalini, 1985). Thanks to this phenomena, the mineral composition reflects the low Na/K and Si/Al ratios of the original volcanic glass and the pH of the circulating pore waters (Passaglia and Vezzalini, 1985; Passaglia et al., 1990). The main zeolite species present in the employed NZ are K-rich/Na-poor chabazite (~68 %), phillipsite (~1.8 %), analcime (~0.6 %) (Table 1) Malferrari et al. (2013). The Apparent Density (AD), Water Retention (WR) and CEC, is reported in Table 2.

Fig 3: NZ from the quarry located near Sorano (Gr, Italy).

Table 1: Quantitative Phase Analysis of the employed NZ. "TZC" refers to Total Zeolitic Content. Data from Malferrari et al. (2013).

	wt%
chabazite	68.5 (0.9)
phillipsite	1.8 (0.4)
analcime	0.6 (0.3)
TZC	70.9
mica	5.3 (0.6)
K-feldspar	9.7 (0.7)
plagioclase	-
pyroxene	2.9 (0.4)
calcite	-
volcanic glass	11.2 (1.0)

Table 2: Apparent Density (AD), Water Retention (WR), and Cation Exchange Capacity (CEC) of the employed NZ. Standard deviation between brackets. Data from Malferrari et al. (2013).

	NZ
AD (g cm^{-3})	0.56
WR (wt%)	34.2
CEC (meq g^{-1})	
Ca	1.46
Mg	0.04
Na	0.07
K	0.6
Total	2.17

Part of the NZ was subjected to an enrichment process, which allowed the enrichment (but not the saturation) with NH_4^+ ions. In this way, an **NH_4^+-enriched form of NZ was obtained (CZ)**. The enrichment process was carried out by static mode in a prototype tank where NZ was mechanically mixed with pig slurry (average NH_4^+ content of 2000 mg l^{-1}) at specific solid/liquid ratio, stirring and

resting times (Faccini et al., 2015). The main chemical characteristics and N and C pools of NZ and CZ are reported in Table 3.

Table 3: Main properties of the employed natural zeolites (NZ) and NH_4^+-enriched zeolites (CZ). GWC refers to gravimetric water content. TN and TOC refers to total N and total organic C, respectively. $\delta^{15}N$ refers to the delta notation for N isotopic signature. TDN and DOC refers to total dissolved N and total dissolved organic C, respectively. MB-C and MB-N refers to C and N immobilized by soil microbial biomass, respectively. Exch and Fix NH_4^+-N refers to exchangeable and fixed NH_4^+ fractions, respectively (see chapter 3.2 for method descriptions). The presence of "BDL" refers to "below detection limits".

	NZ	CZ
Grain Size (mm)	3-5	3-5
Air-dry GWC (wt%)	12.8	22.8
TN (g kg^{-1})	0.01	4.27
TOC (g kg^{-1})	0.08	1.24
$\delta^{15}N$ (‰)	BDL	18 - 43.6
TDN (mg kg^{-1})	14.6	3611
DOC (mg kg^{-1})	BDL	118
TOC/TN	8.42	0.29
pH	7.58	6.95
EC (mS cm^{-1})	0.542	15.0
MB-C (mg kg^{-1})	22.2	23.8
MB-N (mg kg^{-1})	9.69	388
MB C/N	2.29	0.06
NO_3^--N (mg kg^{-1})	BDL	146
Exch NH_4^+-N (mg kg^{-1})	BDL	3014
Fix NH_4^+-N (mg kg^{-1})	BDL	343
ergosterol (mg kg^{-1})	BDL	BDL

NZ was characterized, by very low TN and TOC content, as well as low TDN and DOC. However, as evidenced by MB-C and MB-N measurements, the mineral was slightly colonized by microorganisms. The pH of NZ was neutral to sub-alkaline and the EC was very low.

After the enrichment process with pig slurry, CZ minerals gained a total N load of 4.27 g kg^{-1} (Table 3). Most of the N was present in the form of exchangeable NH_4^+-N, while Fix[2] NH_4^+-N was considerably lower. NO_3^--N load was on average 148 mg kg^{-1}. CZ was characterized by a very high MB-N and a low MB-C, resulting in a MB-C/N ratio lower than 1. TOC content was quite low (1.24 g kg^{-1} on average) resulting in a low C/N. The pH of CZ was neutral and the EC was very high.
In this thesis, the superficially adsorbed NH_4^+ ions were not differentiated from the extra framework adsorbed NH_4^+.

2.2 ZeoLIFE: brief project explanation and experimental field description

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 NO_3^- pollution (Nitrate Vulnerable Zone, NVZ) such as the whole Ferrara Province (Emilia Romagna, Italy), in which the maximum N input per year in agricultural fields is limited to 170 kg N ha^{-1}. The Ferrara province 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 and the consolidate agricultural practice of zoo-technical slurry spreading further emphasize the vulnerability of this province (Arcara et al., 1999).
In this framework, the ZeoLIFE project was conceived to test different CHA zeolitite[3] amendments at open-field scale, aiming at reducing the N inputs from fertilizers and the irrigation water in agriculture. This project focus on exploiting the high CEC of this material in two different ways:

1) **Apply NZ** in the soil directly at its natural state, in order to catch and adsorb the NH_4^+ ions formed after the addition of chemical or organic fertilizers, **increasing the NH_4^+ retention in the soil and thus increasing fertilization efficiency by reducing N losses**.

[2] Fix NH_4^+-N is a more stable and hardly exchangeable form of NH_4^+, generally associated with the adsorption inside clay mineral interlayers and not replaceable by ion exchange with K^+ (Silva, 1964).
[3] Rock constituted by more than 50% of zeolite minerals (Galli and Passaglia, 2011).

2) **First create a CZ** by up-taking NH_4^+ from a zoo-technical waste **(pig slurry)** with high-environmental impact, using a specifically designed prototype tank (Faccini et al., 2015). The material is added then to the soil in order to **create a "slow-release" N pool for plants** which should be **not significantly affected by N leaching**. Concomitantly, the **environmental impact of the slurry should be decreased** because of the reduction of its NH_4^+ load after the treatments with zeolites. After the complete release of the N adsorbed from the pig-slurry, CZ will have the same function as NZ.

The reversible sorption of NH_4^+ ions and the long term structural stability of this mineral component (Baerlocher et al., 2001; Gualtieri and Passaglia, 2006), will reflect in a long-term soil amelioration. With both this practices (amendments with natural or NH_4^+-enriched CHA), the final aim is to reduce the input of fertilizers by increasing their efficiency, allowing a higher NH_4^+ retention in the soil system and thus a more efficient NUE by crops (and consequently lower N losses).

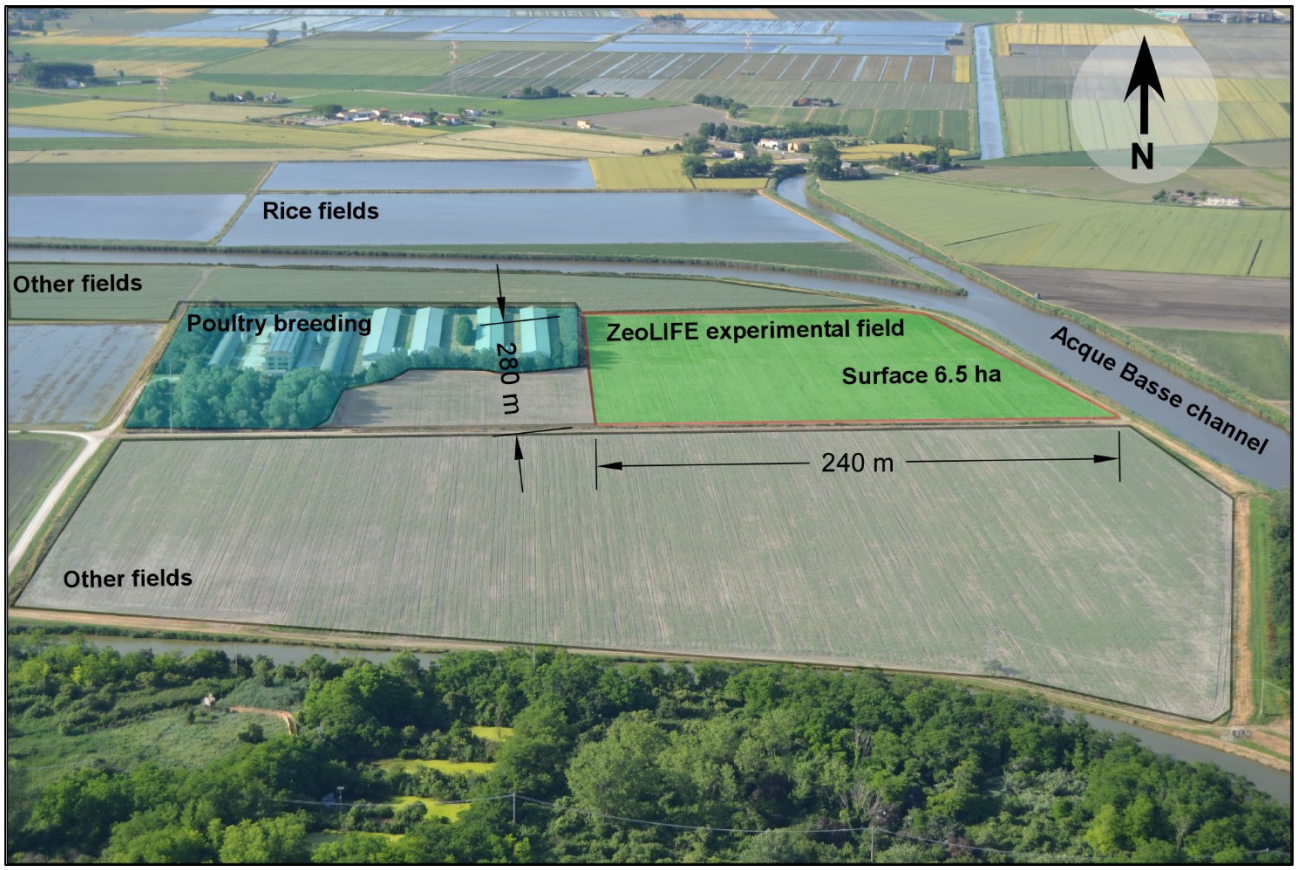

Fig 4: ZeoLIFE experimental field location and dimensions.

The experimental field of the ZeoLIFE project is located near Codigoro town (Italy) (Fig 4), 40 km eastward of Ferrara (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 range 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 inter-distributary bays and brackish marshes were recently (1860-1960) reclaimed (Mastrocicco et al., 2013).

The soil of the experimental field belongs to the cartographic unit FOR1-LCO1 of the Emilia Romagna 1:50,000 Soil Map, and it is classified as a clayey-silt Calcaric Gleyic Cambisol (Di Giuseppe et al. 2014; IUSS 2014). Soil mineralogical composition is characterized by quartz, feldspar, calcite and clay minerals (illite, chlorite, kaolinite) (Fig 5) (Malferrari et al., 2013).

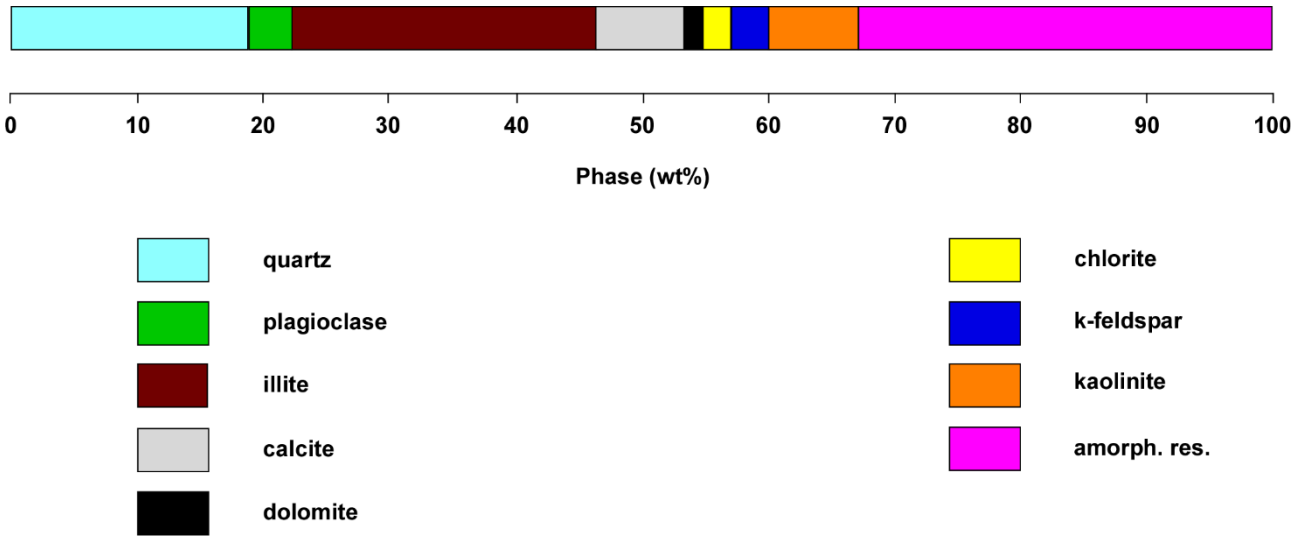

Fig 5: Quantitative Phase Analysis (QPA) of the experimental field soil. The analysis refers to the 0-20 cm soil layer. Modified from Malferrari et al., 2013.

The main soil properties are listed in Table 4. As visible, the soil reaction is sub-alkaline with a high carbonate content and a quite high salt content. The CEC is relevant because of clay minerals presence (mainly illite) but it is almost five time lower than that of NZ (Table 2 and 4). The total N of the soil is very high as well as the organic C content.

Table 4: Main properties of the experimental field soil. EC refers to Electrical Conductivity, TN to Total Nitrogen and TOC to Total Organic Carbon. Standard deviation within brackets.

	Soil
pH	7.6 (0.2)
EC (mS cm^{-1})	1.44 (0.04)
$CaCO_3$ (g kg^{-1})	64.5 (3.5)
CEC (meq g^{-1})	0.45 (0.02)
TN (g kg^{-1})	2.33 (0.31)
TOC (g kg^{-1})	22.76 (3.2)
Soil TOC/TN ratio	9.76 (0.34)
Bulk Density (kg m^{-3})	1250

3.0 ^{15}N natural abundance, N and C pools in soil-sorghum system amended with natural and NH_4^+-enriched zeolitites

3.1 Introduction

As already anticipated in the previous chapter, a detailed investigation about the dynamics of different N species in a zeolite-amended agricultural field is still lacking in the scientific literature. Therefore, in this chapter, a general overview on the dynamics of many N (and C) pools in soils amended with NZ and CZ (e.g. total N, exchangeable and fixed NH_4^+, NO_3^-, microbial biomass N and C, total organic C) over one year of monitoring in the ZeoLIFE experimental field is presented.

By studying the dynamics of N pools in the soil-plant system it should be possible to gain information about how this mineral component affects the N cycle.

The unamended soil of the experimental field was compared with both NZ and CZ amended soils from November 2012 to September 2013. Sorghum (*Sorghum vulgare Pers*.) was cultivated during the experimentation (from June to September 2013).

In this context we tried also to perform some preliminary measurements of the ^{15}N natural abundance in soil and plants in order to obtain insights about the fate of the N introduced with CZ in the soil-plant system. The measurement of the N isotopic signature in soils and associated plants is in fact recognized as very powerful tools that allows a better understanding of the plant-soil N dynamics (Högberg, 1997; Szpak, 2014). It is well known that variations in the N stable isotopic ratio can give robust information about N fluxes and plant N sources. Many studies consider that no variation of the isotopic ratio occurs after the absorption of N by the plant, thus, foliar or stems $\delta^{15}N$ can act as tracer, reflecting the isotopic ratio of the N source in the soil (Handley and Raven, 1992; Högberg, 1997; Evans, 2001). However, this assumption is not completely correct due to possible fractionations during N uptake, intra-plant N re-allocation, physiological factors and fractionations due to mycorrhizal associations (Evans, 2001; Szpak, 2014). Zootechnical effluents, such as pig-slurry that is commonly used as organic fertilizer, are in fact strongly enriched in the heavier ^{15}N isotope due to

NH_3 volatilization that cause depletion in the lighter ^{14}N, resulting in a $\delta^{15}N$ generally > 10 ‰ or even > 20 ‰ (Högberg, 1997; Dittert et al., 1998; Schmidt and Ostle, 1999; Lim et al., 2007). This in turn implies that plants grown with N inputs from zootechnical effluents (such as pig-slurry) will have significantly higher $\delta^{15}N$ with respect to plants grown under unfertilized or with N inputs from chemical fertilizers (Choi et al., 2002, 2003; Bateman et al., 2005; Yun et al., 2006; Szpak et al., 2012,2014).

The aim of this monitoring campaign was therefore to asses if the amendments performed with NZ and CZ influenced the N pool dynamics in the ZeoLIFE experimental field and concomitantly to gain preliminary information about the possible N transfer from CZ to the cultivated sorghum plants by the mean of isotopic techniques at natural abundance.

3.2 Materials and Methods

3.2.1 Experimental field setting

The ZeoLIFE experimental field (Fig 4) was subdivided in 4 plots between October and November 2012. The various plots were designed linear and continuous in order to facilitate the movements of farm machines. One plot of 0.5 ha was amended with 7 kg m^{-2} of CZ (7CZ). Two plots, of 1 ha each, were amended with 5 and 15 kg m^{-2} of NZ (5NZ and 15NZ), respectively. Finally, 3.5 ha were left unamended (UA). The smaller size of 7CZ plot was due to the long time required to produce CZ by the ZeoLIFE prototype tank, which has a limited production rate of about 250 kg day^{-1}. Addition and spreading into the field of NZ and CZ was carried out between October 7th and November 6th, 2012. CZ supplied approximately 299 kg N ha^{-1} in the 7CZ plot (as total N). Immediately after spreading, the field was ploughed and the zeolites were homogenized in the first 30 cm soil layer. According to ZeoLIFE plans, sorghum (*Sorghum vulgare Pers*.) was sowed on May, 9th, 2013. N fertilizers have

been distributed in two steps: di-ammonium phosphate during the sowing, with an application of 21.6 kg N ha^{-1} in all plots and urea on June, 3rd, 2013, applying reduction in all zeolite amended plots.

In the UA plot, a total amount of 170 kg N ha^{-1} (di-ammonium-phosphate + urea) was applied (maximum admitted in a NVZ zone). A reduction of 30 % and 50 % of urea in NZ and CZ plots was applied, respectively. By consequence, the total amount of chemical fertilizers applied in 5NZ and 15NZ was 127 kg N ha^{-1}, while in 7CZ a total amount of 81 kg N ha^{-1} was applied.

Harvest occurred in September 23rd, 2013 and the yield was separately evaluated for each treatment.

This thesis developed within the ZeoLIFE project and thus was strictly constrained to the project experimental set-up and agricultural managements, such as fertilization plans. For this reason, unfortunately, it was not possible to build a "negative control" (with no N inputs) and controls with the same N inputs reductions as in zeolite treated plots, as well as replicates of the different plots.

3.2.2 Soil and plant sampling

Soil sampling campaigns were carried out in four periods: (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), during the sorghum growing season and after chemical fertilization; (4) September 2013 (Sep-2013), at the harvest.

Soil samples (from 0 to 30 cm depth) were collected in the three replicates from each treatment by manual drilling using an Ejielkamp Agrisearch auger. Soil samples collected at the harvest (Sep-2013) were took both away from the roots zone (Sep-2013 Bulk) and in the rhizosphere (Sep-2013 Rhizo). The rhizosphere was separated by gently shaking plant roots.

Three representative plants from each treatment were sampled during the harvest. Each plant was subdivided in roots, stems, leaves and panicles that were subsequently dried at 50°C and milled.

3.2.3 Analytical techniques

CEC and exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+) were determined using the $Co(NH_3)_6Cl_3$ method (Orsini and Rèmy 1976; Ciesielsky and Sterckeman 1997) on both bulk soil and rhizosphere samples collected in Sep-2013.

Soil pH was determined on MQ-water extracts (in 1:5 w:v ratio) using an Orion 9102BNWP pH-meter connected to an Orion 4star pH – ISE benchtop (Thermo Fisher).

Total N (TN) and organic C (TOC) of soil and plant samples were determined by an elemental CHNS-O 1110 Thermo Fisher Scientific elemental analyser (EA) coupled with mass spectrometry (IRMS) (Delta Plus, Finnegan, Thermo-Fisher) at the University of Bologna (Italy). Soil samples were weighted in silver pots and treated with HCl in order to eliminate the inorganic C, while plant samples were weighted in tin pots and directly analysed.

Exchangeable NH_4^+-N was extracted with 1 M 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^- and K^+ ions were verified and excluded by comparing measurements made with NH_4Cl, CH_3COONH_4 + 1 M KCl and CH_3COONH_4 + H_2O standard solutions.

Fixed NH_4^+-N (Fix NH_4^+-N) was determined according to the method proposed by Silva and Bremner (1966) with some modifications. 20 ml of KBrO were added to 2 g of air-dried soil samples then, after 2 hours, 40 ml of distilled water were added and a heating cycle of about 10 minutes in a microwave oven was applied. Successively, 20 ml of distilled H_2O were added and samples were left still for 24 hours. Finally, samples were centrifuged at 5000 rpm for 10 minutes and washed 2 times with 0.5 M KCl and analysed by EA-IRMS for the evaluation of TN and $\delta^{15}N$. With this method, all the organic and exchangeable N are eliminated and only the deeply fixed NH_4^+ ions remain before the analysis.

NO_3^--N was extracted with Milly-Q (Millipore USA) water in a 1:5 (w:v) ratio, the solution was shaken for 1 h and then filtered. NO_3^--N was determined by ion chromatography with an isocratic

dual pump (ICS-1000 Dionex), equipped with an AS9-HC 4 × 250 mm high-capacity column and an ASRS-Ultra 4-mm self-suppressor. An AS-40 Dionex auto-sampler was employed to run the analysis. Quality control (QC) samples were run every ten samples.

The ^{15}N natural abundance has been measured in TN and Fix NH_4^+-N pools, all sorghum organs and all N inputs (chemical fertilizers and CZ). The ^{15}N natural abundance has been expressed using the delta notation (δ ‰) (Eq. 1, Mariotti, (1984)), where δ represents the difference from the standard, represented by atmospheric N_2 (0.3663 atom% ^{15}N):

(1) $$\delta^{15}N\ ‰ = \left[\frac{\left(\frac{^{15}N}{^{14}N}\right)sample - \left(\frac{^{15}N}{^{14}N}\right)standard}{\left(\frac{^{15}N}{^{14}N}\right)standard} \right] * 1000$$

C and N immobilized by soil microbial biomass (MB-C and MB-N) were obtained by chloroform fumigation-extraction method. According to Vance et al. (1987), 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 periods, two replicates were extracted with 0.5 M K_2SO_4, representing the non-fumigated samples (NF). On the other two samples, $CHCl_3$ was added in a closed drier equipped with a void pump to allow $CHCl_3$ volatilization at room temperature. In these fumigate (F) samples the presence of $CHCl_3$ atmosphere guarantees the death of the microorganisms and allow the release of the immobilized C and N. After the fumigation, the same extraction with 0.5 M K_2SO_4 was performed. Finally, both NF and F samples were analysed with a Shimadzu Total Organic Carbon Analyser TOC-V CPN coupled with a TN unit TNM-1. The C and N extracted from F samples minus that extracted from NF samples represent the C and N immobilized by soil microorganisms. A correction factor of 0.45 and 0.54 was employed according to Beck et al. (1997) and Brookes et al. (1985) in order to determine the extractable part of microbial C-N.

3.2.4 Statistical analysis

For evaluating significant differences between the treatments, parametric statistic was applied to the dataset. Two-Way ANOVA was employed 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=0.05. Correlation matrixes were also performed (using Pearson coefficient "ρ") in order to highlight positive or negative correlations between variables for each treatment.

3.3 Results

For the main soil properties and zeolites (NZ and CZ) N-C pools, the reader must refer to chapters 2.0.

Soil pH was not affected by the addition of both NZ and CZ ($p>0.05$). It remained very close to neutral values in all treatments, with exception of Jun-2013 where after urea addition an increase toward sub-alkaline values was well visible ($p<0.05$) (Fig. 6).

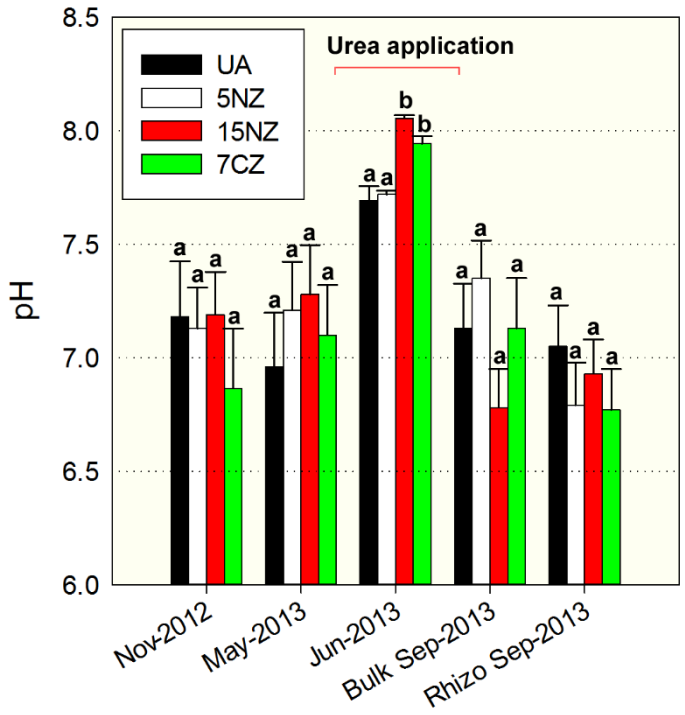

Fig 6: Soil pH among the experimental period. Different letters indicate significant differences at p=0.05. Error bars represent standard deviation.

As visible from Table 5, CEC increased significantly ($p<0.05$) in the bulk soil of all zeolite amended plots, however in the rhizosphere no significant differences between the treatments were observed ($p>0.05$). In general, Ca^{2+} and Mg^{2+} were the major exchangeable bases, followed by K^{+} and Na^{+}, with the latter always higher in 7CZ than in the other treatments. No significant differences were found between the various plots concerning exchangeable K^{+} ($p>0.05$), while Ca^{2+} and Mg^{2+} were generally higher in all zeolite amended plots (especially in the bulk soil with respect to rhizosphere) ($p<0.05$).

Table 5: Average of exchangeable bases and CEC of soil samples, both bulk soil and rhizosphere. Standard deviation within brackets. Different upper case letters indicate significant differences (at p=0.05) as result of ANOVA and Tukey HSD test.

Time	Treatment	Ca $cmol_{(+)}kg^{-1}$	K $cmol_{(+)}kg^{-1}$	Mg $cmol_{(+)}kg^{-1}$	Na $cmol_{(+)}kg^{-1}$	CEC $cmol_{(+)}kg^{-1}$
Sep-2013 Rhizo	UA	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	UA	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)^{b}
	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}

3.3.1 Soil N and C pools

Soil N pools significantly differed between the treatments and among the sampling time ($p<0.05$), with the exception of TN that showed significant differences among sampling time only ($p<0.05$) (Table 6). High TN values were detected in Nov-2012 and Sep-2013, while a depletion occurred in May-2013 and Jun-2013 ($p<0.05$).

A similar behaviour was recorded for Fix NH_4^+-N pool (Fig 7A), but in this case, all zeolite amended soils showed slightly lower values ($p<0.05$) in Nov-2012 with respect to the UA. In correspondence of May-2013 and Jun-2013, Fix NH_4^+-N pool significantly decreased ($p<0.05$), but generally no significant differences between the treatments were observed ($p>0.05$) (except for 15NZ in Jun-2013). The total reserve of this pool increased at the end of the growing season, both in the bulk soil and in the rhizosphere, with no significant differences between the treatments ($p>0.05$)

Table 6: soil TN, TOC and C/N ratio during the experimental period. Standard deviation within brackets. Different upper case letters indicate significant differences at each sampling time (at p=0.05) as result of ANOVA and Tukey HSD tests.

Time	Treatment	TN	TOC	C/N
		g kg^{-1}	g kg^{-1}	
Nov-12	UA	2.65 (0.07)^{a}	25.3 (1.2)^{b}	9.55 (0.27)^{b}
	5NZ	2.60 (0.01)^{a}	24.7 (0.9)^{ab}	9.51 (0.30)^{b}
	15NZ	2.71 (0.09)^{a}	27.3 (0.1)^{b}	10.1 (0.3)^{b}
	7CZ	2.77 (0.35)^{a}	20.7 (1.9)^{a}	7.49 (0.25)^{a}
May-2013	UA	2.42 (0.03)^{a}	24.6 (0.3)^{a}	10.2 (0.03)^{a}
	5NZ	2.66 (0.07)^{a}	25.8 (0.04)^{a}	9.73 (0.23)^{a}
	15NZ	1.68 (0.80)^{a}	22,0 (3.5)^{a}	14.2 (4.7)^{a}
	7CZ	1.98 (0.09)^{a}	19.2 (0.1)^{a}	9.67 (0.50)^{a}
Jun-2013	UA	2.04 (0.23)^{a}	19.7 (2.1)^{ab}	9.68 (0.14)^{a}
	5NZ	2.16 (0.04)^{a}	20.3 (0.6)^{ab}	9.41 (0.44)^{a}
	15NZ	1.83 (0.06)^{a}	17.6 (1.0)^{a}	9.61 (0.19)^{a}
	7CZ	2.42 (0.07)^{a}	23.4 (1.3)^{b}	9.69 (0.25)^{a}
Sep-2013 Bulk	UA	2.13 (0.03)^{a}	20.6 (1.4)^{a}	9.68 (0.51)^{a}
	5NZ	2.47 (0.07)^{a}	23.1 (0.3)^{a}	9.33 (0.38)^{a}
	15NZ	2.43 (0.12)^{a}	23.2 (0.4)^{a}	9.58 (0.31)^{a}
	7CZ	2.32 (0.13)^{a}	22.8 (1.1)^{a}	9.81 (0.08)^{a}
Sep-2013 Rhizo	UA	2.58 (0.10)^{a}	25.8 (2.4)^{a}	9.98 (0.6)^{a}
	5NZ	2.83 (0.13)^{a}	29.1 (2.2)^{ab}	10.3 (0.4)^{a}
	15NZ	2.88 (0.20)^{a}	31.5 (2.6)^{b}	11.0 (0.8)^{a}
	7CZ	2.80 (0.04)^{a}	28.1 (0.3)^{ab}	10.0 (0.03)^{a}
	Treatment	x	*	*
	Time	*	*	*
	Treatment*Time	*	*	*

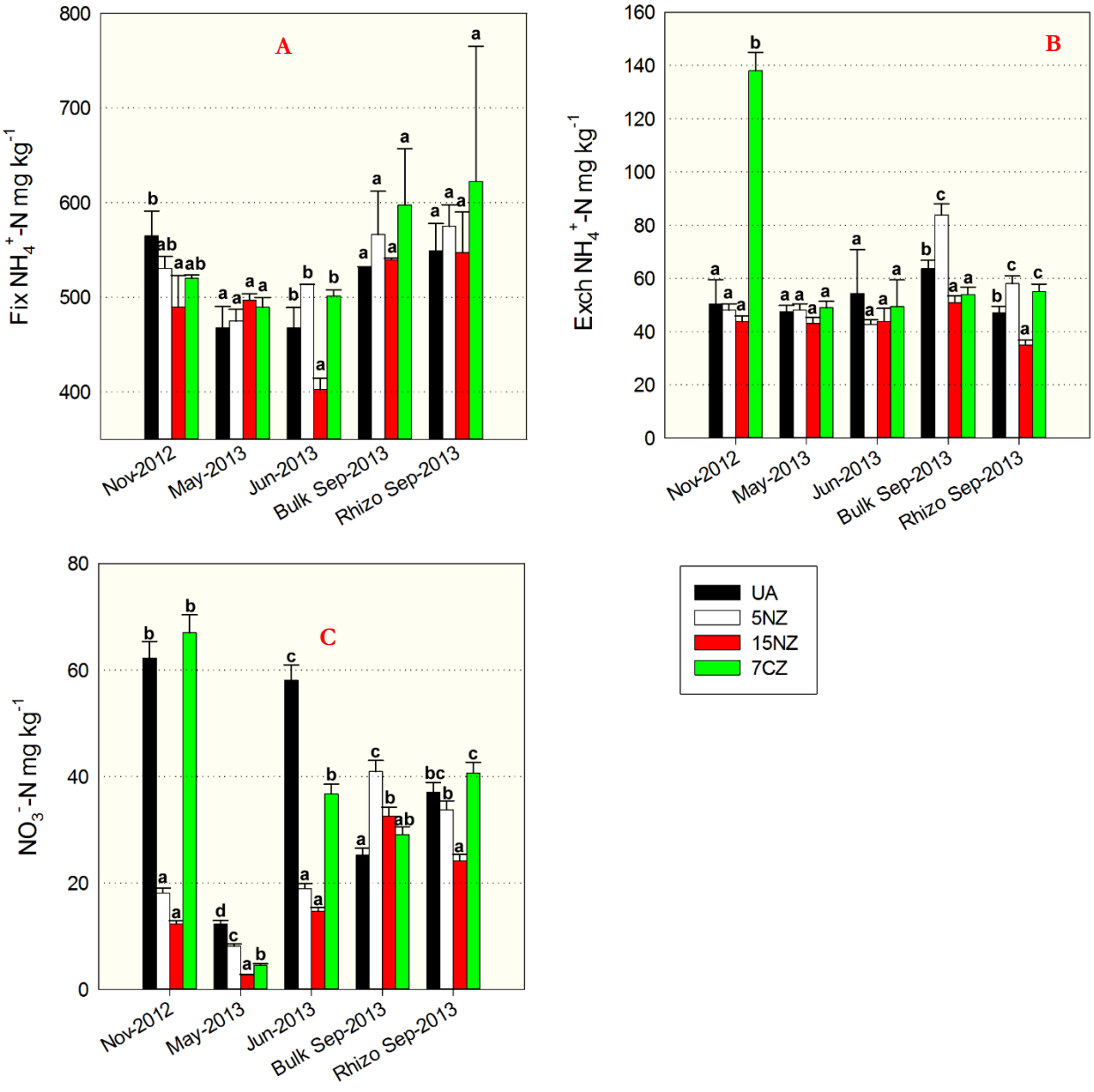

Fig 7: Fix NH_4^+-N (A), Exch NH_4^+-N (B) and NO_3^--N pools during the experimental period. Different letters indicate significant differences at p=0.05 at each sampling time as results of ANOVA and Tukey HSD tests. Error bars represent standard deviation.

Exch NH_4^+-N (Fig 7B) was significantly affected by CZ addition in Nov-2012 ($p<0.05$) (138 mg kg^{-1} vs 50 mg kg^{-1} of 7CZ and UA, respectively) while starting from May-2013 these differences completely disappeared ($p>0.05$). In Jun-2013, no differences between the treatments were found ($p>0.05$).

A relative enrichment of the bulk soil Exch NH_4^+-N with respect to the rhizosphere was recorded in Sep-2013.

At the beginning of the experiment, NO_3^--N content (Fig 7C) was significantly higher in the UA and 7CZ plots rather than in those amended with NZ (5NZ and 15NZ, respectively) ($p<0.05$). In May-2013, a general depletion of NO_3^--N pool occurred in all the treatments, with UA plot showing significantly higher values in comparison to all zeolite amended plots ($p<0.05$). NO_3^--N increased strongly in Jun-2013, after the addition of urea fertilizer, especially in the UA plot. NO_3^--N values of all zeolite amended soils (especially 5NZ and 15NZ) were remarkably lower in this period with respect to the UA ($p<0.05$).

At the harvest, NO_3^--N content in the bulk soil (Sep-2013 Bulk) was significantly different between the treatments ($p<0.05$) with 5NZ showing the highest values and UA-7CZ the lowest while in the rhizosphere, NO_3^--N values were higher in 7CZ and UA while considerably lower in 15NZ ($p<0.05$) (Fig 7C).

During the monitored period, MB-C (Fig 8A) ranged from 43.2 to 432 mg kg^{-1}. At the beginning of the experiment huge differences ($p<0.05$) were detected among the various treatments. In this period, MB-C content decreased following the order UA (148 mg kg^{-1}) > 5NZ (101 mg kg^{-1})> 15NZ (98.5 mg kg^{-1}) > 7CZ (43.2 mg kg^{-1}). In May-2013, MB-C increased in all treatments, and decreased again in Jun-2013. As expected, MB-C content in the rhizosphere was higher than that determined in the bulk soil.

Generally, at the beginning of the experiment all the treatments showed a low MB-N content (Fig 8B), following the order 7CZ>UA>5NZ=15NZ, suggesting that zeolite amendments differently affected the MB-N pool.

The MB-N pool increased in May-2013, while it decreased in June-2013 after the chemical fertilization. As expected, MB-N content in rhizosphere soil was higher than that determined in the bulk soil at the harvest. A remarkable increase of MB-C and MB-N and a contemporaneous decrease of NO_3^--N in all the treatments was observed in May-2013.

The average MB-C/N ratio ranged from 3.1 to 94 (Fig 9). In Nov-2012, lower values of MB-C/N were observed in 5NZ and 7CZ treatments. Concerning 5NZ, the lower ratio was due to the lower MB-C with respect to the other treatments, while for 7CZ the lower ratio was caused by the high MB-N.

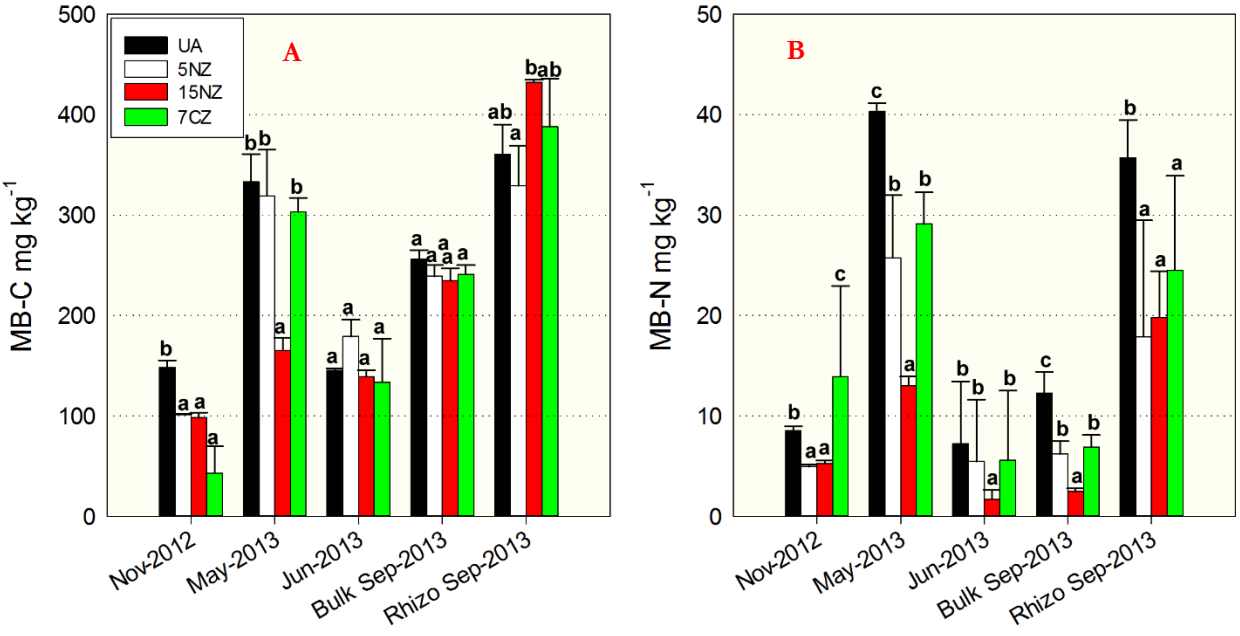

Fig 8: MB-C (A) and MB-N (B) among the experimental period. Different letters indicate significant differences at p=0.05 as results of ANOVA and Tukey HSD tests. Error bars represent standard deviation.

All zeolite amended soils showed a higher MB-C/N ratio than UA after May-2013 because of the lower MB-N. On average, MB-C/N ratio increased according to the order 15NZ>5NZ>7CZ>UA.

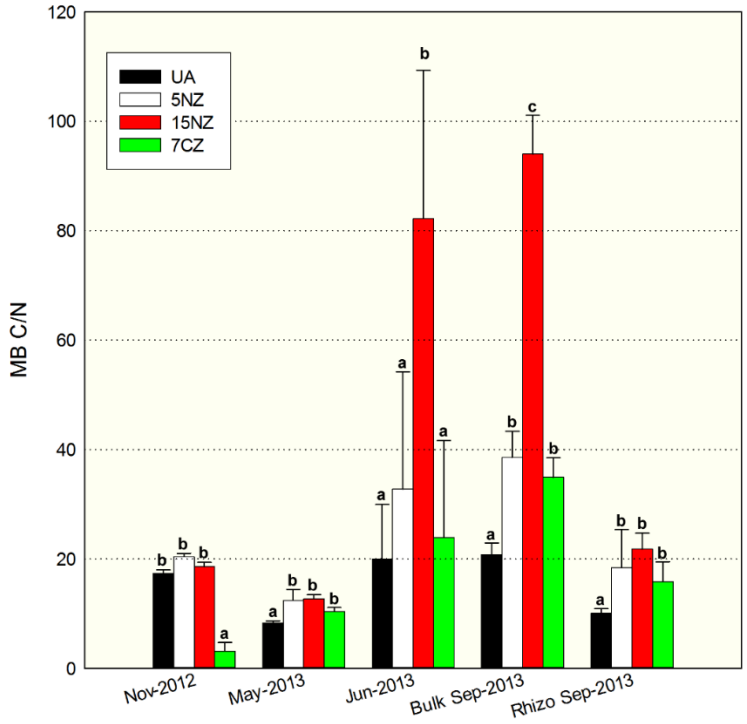

Fig 9: MB C/N among the experimental period. Different letters indicate significant differences at p=0.05 at each sampling time as result of ANOVA and Tukey HSD tests. Error bars represent standard deviation.

Soil TOC content was significantly different both 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 6). As expected, TOC content in Sep-2013 was higher in the rhizosphere than in the bulk soil. A clear direct correlation between TN and TOC was well visible (ρ=0.83). 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 always constant around values of 10 with no significant differences between the treatments ($p>0.05$) (Table 6).

3.3.2 Total N and C content in sorghum organs

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 7). Generally, leaves and panicles

were higher in 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.

Table 7: Average of TN, TOC and C/N of the various sorghum organs. Different letters indicate significant differences at p=0.05. Standard deviation within brackets.

Plant organ	Treatment	TN	TOC	C/N
		g kg^{-1}	g kg^{-1}	
Roots	UA	13.3 (4.2)[a]	422 (12)[a]	31.8 (7.1)[a]
	5NZ	11.4 (2.6)[a]	419 (5)[a]	37.8 (9.0)[a]
	15NZ	9.4 (0.72)[a]	420 (8)[a]	45.1 (3.8)[a]
	7CZ	13.6 (1.6)[a]	413 (6)[a]	30.7 (4.2)[a]
Stems	UA	8.01 (3.27)[a]	414 (8)[a]	50.6 (6.1)[a]
	5NZ	10.4 (3.1)[a]	412 (5)[a]	41.5 (12.0)[a]
	15NZ	6.75 (1.31)[a]	401 (9)[a]	61.2 (9.3)[a]
	7CZ	6.17 (0.59)[a]	403 (5)[a]	66.2 (6.5)[a]
Leaves	UA	24.4 (2.2)[a]	414 (15)[a]	17.3 (5.7)[a]
	5NZ	25.1 (1.8)[a]	424 (1)[a]	16.9 (1.2)[a]
	15NZ	18.8 (5.1)[a]	433 (13)[a]	23.2 (5.7)[a]
	7CZ	23.1 (1.1)[a]	408 (7)[a]	17.5 (1.0)[a]
Panicles	UA	19.5 (6.5)[a]	429 (6)[a]	22.0 (4.2)[a]
	5NZ	21.2 (3.2)[a]	430 (1)[a]	20.6 (3.0)[a]
	15NZ	18.0 (1.2)[a]	423 (1)[a]	23.9 (1.8)[a]
	7CZ	20.9 (2.1)[a]	431 (2)[a]	20.7 (2.3)[a]

3.3.3 ^{15}N natural abundance of the main N inputs, soil and sorghum organs

The N isotopic signature ($\delta^{15}N$) of the "pure" CZ mineral (enriched with pig-slurry derived NH_4^+) was characterized by a $\delta^{15}N$ variable from 18 to 43.6 ‰ (Table 3), indicating a very high ^{15}N natural abundance. On the other hand, the $\delta^{15}N$ of the two types of chemical fertilizers added during the

experimentation (di-ammonium phosphate and urea) were slightly negative with values of -1.18 ‰ and -0.89 ‰, respectively.

The $\delta^{15}N$ of TN and Fix NH_4^+-N pools differed significantly ($p<0.05$) among treatments and during experimental time (Fig 10 A,B). At the beginning of the experiment (Nov-2012), 7CZ showed a remarkably higher isotopic signature with respect to the other treatments ($p<0.05$) ($\delta^{15}N$ of 7.46 ‰ and 8.27 ‰ for TN and Fix NH_4^+-N, respectively).

Furthermore, without considering 7CZ treatment, Fix NH_4^+-N pool showed a high $\delta^{15}N$ variability (from 4.55 ‰ to 6.00 ‰) with respect to the TN pool (from 3.38 ‰ to 3.86 ‰). In May-2013, TN $\delta^{15}N$ was still significantly higher ($p<0.05$) in 7CZ treatment, while no significant differences were observed regarding Fix NH_4^+-N pool. In Jun-2013 (after the chemical fertilization), all treatments showed a homogeneous $\delta^{15}N$ in both TN and Fix NH_4^+-N pools ($p>0.05$). In Sep-2013, rhizosphere samples showed higher $\delta^{15}N$ with respect to the bulk soils in both TN and Fix NH_4^+-N pools (especially in the latter).

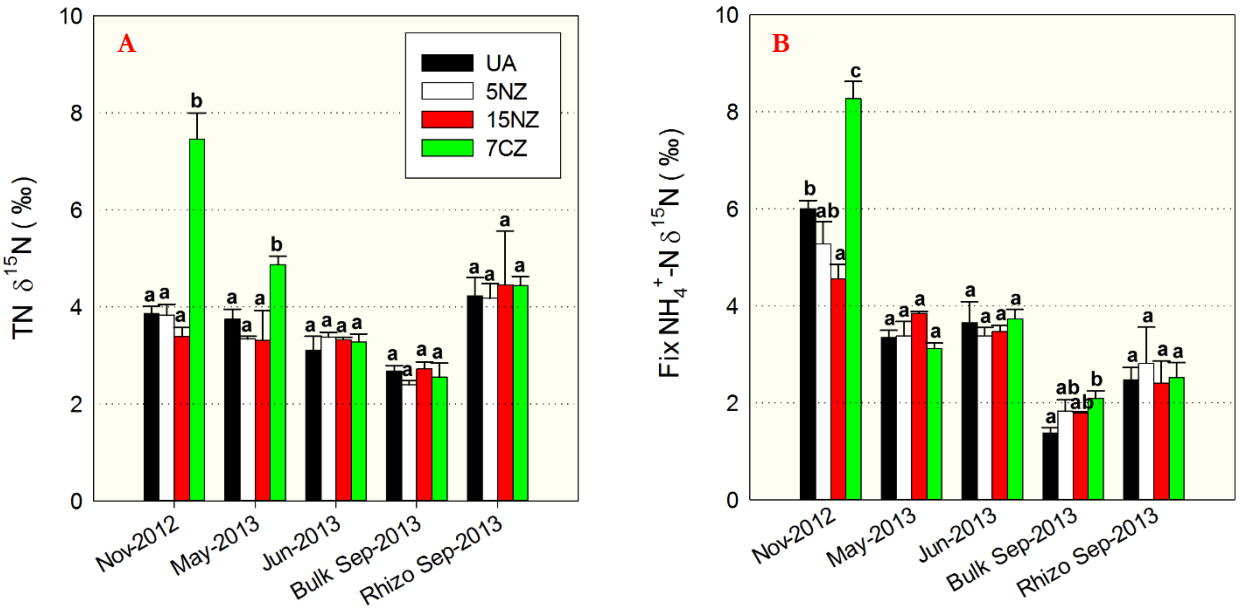

Fig 10: N isotopic signature of TN (A) and Fix NH_4^+-N (B) pools. Different letters indicate significant differences at p=0.05 at each sampling time as result of ANOVA and Tukey HSD tests. Error bars represent standard deviation.

Isotopic analysis on the harvested plants indicated that the $\delta^{15}N$ of plant tissues was influenced by the different treatments (Fig 11). The $\delta^{15}N$ was significantly higher in 7CZ sorghum leaves ($p<0.05$) and significantly lower in all 5NZ plants organs with respect to the UA plants ($p<0.05$). 15NZ roots, stems, panicles and leaves were quite close to the values showed by UA organs ($p>0.05$).

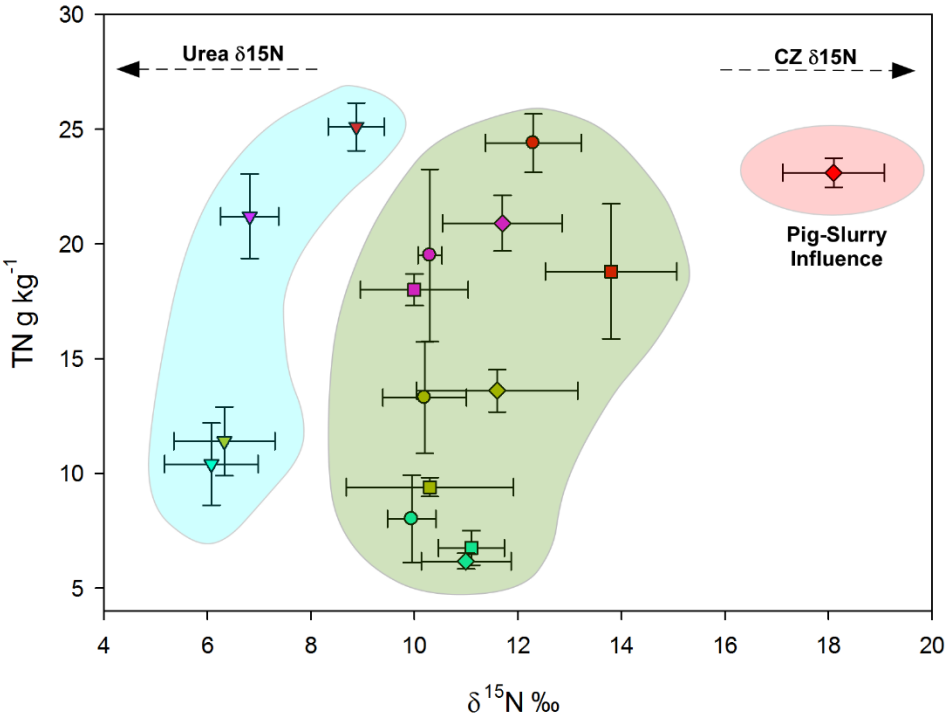

Fig 11: TN vs $\delta^{15}N$ plot of the various sorghum organs. Circles, reverse triangles, squares and rhombus represent UA, 5NZ, 15NZ and 7CZ plants respectively. Red, purple, brown and green fills represents leaves, panicles, roots and stems, respectively. Vertical and horizontal error bars indicate standard errors.

3.3.4 Sorghum yield

The UA plot obtained a yield of 5,818 kg ha^{-1}. The two NZ obtained an equal yield of 6,032 kg ha^{-1} each, while 7CZ plot yield was of 6,627 kg ha^{-1}. The yield increment with respect to the UA, varied from +3.7 to +13.9 % although not statistically significant because of the lacks of replicates.

3.4 Discussions

Zeolite addition to soil raised significantly CEC with respect to the UA in the bulk soil. Gholamhoseini et al. (2013) and Ozbahce et al. (2015) found a similar tendency after mixing zeolite in the first 30 cm of soil or after the spreading in combination with composted cattle manure. However, in the rhizosphere, soil CEC was not significantly different and the variability within treatments was greatly higher (Table 5).

Both bulk and rhizosphere soils in the 7CZ plots showed higher exchangeable Na^+ with respect to the other treatments, likely because of the adsorption of some Na^+ ions during the CZ enrichment process with pig slurry (whose Na^+ content was quite high, data not shown).

Exch NH_4^+-N, NO_3^--N and MB-N pools were significantly influenced by CZ introduction. Through the application of a simple mass balance equation, it can be estimated that the increment in 7CZ Exch NH_4^+-N pool correspond with a good approximation to the amount of NH_4^+ introduced with CZ in the first 30 cm of soil (Eq. 2, 3 and 4 and Fig 7 B).

(2) $$1m*1m*0.3m \text{ soil layer weight} = 1250 \text{ kg m}^{-3} * 0.3 \text{ m}^3 = 375 \text{ kg}$$

(3) $$\text{Exch } NH_4^+\text{-N input from CZ} = 3014 \text{ mg kg}^{-1} * 7 \text{ kg} = 21098 \text{ mg}$$

(4) $$\text{CZ contribution} = \frac{21098 \text{ mg}}{375 \text{ kg}} = 56 \text{ mg kg}^{-1}$$

Where 1250 kg m^{-3} is the average soil bulk density, 3014 mg kg^{-1} is the average Exch NH_4^+-N of CZ, 7 kg is the amount of CZ applied per m^{-2} of soil. This evidence confirms that the NH_4^+ adsorbed by CZ consist in a “high mobility” form of NH_4^+, stocked almost entirely as Exch NH_4^+ and not in a “low mobility” form as the Fix NH_4^+ pool. The Exch NH_4^+ generally represent an easily exchangeable fraction of NH_4^+ that can be obtained by a 1 M extraction with KCl. On the other hand, fixed NH_4^+ is a more stable and hardly exchangeable form of NH_4^+, generally associated with the adsorption inside clay mineral interlayers and not replaceable by ion exchange with K^+ (Silva and Bremner, 1966). For this reason, the dynamics and the turnovers of fixed ammonium are generally slower than that of the exchangeable NH_4^+.

The decrease of Fix NH_4^+-N during the growing season suggest an exploitation by plants, confirming the active role of this pool in crop nutrition (Li et al., 1990; Marzadori et al., 1994). However, it was not visible any $\delta^{15}N$ turnover in this specific pool immediately after the chemical fertilization applied in Jun-2013, while the addition of urea was well marked by a typical increase of the soil pH (Martens and Bremner, 1984). On the other hand, a clear $\delta^{15}N$ turnover was visible in Sep-2013, where the significantly lower $\delta^{15}N$ of bulk soil Fix NH_4^+-N may indicate a storage of N from chemical fertilizers (which have negative isotopic signature) that were reasonably left unexploited by plants during the growing season. In the rhizosphere, the $\delta^{15}N$ of Fix NH_4^+-N pool was significantly higher, probably as a consequence of a faster turnover rather than the bulk soil because of roots influence (plants uptake).

Beside the dynamics of this pool during the experimental period, no significant treatment-derived effects were found, confirming that both NZ and CZ have not influenced the Fix NH_4^+-N pool.

As already indicated by some scientists, NH_4^+-enriched zeolites seems to be able to act as slow-release fertilizer (Barbarick and Pirela, 1984; Lewis et al., 1984; Dwairi, 1998). In our experiment this behaviour seems to be supported by the isotopic signature of the TN pool during the monitored period. The higher $\delta^{15}N$ of TN pool in 7CZ with respect to that found in the other treatments in Nov-2012, was in fact due to the high ^{15}N natural abundance of the pig slurry contained into the CZ. Dittert et al. (1998) found a $\delta^{15}N$ of 5.90 ‰ in agricultural soil treated with slurry while a $\delta^{15}N$ of 3.90 ‰ was found in soils treated with inorganic fertilizers. Pig slurry has usually high $\delta^{15}N$ values, as a result of the fractionation processes occurring during volatilization of NH_3 after the excretion, which causes an enrichment in the heavier ^{15}N atoms in the substrate (slurry) with respect to the product (NH_3 gas) (Högberg, 1997; Dittert et al., 1998; Schmidt and Ostle, 1999; Lim et al. 2007). For this reason, the still significantly higher $\delta^{15}N$ of TN pool in May-2013 suggests that a significant amount of N introduced with CZ was still present in the soil before the sowing.

At the beginning of the experimentation, the organic C cycle in 7CZ treatment was probably affected by the high N input provided by CZ, resulting in a decrease of TOC content and thus of the soil C/N ratio.

Concomitantly, the increase in N immobilization by soil MB in 7CZ plot was not counterbalanced by a parallel MB-C increment, resulting in a strong decline of the MB-C/N ratio. These evidences, suggest that with all probability organic matter mineralization processes were enhanced (Kitayama and Iwamoto, 2001) and that soil MB was bacteria-dominated (Strickland and Rousk, 2010). It is widely known that C/N ratio of bacteria is expected to be ~6 while the one of fungi is expected to be ~5-15 resulting in a significantly lower C/N of bacteria with respect to fungi (McGill et al., 1981; Strickland and Rousk, 2010).

However, these two evidences (decrease of soil TOC and increase in MB-N) suggest that soil MB interacted in the short term period with the N introduced with CZ.

After the amendment, NO_3^--N content in 7CZ plot was very similar to the UA plot notwithstanding the large N input provided by CZ spreading. This may indicate that nitrification processes were not significantly stimulated after the addition of CZ. This behavior seems to agree with what already stated by some authors in the scientific literature (Mercer et al., 1970; Ferguson and Pepper, 1987; Perrin et al., 1998; Ippolito et al., 2011; Malekian et al., 2011), concerning the reduction of nitrification processes in presence of zeolites because of the unavailability of the adsorbed NH_4^+ to nitrifying bacteria. However, the initial increase of MB-N clearly indicate that part of the N supplied with CZ was immobilized by soil MB.

In correspondence of May-2013 sampling, MB-C and MB-N increased significantly, while NO_3^--N significantly decreased in all the treatments (Fig 7C and 8 A,B), suggesting that probably in this period MB immobilization prevailed over mineralization processes (Azam et al., 1986). In addition to NO_3^--N decrease, in 7CZ treatment a reduction of Exch NH_4^+-N pool was clearly detected from Nov-2012 to May-2013, where values decreased by an aliquot correspondent with a good approximation to the amounts of the NH_4^+-N supplied with CZ mineral (see Eq. 4 and Fig 7 B).

Therefore, considering the following evidences:

1. higher MB-N immediately after the amendment with CZ and the further increase in May-2013;
2. decrease in Exch NH_4^+-N and in NO_3^--N from Nov-2012 to May-2013;
3. presence of high amounts of ^{15}N in May-2013;

it is possible that a considerable fraction of the N contained into CZ has been immobilized by the soil MB. However, a fraction of this mineral N depletion (decrease in NO_3^- and NH_4^+) can be also attributed to NO_3^- leaching and/or to different pathways of gaseous losses (e.g. NH_3, NO, NO_2, N_2O, N_2, not measured in this investigation).

Concerning the effects of NZ amendments, the decrease of NO_3^--N content in the two NZ amended plots and the decrease of Fix NH_4^+-N observed in 15NZ at the begin of the experimentation was probably due to a dilution effect operated by the high amount of NZ (devoid of N) introduced in the soil system.

On the other hand, the generally lower NO_3^--N content observed in all zeolite amended plots during the growing season (June-September) may be principally attributed to the effective lower amount of chemical fertilizers applied (-30 % with respect to that applied in the UA).

In this respect, the most interesting evidence was that notwithstanding the strong chemical fertilization reduction, crop yield was slightly higher with respect to the UA plot, suggesting that in these particular treatments, the Fertilizers Use Efficiency (FUE) was probably higher. The possibility to adsorb cations inside zeolite extra-framework sites should allow a higher retention time of NH_4^+ in the soil (Latifah et al., 2011). The higher retention time of NH_4^+ should lead to lower opportunities for nitrification[4] (Zaman et al., 2007; Ippolito et al., 2011) and hence to a higher FUE.

[4] Notwithstanding many authors stated this assumption, studies in which an accurate measurements of nitrification rates in soil amended with natural zeolites actually lacks in the scientific literature.

Moreover, it is well known that chemical fertilizers (especially urea and all NH_4^+ based fertilizers), are subjected to important N losses through NH_3 volatilization immediately after their application to the soil and, if the conditions are favorable (sub-alkaline soil pH, moist soil, high wind speed and temperatures), these losses may be far more than 30 % of the applied N (Soares et al., 2012). In this optic, it is known that mixing zeolites with urea fertilizers will result in lower NH_3 losses. For example, Haruna Ahmed et al. (2008a) found that urea-N use efficiency can be increased while reducing gaseous ammonia (NH_3) losses if urea is combined with natural zeolites, while Latifah et al. (2011) demonstrated that zeolite application together with urea decreased NH_3 losses and increased NH_4^+ retention in the soil retarding NO_3^- formation. For this reason, the possibility of decreasing also NH_3 losses may have contributed to increase additionally FUE during our experimentation and guarantee a good crop yield even applying 30 % less urea with respect to the UA plot.

In Nov-2012, MB-C and MB-N were lower in 5NZ and 15NZ with respect to UA, suggesting that NZ introduction caused an initial disequilibrium in MB-C immobilization, however, the MB C/N was not altered. At the begin of the sorghum growing season (Jun-2013), soil MB was probably influenced by the competition with the plants, resulting in a remarkable decrease in MB-C and MB-N in all the treatments. Apparently, 15NZ treatment had a stronger influence on MB-N, resulting in a lower N immobilization that was already visible in May-2013 (in this case even MB-C pool was affected) and lasted until Sep-2013. This evidence supports the hypothesis that probably the more NZ is in the soil the less N is easily available to soil microorganisms. The C/N ratio of soil MB may give information regarding bacterial or fungal prevalence in the microbial community (Hodge et al., 2000; Nannipieri et al., 2003). As already previously stated, fungi are characterized by a wider C/N ratio and thus to a higher tolerance to N deficiency. In this respect, the tendency of a greater MB-C/N ratio found in all zeolite-amended soils starting from May-2013 may be indicative of a relative stronger fungal prevalence in the soil microbial community (Vries et al., 2006; Strickland and Rousk, 2010). It is plausible that the combination of the lower N inputs applied and zeolite introduction, especially in 5NZ and 15NZ, caused a lower N availability and accessibility to soil microorganism, favoring the

development of fungal biomass. A greater fungal/bacteria ratio can be often understood as marker of a more sustainable agricultural system, where the nutrient required for the plant growth are supplied by organic matter decomposition and N mineralization, with probably lesser N losses in the environment (Vries et al., 2006; Strickland and Rousk, 2010).

At the harvest, in general the rhizosphere was depleted in Exch NH_4^+-N with respect to the bulk soils but significantly enriched in MB C-N, TOC and TN. It is reasonable that the decrease in the exchangeable pool was due to an exploitation by plant roots while bulk soil resulted 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 MB, TOC and TN (Merckx et al., 1986; Liljeroth et al., 1990) and also the slight different N isotopic signature of TN pool. 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 UA. However, this evidence is not supported by a significant increase in MB-C or MB-N. Ozbahce et al. (2015) found that the application of NZ mixed in the first 30cm soil layer increased organic matter content, although, a clear explanation of this phenomenon was not given. Capasso et al. (2005) found that Italian zeolitic tuffs can adsorb significant amounts of humic substances from solutions especially when divalent cations (such as Ca^{2+}) act as bridge between the negatively charged mineral surface and the organic phase. A plausible hypothesis is that zeolite had a retention effect on humic substances resulting in an increase of TOC in the soil, as Ca^{2+} was the major exchangeable base, reducing in this way organic carbon losses. Further studies are required to confirm this very important aspect.

3.4.1 Plant-zeolite interactions

N was preferentially allocated in sorghum leaves and panicles and the lack of significant differences in N content respect to the distinct treatments suggests that, notwithstanding the fertilization reductions in the zeolite amended plots, plants up-took similar N amounts. This may reflect in a

probable higher fertilizer recovery or NUE (it was not possible to calculate NUE because of the absence of a "negative" control). As reported by Evans et al. (1996) and Evans (2001), the $\delta^{15}N$ of plant tissues is determined by the physiological mechanism within the plants and by the isotopic ratio of external N sources. Moreover, intra-plant N isotopic variations, for example between roots and leaves, can be due to different patterns of N assimilation or reallocation. These intra-plant $\delta^{15}N$ variations are well recognizable also in the studied sorghum plants, where leaves $\delta^{15}N$ was generally greater (from 2 to 3 ‰) than that determined in roots, except for 7CZ where the differences were even greater (~ 6 ‰). Many authors used ^{15}N natural abundance of leaves or stems for tracing the main soil N source undermined by the plants during the growing season (Evans et al., 1996; Högberg, 1997; Erskine et al., 1998; Evans, 2001; Hulton et al., 2007; Kahmen et al., 2008). It is well visible that average $\delta^{15}N$ of 7CZ leaves was significantly higher and closer to CZ $\delta^{15}N$ rather than the plants from the other plots (Fig 11). The remarkably higher isotopic signature of 7CZ leaves indicate that during the growing season, an uptake from a N source characterized by a significantly higher $\delta^{15}N$ occurred. The main N source was probably represented by CZ-N since 7CZ was the only plot where a high $\delta^{15}N$ 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 $\delta^{15}N$ with respect to plants grown under organic fertilizers (like pig-slurry, which have usually higher $\delta^{15}N$) (Choi et al., 2003; Bateman et al., 2005; Yun et al., 2006). It is important to remind that the other N inputs were represented only by urea and di-ammonium phosphate, which have a negative $\delta^{15}N$ and were applied to all treatments (including 7CZ). These evidences suggest that the N input from CZ was still present during the growing season and that was highly available for plant uptake even after several months after the application to the soil.

It is known that most of the N in the soil is bound in forms not immediately available to plants (Högberg, 1997; Bateman et al., 2005), for this reason, usually the $\delta^{15}N$ of TN is not a good approximation of the isotopic signature of the N source preferentially used by the plants. Anyhow, ^{15}N natural abundance in leaves of UA, 15NZ and especially 5NZ reflected the use of an N source

characterized by a lower $\delta^{15}N$, probably more affected by chemical fertilizers signature. It is important to note that notwithstanding the lower urea application (-30 % with respect to the UA), in 5NZ and 15NZ treatments, leaves $\delta^{15}N$ was similar or even lower with respect to UA. In particular, as visible from Fig 11, 5NZ plants showed the lowest $\delta^{15}N$ not only in leaves but also in roots, stems and panicles, suggesting a higher uptake of N from a source with lower $\delta^{15}N$, i.e. chemical fertilizers.

3.5 Conclusions

A detailed investigation of the N-C pools and a preliminary investigation of $\delta^{15}N$ dynamics in the soil-sorghum system have been performed in a field amended with natural and NH_4^+-enriched zeolites, under fertilization reductions.

The N introduced in the soil system with CZ increased the Exch NH_4^+-N reserve, suggesting that N adsorbed by zeolites affects preferentially this pool. On the other hand, zeolite amendments generally had not significantly influenced the total reserve and the dynamics of Fix NH_4^+-N pool.

The generally lower NO_3^--N cannot be entirely attributed to zeolite influence since a lower fertilizer N input was applied in all zeolite treated plots but, in this respect, the interesting evidence is that notwithstanding the strong chemical fertilization reduction applied, crop yield was slightly higher with respect to the UA plot, suggesting that in these particular treatments FUE was probably higher. The $\delta^{15}N$ of plants grown in plots amended with NZ support this hypothesis.

Microbial activity was strongly affected by CZ and NZ introduction which seems to cause at first a disorder (decrease of MB-C and MB-N immobilization), then a possible change in microbial population towards a fungal prevalence, usually indicative of a system with lower N accessible to microorganisms.

^{15}N natural abundance in soils and plants, together with the positive yield results, prove that plants benefited of the N derived from CZ. However, it has to be underlined that in all likelihood, plants do not directly exploit N from CZ, but the transfer involves interactions with the soil microbial biomass.

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

4.1 Introduction

In this fourth chapter, the attention is focused on the interaction between zeolites and plants by the mean of an isotopic investigations at natural abundance.

In the chapter 1, it has been showed that probably during sorghum cultivation a N transfer from CZ to plants occurred and that the main organ in which this N was stocked were the leaves. Moreover, plants grown in 5NZ plot seemed to have more efficiently exploited N from chemical fertilizers while plants grown in 15NZ plot did not differed significantly from the UA plants.

After this first and preliminary investigation, we prolonged the elemental and isotopic measurements in the ZeoLIFE experimental field also for the two following cultivation cycles (maize and wheat), in order to get more consistent information about the observed behaviors.

The goal of this chapter is thus to verify, by acquiring more consistent data, if soil amendments with zeolites at natural state can effectively increase chemical fertilization efficiency and to trace the N transfer from CZ to plants by taking into consideration the N and C elemental and isotopic distribution in the soil-plant system.

The hypothesis is that in soils amended with zeolites at natural state, plants are more influenced by chemical fertilizers isotopic signature with respect to plants grown on an unamended soil, since natural 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 natural 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.

4.2 Materials and methods

4.2.1 Experimental field setting

This study has been carried out in the ZeoLIFE experimental field. The experimental set-up was slightly different with respect to the one described in the previous chapter for sorghum cultivation, because of internal decision took in the framework of the ZeoLIFE project. The main difference was that after sorghum harvesting, another plot amended with 10 kg m^{-2} of CZ was created (10CZ). The experimental set up for these two cultivation cycles consisted thus in five plots, one without zeolite addition (UA), two amended with NZ (5NZ and 15NZ) and two amended with CZ (7CZ and 10CZ) (see Table 8 for the experimental set-up description).

4.2.2 Agricultural management

The experimentation was carried out for two consecutive cultivation cycles (maize during 2014 and winter wheat during 2014-2015). Maize (*Zea mays*) was sowed on March 28th, 2014 and harvested on September 7th, 2014, while winter wheat (*Triticum durum*) was sowed on November 11th, 2014 and harvested on June 30th, 2015.

In accordance with ZeoLIFE project fertilization plans, even for these two cultivation cycles chemical fertilization was reduced in all zeolite amended plots with respect to the fertilization rate applied in the UA plot (Table 8). Urea was applied in 2 steps on April 18th and May 13th, 2014, during maize cultivation, while NH_4NO_3 was applied in two steps on March 04th and April 29th, 2015, during wheat cultivation (Table 8). It is very important to specify, that the two CZ plots were the only one subjected to a N input derived from pig-slurry (supplied with CZ itself) since November 2012.

Table 8: Experimental set-up for maize and winter wheat cultivation cycles. *Chemical fertilization was performed following ZeoLIFE project plans (~30 % fertilization reduction in NZ plots and ~50 % in CZ plots during maize cultivation and ~30 % in all plots during wheat cultivation). Values represent the exact amount of fertilizer applied by the farmer

Treatments	Surface (ha)	Zeolite amount (kg m^{-2})	Zeolite type	N input from Pig-slurry (as CZ) (kg-N ha^{-1})		N from chemical fertilizers (kg-N ha^{-1})		Crop Yield (q ha^{-1})	
				Maize	Wheat	Maize	Wheat	Maize	Wheat
UA	1.5	0	/	0	0	241	141	94.9	63.7
7CZ	0.5	7	CZ	0	0	123*	101*	97.1	70.6
5NZ	1	5	NZ	0	0	179*	114*	103	72.0
10CZ	0.5	10	CZ	427	0	123*	99*	100	65.3
15NZ	1	15	NZ	0	0	175*	113*	118	70.6

4.2.3 Plants and rhizosphere sampling

Six representative plants of maize and wheat from each treatment were sampled before the harvest (Fig 12). As representative of the above ground biomass, maize plants were subdivided in stems, leaves and grains while wheat plants were subdivided in stems and grains only. The plant tissues were 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.

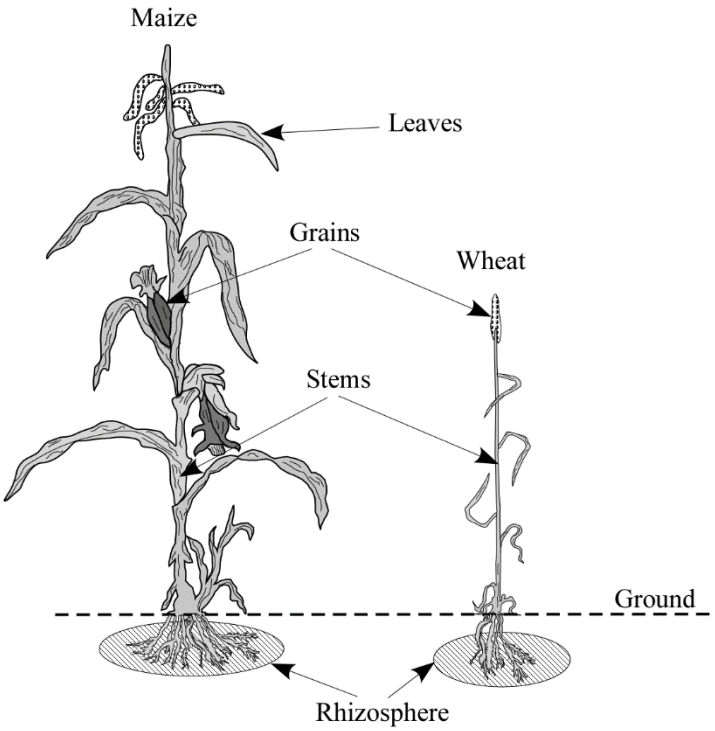

Fig 12: Sketch draw of maize and wheat plants cultivated during the experimentation.

4.2.4 C-N elemental and isotopic analysis

Measurements of the elemental and isotopic C and N compositions have been carried out on samples of the various N inputs (CZ and chemical fertilizers) and on all soil/plant samples. An Elementar Vario Micro Cube Elemental Analyzer in line with an ISOPRIME 100 Isotopic Ratio Mass Spectrometer operating in continuous-flow mode was employed for run the analysis. 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) O_2 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 N_2. The formed analyte gases (N_2, H_2O and CO_2), 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 N_2 goes directly to the interfaced IRMS for isotopic composition determination, while CO_2 is held by the TPD column, kept at room temperatures 20–25 °C. When N_2 isotopic analysis is over, CO_2 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 N_2 and CO_2 (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 standard analyses, 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 $\delta^{13}C$ and $\delta^{15}N$ 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.

4.2.5 Statistical analysis

For evaluating significant differences between the treatments, parametric and non-parametric statistic 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 assumption 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.

4.3 Results

4.3.1 Elemental and isotopic composition of the N inputs

The elemental and isotopic composition of both NZ and CZ is reported in Table 3 (Chapter 2.0). Urea and NH_4NO_3 fertilizers are characterized by 46 wt% and 35 wt% of N content, respectively, ad by a very similar and slightly negative $\delta^{15}N$ of -0.1 (±0.8) ‰.

4.3.2 Soil rhizosphere

The results of the elemental and isotopic C and N analyses of rhizosphere samples are reported in Table 9. All the plots appeared very homogeneous in terms of total C-N contents and $\delta^{15}N$ in both cultivation cycles, showing apparently no differences between the distinct fertilization strategies. Average C content ranged from 31.9 (7CZ) to 34.5 (UA) g kg^{-1} during maize cultivation and from 32.5 (15NZ) to 34.2 (10CZ) g kg^{-1} during wheat cultivation while average N contents ranged from 2.22 (7CZ) to 2.51 (UA-5NZ) g kg^{-1} during maize cultivation and from 2.04 (15NZ) to 2.33 (10CZ) g kg^{-1} during wheat cultivation. The $\delta^{15}N$ of the rhizosphere soils was very similar in both cultivation cycles and values ranged between 4.89 ‰ (15NZ) to 5.66 ‰ (UA), showing comparable N isotopic fingerprint in all the parcels.

On the other hand, the $\delta^{13}C$ measured on maize and wheat rhizospheres showed systematic differences, with wheat rhizosphere generally displaying less negative $\delta^{13}C$ values (on average by 1.3 ‰).

Table 9: Elemental and isotopic C-N composition of rhizosphere soils during maize and wheat cultivation cycles.

	Treatment	C	N	$\delta^{13}C$	$\delta^{15}N$
		g kg^{-1}		‰	
Maize (2014)	UA	34.5	2.51	-18.34	5.66
	7CZ	31.9	2.22	-16.34	5.47
	5NZ	33.6	2.51	-18.43	4.91
	10CZ	34.0	2.25	-17.93	5.27
	15NZ	33.4	2.45	-17.20	5.38
Wheat (2014-2015)	UA	34.1	2.30	-17.05	5.18
	7CZ	33.6	2.27	-16.95	4.94
	5NZ	32.8	2.22	-16.22	5.08
	10CZ	34.2	2.33	-15.72	5.01
	15NZ	32.5	2.04	-15.77	4.89

4.3.3 Plant organs

The results of the elemental and isotopic C and N analyses with the associated statistical report are listed in Table 10.

C elemental and isotopic composition

Concerning maize (cropped in 2014), the only significant differences ($p<0.05$) between the treatments were observed in the stems C contents, which varied from median values of 39.6 wt% (7CZ and 10CZ) to 42.5 wt% (UA). The grains and leaves total C content was very similar and no significant differences were observed between the treatments ($p>0.05$), values were included between 36.4 and 39.7 wt% for both grains and leaves. The relative C isotopic signature ranged from -14.1 to -14.5 ‰ in the stems and leaves, with no significant differences between the treatments ($p>0.05$) while it

ranged from -13.0 to -13.4 ‰ in the grains with slight but significant differences between the treatments ($p<0.05$).

Concerning wheat (cropped in 2015), the C contents ranged from median values of 41.0 to 42.6 wt% (5NZ and UA, respectively) in stems and from 39.9 to 40.7 wt% in grains (UA and 5NZ, respectively) with no significant differences between the treatments ($p<0.05$). The relative C isotopic composition in wheat stems varied from -27.9 to -28.9 ‰ (15NZ and UA, respectively) with slight but significant differences between the treatments ($p<0.05$) and from -26.1 to -26.9 ‰ (15NZ and 5NZ, respectively) in the grains, with no significant differences between the treatments ($p>0.05$).

N elemental and isotopic composition

Generally, the N content of all plant tissues was more variable than C content. The N content of maize stems ranged from median values included between 3.6 and 8.6 g kg^{-1} (showed by 7CZ and 10CZ, respectively) with significant differences between the treatments ($p<0.05$). The N content of grains was higher than that of the stems and ranged from values included between 11.3 and 15.8 g kg^{-1} (of 10CZ and UA, respectively) with significant differences between the treatments. Concerning maize leaves, the elemental N content was greater than that recorded in stems and grains and the median values ranged from 18.6 to 31.8 g kg^{-1} (of 7CZ and 10CZ, respectively) with significant differences between the treatments ($p<0.05$). The relative N isotopic fingerprint was highly variable in the distinct plots of the experimental field highlighting always significant differences between the treatments ($p<0.05$) with generally higher values in CZ amended plots (except 7CZ grains) and lower for NZ amended plots. The $\delta^{15}N$ varied between 8.47 (5NZ) and 25.4 ‰ (10CZ) in stems and between 9.0 (7CZ) and 18.1 ‰ (10CZ) in grains while concerning leaves it ranged from 10.1 to 22.1 ‰ (of 15NZ and 7CZ, respectively). On average, if plants from different plots are compared it can be observed that 10CZ > 7CZ (except in grains)> UA > 15NZ ≥ 5NZ in terms of $\delta^{15}N$.

Table 10: Descriptive statistic of C, N, $\delta^{13}C$ and $\delta^{15}N$ of maize and wheat organs. Different letters near median values indicate significant differences (at p=0.05) as result of ANOVA and Fisher (LSD) or Student-Newman-Keuls Pairwise Multiple Comparison tests.

	C wt %		N g kg^{-1}		$\delta^{13}C$ ‰		$\delta^{15}N$ ‰	
	median	st. error	median	st. error	median	st. error	median	st. error
Maize Stems								
UA	42.5^{c}	0.7	6.1^{b}	0.53	-14.3^{a}	0.1	12.3^{b}	0.9
7CZ	39.6^{a}	0.56	3.7^{a}	0.21	-14.3^{a}	0.1	15.7^{c}	0.2
5NZ	41.3^{b}	0.7	4.0^{a}	0.26	-14.3^{a}	0.1	8.81^{a}	0.6
10CZ	39.6^{a}	0.8	8.6^{c}	0.48	-14.5^{a}	0.2	25.4^{d}	0.8
15NZ	41.6^{bc}	0.6	3.9^{a}	0.18	-14.1^{a}	0.2	8.47^{a}	0.2
Maize Grains								
UA	39.3^{a}	0.7	15.8^{c}	1.32	-13.2^{ab}	0.1	14.8^{d}	0.6
7CZ	39.2^{a}	0.4	11.6^{a}	0.66	-13.4^{b}	0.1	9.0^{a}	0.2
5NZ	39.0^{a}	0.3	14.8^{bc}	0.64	-13.4^{b}	0.1	10.8^{b}	0.6
10CZ	39.7^{a}	0.2	11.3^{a}	0.48	-13.0^{a}	0.1	18.1^{e}	1.3
15NZ	38.9^{a}	0.3	13.0^{b}	0.47	-13.2^{ab}	0.1	12.7^{c}	0.6
Maize Leaves								
UA	39.7^{a}	0.3	20.7^{b}	0.9	-14.2^{a}	0.1	11.1^{b}	0.6
7CZ	37.6^{a}	2.2	18.6^{ab}	3.2	-14.4^{a}	0.1	22.1^{d}	1.0
5NZ	36.4^{a}	1.4	16.4^{a}	0.3	-14.4^{a}	0.1	10.4^{a}	0.4
10CZ	39.6^{a}	0.8	31.8^{c}	1.9	-14.5^{a}	0.1	16.2^{c}	0.4
15NZ	39.4^{a}	0.9	20.1^{b}	0.4	-14.1^{a}	0.2	10.1^{a}	0.6
Wheat Stems								
UA	42.6^{a}	0.7	3.5^{a}	0.3	-28.9^{b}	0.2	2.97^{b}	1.0
7CZ	41.8^{a}	0.3	3.7^{a}	0.3	-28.0^{a}	0.09	6.4^{c}	1.6
5NZ	41.0^{a}	0.6	3.1^{a}	0.3	-28.6^{b}	0.2	0.58^{a}	0.2
10CZ	41.9^{a}	0.8	3.6^{a}	0.1	-28.1^{a}	0.1	13.0^{d}	1.2
15NZ	41.5^{a}	0.5	2.6^{a}	0.4	-27.9^{a}	0.1	3.53^{b}	0.9
Wheat Grains								
UA	39.9^{a}	0.3	19.6^{a}	0.5	-26.3^{a}	0.1	7.93^{b}	0.8
7CZ	39.9^{a}	0.2	18.2^{a}	0.4	-26.6^{a}	0.02	7.90^{b}	0.9
5NZ	40.7^{a}	0.1	21.4^{b}	0.3	-26.9^{a}	0.1	3.36^{a}	0.04
10CZ	40.3^{a}	0.3	22.0^{b}	0.5	-26.4^{a}	0.3	17.0^{c}	0.6
15NZ	40.1^{a}	0.2	20.5^{b}	1.8	-26.1^{a}	0.2	7.40^{b}	1.0

Regarding wheat, N elemental content was more homogeneous and varied between 2.6 to 3.7 g kg^{-1} in stems, with no significant differences between the treatments ($p>0.05$) and between 18.2 and 22.0

g kg^{-1} in the grains, with higher values showed by 5NZ, 10CZ and 15NZ ($p<0.05$). The restricted elemental variation was associated with remarkable $\delta^{15}N$ heterogeneity. In particular, $\delta^{15}N$ varied between 0.58 ‰ (5NZ) and 13.0 ‰ (10CZ) in stems, and between 3.36 ‰ (5NZ) and 17.0 ‰ (10CZ) in grains. In this case, on average, the $\delta^{15}N_{grains}>\delta^{15}N_{stems}$. A comparison with the comprehensive dataset provided by Longobardi et al. (2015), highlights that the $\delta^{15}N$ values obtained in this study are generally high, thus reflecting a significant signal of animal manure which was preponderant in the 10CZ samples.

Resuming, the median $\delta^{15}N$ values were higher in 10CZ for all the investigated plant parts, whereas 7CZ was higher only in maize stems and leaves and only in wheat stems. The lowest median values have been generally recorded especially in 5NZ. Isotopic differences between distinct parcels ($\Delta^{15}N$) 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 $\Delta^{15}N$ are recorded in 10CZ and account for 3.1 in grains and 13.01 in the stems of maize, and 9.11 in grains and 10.2 in the stems of wheat.

4.3.4 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 8) albeit not statistically significant because of the lacks of replicates. During maize cultivation, the higher crop yield was obtained in 15NZ plot (118 q ha^{-1}) and the lower was recorded in the UA plot (94.9 q ha^{-1}) while 5NZ and 10CZ yield was very similar (103 and 100 q ha^{-1}, respectively). During wheat cultivation, crop yield was more similar between the various plots. The higher yield was obtained in 5NZ (72 q ha^{-1}) and the lower in the UA plot (63.7 q ha^{-1}). 7CZ and 15NZ obtained the same crop yield (70.6 q ha^{-1}) while 10CZ was close to the UA (65.3 q ha^{-1}).

4.4 Discussions

The $\delta^{13}C$ values registered by the cultivated maize plants are conforming with those typical of C4 photosynthetic pathways worldwide, and particularly with those of maize plants from other Mediterranean occurrences (Lasa et al., 2011). 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 $\delta^{13}C$ 1.0-1.5 ‰, comparable to what observed by Lasa et al. (2011).

Concerning wheat, the obtained results, in terms of $\delta^{13}C$, 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). Results highlight physiological differences between stems and grains, with the latter generally ^{13}C-enriched, leading to intra-plant isotopic fractionation in the order of $\delta^{13}C$ 1.3-2.4 ‰ comparable to what observed by Sanchez-Bragado et al. (2013).

The differences observed between maize and wheat $\delta^{13}C$ (Table 10) are due to the distinct fractionation attitude of the two crop species as a consequence of their photosynthetic pathways (C3 and C4). In fact, it is well known that wheat (a C3 plant) develop significantly more negative $\delta^{13}C$ values with respect to maize (a C4 plant) (O'Leary, 1988; Staddon, 2004). In this respect, the slight differences in wheat and maize rhizospheres are noticeable (Table 9). These differences may be due to different isotopic composition 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).

While elemental C and N contents do not appear to be strictly 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 $\delta^{15}N$ ca. 0 ‰; Freyer and Aly, 1974; Shearer et al., 1974; Vitòria et al., 2004), variously applied in the distinct plots, and CZ enriched with pig-slurry derived NH_4^+($\delta^{15}N$ variable from +18 ‰ to +43.6 ‰) applied only in 7CZ (end of year 2012, before

sorghum cultivation) and 10CZ (year 2014, before maize cultivation) (Table 8). In spite of a remarkable N-inputs reduction (-30 % in 5NZ and 15NZ plots), yields were generally slightly higher in all zeolite-amended plots with respect to UA, even if these results are not statistically supported by replicates of the different plots (Table 8). In this respect, while during maize cultivation (and also sorghum cultivation, see chapter 3) no particular trends were observed in terms of plant tissues N content between the treatments, during wheat cultivation all the plants grown on zeolite amended soils showed significantly higher (or very similar in the case of 7CZ) total N content in grains with respect to UA plants. This may suggest that in this kind of soil, common fertilizers application exceed the real plant N demand, considering that in both 5NZ and 15NZ plots, by reducing N inputs by 30 % for three consecutive years, plant N content was still similar or even higher with respect to the UA. This evidence may also suggest that the presence of zeolites in the soil probably favoured the preservation of plant nutrient budget and NUE (Gholamhoseini et al., 2013).

It is evident that in the two agronomic years, plants grown on 5NZ and 15NZ plots showed significantly lower $\delta^{15}N$ with respect to UA plants in most of the organs (Fig 13 and 14). This behavior can be attributed to a stronger influence of the chemical fertilizers isotopic signature in the mentioned plots. It is plausible that natural zeolites have captured part of NH_4^+ ions formed after urea and NH_4NO_3 hydrolysis because of their very high CEC and ammonium affinity, avoiding thus part of the N losses that normally occurs after the application of fertilizers (nitrate leaching after nitrifications, denitrification and ammonia volatilization) and increasing NH_4^+ retention time in the upper soil layer (Latifah et al., 2011). The retention of these ions probably allowed plants to uptake higher amounts of N from this specific source, resulting in a $\delta^{15}N$ significantly closer to the one typical of the chemical fertilizers. It is interesting to note that this behavior was more visible in 5NZ plants instead of 15NZ one. It is possible that the addition of so high amounts of natural zeolites in 15NZ plot probably induced a relevant competition for the neo-formed NH_4^+ ions from the fertilization between zeolites and plants, reflecting in a relatively lower N uptake from this specific N pool.

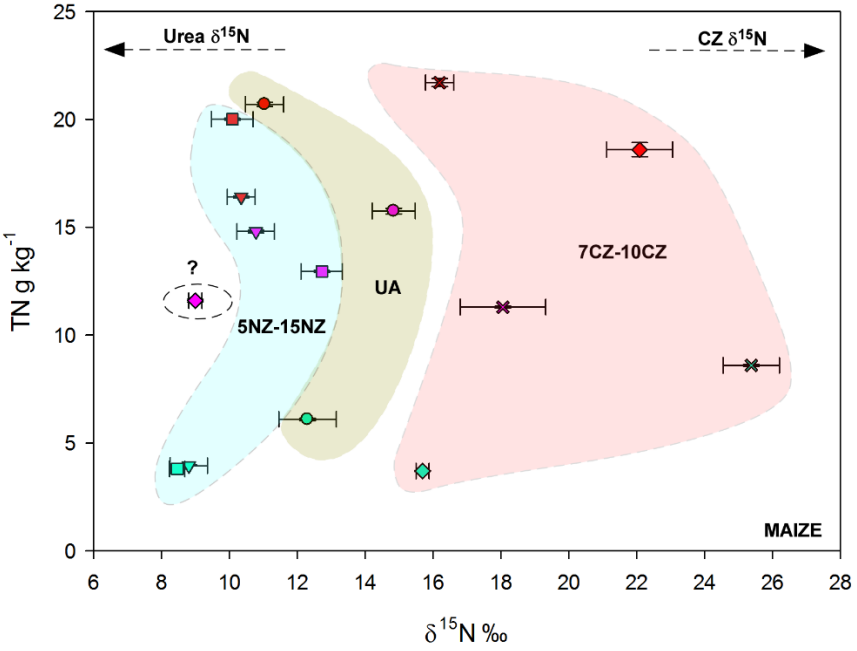

Fig 13: TN vs $\delta^{15}N$ plot of maize plant tissues. Different symbols refer to plants grown in different plots, respectively: circles (UA), reverse triangles (5NZ), squares (15NZ), rhombus (7CZ) and crosses (10CZ). Different colors represent different plant organs, respectively: green (stems), purple (grains), red (leaves).

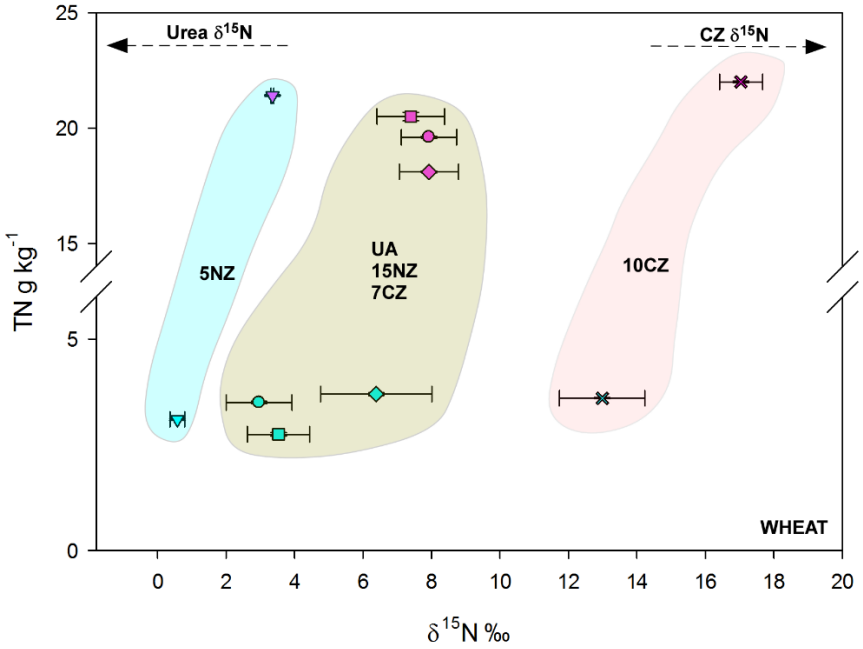

Fig 14: TN vs $\delta^{15}N$ plot of wheat plant tissues. Different symbols refer to plants grown in different plots, respectively: circles (UA), reverse triangles (5NZ), squares (15NZ), rhombus (7CZ) and crosses (10CZ). Different colors represent different plant organs, respectively: green (stems), purple (grains).

On the other hand, despite the different physiology and photosynthetic pathway of the investigated plants (C4 for maize, C3 for wheat), the $\delta^{15}N$ values recorded in the different organs revealed that pig-slurry signature has clearly influenced 7CZ and 10CZ plants (Fig 13 and 14). It has been demonstrated that generally plants $\delta^{15}N$ increases in parallel with fertilizer $\delta^{15}N$, and that plants grown in soils amended with pig-slurry can show a $\delta^{15}N$ up to +25 ‰ (Szpak, 2014). This evidence confirms that plants have mined the CZ pool, which in the considered experimental setup provided a source of nutrient for at least two cultivation cycles. It is evident that the pig-slurry influence was still visible also in 7CZ plot (in which CZ was added before sorghum cultivation in November 2012) during maize cultivation especially in stems and leaves, and lasted (even if clearly diminished) until wheat cultivation only in stems, while in the grains the influence was not visible anymore. Concerning 10CZ, the isotopic signature was clearly more influenced because of the "fresh" CZ addition during 2014 and affected all the plants organs in both cultivation cycles. Of course the addition and the uptake of also chemical fertilizers derived N by cultivated plants may have diluted their isotopic signature. However, these evidences confirm that an uptake from a significantly more enriched ^{15}N source (pig-slurry) occurred in both cultivation cycles only in CZ amended plots.

It has been demonstrated that soil solution, together with soil microbial biomass, can interact with NH_4^+-enriched zeolites once added into the soil (Leggo, 2000), thus it is highly possible that the process does not involve a simple N transfer from zeolites to plants, but a complex interaction with soil solution and soil microbial biomass that may have mediated the zeolite-plant N transfer in the investigated cultivation cycles.

Although the relationships between $\delta^{13}C$-$\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ variation in maize and wheat grains can be observed in our case study (Table 8).

Distinct fertirrigation practices, in fact, influence the C discrimination factor ($\Delta^{13}C$) (Lasa et al., 2011; Bogaard et al., 2013; Maxwell et al., 2014) expressed as [(Ra-Rp)/(1+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 $\Delta^{13}C$ has been identified as a suitable indicator of the plant physiological status, especially in C3 plants where the variation in the $\Delta^{13}C$ is generally higher (Farquhar et al., 1989; Lasa et al., 2011; Maxwell et al., 2014).

In Fig 15, the $\Delta^{13}C$ - $\delta^{15}N$ relationship found in maize and wheat grains are reported.

Contrary of what expected, a very clear trend was recorded in maize instead of wheat. C3 plants (wheat) are in fact expected to have higher variation in C isotope composition rather than C4 plants (maize), reflecting in a more recognizable C discrimination factor (Farquhar et al., 1989). It is well visible that maize $\Delta^{13}C$ decreased not only when $\delta^{15}N$ of plant tissues (and thus of the N source) increased, but it seems also to be related with the amount and type of zeolites applied in the soil. In fact, $\Delta^{13}C$ was lower in 10CZ plot during maize cultivation when a "fresh" pig-slurry input was just supplied with CZ before the sowing in 2014. This trend seems to be coherent with those obtained in experimental studies where relationships between $\Delta^{13}C$-$\delta^{15}N$ and manure application have been observed (Maxwell et al., 2014).

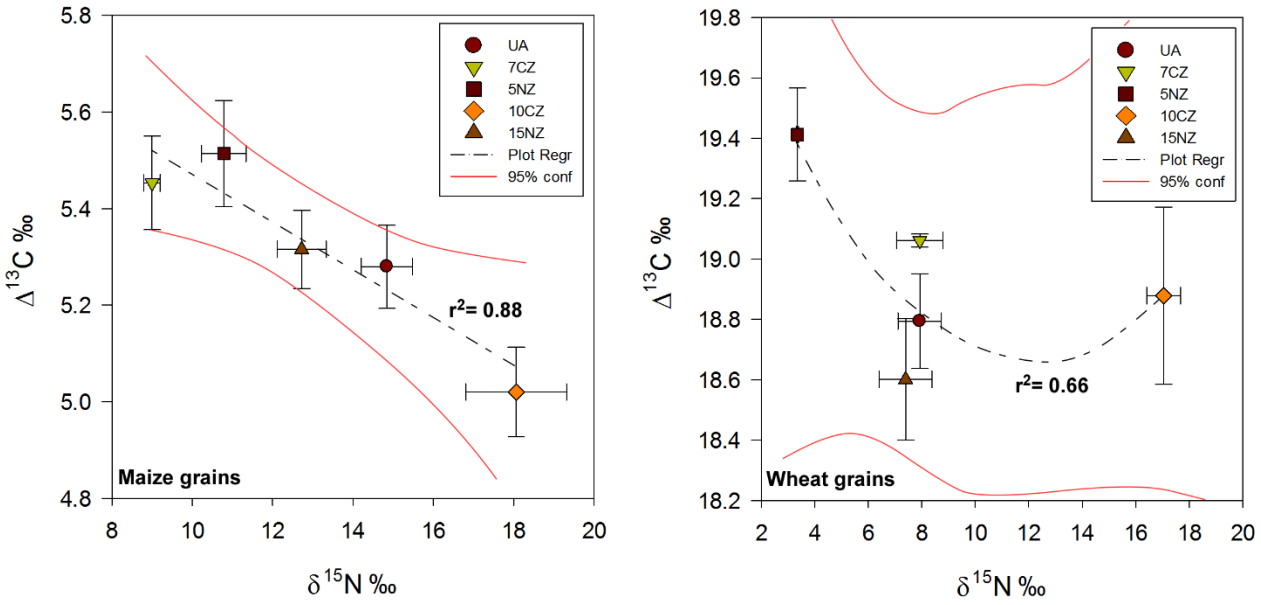

Fig 15: $\Delta^{13}C$ - $\delta^{15}N$ relationships in maize and wheat grains, respectively. Error bars represent standard errors.

On the contrary, in 7CZ plot this behavior was not observed probably because of a lower pig-slurry influence since it was already the second cultivation cycle after CZ application in that particular plot. 15NZ was very similar with respect to UA, while concerning 5NZ, the decrease in $\delta^{15}N$ was accomplished by an increase in the $\Delta^{13}C$. The same trend was not maintained in wheat plants, were the $\Delta^{13}C$ did not changed significantly between the treatments, apart for 5NZ that maintained always higher $\Delta^{13}C$ (and lower $\delta^{15}N$).

These differences in $\Delta^{13}C$ can be due to slight changes in photosynthetic rates between the treatments as consequence of the type of N source and amount of applied N (Maxwell et al., 2014). Generally, a decrease in C isotope discrimination may be attributable to an increase in salinity, a decreased in water availability, to soil compaction and to an increase in vapor-pressure deficit (Lasa et al., 2011 and references therein). In this optic, it is difficult to find the exact reason why 5NZ plants showed that increase in $\Delta^{13}C$ since the mentioned parameters were not measured in the present experiment. However, in the study performed 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. Changes in soil volumetric water content may have also contributed to

influence plant water availability. For this reason, it cannot be excluded that together with the influence of the different N sources, also soil water availability may have played a role in these $\Delta^{13}C$ variations.

4.5 Chapter conclusions

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

We showed that zeolite amendment practices influenced N and C isotopic compositions in the soil-plant system. $\delta^{15}N$ results showed that the addition of natural 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 cycle. The exact N transfer dynamics from zeolites to plants, which may have involved interactions with other soil N pools (like soil microbial biomass), require a more in deep and specific investigation to be understood. The C discrimination factor ($\Delta^{13}C$) was 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 was obtained for the 5NZ parcel, which coherently showed the higher agronomic yields.

5.0 High resolution short-term investigation of soil CO_2, N_2O, NO_x and NH_3 emissions after different chabazite zeolite amendments

5.1 Introduction

Notwithstanding the increasing number of climate change mitigation policies, the annual emissions of greenhouse gases (GHGs) keeps rising, leading to high costs in terms of environmental quality (Edenhofer et al., 2014). In particular, the agricultural sector accounts for an estimated emission of 10-12 % of the total global anthropogenic GHGs production (Smith et al., 2007).

CO_2 and N_2O are potent GHGs whereby N_2O has a 298-fold global warming potential "GWP" of CO_2 (Edenhofer et al., 2014). In addition, nitrogen oxides (NO_x) and ammonia (NH_3), even if not considered GHGs, can cause indirect N_2O emissions, increase acidity of precipitations and strongly influence tropospheric O_3 formation (Akiyama et al., 2004; Schindlbacher and Zechmeister-Boltenstern, 2004). Moreover, all N gaseous losses cause remarkable reductions in fertilization efficiency, representing thus an economic loss for the farmers (Smil, 1999).

It is known that the great majority of gaseous N losses occurs during the firsts days after fertilizer application, in particular, NH_3 emission have been found to account up to 75 % of the applied N within the first 24 h (Stevens and Logan, 1987). For this reason, finding new methodologies that can delay immediate gaseous N-losses may lead to an increase of fertilizer use efficiency and thus to a reduction of their environmental impacts.

Concerning the utilization of natural and NH_4^+-enriched zeolites in agriculture, up to now, very few studies investigated the effects of zeolite amendments on soil gaseous emissions, especially concerning zeolites of the chabazite type. On the other hand, natural clinoptilolite zeolite as amendment to soils have been described to reduce respiratory activity probably because of the adsorption capacity of zeolites for CO_2 (Mühlbachová and Šimon, 2003). Kučić et al. (2013) showed that natural clinoptilolite zeolite adsorption potential for CO_2 and NH_3 evolved during co-composting

of grape and tobacco waste was 31% and 100%, respectively. In addition, pasture and wetland soils amended with clinoptilolite zeolite showed a reduction in daily and total N_2O emissions during a 90-day incubation compared to unamended soils (Zaman et al., 2007). These reductions are due to the sorption of NH_4^+ by zeolite, lowering the available substrate for microbial nitrification and hence potential losses of N_2O from nitrification and denitrification. While the literature about the mitigation potential of zeolite application for NH_3 emissions is more consistent (He et al., 2002; Haruna Ahmed et al., 2008a,b; Bundan et al., 2011; De Campos Bernardi et al., 2013; Kučić et al., 2013), the majority of these studies has been conducted on surface applied zeolite in combination with fertilizer and not as amendment well homogenized in the soil profile. Yet to our best knowledge, the effects of zeolite as soil amendments on NO_x emissions have not been reported before. Besides, the majority of studies were conducted employing clinoptilolite zeolites, with so far unknown effects of the less abundant chabazite zeolite, the main natural zeolite occurring in the Italian peninsula (Malferrari et al., 2013). Moreover, the effects of NH_4^+-enriched zeolites on soil gaseous emissions are completely unknown. Finally, there is a lack of studies applying different type of zeolites as soil amendment that simultaneously investigate a wide range of gaseous species focusing on a high resolution investigation of the immediate emissions after fertilizer applications.

To this end, the present chapter aims at elucidating the effects of different chabazite zeolites amendments on soil CO_2, N_2O, NO_x and NH_3 emissions evolved during the first 24 h after fertilizer application from an agricultural soil in high resolution.

The experimentation was designed in order to mimic the same treatments and condition of the ZeoLIFE experimental field, which consist in an EU funded project that is testing different chabazite zeolite amendments at the field scale in a nitrate vulnerable zone (NVZ) (Nitrate Directive 91/676/EEC).

We tested chabazite zeolites amendments both at natural and NH_4^+-enriched state in comparison to an unamended soil under an N input of 170 kg N ha^{-1} (maximum amount of N applicable in a NVZ)

and without any further N input (unfertilized conditions). Considering the actual knowledge about zeolite amendments, we built our experiment in order to test the following hypotheses:

1) The zeolite-derived effects on the dynamics of NH_4^+ ions (Leyva-Ramos et al., 2010) are expected to decrease short-term N_2O, NO_x and NH_3 emissions after fertilizer application especially in soil amended with zeolites at natural states;
2) The slow NH_4^+ release behavior of NH_4^+-enriched chabazite zeolites is expected to not influence significantly soil N fluxes in the first 24 h, if no further fertilizer is applied;
3) The possibility of a CO_2 sorption and the probable influence on microbial activity (Mühlbachová and Šimon, 2003; Reháková et al., 2004; Bonenfant et al., 2008; Ridha and Webley, 2009; Kučić et al., 2013) are expected to decrease the short-term soil CO_2 emissions.

5.2 Material and methods

Soil samples (Clayey-Silt Calcaric Gleyic Cambisol) were taken from the ZeoLIFE project experimental field (Ferrara province, Italy, 44°50′33″ N and 12°05′40″E) where both Natural chabazite Zeolites (NZ) and NH_4^+-enriched chabazite Zeolites (CZ) were added to the soil as amendments at the field scale (www.zeolife.it) (See chapter 2.0 for zeolites and starting soil characterization). Soil samples were taken from an unamended plot and zeolites were added in the laboratory immediately before the begin of the experiment, in order to reproduce the same conditions immediately after the amendment. NZ and CZ were added to air dry sieved soil (≤ 5 mm) in different proportions, in order to obtain various soil-zeolite mixtures (see Table 11 for the experimental set up) as in the ZeoLIFE experimental field.

Table 11: Experimental set-up

Treatments	Zeolite amount (wt%)	Zeolite type	N° of replicates	Amount of soil incubated (g)	N° hours(h)	Analysed gaseous species
UA	0	/	3	60	24	CO_2, NO_x ($NO + NO_2$) N_2O NH_3
5NZ	5	NZ				
15NZ	15	NZ				
10CZ	10	CZ				

60 g of each treatment were incubated at 20°C (well representative of average field temperatures) for 24 h in three replicates. Water filled pore space (WFPS) level was adjusted according to Haney and Haney (2010) to 65 % accounting for changes in bulk density after the amendment. This WFPS level is considered a threshold between the relative prevalence of nitrification/denitrification processes (Bateman and Baggs, 2005). In order to check the moisture level reached, soil water content was also measured by oven-drying soils at 105°C overnight and the difference between the adjusted and measured WFPS was < 5 %. No rewetting operations were performed during the incubation.

The experiment was conducted with and without urea input equivalent to 170 kg-N ha^{-1}, (in liquid form, maintaining 65 % WFPS) representing fertilized and unfertilized conditions, respectively. The employed fertilizer rate was the same as in the ZeoLIFE experimental field. The site area is in fact defined a "Nitrate Vulnerable Zones" according to the Nitrate Directive (91/676/EEC) and the maximum amount of N input from fertilizer is limited to 170 kg-N ha^{-1} $year^{-1}$.

CO_2 and NO_x ($NO + NO_2$) were measured in a fully automated continuous flow laboratory system, well described in the work of Schindlbacher and Zechmeister-Boltenstern (2004), with doubled acquisition time of each chamber.

N_2O was sampled by closing the chambers and taking samples with a gas tight syringe after 0, 15, 30 and 45 minutes of the closure of the chamber. Samples were injected in a pre-evacuated 10 ml glass vials (Agilent Technologies) and then analysed with a GC-System (Agilent Technologies) equipped with a headspace auto-sampler and an electron capture detector (ECD), for more information see also

Leitner et al. (2016). N_2O measurements were performed once after approx. 4 hours of incubations and fluxes were calculated according to Metcalfe et al. (2007).

NH_3 measurements were conducted with a fully automated device composed of an incubation system with 6 chambers (ø 7.8 cm and 7.8 cm height) connected to a Cavity Ring-Down Spectrometer (CRDS, Picarro G2103). Chambers and tubes of the incubation device are built of Polytetrafluoroethylene (PTFE) in order to avoid any NH_3 retention. Dried compressed air was used as carrier gas with a constant flow of 1 l min^{-1}. One chamber was used as blank in order to measure NH_3 background air concentrations (Haller, 2015).

CO_2, NO_x and NH_3 fluxes were calculated according to Schindlbacher and Zechmeister-Boltenstern (2004) and represented as cumulative emissions by integrating over the 24 h of incubation.

NO_3^--N was extracted with Milly-Q (Millipore USA) water in a 1:10 (w/v) ratio, the solution was shaken for 1 h and then filtered. NO_3^--N was determined by ion chromatography with an isocratic dual pump (ICS-1000 Dionex), equipped with an AS9-HC 4 × 250 mm high-capacity column and an ASRS-Ultra 4-mm self-suppressor. An AS-40 Dionex auto-sampler was employed to run the analysis; quality control (QC) samples were run every ten samples. pH was measured on the same extracts with an Orion 9102BNWP pH-meter connected to an Orion 4star pH – ISE benchtop (Thermo Fisher).

Exchangeable NH_4^+-N was extracted with 1 M KCl in a 1:10 (w/v) ratio, the solution was shaken for 1 h and then filtered. The solution was diluted and analyzed with an Ion Selective Electrode (ISE) Orion 95-12 connected to an Orion 4star pH – ISE benchtop (Thermo Fisher).

Shapiro-Wilk and Levene's Test were performed for testing data normality and homogeneity of variance. One-way ANOVA (or Kruskal-Wallis test if ANOVA assumption were not met) and Fisher (LSD) tests were then employed for evaluating significant differences between the treatments at p=0.05 level. SigmaPlot 12.0 was used for statistical analysis.

5.3 Results and discussions

Cumulative CO_2, NO_x and NH_3 emissions over the 24 h are presented in Fig 16. All zeolite amended soils (see Table 11 for treatments description) resulted in significantly lower ($p<0.05$) CO_2 emissions compared to the unamended soils (UA) under fertilized conditions (Fig 16 a). These preliminary results show a reduction in CO_2 emissions with the addition of zeolites regardless of zeolite type and application rate, and may indicate a CO_2 sorption effect by zeolites after the fertilization because of their affinity to polar molecules (Mühlbachová and Šimon, 2003; Kučić et al., 2013) or, alternatively, to a higher Carbon Use Efficiency (CUE) (Keiblinger et al., 2010; Blagodatskaya et al., 2014). On the other hand, regarding CO_2 emissions under unfertilized conditions (Fig 16 b), CZ amended soils showed higher fluxes, albeit not significantly ($p>0.05$) by comparing to the other treatments.

It is possible that, without the addition of urea, the substrate C:N ratio was higher, resulting in a higher amount of respired CO_2 to meet metabolic requirements (Russell and Cook, 1995; Schimel and Weintraub, 2003) and hence a lower CUE (Keiblinger et al., 2010). In the present work it has to be noticed that cumulative CO_2 emissions were similar between fertilized and unfertilized conditions, highlighting that microbial activity was high in this agricultural soil even without adding a fresh N-C input. Concerning this aspect, contrasting effects were reported in the scientific literature; while urea fertilization was found to increase soil CO_2 emissions because of increased mineralization of soil organic carbon by heterotrophic bacteria (Choi et al., 2011), the opposite effect was reported by Wilson and Al-Kaisi (2008) after fertilization with NH_4NO_3. However, the reason why no significant differences in CO_2 emissions were observed under unfertilized conditions is hard to explain without a more detailed investigation on treatment specific microbial CUE and CO_2 adsorption mechanisms. Concomitantly, also the cation exchange processes between the soil solution and zeolite minerals were probably different without and with the addition of fertilizers, since urea dissociates into NH_4^+ cations that are potentially adsorbable. It is in fact widely known that the CO_2 adsorption capacity of zeolites depends on many factors including also the distribution and the number of exchangeable cations presents in their cavities (Bonenfant et al., 2008).

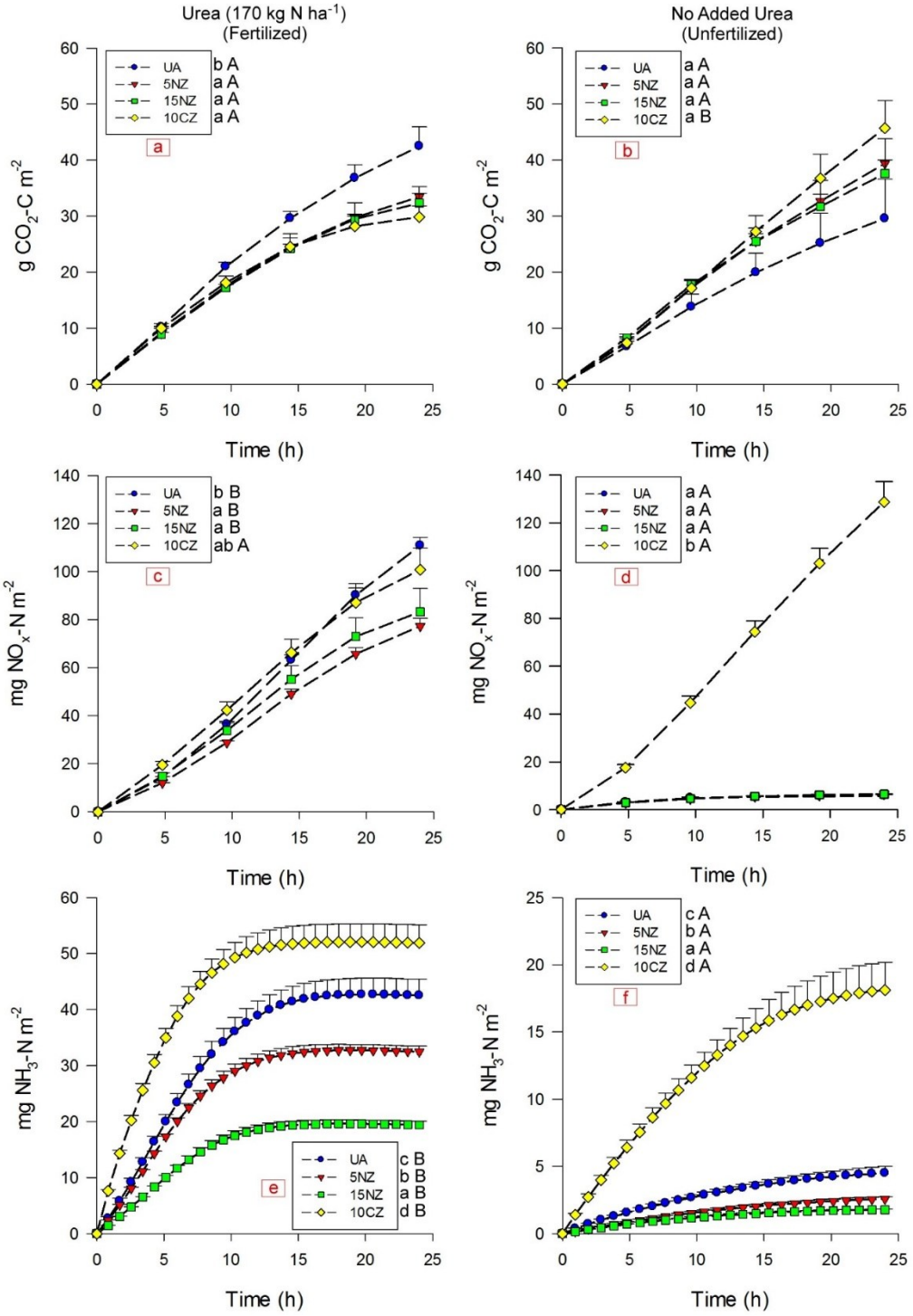

Fig 16: Cumulative emissions with standard error (error bars) over the incubation of both fertilized and unfertilized treatments. Graphs "a" and "b" refers to soil CO_2 emissions, graphs "c" and "d" to soil NO_x emissions and graphs "e" and "f" to soil NH_3 emissions. Different lowercase letters next to the legend indicate significant differences between the treatments while different capital letters indicate significant differences between fertilized and unfertilized conditions of each gas species, as results of statistical analysis (ANOVA and multi-comparison Fisher LSD test at p=0.05).

N_2O fluxes of fertilized soils were significantly lower ($p<0.05$) in both 5NZ (soil plus 5% in weight of NZ) and 10CZ (soil plus 10 % in weight of CZ) with respect to UA, while 15NZ treatment (soil plus 15% in weight of NZ) tended to be slightly lower albeit not statistically different (Table 12). N_2O is produced by both nitrification and denitrification processes, where the main substrate for nitrification is NH_4^+ while for denitrification is NO_3^-. However, these processes are strongly influenced by WFPS level, substrate and oxygen availability (Akiyama et al., 2004; Schindlbacher and Zechmeister-Boltenstern, 2004; Stevens et al., 1997).

Table 12: Soil pH, exchangeable NH_4^+-N, NO_3^--N mean values (measured at the end of the incubation) and N_2O-N fluxes (measured after 4 h of incubation) with the associated standard errors between brackets. Different upper case letters indicate significant differences ($p<0.05$) as results of statistical analysis (ANOVA and multi-comparison Fisher LSD test at $p=0.05$). The presence of "n.m" indicate not measured values.

	Treatments	pH	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	N_2O-N (μg m^{-2} h^{-1})
Unfertilized	UA	7.73 (0.04)^a	10.1 (0.4)^a	54.3 (0.7)^b	n.m
	5NZ	7.80 (0.04)^a	9.7 (0.3)^a	47.9 (0.4)^a	n.m
	15NZ	7.93 (0.06)^a	9.5 (0.2)^a	53.7 (0.9)^b	n.m
	10CZ	7.79 (0.04)^a	79.6 (6.9)^b	309 (6.4)^c	n.m
Fertilized with Urea (170 kg-N ha^{-1})	UA	7.94 (0.02)^a	9.7 (0.3)^a	97.4 (0.9)^b	358 (69)^b
	5NZ	7.99 (0.06)^a	10.1 (0.2)^a	84.7 (0.5)^a	165 (37)^a
	15NZ	8.12 (0.09)^a	9.9 (0.4)^a	83.6 (1.0)^a	281 (21)^{ab}
	10CZ	8.11 (0.10)^a	81.6 (8.3)^b	347 (12)^c	155 (11)^a

The WFPS used in this experiment should be the critical threshold between these two processes (Bateman and Baggs, 2005). Since WFPS and oxygen availability are closely related, the observed differences regarding N_2O emissions can be mainly attributed to a change in soil physical properties operated by zeolite amendments. Zeolite amendment (in a 3-5 mm size) has probably increased soil

aeration and thus reduced the presence of anaerobic microsites in which denitrification can occur at 65 % WFPS, consequently reducing total N_2O emissions with respect to UA. Another explanation can be that the NH_4^+ adsorption, managed especially by the initially N-devoid NZ, might have reduced the immediate availability of the substrate required for nitrification, thus reducing N_2O emissions (Zaman et al., 2007). The latter hypothesis is supported by our results from NZ amendments were in fact NO_3^--N was generally lower, but not for CZ amendment, where on the contrary NO_3^--N was strongly higher than in UA (Table 12).

NO_x are intermediate reactive volatile compounds of both nitrification and denitrification processes and mainly produced after the addition of fertilizers (Skiba et al., 1997). Here, cumulative NO_x emissions after fertilization were significantly lower ($p<0.05$) in 5NZ and 15NZ treatments compared to UA (Fig 16 c) probably because NH_4^+ ions have been partly adsorbed by the initially N-devoid NZ mineral, retarding NH_4^+ transformation and thus potential substrate for NO_x volatilization. This evidence is supported also by a lower NO_3^--N contents in both 5NZ and 15NZ treatments, suggesting that some NH_4^+ ions were probably protected from nitrification (Table 12) in this time frame (Zaman et al., 2007). After fertilization, NO_3^--N content of 10CZ was extremely high, but in this case, NO_x emissions were not significantly different from UA and both NZ treatments ($p>0.05$). On the other hand, 10CZ NO_x emissions of the unfertilized soils (Fig 16 d) were comparable to the fertilized one, while for the other treatments, unfertilized NO_x emissions were very low. These higher emissions in 10CZ can be attributed to an immediate transformation of the N supplied by the already N-saturated CZ. In fact, the extremely high NO_3^--N contents suggests strongly enhanced nitrification (biotic or abiotic) in this particular treatment. Commonly, N-enriched zeolites are considered as “slow release” fertilizers (Barbarick and Pirela, 1984; Dwairi, 1998; Lewis et al., 1984) but Leggo (2000) found a strong increase in NO_3^--N content immediately after the amendment with N-enriched zeolites (starting from the first week). In that work, the increase in NO_3^--N was attributed to an exchange reaction with soil cations (which have substituted the NH_4^+ inside the mineral lattice) and subsequently NH_4^+ oxidation by nitrifying bacteria. This may have occurred also in the present study

since the soil is characterized by a high natural salinity (Di Giuseppe et al., 2014) and a high CEC, which has been further increased by the zeolite amendment (Gholamhoseini et al., 2013; Ferretti et al., 2015).

In both fertilized and unfertilized conditions, a clear reduction in NH_3 emissions was found (Fig. 12 e-f) in NZ treatments with respect to UA ($p<0.05$). The entity of reduction after fertilization was of -24 and -54 % in 5NZ and 15NZ, respectively, while unfertilized conditions show a reduction of -40 and -61 % respectively. Since the pH was sub-alkaline in all the treatments (Table 12) and thus favorable to NH_3 volatilization, this behavior is attributable to the NH_4^+ ions adsorption inside NZ after urea hydrolysis, which can physically protect NH_4^+ from NH_3 volatilization (De Campos Bernardi et al., 2013). However, 10CZ treatment showed higher NH_3 emissions in both fertilized and unfertilized conditions, suggesting that some residual NH_4^+ ions from the enrichment process were available for volatilization. It has to be noticed that for all the other gaseous species, 10CZ (without further N input from urea), showed higher or similar emissions with respect to the urea-fertilized UA. While regarding cumulative NH_3 emissions, those of urea-fertilized UA were about twice of unfertilized 10CZ (Fig 1e-f). This means that urea-N contribute much more to NH_3 emissions than CZ-N.

5.4 Chapter conclusions

After 24 h of monitoring, NZ amendments seemed to reduce the immediate production of CO_2, N_2O, NO_x, and especially NH_3 emissions after the application of urea fertilizer, constituting thus a potential valuable material for reducing short-term soil gaseous C and N losses. On the other hand, CZ amendment lowered CO_2 and N_2O emissions evolved during the first 24 h after fertilizer application, however, nitrification was stimulated and NO_x emission were significantly high even without the addition of urea fertilizer, indicating that part of the N supplied with CZ was immediately transformed biotically or abiotically. Measured NH_3 emissions were higher in NH_4^+-enriched zeolites amended soil, but if the amendment is performed without further N inputs, the emissions can be significantly

lowered with respect to a conventional urea fertilization. However, we are aware of the fact that our short-term, high resolution experiment is limiting the potential to draw conclusions on long-term mitigation strategies, in terms of immediate gaseous N losses.

6.0 Short-term response of soil microbial biomass to different chabazite zeolite amendments

6.1 Introduction

The use of different kinds of natural and modified zeolites as soil amendment has been studied extensively in terms of soil physicochemical characteristics modification (Colombani et al., 2015,2016; Passaglia, 2008), reduced N leaching, increased N use efficiency, water use efficiency and crop yield (Reháková et al., 2004; Sepaskhah and Barzegar, 2010; De Campos Bernardi et al., 2013; Gholamhoseini et al., 2013; Li et al., 2013; Di Giuseppe, 2015). Some authors defined NH_4^+-enriched zeolites as "slow release fertilizers", that can gradually release their NH_4^+ load over time, without significant leaching losses (Barbarick and Pirela, 1984; Dwairi, 1998; Lewis et al., 1984). However, the effects of zeolite amendments on soil microbial biomass (MB) are largely unexplored and only few studies were conducted on the effects of clinoptilolite zeolite amendments on soil MB and respiratory activity (Mühlbachová and Šimon, 2003). Concerning amendments with NH_4^+-enriched zeolite, Leggo (2000) carried out an investigation of plant growth in an organo-zeolitic substrate in which he found an increase in NO_3^- after the use of natural clinoptilolite enriched by composting with poultry manure. He concluded that the Ca^{2+} present in the soil solution have probably exchanged the NH_4^+ adsorbed by the zeolites, making it immediately available to nitrifier microorganisms. This outcome is in contrast with the view of N modified zeolites as slow release fertilizer. However, to our knowledge no studies exists for natural and NH_4^+-enriched CHA zeolites and their effects on soil MB are still largely unexplored.

The aim of this chapter was to investigate the short-term effects of different CHA zeolite amendments on soil MB (and activity) by measuring soil MB C-N immobilization and C/N ratio, soil dissolved organic C (DOC), total dissolved N (TDN), ergosterol, soil NO_3^--N, NO_2^--N, NH_4^+-N and MB $\delta^{15}N$. To this end we designed our experiment for testing the following hypotheses:

H1) Amendments with CHA at natural state will influence N availability to soil MB, favoring the development of fungi rather than bacteria because of their lower nutrient requirements (McGill et al., 1981; Strickland and Rousk, 2010).

H2) NH_4^+-enriched CHA amendment is not immediately affecting soil MB if it acts as slow release fertilizer.

We designed our treatments in order to reproduce the same condition occurring in the experimental field of the ZeoLIFE project**.**

6.2 Material and Methods

6.2.1 Soil

Soil samples were taken from ZeoLIFE experimental field during spring 2015. The soil samples for this experiment were taken in the unamended plot from the first 30 cm depth layer and amended with different type of CHA in the laboratory immediately before the begin of the experiment in order to reproduce the short-term effects of zeolite application. The soil (around 5 kg) was brought to the laboratory immediately after the sampling, sieved to 5 mm and air-dried before the begin of the experiment. The main properties of the starting soil are described in chapter 2.2.

6.2.2 Employed natural and NH_4^+-enriched CHA

The main physicochemical properties of the employed zeolites are well described in chapter 2.1.

6.2.3 Experimental set-up

The experimental set-up in the laboratory was conducted in order to mimic the treatments and conditions as in the ZeoLIFE experimental field immediately after the application of the different type of CHA, resulting in four different treatments replicated three folds. Two treatments were

composed by a mixture of soil and NZ in the proportion of 5 wt% and 15 wt% respectively, further referred to 5NZ and 15NZ. One treatment was composed by a mixture of soil and CZ in the proportion of 10 wt% (10CZ) and one treatment served as control without zeolite addition (UA). For each treatment, 1 kg of 5 mm sieved material was incubated in open ceramic jars (ø 20 cm) for 16 days at room temperature (~20°C) adjusting the moisture level to 45 % of water filled pore space (WFPS) with MQ-water. These conditions reflect the ZeoLIFE field average temperatures and moisture levels, based on 4 years monitoring records, to reproduce conditions similarly to the field. Since the aim of this experiment was to verify the immediate effects after the amendments with NZ and CZ, no further N inputs were applied in UA, 5NZ and 15NZ.

At day 2, 7, 9, 11 and 16 of incubation, a representative subsample was used to analyze a set of parameters mentioned below.

6.2.4 Analytical techniques

Analysis were performed both on pure zeolite minerals before the amendment and in the soil-zeolite mixtures.

Soil pH and substrate total organic C and total N

Soil pH was determined in 0.01 M $CaCl_2$ extract in a 1:10 (w/v) ratio with a Lab pH meter inoLab® pH 196 Level 2 (WTW, Weilheim, Germany).

The total organic C (TOC) and total N (TN) of each treatment have been determined at the begin of the incubation (in 4 replicates) by Elemental Analysis on an Elementar Vario Micro Cube Elemental Analyser. The inorganic C was eliminated according to the method described by Natali and Bianchini (2015).

NH_4^+-N, NO_3^--N and NO_2^--N

NH_4^+-N was extracted with 1 M KCl in a 1:10 (w/v) ratio, the solution was shaken for 1 h and then filtered with Whatman #40 filters. 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.

NO_3^--N and NO_2^--N were extracted with Milly-Q (Millipore USA) water in a 1:10 (w/v) ratio, the solution was shaken for 1 h and then filtered with Whatman #40 filters. NO_3^--N and NO_2^--N were determined by ion chromatography with an isocratic dual pump (ICS-1000 Dionex), equipped with an AS9-HC 4 × 250 mm high-capacity column and an ASRS-Ultra 4-mm self-suppressor. An AS-40 Dionex auto-sampler was employed to run the analysis; quality control (QC) samples were run every ten samples. The standard deviation for all QC samples run was better than 4 %.

Ergosterol determination

Ergosterol was determined following the method proposed by Gong et al. (2001) with some modifications. Zeolite and soil samples were previously freeze-dried at -50°C. 6 ml of Me-OH were added to 4 g of sample, the suspension was homogenized with a hand vortex and then placed in an ultrasound bath for 15 minutes, centrifuged at 10,518 g for 15 minutes and finally the supernatant was filtered with a 4 mm, 0.45 µm PTFE Membrane syringe filter (Whatman). Samples were kept in the dark prior to analysis with an Agilent Technologies Infinity 1290 High Performance Liquid Chromatographer (HPLC) system. The injection volume of the sample was of 5 µl. While the flux was of 0.5 ml min^{-1} using 95 % Me-OH in H_2O as eluent phase and a Zorbax Eclipse Plucs C18 rapid resolution 2.1 x 50 mm column with 1.8 µm porosity as a solid phase was employed. Ergosterol was determined with an UV detector at 254 nm.

Dissolved organic C (DOC), total dissolved N (TDN) and microbial biomass C-N (MB-C, MB-N)

Chloroform Fumigation-Extraction method (CFE) was employed according to Brandstätter et al. (2013) and Öhlinger (1996), for the measurement of MB-C, MB-N, DOC and TDN.

Fumigated and non-fumigate samples were prepared with 1 M KCl in a 1:10 (w/v) ratio. The suspension was shaken for 1 h and then filtered through an N-free filter paper. Filtrates were stored frozen at -20°C prior to analysis with a TOC-L TNM-L Shimadzu Analyzer equipped with an ASI-L auto sampler. Inorganic C was eliminated after acidification before the analysis; the C and N from non-fumigated samples represent DOC and TDN, respectively.

The C and N extracted from chloroform-fumigated soils minus that extracted from non-fumigated soil represent the C and N immobilized by soil microorganisms. A correction factor of 0.45 and 0.54 was employed according to Beck et al. (1997) and Brookes et al. (1985) in order to determine the extractable part of microbial C-N (MB-C and MB-N).

Microbial $\delta^{15}N$ and net ^{15}N microbial immobilization rates calculations

Since it is possible to measure the N isotopic signature of soil MB (Dijkstra et al., 2006), we have taken advance of the very high $\delta^{15}N$ of the pig-slurry employed in the enrichment processes of NH_4^+-enriched zeolites in order to trace and further verify the interactions with MB.

MB isotopic signature was determined only for UA (at the begin of the incubation) and CZ treatments (for day 2, 9 and 16) since no differences in terms of isotopic signature were expected between UA, 5NZ and 15NZ treatments. Extraction-fumigation-extraction (EFE) method was employed for the determination of microbial C-N isotopic signature. Briefly, 30 ml of 0.1 M K_2SO_4 were added to 2 g of soil, the suspension was shaken for 1 h and then filtered through ash-free paper. The rest of soil remained into the vial was then transferred to the filter by adding new extractant shaking and pouring the suspension to the same filter. The soil was then re-extracted by adding 15 ml of 25 mM K_2SO_4 plus 1 ml of $CHCl_3$ shaking for 1 h and filtering with ash-free filter paper. The extracts were then freeze-dried and analyzed with an Elementar Vario Micro Cube Elemental Analyzer in line with an ISOPRIME 100 Isotopic Ratio Mass Spectrometer operating in continuous-flow mode. N isotope ratios are expressed in the standard (δ) notation in per mil (‰) relative to the atmospheric air (AIR)

isotope standard, respectively (Gonfiantini et al., 1995). The amount of ^{15}N incorporated by MB over time was back calculated from δ notation and the amount of MBN.

We calculated also microbial ^{15}N net immobilization rates as in eq. (1):

(1) $^{15}N_{imm} = (MB\ ^{15}N\ t_x - MB\ ^{15}N\ t_i)/\ d$

Were $^{15}N_{imm}$ (in μg ^{15}N day^{-1}) is the net ^{15}N immobilization rate, MB ^{15}N t_x and t_i are the amounts of ^{15}N atoms assimilated at the final and initial considered time points, respectively, by soil MB, and d indicate the days interworked between t_x and t_i.

Stoichiometric imbalances

The theory of environmental stoichiometry has emerged as a powerful tool to study the functioning of terrestrial ecosystems (Sterner and Elser, 2002). In this theory, elemental ratios are used for explaining ecological dynamics by knowing chemical constraints on physiologic and metabolic functions of organisms (Sterner and Elser, 2002; Mooshammer et al., 2014). Homeostasis is a term that has been defined as a constrained elemental ratio (C/N or to some extent also C/P) of microorganisms biomass that keeps constant regardless to changes in substrate C-N (and P) availability. Based on homeostatic soil microbial community, the stoichiometric imbalance (Mooshammer et al., 2014) is driven by the substrate C/N ratio.

For the present study, the bulk substrate stoichiometric imbalances was calculated as the ratios between bulk substrate TOC/TN ratio and MB C/N ratio (Mooshammer et al., 2014) (Table 13). Due to the fact that short-term effects might be reflected by the available nutrients, and their respective ratios, we also calculated the imbalance of the labile substrate using the DOC/TDN ratio instead of TOC/TN ratio. Finally, for the 10CZ treatment, we tried to explain the development of the MB by considering only the imbalance with respect to the pure CZ mineral, by assuming that the charged mineral provides a large extent of available nutrients (Table 13).

While the stoichiometric imbalance of the bulk substrate generally results in ratios > 1, the higher the values the larger is the imbalance, with no imbalance at a ratio of 1. This is in contrast to the imbalance

using labile substrate, where the higher is the ratio the lower the imbalance is, since both DOC/TDN and CZ TOC/TN are a < 1 ratios.

Table 13: Stoichiometric imbalances among the incubation experiment. The Bulk substrate C/N imbalance was calculated as bulk substrate TOC/TN ratio divided by the respective soil MB C/N ratio. The Bulk CZ C/N imbalance was calculated only for 10CZ treatment as the bulk TOC/TN ratio of CZ mineral divided by soil MB C/N. Labile substrate C/N imbalance was calculated as DOC/TDN ratio divided by soil MB C/N ratio. Standard error within brackets. Different upper case letters indicate significant differences at each sampling day (at p=0.05) as result of ANOVA and Fisher LSD test.

		Bulk substrate C/N imbalance	Bulk CZ C/N imbalance	Labile substrate C/N imbalance
Day 2	UA	1.33 (0.28)^{a}	-	0.06 (0.02)^{a}
	5NZ	1.05 (0.09)^{a}	-	0.05 (0.004)^{a}
	15NZ	1.29 (0.01)^{a}	-	0.05 (0.001)^{a}
	10CZ	4.50 (0.26)^{b}	0.10 (0.004)	0.05 (0.004)^{a}
Day 7	UA	1.14 (0.07)^{a}	-	0.05 (0.004)^{bc}
	5NZ	1.5 (0.17)^{a}	-	0.07 (0.01)^{c}
	15NZ	1.18 (0.11)^{a}	-	0.04 (0.004)^{b}
	10CZ	2.19 (0.68)^{b}	0.05 (0.01)	0.02 (0.01)^{a}
Day 9	UA	0.80 (0.27)^{a}	-	0.05 (0.02)^{a}
	5NZ	0.92 (0.05)^{a}	-	0.04 (0.02)^{a}
	15NZ	0.52 (0.15)^{a}	-	0.02 (0.003)^{a}
	10CZ	12.87 (3.92)^{b}	0.27 (0.08)	0.18 (0.09)^{b}
Day 11	UA	1.31 (0.24)^{a}	-	0.06 (0.01)^{a}
	5NZ	0.94 (0.20)^{a}	-	0.04 (0.01)^{a}
	15NZ	1.09 (0.13)^{a}	-	0.04 (0.004)^{a}
	10CZ	16.49 (2.91)^{b}	0.35 (0.06)	0.14 (0.01)^{b}
Day 16	UA	1.29 (0.21)^{a}	-	0.07 (0.01)^{ab}
	5NZ	1.55 (0.22)^{ab}	-	0.05 (0.01)^{a}
	15NZ	2.05 (0.05)^{b}	-	0.08 (0.001)^{b}
	10CZ	17.09 (0.25)^{c}	0.36 (0.01)	0.22 (0.01)^{c}

6.2.5 Statistical analysis

For evaluating significant differences between the treatments, data were checked to meeting parametric statistic assumption, then two-way ANOVA was employed for testing significant differences within factors time and treatments and for testing interactions between factors (treatments*time). Successively, repeated measurement ANOVA and Fisher (LSD) tests were applied at p level of 0.05. Correlation matrixes were built for each treatment using Pearson coefficient "ρ" in order to find moderate to strong correlations between variables (Supplementary Material, Table Sa, Sb, Sc, Sd). SigmaPlot 12.0 was employed for running statistical analysis.

6.3 Results

6.3.1 pH and elemental TOC an TN content

Soil pH ranged from 7.73 and 7.90 with no significant differences between the treatments ($p>0.05$) and no variation during the incubation period ($p>0.05$) (Table 14). TOC of soil and soil-zeolite mixtures was significantly different (Fig 17 A) ($p<0.05$). UA showed the highest TOC content (32.2 ± 0.3 g kg^{-1}) and 15NZ the lowest (28.8 ± 0.3 g kg^{-1}) ($p<0.05$), while 5NZ and 10CZ showed similar amounts ($p>0.05$) of 30.1 ± 0.5 and 30.9 ± 0.8 g kg^{-1}, respectively. In contrast, soil TN (Fig 17 B) was higher in 10CZ, with values of 2.30 ± 0.06 g kg^{-1}, followed by the UA (2.05 ± 0.03 g kg^{-1}), 5NZ (1.86 ± 0.05 g kg^{-1}) and 15NZ (1.81 ± 0.01 g kg^{-1}) ($p<0.05$). Consequently, the soil TOC/TN ratio (Fig 17 C) was very similar ($p>0.05$) between UA, 5NZ and 15NZ with values close to 16, and significantly lower ($p<0.05$) in 10CZ, with an average ratio of 13.5 ± 0.5.

Table 14: Soil pH, NH4+-N, NO3--N and NO2--N during the incubation period. Different upper case letters indicate significant differences at each sampling day (at p=0.05) as result of ANOVA and Fisher LSD test. The presence of "BDL" refers to "below detection limits".

		pH	NH_4^+-N	NO_3^--N	NO_2^--N
				mg kg^{-1}	
Day 2	UA	7.74 (0.05)^a	13.9 (3.4)^a	30.2 (1.7)^a	0.7 (0.1)^a
	5NZ	7.88 (0.01)^a	12.2 (1.0)^a	35.9 (1.2)^b	BDL
	15NZ	7.90 (0.02)^a	10.9 (0.5)^a	34.7 (0.9)^{ab}	BDL
	10CZ	7.84 (0.03)^a	68.7 (4.1)^b	151 (4)^c	20.0 (0.5)^b
Day 7	UA	7.84 (0.03)^a	7.6 (0.7)^a	39.5 (2.6)^b	0.2 (0.1)^a
	5NZ	7.78 (0.01)^a	11.0 (1.0)^b	33.9 (0.9)^a	0.6 (0.2)^a
	15NZ	7.85 (0.03)^a	10.8 (0.2)^b	43.8 (2.7)^b	0.4 (0.2)^a
	10CZ	7.73 (0.06)^a	68.8 (4.0)^c	145 (2)^c	4.3 (0.3)^b
Day 9	UA	7.84 (0.04)^a	9.7 (0.7)^b	25.0 (4.6)^a	7.6 (1.0)^c
	5NZ	7.76 (0.07)^a	6.2 (0.7)^a	29.4 (0.9)^a	0.3 (0.1)^a
	15NZ	7.86 (0.03)^a	7.7 (1.0)^{ab}	24.2 (0.3)^a	BDL
	10CZ	7.86 (0.01)^a	59.1 (2.7)^c	190 (3.5)^b	1.9 (0.1)^b
Day 11	UA	7.78 (0.06)^a	4.8 (0.5)^a	29.5 (0.4)^a	0.3 (0.2)^a
	5NZ	7.85 (0.01)^a	4.3 (0.8)^a	31.4 (0.3)^a	0.3 (0.1)^a
	15NZ	7.88 (0.01)^a	5.1 (0.9)^a	30.2 (0.7)^a	0.2 (0.1)^a
	10CZ	7.84 (0.03)^a	46.7 (2.5)^b	301 (6)^b	2.4 (0.2)^b
Day 16	UA	7.87 (0.04)^a	5.0 (0.7)^a	36.5 (0.1)^a	BDL
	5NZ	7.77 (0.05)^a	5.4 (0.5)^a	49.8 (0.2)^b	3.3 (0.3)^b
	15NZ	7.84 (0.03)^a	6.5 (0.5)^a	40.1 (0.1)^a	BDL
	10CZ	7.80 (0.02)^a	29.0 (0.8)^b	274 (10)^c	2.2 (0.1)^a

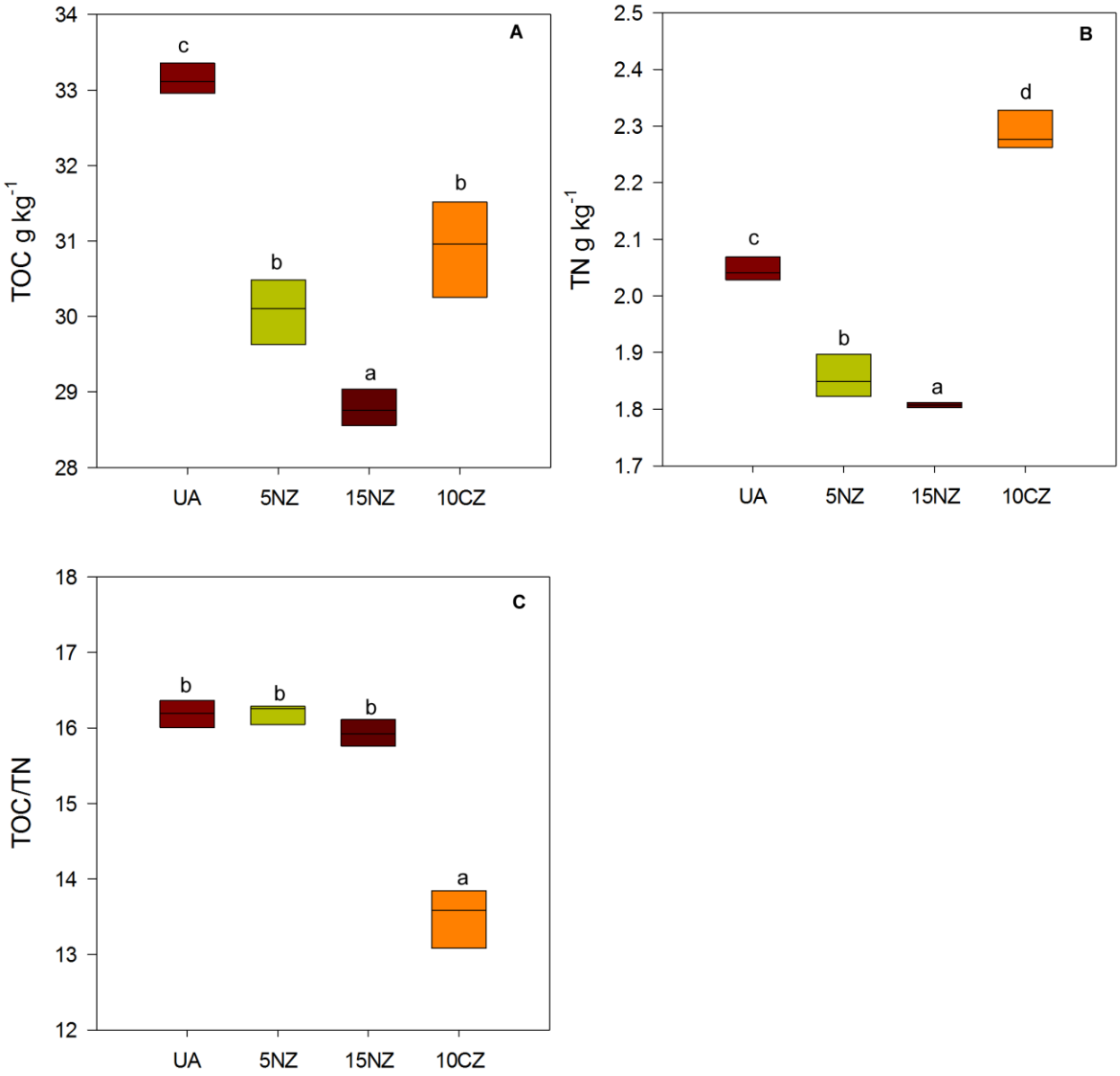

Fig 17: Substrate TOC (A), TN (B) and TOC/TN ratio (C) at the begin of the incubation. Boxes represent minimum, maximum and median of the data distribution. Different letters indicate significant differences at p=0.05 as result of ANOVA and Fisher (LSD) tests.

6.3.2 Soil NH_4^+-N, NO_3^--N and NO_2^--N

NH_4^+-N varied significantly between the treatments and among the incubation period, with significant "treatments*time" interactions ($p<0.05$) (Table 14). Values ranged from a minimum of 4.3 mg kg^{-1} to a maximum of 68.8 mg kg^{-1} in 5NZ and 10CZ, respectively. NH_4^+-N was very similar during the whole incubation between UA, 5NZ and 15NZ with generally no significant differences ($p>0.05$),

with the exception of day 7, where UA was slightly lower ($p<0.05$) and day 9 where 5NZ was slightly lower with respect to UA ($p<0.05$). Concerning 10CZ treatment, exchangeable NH_4^+-N was always much higher with respect to the other treatments ($p<0.05$) because of the N adsorbed by the CZ. A decrease in NH_4^+-N in CZ was observed starting from day 9 ($p<0.05$) since day 16, reaching half of the initial amount.

NO_3^--N varied significantly between the treatments and among the incubation period, with significant "treatments*time" interactions ($p<0.05$) (Table 14). Values ranges from a minimum of 24.2 mg kg^{-1} to a maximum of 301 mg kg^{-1} in 15NZ and 10CZ, respectively. It has to be noticed that 10CZ NO_3^--N content was completely out of scale if compared to the other treatments for the whole duration of the incubation experiment. In fact, after just 2 days of incubation, 10CZ had already a very high NO_3^--N content (151 mg kg^{-1}), approximately five times higher than that of the other treatments ($p<0.05$). NO_3^--N of 10CZ treatment was slightly lower at day 7 ($p<0.05$) and from that time point started to increase strongly from day 9 until day 16, doubling its initial amounts. Concerning the other treatments, no particular trend was observed, but generally average NO_3^--N content varied during the time with higher values at day 7 and 16 and minimum values at day 9, with minor but significant differences between the treatments ($p<0.05$).

NO_2^--N varied significantly between the treatments and among the incubation period, with significant "treatments*time" interactions ($p<0.05$) (Table 14). NO_2^--N values were sometimes below the detection limit, but on average they ranged from <1 to <5 mg kg^{-1} with only one isolated peak of 20 mg kg^{-1}. After an incubation time of two days, 5NZ and 15NZ NO_2^--N content was significantly lower compared to UA and 10CZ ($p<0.05$) while the latter showed the highest values registered during the experiment ($p<0.05$). At day 7, no significant differences were found between UA, 5NZ and 15NZ ($p>0.05$) where values were very low (<1 mg kg^{-1}), while 10CZ NO_2^--N content was still higher ($p<0.05$). NO_2^--N increased in UA at day 9, surpassing even 10CZ treatment ($p<0.05$) while 5NZ and 15NZ remained lower with respect to both UA and 10CZ ($p<0.05$). At day 11, no significant differences were found between UA, 5NZ and 15NZ while 10CZ was still higher even if the absolute

values decreased with respect to the previous days ($p<0.05$). At day 16, NO_2^--N was below the detection limit in UA and 15NZ treatment while 5NZ was higher than the previous days, exceeding also 10CZ ($p<0.05$).

Table 15: Soil DOC, TDN, MB-C, MB-N and MB C/N during the incubation period. Standard deviation within brackets. Different upper case letters indicate significant differences at each sampling day (at p=0.05) as result of ANOVA and Fisher LSD test.

		DOC mg kg^{-1}	TDN mg kg^{-1}	DOC/TDN	MB-C mg kg^{-1}	MB-N mg kg^{-1}	MB C/N
Day 2	UA	35.0 (5.1)^{b}	49.8 (2.4)^{a}	0.70 (0.10)^{c}	233 (16)^{a}	19.1 (7.0)^{a}	12.2 (4.6)^{b}
	5NZ	38.9 (0.3)^{b}	48.7 (0.6)^{a}	0.80 (0.01)^{d}	257 (47)^{a}	16.3 (1.8)^{a}	15.8 (3.4)^{b}
	15NZ	27.6 (2.0)^{a}	48.9 (1.2)^{a}	0.56 (0.03)^{b}	231 (21)^{a}	18.6 (1.5)^{a}	12.4 (1.5)^{b}
	10CZ	47.7 (5.4)^{c}	321 (29)^{b}	0.15 (0.01)^{a}	226 (15)^{a}	74.0 (3.0)^{b}	3.1 (0.2)^{a}
Day 7	UA	36.3 (3.9)^{b}	48.5 (2.6)^{a}	0.75 (0.05)^{c}	347 (93)^{b}	24.0 (6.1)^{a}	14.5 (5.3)^{b}
	5NZ	34.8 2.3)^{b}	47.0 (1.1)^{a}	0.74 (0.05)^{c}	212 (11)^{a}	19.5 (2.9)^{a}	10.9 (1.7)^{ab}
	15NZ	28.9 (1.3)^{a}	57.8 (3.0)^{a}	0.50 (0.02)^{b}	252 (58)^{a}	19.0 (7.4)^{a}	13.3 (6.0)^{b}
	10CZ	45.2 (2.5)^{c}	382 (8)^{b}	0.12 (0.01)^{a}	237 (43)^{a}	40.1 (28.1)^{a}	5.9 (4.3)^{a}
Day 9	UA	48.7 (27.4)^{ab}	50.5 (3.4)^{a}	0.96 (0.52)^{b}	194 (61)^{a}	11.0 (3.9)^{a}	17.6 (8.4)^{b}
	5NZ	40.0 (5.4)^{a}	47.5 (0.9)^{a}	0.84 (0.10)^{b}	202 (7)^{a}	9.71 (5.9)^{a}	20.8 (12.7)^{c}
	15NZ	38.8 (16.2)^{a}	52.1 (0.2)^{a}	0.74 (0.31)^{b}	197 (48)^{a}	6.9 (4.2)^{a}	28.6 (18.8)^{d}
	10CZ	66.7 (32.5)^{b}	406 (80)^{b}	0.16 (0.07)^{a}	165 (70)^{a}	132 (6)^{b}	1.2 (0.5)^{a}
Day 11	UA	30.1 (2.2)^{a}	39.2 (2.7)^{a}	0.77 (0.02)^{c}	260 (82)^{a}	22.2 (13.6)^{a}	11.7 (8.0)^{b}
	5NZ	32.3 (1.2)^{a}	50.1 (14.4)^{a}	0.64 (0.17)^{bc}	191 (9)^{a}	11.1 (4.3)^{a}	17.3 (6.7)^{b}
	15NZ	26.7 (2.6)^{a}	46.4 (1.3)^{a}	0.58 (0.04)^{b}	207 (12)^{a}	14.1 (3.3)^{a}	14.7 (3.6)^{b}
	10CZ	60.4 (0.6)^{b}	535 (154)^{b}	0.11 (0.03)^{a}	184 (12)^{a}	220 (62)^{b}	0.8 (0.2)^{a}
Day 16	UA	47.6 (16.8)^{a}	49.4 (2.8)^{a}	0.96 (0.28)^{c}	240 (102)^{a}	20.6 (14.8)^{a}	11.6 (9.7)^{d}
	5NZ	39.7 (6.1)^{a}	68.0 (0.1)^{a}	0.58 (0.09)^{b}	227 (9)^{a}	21.7 (5.9)^{a}	10.5 (2.9)^{cd}
	15NZ	34.3 (2.8)^{a}	54.7 (2.8)^{a}	0.63 (0.02)^{b}	330 (58)^{b}	42.0 (5.3)^{a}	7.8 (1.7)^{bc}
	10CZ	67.9 (10.5)^{b}	376 (7)^{b}	0.18 (0.03)^{a}	190 (8)^{a}	238 (16)^{b}	0.8 (0.1)^{a}

6.3.3 Soil DOC and TDN

DOC content varied significantly between the treatments and among the incubation period ($p<0.05$) with no significant "treatment*time" interactions ($p>0.05$) (Table 15). Values ranged from a minimum of 26.7 mg kg^{-1} to a maximum of 67.9 mg kg^{-1} registered in 15NZ and 10CZ, respectively. DOC was always higher in 10CZ treatment with an increase over the time starting from day 9 until the end of the experimentation ($p<0.05$). The other treatments did not show significant differences in DOC ($p>0.05$) except for day 2 and 7, where 15NZ was lower with respect to UA and 5NZ.

TDN varied significantly between the treatments ($p<0.05$) and showed significant "treatment*time" interactions ($p<0.05$) (Table 15). Overall values ranged from a minimum of 39.2 mg kg^{-1} to a maximum of 534 mg kg^{-1} registered in UA and 10CZ, respectively. No significant differences were found between UA, 5NZ and 15NZ ($p>0.05$) while 10CZ was much higher throughout the whole incubation period ($p<0.05$).

6.3.4 Ergosterol

Ergosterol measurement is commonly employed as a marker of fungal biomass and used to study fungi for economic and ecological reasons (Gessner and Chauvet, 1993; Johnson and McGill, 1990). Ergosterol varied significantly between the treatments ($p<0.05$) and showed significant "treatment*time" interactions ($p<0.05$) (Fig 18).

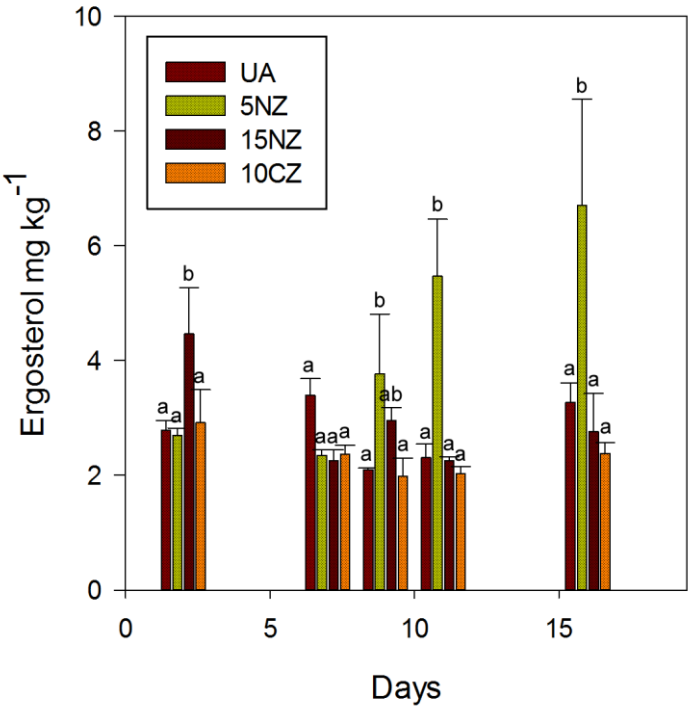

Fig 18: Ergosterol (mg kg-1) during the incubation. Error bars represent the standard error (SE). Different letters indicate significant difference at each sampling day ($p<0.05$) as result of ANOVA and Fisher LSD test.

Generally, ergosterol was quite similar between UA, 15NZ and 10CZ with no significant differences ($p>0.05$) with the exception of day 2, in which 15NZ was significantly higher ($p>0.05$). Starting from day 9, ergosterol significantly increased in 5NZ until the end of the incubation ($p<0.05$), reaching the highest values at day 16. It is interesting to note that only the soil amended with the lower application rate of NZ showed an increase in ergosterol, while the high NZ application rate did not show a significant change in ergosterol content compared to UA.

6.3.5 MB-C, MB-N and MB C/N ratio

MB-C varied significantly between treatments and among the incubation period ($p<0.05$) but with no significant "treatment*time" interactions ($p>0.05$) (Table 15). Values were included between a minimum of 164 mg kg^{-1} to a maximum of 348 mg kg^{-1} in 10CZ and UA, respectively. At day 2 of

incubation, no significant differences were found between the treatments ($p>0.05$), while during day 7, UA MB-C was significantly higher with respect to the other treatments. As in day 2, no significant differences between the treatments ($p>0.05$) were found at day 9 and 11, while at the end of the incubation 15NZ MB-C increased significantly ($p<0.05$).

Concerning MB-N, significant differences were found both between the treatments and among the incubation period, with significant "treatment*time" interactions ($p<0.05$) (Table 15). Values were included between a minimum of 6.9 mg kg^{-1} to a maximum of 238 mg kg^{-1} in 15NZ and 10CZ, respectively. During the whole incubation period, 10CZ was the only treatment which showed significant differences with respect to the others ($p<0.05$). At day 2 of incubation, MB-N was already significantly higher in 10CZ treatment with respect to UA and both NZ treatments ($p<0.05$) but at day 7, albeit average values remain still very high, the high variability of the three analyzed replicates resulted in a non-significant differences between the treatments ($p>0.05$). Starting from day 9, MB-N of 10CZ increased strongly to values higher than 130 mg kg^{-1} differing significantly from the other treatments ($p<0.05$), and further increasing at day 11 and 16 reaching the maximum values registered during the incubation. 10CZ MB-N was strongly related with NO_3^--N and NH_4^+-N dynamics over the incubation period (Fig 19).

MB C/N ratio (Table 15) is a valuable parameter that may be indicative of fungal or bacteria prevalence in the microbial community since fungi and bacteria present different C/N ratios and thus different nutrient requirements (Strickland and Rousk, 2010). The very high MB-N of 10CZ resulted in a constant significantly lower MB C/N ratio in this particular treatment ($p<0.05$), with values ranging from 0.8 to 5.9 (with a strong decline starting from day 9). Concerning the other treatments, no particular trends were found, since there were no strong differences in MB-C and MB-N between them.

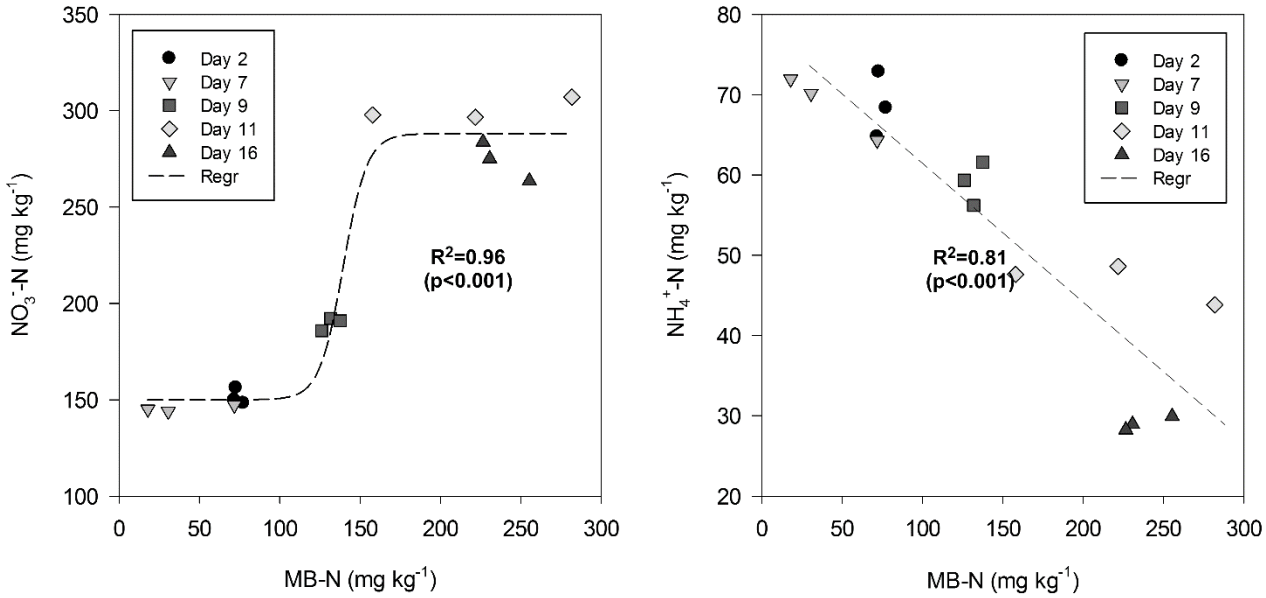

Fig 19: Relationship of MB-N with NO_3^--N and NH_4^+-N in 10CZ soil during the incubation. Dotted lines represent sigmoidal (for NO_3^--N) and linear (for NH_4^+-N) regressions.

6.3.6 Microbial $\delta^{15}N$ and net ^{15}N immobilization rates

The analysis of MB isotopic signature through the EFE method evidenced significant differences ($p<0.05$) between UA and 10CZ. UA MB had a slight negative $\delta^{15}N$ of -4.2 (± 0.49) ‰ and it was assumed constant over the course of incubation since no further N inputs were added, while the isotopic signature of the pure CZ used in this incubation experiment was 43.6 (± 0.6) ‰. At day 2, 10CZ MB $\delta^{15}N$ was highly variable but already significantly higher ($p<0.05$) with respect to the UA with an average value of 12.9 (± 5.7) ‰. At day 9, 10CZ MB $\delta^{15}N$ increased reaching values of 25.6 (± 3.9) ‰, while at day 16 the isotopic signature decreased to values of 15.3 (± 2.0) ‰, thus still significantly higher with respect to the UA MB ($p<0.05$).

The rate of ^{15}N immobilization in 10CZ MB resulted in 31.5 ± 1.9 µg ^{15}N day^{-1} between day 2 and day 9, while the rate almost doubled between day 9 and 16 with values of 55.4 ± 4.3 µg ^{15}N day^{-1}.

6.3.7 Stoichiometric imbalances

The stoichiometric imbalances calculated with respect to the bulk substrate were always very low for UA, 5NZ and 15NZ, with values close to 1 and generally not significantly different between each other ($p>0.05$) except for a slight 15NZ imbalance at day 16 ($p<0.05$) (Table 13). On the contrary, 10CZ bulk substrate imbalance was always significantly higher ($p<0.05$) compared to the other treatment, and it increased over time since day 2 of incubation (Table 13).

In our particular experiment the imbalance calculated as DOC/TDN divided by MB C/N resulted in a <1 ratio; for this reason, the higher the values are, the smaller the imbalance is. The imbalance calculated with respect to the labile substrate was high (low ratios) and very similar ($p>0.05$) for all the treatments at day 2 of incubation. At day 7, the imbalance remains very high but 10CZ showed the highest while 5NZ the lowest ($p<0.05$). Starting from day 9, 10CZ decreased strongly and progressively over time its labile substrate imbalance ($p<0.05$) while for UA, 5NZ and 15NZ the imbalance remained high and generally not significantly different ($p>0.05$) with the exception of a slight decrease of 15NZ at day 16. On the other hand, the imbalance calculated with respect to pure CZ mineral (thus only for 10CZ treatment) (Table 13), increased at day 7, and then gradually decreased from day 9 (with values doubled compared to day 2) until day 16. Concerning 10CZ treatment, it is evident that the imbalance is higher if calculated with respect to the bulk substrate, while lower if calculated with respect to the labile substrate and especially if calculated with respect to the pure CZ mineral.

6.4 Discussion

6.4.1 NZ effects

Pure NZ is characterized by a very low MB-C and MB-N, indicating a poor colonization of zeolites at natural state by microorganisms. Ergosterol was not detected in NZ minerals (Table 2), thus fungi have not been transferred in the soil from NZ amendment.

It is evident that the addition of both 5 wt% and 15 wt% of NZ to the soil caused a relative decrease of TOC and TN of the substrate because of a dilution effect by the added mineral which is very low in TOC and TN content at the natural state (Table 2), however, the TOC/TN ratio was not altered with respect to the UA soil.

MB-C and MB-N dynamics over the 16 days of incubation suggest that microbial immobilization was not influenced in the short term by the addition of NZ (Table 15).

However, ergosterol measurements suggest that the relative amount of fungi compared to the total MB seems to be influenced by NZ amendments, especially in the lower application rate (5NZ) as indicated by ergosterol and MB C/N (Fig 20). It is in fact evident that especially 5NZ was characterized by a higher ergosterol and/or higher MB C/N ratio starting from day 9, suggesting an increase in fungal population. Fungi are known to have a lower nutrient (N) requirement compared to bacteria (Güsewell and Gessner, 2009) because of their higher cellular C/N ratio. As a large set of factors that are known to influence fungal abundance in agricultural soils (i.e. agricultural management, soil pH, soil moisture, temperature and atmospheric CO_2) (Strickland and Rousk, 2010), which were kept constant in the present experiment, a possible explanation can reside in a lower immediate nutrient availability because of:

1) a dilution effect operated by the amendment with a significant amount of NZ (which is a low-N bearing mineral) that have reduced the total C-N pools available in the substrate;
2) a “competition” for the dissolved mineral N species between NZ (adsorption) and MB (assimilation) in the short-term.

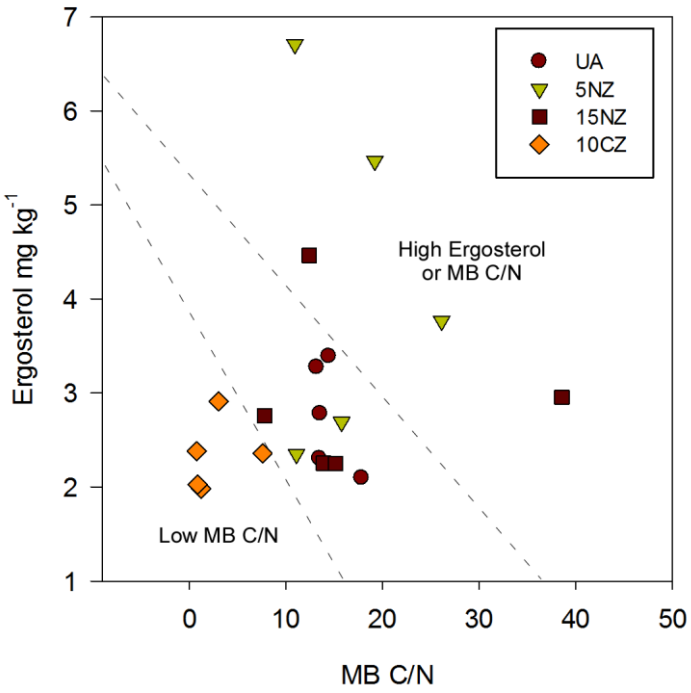

Fig 20: Scatter plot of ergosterol vs MB C/N. Short dashed lines separate samples with low MB C/N (10CZ soils) and samples with high ergosterol or MB C/N ratio (mainly 5NZ or 15NZ).

It is thus plausible that the addition of zeolites (CEC equal to 2.17 meq g^{-1}) increased the soil CEC (0.45 meq g^{-1}), as already found by Gholamhoseini et al. (2013) after using zeolite-amended cattle manure on sunflower field and by Ferretti et al. (2015) directly in the ZeoLIFE experimental field, where in the zeolite amended soils, CEC raised up to 0.51 meq g^{-1}. However, CEC was not measured in the employed soil-zeolite mixtures. The addition of an initially N-deficient mineral with a very high CEC and affinity for NH_4^+ can have established a sort of "competition" with soil microorganisms in the short term period for the dissolved mineral N.

It is very interesting to note that this increase in ergosterol was not found in 15NZ treatment despite the very high NZ application rate. Following the previous hypothesis, the more NZ is in the soil the more fungi should develop because of lower microbial available mineral N. In fact, ergosterol content was higher in 15NZ only at day 2, while MB C/N was much higher only at day 9 with a lack of any particular trend. Moreover, the substrate TOC/TN ratio was lower, albeit not significantly different from 5NZ (Fig 17 C), but both TOC and TN pools were slightly lower (Fig 17 A,B). Additionally,

the amount of DOC was significantly lower at the begin of the incubation (day 2 and 7) in 15NZ compared to 5NZ, but the TDN content was similar, resulting in a wider DOC/TDN ratio and in more available C for fungal biomass in 5NZ treatment. This may indicate that the amount of NZ can influence nutrients availability in the short period with different effects on fungal biomass depending on the NZ application rate. Probably, the optimal equilibrium between DOC and TDN availability for fungal biomass was met in 5NZ but not in 15NZ.

The outcomes of this experiments indicate that our first working hypothesis (H1) can be accepted for 5NZ but not for 15NZ amendment.

6.4.2 CZ effects

Pure CZ was characterized by a very high MB-C and MB-N, indicating a strong colonization of NH_4^+-enriched zeolites by microorganisms. Ergosterol was not detected on pure CZ (Table 2), thus fungi have not been introduced into the soil from CZ amendment. However, the MB C/N ratio of the microorganisms that colonized CZ was extremely low and very close to the C/N ratio of the mineral itself (Table 2). This extremely low MB C/N suggests that CZ was mainly colonized by bacteria (Zechmeister-Boltenstern et al., 2015).

CZ amendment has decreased soil TOC (Fig 17 A) but has increased soil TN (Fig 17 B), reflecting a significant decline of the soil TOC/TN ratio (Fig 17 C). This is obviously attributable to the N input of CZ, which significantly increased the substrate N pool. The very high TDN found in 10CZ is caused by the 1 M KCl extraction that have completely exchanged the NH_4^+ ions previously adsorbed by CZ.

High NO_2^--N content at day 2 suggests that ammonia oxidation occurred (the first step in nitrification) (Ruiz et al., 2003). These high NO_2^--N accumulation might indicated that the total nitrification process, and therefore the production of NO_3^-, might have been inhibited at the early stages of incubation. It is plausible that this inhibitory effect was due to high NH_3 levels (favored also by the

sub-alkaline pH) that decreased nitrite oxidizing bacteria (NOB) activity giving rise to NO_2^- accumulation (Stojanovic and Alexander, 1958; Morrill and Dawson, 1967; McGilloway et al., 2003). Considering that the amount of CZ added in the soil batch was 100 g (10 % of 1 kg) and its residual NO_3^--N load (146 mg kg^{-1}, Table 2), the addition of CZ brought to the soil a total amount of 14.6 mg of NO_3^--N which are only the 9.6 % of the total soil NO_3^--N of 10CZ at day 2 of incubation. This little addition of residual NO_3^--N may have partially contributed to stimulating microbial growth and decomposition processes. Moreover, CZ was already colonized before the insertion in the soil as well visible from CZ MB-N and MB-C (Table 2).

It is well visible that after 9 days, MB-N started to further increase in CZ treatment as well as NO_3^--N with a contemporaneous decline of NH_4^+-N concentrations, suggesting an increase in nitrification, in fact, MB-N, NO_3^--N and NH_4^+-N were highly correlated during the incubation (Fig 19).

These processes are further supported by ρ coefficients (see Supplementary Material Sd). However, this high availability of NH_4^+ ions may have not only stimulated NO_3^- production, but also microbial immobilization into biomass. This statement is supported by the isotopic analysis conducted in three different time steps (day 2, day 9 and day 16) on 10CZ MB. The $\delta^{15}N$ of CZ was 43.6 (± 0.6) ‰ and it is well representative of the pig-slurry isotopic signature employed in the enrichment process, while the UA MB $\delta^{15}N$ was on average -4.2 (± 0.49) ‰ at the begin of the incubation. In this respect, it is clear that the $\delta^{15}N$ of 10CZ MB was strongly influenced by CZ isotopic signature since day 2, but especially at days 9 and 16 and thus that high amounts of ^{15}N were assimilated by MB (Dittert et al., 1998). However, this evidence become more clearly visible if the amount of ^{15}N present in the soil MB is considered (Fig 21). It is interesting to note that the rates at which ^{15}N atoms that were incorporated, almost doubled ($p<0.05$) starting from day 9, concomitantly with the strong net NH_4^+ decrease.

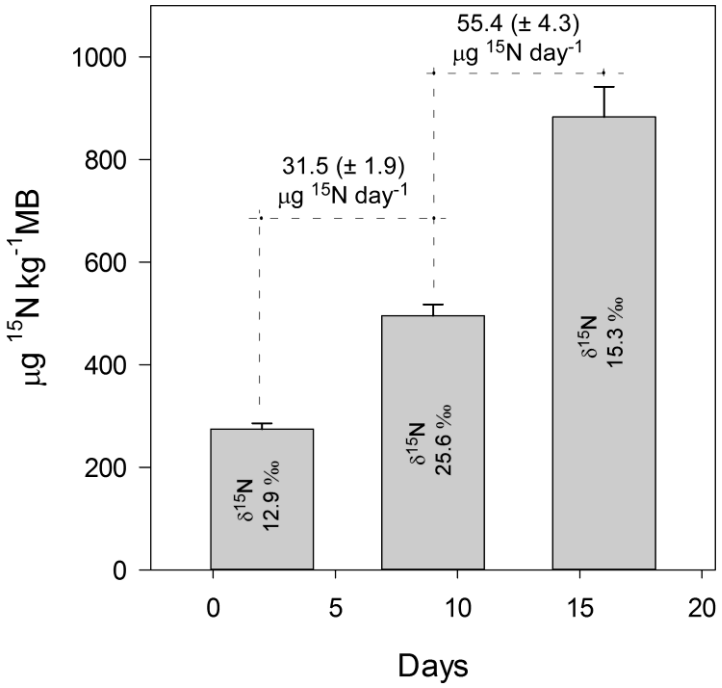

Fig 21: Amounts of ^{15}N immobilized by 10CZ MB over the incubation period and immobilization rates between day 2-9 and days 9-16. The $\delta^{15}N$ of MB is indicated inside each column. Error lines and number within brackets represent standard deviation.

Moreover, the reduction in NH_4^+ levels by microbial immobilization might have also reduced the substrate for NH_3 production, resulting in a lower inhibitory effects on NOB and thus in a more favorable condition for NO_3^- production.

Notwithstanding the high nitrification occurred in this treatment, it is interesting to note that soil pH did not decrease, suggesting a good buffering capacity of CZ (Colella, 1999; Rădulescu, 2013) together with soil carbonates.

These results partially agree with the work of McGilloway et al. (2003), where they found that in a zeoponic substrate with NH_4^+-enriched clinoptilolite zeolites, nitrification was higher than in soil systems and that ammonium oxidizing bacteria (AOB) were higher than NOB, causing NO_3^- accumulation, but they did not found a good buffering effect of the substrate. Leggo (2000), which used NH_4^+ enriched clinoptilolite after the mixing with poultry manure for the creation of an organo-zeolitic substrate, stated that a possible explanation for the high NO_3^--N concentration visible since

the very first day after the application might reside in the interactions of CZ with the soil solution. The high natural salinity of the soil employed in the present experiment (Di Giuseppe et al., 2014) may have induced cation exchange reactions with the NH_4^+ adsorbed into the zeolites, thus increasing the availability of the substrate required for nitrification.

Moreover, it is interesting to note that NO_3^--N decreased significantly ($p<0.05$) from day 11 to day 16. Probably, since the soil in the jar was not continuously mixed and aerated, the presence of anaerobic microniches might have favored denitrification processes towards the end of the incubation (Mastrocicco et al., 2011).

The increase in soil DOC visible at day 2 is with all probability due to the residual DOC brought by CZ amendment, since the addition of 10 % of CZ should have raised soil DOC by around 12 mg kg^{-1}. However, when NO_3^--N started to increase at day 9, also DOC increased, confirming an enhancement of mineralization processes. This evidence further proves that the addition of CZ gives immediately accessible N to microorganisms that can trigger degradation of soil organic matter, thus increasing soil DOC significantly (Jokubauskaite et al., 2015).

Considering the abovementioned points, the combination of the following events: i) the supply of a little amount of residual NO_3^- and DOC, ii) the probable exchange processes with soil cations, and iii) the colonization of CZ, might have caused a positive priming effect on 10CZ MB (Kuzyakov et al., 2000), an initial NO_2^- accumulation and then a strong net NO_3^- increase/NH_4^+ decline, well visible especially from day 9 of incubation (Fig 19).

Since MB-N was significantly affected by CZ amendment, also MB C/N was influenced by this practice. Our results therefore suggest that the microbial community in 10CZ treatment was not homeostatic (Sterner and Elser, 2002). The imbalance with respect to the bulk substrate was in fact largely higher in 10CZ rather than in the UA and both soils amended with NZ, moreover, this imbalance even increased over time (Table 13). The latter indicates that 10CZ MB was adjusting its C/N ratio not for meeting the bulk soil C/N ratio, which was in fact higher if compared to its MB C/N ratio. On the contrary, if the TOC/TN ratio of the "pure" CZ mineral is considered in the calculation,

it results in a much lower imbalance (Table 13). For this reason, we proposed the calculation of the stoichiometric imbalance considering the labile C/N fractions of the substrate (DOC and TDN) with respect to MB C/N ratio (Table 13), since the strong majority of CZ-N is in the form of labile TDN (Table 2). In the present study, if the labile C/N fraction is considered, the lower imbalance (ratios closer to 1) was found for 10CZ, while UA, 5NZ and 15NZ showed higher ($p<0.05$) and often similar imbalances between each other ($p>0.05$). The imbalance with respect to the labile fraction decreased progressively over the incubation period starting from day 9 until day 16, indicating that MB adjusted its stoichiometric C/N ratio in order to meet preferentially the main labile N source in the substrate, which during the incubation period was represented by CZ mineral. This statement is further supported by the strong direct correlation found between bulk substrate and labile C/N imbalances ($\rho=0.87$), that indicates a progressive drift of MB C/N from bulk substrate C/N in order to meet the labile C/N ratio.

Plotting the labile C/N imbalance vs NH_4^+-N and NO_3^--N of 10CZ treatment (Fig 22), it is possible to individuate a threshold imbalance value (~0.09) which delimitate the strong increase in nitrification process. This threshold has been reached at day 9 of incubation, and coherently coincide with the increase in DOC of 10CZ treatment.

It is widely known that C/N ratio of bacteria is expected to be ~6 while the one of fungi is expected to be ~5-15 resulting in a significantly lower C/N of bacteria with respect to fungi (McGill et al., 1981; Strickland and Rousk, 2010). In this respect, MB C/N ratio indicates a clear bacteria prevalence in the microbial community of 10CZ treatment (Fig 20) (Carney et al., 2007).

The reason of this decrease was due to the addition of a fresh N input that was immediately accessible to microorganism (CZ-N), resulting in an adequate environment for bacteria development, which are known to have lower cellular C/N ratio and higher nutrient requirements compared to fungi.

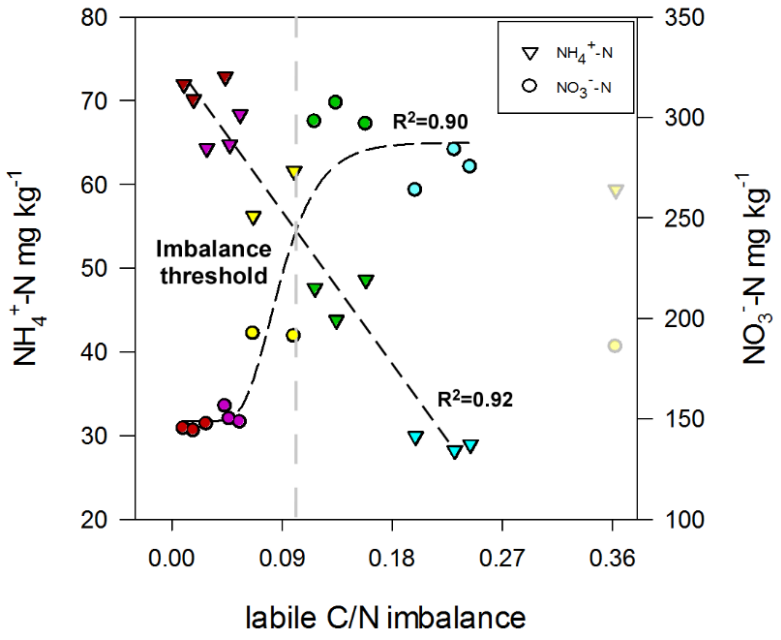

Fig 22: Scatter plot of labile C/N imbalance vs NH_4^+-N and NO_3^--N of 10CZ treatment. The dotted gray line delimitates the imbalance threshold above which a strong NO_3^- increase was recorded. Dotted lines represent sigmoidal (for NO_3^--N) and linear (for NH_4^+-N). Red symbols represent samples from day 2, purple from day 7, yellow from day 9, green from day 11 and azure from day 16.

The amount of ergosterol has not changed significantly over time and with respect to the UA soil, suggesting that in the short period, fungal biomass was not affected by CZ addition.

Concerning CZ amendment, the outcomes of our experiment indicates that our second working hypothesis H2 needs to be rejected since CZ did not act as a slow release N source.

6.5 Chapter conclusions

The amendment employing 5 wt% of NZ increased ergosterol over time, suggesting an increase in fungal biomass and thus may indicate a possible positive practice for increasing soil C-sequestration. However, the same evidence has not been observed when 15 wt% of NZ was employed as amendment. This evidence may suggest that the amount of NZ can influence the nutrients availability to soil microbial biomass in the short period with different effects on fungal biomass development depending on the NZ application rate.

The performed analysis confirmed the immediate availability of the N inserted with NH_4^+-enriched CHA zeolites to soil microorganisms. This immediate availability needs to be taken into account for their potential application in agricultural context since NH_4^+-enriched CHA zeolites will act not as "slow release fertilizers" but as a pool of immediately available N to soil microbial biomass, which can lead to an increase of soil nitrification. This study could serve as a basis to foster long term experiments, both in laboratory and in the field conditions to get new insights on soil microbial response to zeolite amendments.

7.0 General Conclusions

In the present thesis, an overview on the effects of natural and NH_4^+-enriched chabazite zeolite amendments on soil-plant N-C pools, soil gaseous emissions and microbial biomass population/activity have been performed.

The main outcomes of this work revealed that, at the field scale, not all the soil N pool are significantly affected by zeolite addition (i.e fixed NH_4^+ pool). The NH_4^+ adsorbed by the mineral has been proved to be in a very mobile and accessible form, almost entirely definable as exchangeable NH_4^+ (extractable using 1 M KCl).

The N transfer from zeolites to plants have been proved by the mean of isotopic analysis at natural abundance, by exploiting the very high natural $\delta^{15}N$ of the zoo-technical effluent employed for the enrichment process. Moreover, where zeolites at natural state were employed, the information supplied by the plant isotopic signature in combination with the crop yield results suggest that probably FUE was enhanced.

By the measurements of soil gaseous emissions, the use of zeolites at natural state seemed to reduce the immediate production of CO_2, N_2O, NO_x, and especially NH_3 emissions after the application of urea fertilizer, constituting thus a potential valuable material for reducing short-term soil gaseous C and N losses. On the other hand, measurements performed on soil amended with NH_4^+-enriched CHA zeolites indicated that part of the N supplied with this material was immediately transformed biotically or abiotically. However, from this particular short-term experimentation it is not possible to draw conclusions about long-term mitigation strategies.

Lastly, both the amendments with natural and NH_4^+-enriched CHA zeolites affected significantly soil microbial biomass activity and population in the short term period.

Amendments with zeolite at natural state probably favored the development of fungal biomass, however, the effect was with all probability related to the amount of zeolites added to the soil that influenced N and C availability to soil microbial biomass.

The immediate availability of the N supplied with NH_4^+-enriched CZ to soil microbial biomass was finally confirmed by the priming effect encountered immediately after its application to the soil (that favored bacteria development) and by isotopic analysis performed on soil microbial biomass.

From these point of view, this thesis produced new important insights and clues about many aspects that are currently unexplored in the scientific literature.

I am aware that at this point it is still not possible to draw conclusions about the long-term effects of zeolites amendments on soil N fluxes, however, these outcomes may serve as a fundamental "starting point" for future long-term experiments or more specific investigations.

9.0 References

Adesemoye, A.O., Kloepper, J.W., 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85, 1-12.

Akiyama, H., McTaggart, I.P., Ball, B.C., Scott, A., 2004. N2O, NO, and NH3 emissions from soil after the application of organic fertilizers, urea and water. Water Air Soil Pollut. 156, 113–129.

Arcara, P.G., Gamba, C., Bidini, D., Marchetti, R., 1999. 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). Biology and Fertility of Soils. 29, 270-276.

Aulakh, M.S., Singh, B., 1997. Nitrogen losses and fertilizer N use efficiency in irrigated porous soils. Nutr. Cycl. Agroecosys. 47, 197-212.

Azam, F., Malik, K.A., Hussain, F., 1986. Microbial biomass and mineralization-immobilization of nitrogen in some agricultural soils. Biology and Fertility of Soils. 2,157-163.

Baerlocher, C.H., Meier, W.M., Olson, D.H., 2001. Atlas of Zeolite Framework Types, Fifth Revised edition. (Published on behalf of the Structure Commission of the International Zeolite Association by Elsevier).

Barbarick, K.A., Pirela, H.J., 1984. Agronomic and horticultural uses of zeolites: a review. In: Pond, W.G. and Mumpton, F.A. (Eds), Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture, International Committee on Natural Zeolites, pp. 93-103.

Bateman, A.S., Kelly, S.D., Jickells, T.D., 2005. Nitrogen Isotope Relationships between Crops and Fertilizer: Implications for Using Nitrogen Isotope Analysis as an Indicator of Agricultural Regime. J. Agric. Food Chem. 53, 5760-5765.

Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. Biol. Fertil. Soils 41, 379–388.

Beck, T., Joergensen, R.G., Kandeler, E., Makeshin, E., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29, 1023–1032.

Blagodatskaya, E., Blagodatsky, S., Anderson, T.H., Kuzyakov, Y., 2014. Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. PLoS One 9.

Bogaard, A., Fraser, R., Heaton, T.H.E., Wallace, M., Vaiglova, P., Charles, M., Jones, G., Everdhed, R.P., Styring, A.K., Andersen, N.H., Arbogast, R.M., Bartosiewicz, L., Gardeisen, A., Kanstrup, M., Maier, U., Marinova, E., Ninov, L., Schäfer, M., Stephan, E., 2013. Crop manuring and intensive land management by Europe's first farmers. Proc. Natl. Acad. Sci. USA. 110, 12589-12594.

Bonenfant, D., Kharoune, M., Niquette, P., Mimeault, M., Hausler, R., 2008. Advances in principal factors influencing carbon dioxide adsorption on zeolites. Sci. Technol. Adv. Mater. 9, 1468–6996.

Brandstätter, C., Keiblinger, K., Wanek, W., Zechmeister-Boltenstern, S., 2013. A closeup study of early beech litter decomposition: Potential drivers and microbial interactions on a changing substrate. Plant Soil 371, 139–154.

Brescia, M.A., Di Martino, G., Fares, C., Di Fonzo, N., Platani, C., Ghelli, S., Reniero, F., Sacco, A., 2002. Characterization of Italian Durum Wheat Semolina by Means of Analytical and Spectroscopic Determinations. Cereal. Chem. 79, 238-242.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837-842.

Brüggemann, N., Gessler, A., Kayler, Z., Keel, S.G., Badeck, F., Barthel, M., Boeckx, P., Buchmann, N., rugnoli, E., Esperschütz, J., Gavichkova, O., Ghashghaie, J., Gomez-Casanovas, N., Keitel, C., Knohl, A., Kuptz, D., Palacio, S., Salomon, Y., Uchida, Y., Bahn, M., 2011. Carbon

allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosciences. 8, 3457-3489.

Bundan, L., Majid, N.M.A., Ahmed, O.H., Jiwan, M., Kundat, F.R., 2011. Ammonia volatilization from urea at different levels of zeolite. Int. J. Phys. Sci. 6, 7717–7720.

Campisi, T., Abbondanzi, F., Faccini, B., Di Giuseppe, D., Malferrari, D., Coltorti, M., Laurora, A., Passaglia, E., 2016. Ammonium-charged zeolitite effects on crop growth and nutrient leaching: Greenhouse experiments on maize (Zea mays). Catena 140, 66–76.

Capasso, S., Salvestrini, S., Coppola, E., Buondonno, A., Colella, C., 2005. Sorption of humic acid on zeolitic tuff: a preliminary investigation. Applied Clay Science. 28, 159–165.

Carney, K.M., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2007. Altered soil microbial community at elevated CO2 leads to loss of soil carbon. Proc. Natl. Acad. Sci. U. S. A. 104, 4990–4995.

Choi, W.J., Lee, S.M., Ro, H.M., Kim, K.C., Yoo, S.H., 2002. Natural 15N abundances of maize and soil amended with urea and composted pig manure. Plant. Soil. 245, 223–232.

Choi, W.J., Ro, H.M., Hobbie, E.A., 2003. Patterns of natural 15N in soils and plants from chemically and organically fertilized uplands. Soil Biol. Biochem. 35, 1493-1500.

Choi, W.-J., Matushima, M., Ro, H.-M., 2011. Sensitivity of soil CO2 emissions to fertilizer nitrogen species: urea, ammonium sulfate, potassium nitrate, and ammonium nitrate. J. Korean Soc. Appl. Biol. Chem. 54, 1004–1007.

Ciesielsky, H., and Sterckeman, T. 1997. Determination of cation exchange capacity and exchangeable cations in soils by means of cobalt heamine trichloride. Effects of experimental conditions. Agronomie 17, 1-7.

Colella, C., 1999. Natural zeolites in environmentally friendly processes and applications, in: Porous Materials in Environmentally Friendly Processes Proceedings of the 1st International FEZA Conference. pp. 641–655.

Colombani, N., Mastrocicco, M., Di Giuseppe, D., Faccini, B., Coltorti, M., 2014. Variation of the hydraulic properties and solute transport mechanisms in a silty-clay soil amended with natural zeolites. Catena. 123, 195-204.

Colombani, N., Mastrocicco, M., Di Giuseppe, D, D., Faccini, B., Coltorti, M., 2015. Batch and column experiments on nutrient leaching in soils amended with Italian natural zeolitites. Catena. 127, 64-71.

Colombani, N., Di Giuseppe, D., Faccini, B., Ferretti, G., Mastrocicco, M., Coltorti, M., 2016. Estimated Water Savings in an Agricultural Field Amended With Natural Zeolites. Environ. Process. 2011–2014.

Coombs, D., Alberti, A., Armbruster, T., Artioli, G., Colella, C., Galli, E., Grice, J.D., Liebau, F., Mandarino, J.A., Minato, H., Nickel, E., H., Passaglia, E., Peacor, D.R., Quartieri, S., Rinaldi, R., Ross, M., Sheppard, R.A., Tillmanns, E., Vezzalini, G., 1997. Recommended nomenclature for zeolite minerals: report of the subcommittee on zeolites of the international mineralogical association, Commission on New Minerals and Mineral Names. Can. Mineral. 35, 1571-1606.

De Campos Bernardi, A.C., Anchão Oliviera, P.P., De Melo Monte, M.B., Souza-Barros, F., 2013. Brazilian sedimentary zeolite use in agriculture. Microporous Mesoporous Mater. 167, 16–21.

Di Giuseppe, D., Faccini, B., Mastrocicco, M., Colombani, N., Coltorti M., 2014. Reclamation influence and background geochemistry of neutral saline soils in the Po River Delta Plain (Northern Italy). Environ. Earth Sci. 72, 2457-2473.

Di Giuseppe, D., Faccini, B., Melchiorre, M., Ferretti, G., Coltorti, M., Ciuffreda, G., Zago, Alessandro., Pepi, Salvatore., 2015. Yield and quality of maize grown on a loamy soil amended with natural chabazite zeolitite. EQA - Int. J. Environ. Qual. 17, 35–45.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O. V., Hungate, B.A., 2006. 13C and 15N natural abundance of the soil microbial biomass. Soil Biol. Biochem. 38, 3257–3266.

Dittert, K., Georges, T., Sattelmacher, B., 1998. Nitrogen turnover in soil after application of animal manure and slurry as studied by the stable isotope 15N: a review. J. Plant. Nutr. Soil. Sc. 161, 453-463.

Dutta, K., Schuur, E.A.G., Neff J.C., Zimov A., 2000. Potential carbon release from permafrost soils of Northeastern Siberia. Glob. Change. Biol. 12, 1-16.

Dwairi, I.M., 1998. Evaluation of jordanian zeolite tuff as a controlled slow-release fertilizer for NH_4^+. Environ. Geol. 34, 1–4.

Dyer, A., Zubair, M., 1998. Ion-exchange in chabazite. Micropor Mesopor Mat. 22, 135-150.

Eberl, D.D., Barbarick, K.A., Lai, T.M., 1995. Influence of NH4-exchanged clinoptilolite on nutrient concentrations in sorghum-sudangrass. Natural Zeolites ’93. Occurrence, Properties, Use. D.W. Ming & F.A. Mumpton (eds.), International Committee on Natural Zeolites, Brockport, New York 491-504.

Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Minx, J.C., Farahani, E., Susanne, K., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlomer, S., von Stechow, C., Zwickel, T., 2014. Climate Change 2014: Mitigation of Climate Change, Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Erskine, P.D., Bergstrom, D.M., Schmidt, S., Stewart, G.R., Tweedie, C.E., Shaw, J.D., 1998. Subantarctic Macquarie Island ± a model ecosystem for studying animal-derived nitrogen sources using 15N natural abundance. Oecologia. 117, 187-193.

Evans, D.R., Bloom, A.J., Sukrapanna, S.S., Ehleringer, J.R., 1996. Nitrogen isotope composition of tomato (Lycopersicum Esculentum Mill. cv. T-5) grown under ammonium or nitrate nutrition. Plant, Cell & Environment. 19, 1317-1323.

Evans, D.R., 2001. Physiological mechanism influencing plant nitrogen isotope composition. Trends Plan. Sci. 6, 121-126.

Faccini, B., Di Giuseppe, D., Malferrari, D., Coltorti, M., Abbondanzi, F., Campisi, T., Laurora, A., Passaglia, E., 2015. Ammonium-exchanged zeolitite preparation for agricultural uses: From laboratory tests to large-scale application in ZeoLIFE project prototype. Period. di Mineral. 84, 303–321.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 503-537.

Ferguson, G.A., Pepper, I.L., 1987. Ammonium retention in sand amended with clinoptilolite. Soil Science Socieety of America Journal. 51, 231-234.

Ferreras, L., Gomez, E., Toresani, S., Firpo I., Rotondo R., 2006. Effect of organic amendments on some physical, chemical and biological properties in a horticultural soil. Bioresource Technol. 97, 635-640.

Ferretti, G., Faccini, B., Vittori Antisari, L., Di Giuseppe, D., Massimo, C., 2015. Nitrogen and carbon pools in an agricultural soil amended with natural and NH4-enriched K-Chabazite zeolitite. Geophysical Research Abstracts, p. 10790.

Freyer, H.D., Aly, A.I.M., 1974. Nitrogen-15 variations in fertilizer nitrogen. J. Environ. Qual. 3, 405-406.

Galli, E., Passaglia, E., 2011. Natural Zeolites in Environmental Engineering. In: Holzapfel, H. (Ed.), Zeolites in Chemical Engineering. Process Eng Engineering GmbH, Vienna, pp. 392–416.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C., Green, P.A., Holland, E., Karl, D.M., Michaels, A., Porter, J.H., Townsend, A., Vorosmarty, C., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.

Gessner, M.O., Chauvet, E., 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. 59, 502–507.

Gholamhoseini, M., Ghalavand, A., Khodaei-Joghan, A., Dolatabadian, A., Zakikhani, H., Farmanbar, E., 2013. Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and nutrient leaching. Soil Tillage Res. 126, 193–202.

Gonfiantini, R., Stichler, W., Rozanski, K., 1995. Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements, in: Stichler, W. (Eds.), Reference and Intercomparison Materials for Stable Isotopes of Light Elements. Vienna, IAEA, pp. 13-29.

Gong, P., Guan, X., Witter, E., 2001. A rapid method to extract ergosterol from soil by physical disruption. Appl. Soil Ecol. 17, 285–289.

Gualtieri, A.F., Passaglia, E., 2006. Rietveld structure refinement of NH4-exchanged natural Chabazite. Eur. J. Mineral. 18 (3), 351–359.

Güsewell, S., Gessner, M.O., 2009. N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Funct. Ecol. 23, 211–219.

Haller, H., 2015. Ammonia emissions from beech litter as source of reactive nitrogen gas in deciduous forests. Master thesis, Universität für Bodenkultur, Wien.

Handley, L.L., Raven, J.A., 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ. 15, 965-985.

Haney, R.L., Haney, E.B., 2010. Simple and Rapid Laboratory Method for Rewetting Dry Soil for Incubations. Commun. Soil Sci. Plant Anal. 41, 1493–1501.

Haruna Ahmed, O., Husin, A., Husni Mohd Hanif, A., 2008a. Ammonia volatilization and ammonium accumulation from urea mixed with zeolite and triple superphosphate. Acta Agric. Scand. Sect. B - Plant Soil Sci. 58, 182–186.

Haruna Ahmed, O., Hussin, A., Ahmad, H.M.H., Rahim, A.A., Majid, N.M.A., 2008b. Enhancing the Urea-N Use Efficiency in Maize (Zea mays) Cultivation on Acid Soils Amended with Zeolite and TSP. TheScientificWorldJOURNAL. 8, 394-399.

He, Z.L., Calvert, D. V., Alva, A.K., Li, Y.C., Banks, D.J., 2002. Clinoptilolite zeolite and cellulose amendments to reduce ammonia volatilization in a calcareous sandy soil. Plant Soil 247, 253–260.

Hodge, A., Robinson, D., Fitter, A., 2000. Are microorganisms more effective than plants at competing for Nitrogen?. Trends in plant science. 5, 304-308.

Högberg, P., 1997. Tansley review No. 95 15N natural abundance in soil-plant systems. New Phytologist. 137, 179-203.

Howarth, R.W., Walker, D., Sharpley, A., 2002. Sources of nitrogen pollution to coastal waters of the United States. Estuaries 25, 656–676.

Howarth, R.W., Ramakrishna, K., Choi, E., Elmgren, R., Martinelli, L., Mendoza, A., Moomaw, W., Palm, C., Roy, R., Scholes, M., Zhu, Z.-L., 2005. Nutrient management, responses assessment. Ecosystems and Human Well-being, Policy Responses, the Millennium Ecosystem Assessment, vol. 3. Island Press, Washington, DC, Chapter 9, pp. 295–311.

Howarth, R.W., 2008. Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae. 8, 14-20.

Hulton, B.Z., Sigman, D.M., Schuur, E.A.G., Edin, L.O., 2007. A climate-driven switch in plant nitrogen acquisition within tropical forest communities. Proceedings of the National Academy of Sciences of the United States of America. 114, 8902-8906.

Ippolito, J.A., Tarkalson, D.D., Lehrsch, G.A., 2011. Zeolite Soil Application Method Affects Inorganic Nitrogen, Moisture, and Corn Growth. Soil Sci. 176, 136-142.

IUSS Working Group WRB, 2014. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106.

Johnson, B.N., McGill, W.B., 1990. Comparison of ergosterol and chitin as quantitative estimates of mycorrhizal infection and Pinus contorta seedling response to inoculation. Can. J. For. Res. 20, 1125–1131.

Jokubauskaite, I., Slepetiene, A., Karcauskiene, D., 2015. Influence of different fertilization on the dissolved organic carbon, nitrogen and phosphorus accumulation in acid and limed soils 4, 137–143.

Kahmen, A., Wanek, W., Buchmann, N., 2008. Foliar d15N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. Oecologia. 156, 861-870.

Kennedy, B.A., 1990. Surface Mining, Second Edition. Society for Mining, Mettalurgy, and Exploration (U.S.). pp 1194.

Kitayama, K., and K. Iwamoto., 2001. Patterns of natural 15N abundance in the leaf-to-soil continuum of tropical rain forests differing in N availability on Mount Kinabalu, Borneo. Plant and Soil. 229, 203-212.

Kusaka, S., Nakano, T., 2014. Carbon and oxygen isotope ratios and their temperature dependence in carbonate and tooth enamel using GasBench II preparation device. Rapid Commun. Mass. Sp. 28, 563-567.

Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., et al., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiol. Ecol. 73, 430–440.

Kroeze, C., Aerts, R., Breemen, N., Dam, D., Hoek, K., Hofschreuder, P., Hoosbeek, M., Klein, J., et al., 2003. Uncertainties in the fate of nitrogen I: An overview of sources of uncertainty illustrated with a Dutch case study. Nutrient Cycling in Agroecosystems. 66, 43-69.

Kučić, D., Kopčić, N., Briški, F., 2013. Zeolite and potting soil sorption of CO2 and NH3 evolved during co-composting of grape and tobacco waste. Chem. Pap. 67, 1172–1180.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32, 1485–1498.

Latifah, O., Ahmed, O.H., Muhamad, A.M.N., 2011. Ammonia loss, ammonium and nitrate accumulation from mixing urea with zeolite and peat soil water under waterlogged condition. Afr. J. Biotechnol. 10, 3365-3369.

Lasa, B., Iraneta, I., Muro, G., Irigoyen, I., Tejo, P.M.A., 2011. Isotopic composition of maize as related to N-fertilization and irrigation in the Mediterranean region. Sci. Agric. (Priacicaba, Braz.). 68, 182-190.

Leggo, P.J. 2000. An investigation of plant growth in an organo-zeolitic substrate and its ecological significance. Plant. Soil. 219, 135-146.

Leitner, S., Sae-Tun, O., Kranzinger, L., Zechmeister-Boltenstern, S., Zimmermann, M., 2016. Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest. Plant Soil 1–15. doi:10.1007/s11104-015-2771-3

Lewis, M.D., Moore, F.D., Goldsberry, K.L., 1984. Ammonium-exchanged clinoptilolite and granulated clinoptilolite with urea as nitrogen fertilizers. In: Pond, W.G. and Mumpton, F.A. (Eds), Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture; International Committee on Natural Zeolites, pp. 105-111.

Leyva-Ramos, R., Monsivais-Rocha, J.E., Aragon-Pi??a, A., Berber-Mendoza, M.S., Guerrero-Coronado, R.M., Alonso-Davila, P., Mendoza-Barron, J., 2010. Removal of ammonium from aqueous solution by ion exchange on natural and modified chabazite. J. Environ. Manage. 91, 2662–2668.

Li, C., Fan, X., Mengel, K., 1990. Turnover of interlayer ammonium in loess-derived soil grown with winter wheat in the Shaanxi Province of China. Biology and Fertility of Soils. 9, 211-214.

Li, Z., Zhang, Y, Li, Y. 2013. Zeolite as slow release fertilizer on spinach yields and quality in a greenhouse test. J. Plant Nutr. 36, 1496-1505.

Liljeroth, E., Van Veen, J.A., Miller, H.J., 1990. Assimilate translocation to the rhizosphere of two wheat cultivars and subsequent utilization by rhizosphere microorganisms at two soil nitrogen levels. Soil Biology & Biochemistry. 22, 1015-1021.

Lim, S.S., Choi, W.J., Kwak, J.H., Jung, J.W., Chang, S.X., Kim, H.Y., Yoon, K.S., Choi, S.M., 2007. Nitrogen and carbon isotope responses of Chinese cabbage and chrysanthemum to the application of liquid pig manure. Plant. Soil. 295, 67-77.

Lima, D.L.D., Santos, S.M., Scherer, H.W., Schneider, R.J., Duart, A.C., Santos, E.B.H., Esteves, V.I., 2009. Effects of organic and inorganic amendments on soil organic matter properties. Geoderma. 150: 38-45.

Longobardi, F., Sacco, D., Casiello, G., Ventrella, A., Sacco, A., 2015. Characterization of the Geographical and Varietal Origin of Wheat and Bread by Means of Nuclear Magnetic Resonance (NMR), Isotope Ratio Mass Spectrometry (IRMS) Methods and Chemometrics: A Review. Agricultural Sciences. 6, 126-136.

Malekian, R., Abedi-Koupai, J., Eslamian, S.S., 2011. Influences of clinoptilolite and surfactant-modified clinoptilolite zeolite on nitrate leaching and plant growth. Journal of Hazardous Materials. 185, 970-976.

Malferrari, D., Laurora, A., Brigatti, M.F., Coltorti, M., Di Giuseppe, D., Faccini, B., Passaglia, E., Vezzalini, M.G., 2013. Open-field experimentation of an innovative and integrated zeolitite cycle: project definition and material characterization. Rend. Lincei Sci. Fis. 24, 141-150.

Mariotti, A., 1984. Natural N-15 abundance measurements and atmospheric nitrogen standards. Nature. 311, 251-252.

Martens, D.A., and J.M. Bremner. 1984. Urea hydrolysis in soils: factors influencing the effectiveness of phenylphosphorodiamidate as a retardant. Soil Biology & Biochemistry. 16, 515-519.

Marzadori, C., Vittori Antisari, L., Gioacchini, P., Sequi, P., 1994. Turnover of interlayer ammonium in soil cropped with sugar beet. Biology and Fertility of Soils. 18, 27-31.

Mastrocicco, M., Colombani, N., Salemi, E., Castaldelli, G., 2011. Reactive modeling of denitrification in soils with natural and depleted organic matter. Water. Air. Soil Pollut. 222, 205–215.

Mastrocicco, M., Colombani, N., Di Giuseppe, D., Faccini, B., Coltorti, M., 2013. Contribution of the subsurface drainage system in changing the nitrogen speciation of an agricultural soil located in a complex marsh environment (Ferrara, Italy). Agr. Water Manage. 119, 144-153.

Maxwell, T.B., Silva, L.C.R., Horwath, W.R., 2014. Using multielement isotopic analysis to decipher drought impacts and adaptive management in ancient agricultural systems. Proc. Natl. Acad. Sci. USA. 111, E4807-E4808.

McGill, W.B., Hunt, H.W., Woodmansee, R.G., Reuss, J.O., 1981. PHOENIX, a model of the dynamics of carbon and nitrogen in grassland soils, in: Clark, F.E., Rosswall, T. (Eds.), Terrestrial Nitrogen Cycles, Ecological Bullettins, Stockolm, Sweden, pp. 49-116.

McGilloway, R.L., Weaver, R.W., Ming, D.W., Gruener, J.E., 2003. Nitrification in a zeoponic substrate. Plant Soil 256, 371–378.

Mercer, B.W., Ames, L.L., Touhill, C.J., Van Slyke, W.J., Dean, R.B., 1970. Ammonia removal from secondary effluents by selective ion exchange. Journal of the Water Pollution Control Federation. 42, 95-107.

Merckx, R., Van Ginkel, J.H., Sinnaeve, J., Cremers, A., 1986. 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 and Soil. 96, 85-93.

Metcalfe, D.B., Meir, P., Aragão, L.E.O.C., Malhi, Y., da Costa, A.C.L., Braga, A., et al., 2007. Factors controlling spatio-temporal variation in carbon dioxide efflux from surface litter, roots, and soil organic matter at four rain forest sites in the eastern Amazon. J. Geophys. Res. Biogeosciences 112, 1–9.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: Mechanisms and implications of microbial adaptations to their resources. Front. Microbiol. 5, 1–10.

Morrill, L.G., Dawson, J.E., 1967. Patterns observed for the oxidation of ammonium to nitrate by soil organisms. Soil. Sci. Soc. Am. Proc. 31, 757–902.

Mühlbachová, G., Šimon, T., 2003. Effects of zeolite amendment on microbial biomass and respiratory activity in heavy metal contaminated soils. Plant, Soil Environ. 49, 536–541.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Sciences. 54, 655-670.

Natali, C., Bianchini, G., 2015. Thermally based isotopic speciation of carbon in complex matrices: a tool for environmental investigation. Environ. Sci. Pollut. Res. 22, 12162–12173.

Oenema, O., Kros, H., Vries, W., 2003. Approaches and uncertainties in nutrient budgets: implications for nutrient management and environmental policies. European Journal of Agronomy. 20, 3-16.

Öhlinger, R., 1996. Biomass-C by fumigation-extraction technique, in: Schinner, F., Kandeler, E., Öhlinger, R., Margsin, R. (Eds.), Methods in soil biology. Springer, Berlin, pp 54–56.

O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. BioScience. 38, 325-36.

Orsini, L., Rèmy, J.C., 1976. Utilization du chlorure de cobaltihexammine pour la déterminantion simultanée de la capacité d'èchange et des bases échangeables des sols. Sci. Sol. 4, 269-275.

Ozbahce, A., Tari, A.F., Gönülal, E., Simsekli, N., Padem, H., 2015. The effect of zeolite applications on yield components and nutrient uptake of common bean under water stress. Arch Agron Soil Sci. 61, 615-626.

Passaglia, E., Vezzalini, G., 1985. Crystal chemistry of diagenetic zeolites in volcanoclastic deposits of Italy. Contr. Mineral. Petrol. 90, 190-198.

Passaglia, E., Vezzalini, G., Carnevali, R., 1990. Diagenetic chabazites and phillipsites in Italy: crystal chemistry and genesis. Eur. J. Mineral. 2, 827-839

Passaglia, E., 2008. Zeoliti naturali, zeolititi e loro applicazioni. Arvan.

Perrin, T.S., Boettinger, J.L., Drost, D.T., Norton, J.M., 1998. Decreasing nitrogen leaching from sandy soil with ammonium-loaded clinoptilolite. Journal of Environmental Quality. 27, 656-663.

Rădulescu, H., 2013. Soil Treatment Effects of Zeolitic Volcanic Tuff on Soil Fertility 45, 238–244.

Reháková, M., Čuvanová, S., Dzivák, M., Rimár, J., Gaval'ová, Z., 2004. Agricultural and agrochemical uses of natural zeolite of the clinoptilolite type. Curr. Opin. Solid State Mater. Sci. 8, 397-404.

Ridha, F.N., Webley, P.A., 2009. Anomalous Henry's law behavior of nitrogen and carbon dioxide adsorption on alkali-exchanged chabazite zeolites. Sep. Purif. Technol. 67, 336–343.

Riehl, S., Pustovoytov, K.E., Weippert, H., Klett, S., Hole, F., 2014. Drought stress variability in ancient Near Eastern agricultural systems evidenced by δ13C in barley grain. Proc. Natl. Acad. Sci. USA. 111, 12348-12353.

Ruiz, G., Jeison, D., Chamy, R., 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. Water Res. 37, 1371–1377.

Russell, J.B., Cook, G.M., 1995. Energetics of bacterial growth: balance of anabolic and catabolic reactions. Microbiol. Rev. 59, 48–62.

Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., Araus, J.L., 2013. Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: Genotypic and growing conditions effects. J. Integr. Plant Biol. 56, 444-45.

Scherer, H.W., Werner, W., 1996. Significance of soil microorganisms for the mobilization of nonexchangeable ammonium. Biology and Fertility of Soils. 22, 248-251.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. Soil Biol. Biochem. 35, 549–563.

Schindlbacher, A., Zechmeister-Boltenstern, S., 2004. Effects of soil moisture and temperature on NO, NO2, and N2O emissions from European forest soils. J. Geophys. Res. 109, 1–12.

Schlesinger, W.H., 1997. Biogeochemistry: An Analysis of Global Change. Academic Press, NY.

Schmidt, O., Ostle, N.J., 1999. Tracing nitrogen derived from slurry in earthworms using 15N/14N stable isotope ratios at natural abundances. Appl. Soil Ecol. 12, 7–13.

Sepaskhah, A.R., Barzegar, M., 2010. Yield, water and nitrogen-use response of rice to zeolite and nitrogen fertilization in a semi-arid environment. Agric. Water Manag. 98, 38–44.

Silva JA, Bremner J.M., 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 5. Fixed ammonium. Soil Science Society of America, Proceedings. 30, 587-594.

Shearer, G.B., Kohl, D.H., Commoner, B., 1974. The precision of determinations of the natural abundance of nitrogen-15 in soils, fertilizers, and shelf chemicals. Soil. Sci. 118, 308-316.

Skiba, U., Fowler, D., Smith, K. a, 1997. Nitric oxide emissions from agricultural soils in temperate and tropical climates: sources, controls and mitigation options. Nutr. Cycl. Agroecosystems 48, 139–153.

Smil, V., 1999. Nitrogen in crop production: An account of global flows. Global Biogeochem. Cycles 13, 647–662.

Smil, V., 2001. Enriching the Earth. MIT Press, Cambridge, MA.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., 2007. Agriculture. In Climate Change 2007: Mitigation, Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, in: Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. pp. 497–540.

Soares, J.R., Cantarella, H., Menegale, M.L.C., 2012. Ammonia volatilization losses from surface-applied urea with urease and nitrification inhibitors. Soil Biol Biochem. 52, 82-9.

Staddon, P.L., 2004. Carbon isotopes in functional soil ecology. Trends Ecol. Evol. 19, 148-154.

Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere, The New phytologist.

Stevens, R.J., Logan, H.J., 1987. Determination of the volatilization of ammonia from surface-applied cattle slurry by the micrometeorological mass balance method. J. Agric. Sci. 109, 205.

Stevens, R.J., Laughlin, R.J., Burns, L.C., Arah, J.R.M., Hood, R.C., 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Biol. Biochem. 29, 139–151.

Stojanovic, B.J., Alexander, M., 1958. Effect of inorganic nitrogen on nitrification. Soil. Sci. 86, 208–215.

Strickland, M.S., Rousk, J., 2010. Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biol. Biochem. 42, 1385–1395.

Szpak, P., Millaire, J.F., White, C.D., Longstaffe, F.J., 2012. Influence of seabird guano and camelid dung fertilization on the nitrogen isotopic composition of field-grown maize (Zea mays). J. Archaeol. Sci. 39, 3721–3740.

Szpak, P., 2014. Complexities of nitrogen isotope biogeochemistry in plant-soil systems: implications for the study of ancient agricultural and animal management practices. Front. Plant Sci. 5, 1-19.

Tilman, D., 1998. The greening of the green revolution. Nature. 396, 211–212.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature. 418, 671-677.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry. 19, 703-707.

Vitòria, L., Otero, N., Soler, A., Canals, À., 2004. Fertilizer Characterization: Isotopic Data (N, S, O, C and Sr). Environ. Sci. Technol. 38, 3254-3262.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Technical report: human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.

Vitousek, P.M., Naylor, R., Crews, T., David, M.B., et al., 2009. Nutrient Imbalances in Agricultural Development. Science. 324, 1519-1520.

Vries, F.T., Hoffland, E., Eekeren, N., Brussaard, L., Bloem, J., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology & Biochemistry. 38, 2092-2103.

Waltz, F.C.Jr., Quisenberry, V.L., McCarty, L.B., 2003. Physical and hydraulic properties of rootzone mixes amended with inorganics for golf putting greens. Agron. J. 95, 395-404.

Wilson, H.M., Al-Kaisi, M.M., 2008. Crop rotation and nitrogen fertilization effect on soil CO2 emissions in central Iowa. Appl. Soil Ecol. 39, 264–270.

Yun, S.I., Ro, H.M., Choi, W.J., Chang, S.X., 2006. Interactive effects of N fertilizer source and timing of fertilization leave specific N isotopic signatures in Chinese cabbage and soil. Soil Biol. Biochem. 38, 1682-1689.

Zaman, M., Nguyen, M.L., Matheson, F., Blennerhassett, J.D., Quin, B.F., 2007. Can soil amendments (zeolite or lime) shift the balance between nitrous oxide and dinitrogen emissions from pasture and wetland soils receiving urine or urea-N? Aust. J. Soil Res. 45, 543–553.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecol. Monogr. 85, 133-155.

Supplementary Material

Table Sa: UA Correlation Matrix

	TDN	MB-C	MB-N	MB C/N	Ergosterol	NH_4-N	NO_3-N	NO_2-N	pH
DOC	0.49	-0.49	-0.42	0.24	-0.16	0.028	-0.26	0.22	0.09
p	0.06	0.06	0.12	0.39	0.56	0.92	0.36	0.43	0.74
TDN		-0.24	-0.44	0.46	0.18	0.42	0.13	0.32	0.04
p		0.39	0.10	0.09	0.53	0.12	0.66	0.25	0.88
MB-C			0.84	-0.42	0.43	-0.15	0.60	-0.36	0.34
p			<0.001	0.12	0.11	0.59	0.02	0.192	0.21
MB-N				-0.80	0.26	-0.07	0.38	-0.44	0.22
p				<0.001	0.34	0.81	0.17	0.105	0.44
MB C/N					-0.12	-0.10	-0.17	0.46	-0.03
p					0.68	0.73	0.54	0.08	0.93
Ergosterol						-0.19	0.81	-0.57	0.35
p						0.50	<0.001	0.03	0.20
NH_4-N							-0.36	0.25	-0.45
p							0.19	0.37	0.09
NO_3-N								-0.63	0.25
p								0.01	0.37
NO_2-N									0.14
p									0.62

Table Sb: 5NZ Correlation Matrix

	TDN	MB-C	MB-N	MB C/N	Ergosterol	NH_4-N	NO_3-N	NO_2-N	pH
DOC	0.22	0.18	0.08	-0.08	-0.11	0.09	0.28	0.26	-0.53
p	0.44	0.51	0.79	0.79	0.69	0.75	0.31	0.36	0.04
TDN		0.04	0.27	-0.12	0.65	-0.40	0.80	0.80	-0.21
p		0.89	0.34	0.68	0.01	0.14	<0.001	<0.001	0.44
MB-C			0.39	-0.20	-0.19	0.53	0.32	0.07	0.32
p			0.15	0.49	0.50	0.04	0.25	0.80	0.24
MB-N				-0.87	-0.27	0.32	0.63	0.54	0.10
p				<0.001	0.33	0.24	0.01	0.04	0.73
MB C/N					0.29	-0.26	-0.49	-0.36	-0.13
p					0.30	0.35	0.07	0.19	0.64
Ergosterol						-0.66	0.44	0.51	-0.01
p						0.01	0.11	0.05	0.75
NH_4-N							-0.12	-0.37	0.21
p							0.66	0.18	0.46
NO_3-N								0.92	-0.15
p								<0.001	0.59
NO_2-N									-0.33
p									0.23

Table Sc: 15NZ Correlation Matrix

	TDN	MB-C	MB-N	MB C/N	Ergosterol	NH_4-N	NO_3-N	NO_2-N	pH
DOC	0.25	-0.12	-0.06	0.79	-0.01	-0.20	-0.21	0.07	-0.52
p	0.37	0.67	0.83	<0.001	0.97	0.48	0.45	0.83	0.05
TDN		0.46	0.37	-0.07	-0.33	0.38	0.63	0.42	-0.25
p		0.08	0.18	0.82	0.23	0.16	0.01	0.17	0.36
MB-C			0.92	-0.61	-0.12	0.04	0.60	0.38	-0.10
p			<0.001	0.02	0.67	0.90	0.02	0.22	0.71
MB-N				-0.63	-0.05	-0.09	0.65	0.31	-0.19
p				0.01	0.86	0.75	0.01	0.33	0.50
MB C/N					0.05	-0.15	-0.60	-0.01	-0.29
p					0.86	0.60	0.02	0.97	0.30
Ergosterol						0.34	-0.13	-0.44	0.26
p						0.21	0.65	0.15	0.36
NH_4-N							0.41	0.11	0.11
p							0.13	0.73	0.70
NO_3-N								0.55	-0.21
p								0.07	0.46
NO_2-N									-0.66
p									0.02

Table Sd: 10CZ Correlation Matrix

	TDN	MB-C	MB-N	MB C/N	Ergosterol	NH_4-N	NO_3-N	NO_2-N	pH
DOC	0.23	-0.86	0.44	-0.50	-0.35	-0.47	0.41	-0.38	0.20
p	0.41	<0.001	0.10	0.06	0.20	0.08	0.13	0.17	0.47
TDN		-0.35	0.52	-0.26	-0.42	-0.31	0.55	-0.44	-0.05
p		0.20	0.05	0.34	0.12	0.26	0.03	0.10	0.86
MB-C			-0.38	-0.38	0.37	0.34	-0.39	0.39	-0.22
p			0.17	0.17	0.17	0.22	0.15	0.16	0.42
MB-N				0.75	-0.25	-0.90	0.92	-0.48	0.19
p				0.001	0.36	<0.001	<0.001	0.07	0.50
MB C/N					0.22	0.63	-0.63	0.15	-0.45
p					0.44	0.01	0.01	0.59	0.09
Ergosterol						0.20	-0.32	0.52	-0.16
p						0.47	0.25	0.05	0.57
NH_4-N							-0.86	0.53	-0.05
p							<0.001	0.04	0.85
NO_3-N								-0.54	0.19
p								0.04	0.49
NO_2-N									0.13
p									0.65

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