**High resolution short-term investigation of soil CO₂, N₂O, NOₓ and NH₃ emissions after different chabazite zeolite amendments**

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Abstract

Reducing the effects of climate change is a key-point to achieve sustainable development. It is known that fertilizer application stimulates soil gaseous N losses, especially immediately after their application. In this short-term incubation experiment, the effects of different chabazite zeolite amendments on soil gaseous emissions (CO₂, N₂O, NOₓ and NH₃) were evaluated in high resolution as potential mitigation strategy for agricultural soils. Different soil-zeolite mixtures, including both natural and NH₄⁺-enriched chabazite zeolites, were incubated for 24 h both immediately after the application of urea fertilizer and without a further N input in order to carry out a high resolution investigation of soil CO₂, N₂O, NOₓ and NH₃ fluxes, in comparison to an unamended soil. Immediate CO₂, N₂O, NOₓ and especially NH₃ emissions after fertilizer application were generally reduced in soils amended with zeolites at natural state, indicating a potential valuable material for reducing soil C-N gaseous losses. On the other hand, the application of NH₄⁺-enriched zeolites lowered CO₂ and N₂O emissions, but very high NOₓ fluxes occurred during the first 24 h even without applying any further N input. NH₃ emissions were higher in NH₄⁺-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.

Keywords: natural zeolites; NH₃; CO₂; N₂O; NOₓ; soil emissions.

1.0 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).

CO₂ and N₂O are potent GHGs whereby N₂O has a 298-fold global warming potential “GWP” of CO₂ (Edenhofer et al., 2014). In addition, nitrogen oxides (NOₓ) and ammonia (NH₃), even if not considered GHGs, can cause indirect N₂O emissions, increase acidity of precipitations and strongly influence tropospheric O₃ 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 first days after fertilizer application and these "immediate" direct flux rates are not completely known, in particular, NH₃ 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.

Since many years, natural zeolites have been recognized as valuable soil conditioners that can positively affect the soil-plant system. These aluminosilicates are characterized by a high and selective cation exchange capacity (CEC), reversible dehydration and molecular sieving that can strongly influence soil physico-chemical characteristics and N cycling by reducing N leaching, increasing nitrogen use efficiency and crop yield (Colombani et al., 2014; De Campos Bernardi et al., 2013; Gholamhoseini et al., 2013; Malferrari et al., 2013; Reháková et al., 2004). Zeolites can be easily modified from their natural state by enrichment processes which cause the adsorption of specific cations (i.e., NH₄⁺, Na⁺) (Barbarick et al., 1990; Campisi et al., 2016; Dwairi, 1998; Leggo, 2000), for this reason, the use of NH₄⁺-enriched zeolite has been proposed as “slow release fertilizer” (Barbarick and Pirela 1984; Dwairi, 1998; Faccini et al., 2015; Lewis et al. 1984). Up to now, however, very few studies exist on the effects of zeolite amendments on soil gaseous emission especially concerning natural zeolite of the chabazite type. Natural clinoptilolite zeolite as amendment to soils have been described to reduce respiratory activity probably because of the adsorption capacity of zeolites for CO₂ (Mühlbachová and Šimon, 2003). Kučić et al. (2013) showed that natural clinoptilolite zeolite adsorption potential for CO₂ and NH₃ 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 N₂O emissions during a 90-day incubation compared to unamended soils (Zaman et al., 2007). These reductions are due to the sorption of NH₄⁺ by zeolite, lowering the available substrate for microbial nitrification and hence potential losses of N₂O from nitrification and denitrification. The literature about the mitigation potential of zeolite application for NH₃ emissions is more consistent (Bundan et al., 2011; De Campos Bernardi et al., 2013; Haruna Ahmed et al., 2008; He et al., 2002; Kučić et al., 2013), however, 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 NOₓ emissions have not been reported. 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 NH₄⁺-enriched zeolites on soil gaseous emissions are completely unknown and usually the N input added with this material is very high (Ferretti et al., 2017). 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 emission after fertilizer applications.
To this end, the present study aims at elucidating the effects of different chabazite zeolites amendments on soil CO₂, N₂O, NOₓ, and NH₃ 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 treatment and condition of the ZeoLIFE experimental field, which consist in an EU funded project that is testing different chabazite zeolite amendment at the field scale in an Italian nitrate vulnerable zone (NVZ) (Nitrate Directive 91/676/EEC).

For this reason, we decided to test chabazite zeolites amendments both at natural and NH₄⁺-enriched state in comparison to an unamended soil both under an N input from urea 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 builded our experiment in order to test the following hypotheses:

1) The zeolite-derived effects on the dynamics of NH₄⁺ ions (Leyva-Ramos et al., 2010) are expected to decrease short-term N₂O, NOₓ and NH₃ emissions after fertilizer application especially in soil amended with zeolites at natural states;

2) The controlled slow NH₄⁺ release behavior of NH₄⁺-enriched chabazite zeolites is expected to not influence significantly soil N fluxes in the first 24 h, if no further fertilizer is applied notwithstanding its high N content;

3) The possibility of a CO₂ sorption and the probable influence on microbial activity (Bonenfant et al., 2008; Kučić et al., 2013; Mühlbachová and Šimon, 2003; Reháková et al., 2004; Ridha and Webley, 2009) are expected to decrease the short-term soil CO₂ emissions.

2.0 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 NH₄⁺-enriched chabazite zeolites (CZ) (created following the method proposed by Faccini et al., 2015) were added to the soil as amendments at the field scale (www.zeolife.it) (see Electronic Supplementary Material “ESM1” for the main soil properties and additional information on zeolites characteristics). 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 1 for the experimental set up) as in the ZeoLIFE experimental field.
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. These high application rates are common in countries such as Italy (in which fertilization can reach more than 340 kg ha⁻¹ in non-nitrate vulnerable zones) or even lower in comparison to some studies available in the scientific literature (e.g. Min et al., 2011; Ju et al., 2006) in which more than 600 kg N ha⁻¹ have been applied. We choose to reproduce the maximum admissible rates of the nitrate vulnerable zone where the ZeoLIFE experimental field site is located in order to get insights on the amounts of N losses immediately after the application of chemical fertilizers like urea with and without the use of zeolites as soil amendment in this particular area.

The 10CZ treatment that was applied in the ZeoLIFE field experiment was also reproduced in the present study providing an N input equivalent to approximately 497 kg N ha⁻¹. Although, the applied N is unbalanced if compared to the other treatments, this allowed to test the behaviour in presence of an excess of N above the recommendation.

This N input from CZ was mainly constituted by exchangeable NH₄⁺-N and by a little fraction of residual NO₃⁻-N from the enrichment process (ESM 1). The 10CZ treatment was tested with and without urea fertilizer, consequently it is further termed as “unfertilized” when no urea was added, while it is further termed “fertilized” when it received the additional 170 kg N ha⁻¹ from urea as all the other treatments.

CO₂ and NO₃ (NO + NO₂) 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. N₂O 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). N₂O measurements were performed once after approx. 4 hours of incubations and fluxes were calculated according to Metcalfe et al. (2007).

NH₃ 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. Moreover, we considered also the emissions per unit of N added by calculating the ratio between the amount of gas emitted divided by the total N added.

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 autosampler 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 analysed 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.

### 3.0 Results and discussions

Cumulative CO$_2$, NO$_x$ and NH$_3$ emissions over the 24 h are presented in Fig 1. Soil pH, exchangeable NH$_4$$^+$-N, NO$_3$-N mean values and N$_2$O-N fluxes are visible in Table 2, while the total amounts of volatilized gaseous species (expressed in kg N ha$^{-1}$), the reduction/increment % with respect to the CNTR and the effects of the fertilization rates on cumulative gaseous losses are shown in Table 3. The total amounts of volatilized gaseous species (expressed in kg N ha$^{-1}$), the reduction/increment % with respect to the CNTR of the treatments with no N added from fertilization (or from CZ) are visible in the Electronic Supplementary Material “ESM2”.

All zeolite amended soils (see Table 1 for treatments description) resulted in significantly lower (p<0.05) CO$_2$ emissions compared to the unamended soil (CNTR) under fertilized conditions (Fig 1a). 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 (Kučić et al., 2013; Mühlbachová and Šimon, 2003) or, alternatively, to a higher Carbon Use Efficiency (CUE) (Blagodatskaya et al., 2014; Keiblinger et al., 2010). On the other hand, regarding CO$_2$ emissions under unfertilized conditions (Fig 1b), the fluxes were not significantly different between the various treatments (p>0.05).

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$_4$NO$_3$. 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. 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).

The cumulative soil CO$_2$ emissions evolved in 24 h have been reduced by ~21 % to approximately ~30 % with respect to the CNTR soil in presence of urea fertilizer, coherently, the CNTR showed the highest emissions in relation to the total amount of N added (Table 3), even if compared to the very high amount of N supplied by CZ.

N$_2$O 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) treatments with respect to CNTR, while 15NZ treatment (soil plus 15% in weight of NZ) tended to be slightly lower albeit not statistically different (Table 2). N$_2$O 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). 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$_2$O 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 N₂O emissions with respect to CNTR. Another explanation can be that the NH₄⁺ adsorption from soil solution, managed especially by the initially N-devoid NZ, might have reduced the immediate availability of the substrate required for nitrification, thus reducing N₂O emissions (Zaman et al., 2007). The latter hypothesis is supported by our results from NZ amendments were in fact NO₃⁻-N was generally lower, but not for CZ amendment, where on the contrary NO₃⁻-N was strongly higher than in CNTR (Table 2).

NOₓ are intermediate reactive volatile compounds of both nitrification and denitrification processes and mainly produced after the addition of fertilizers (Skiba et al., 1997). Coherently, the amount of N lost as NOₓ was considerably higher after urea application compared to unfertilized conditions (Fig 1c,d). Cumulative NOₓ emissions after fertilization were significantly lower (p<0.05) in 5NZ and 15NZ treatments compared to CNTR (Fig 1c). This evidence can be partially explained by the lower NO₃⁻-N contents in both 5NZ and 15NZ treatments, which suggests that some NH₄⁺ ions were probably protected from nitrification (Table 2) in this time frame (Zaman et al., 2007). The entity of the reduction of the 5NZ and 15NZ with respect to the CNTR soil under fertilized conditions ranged from ~ 25 - 31% (Table 3).

Generally, urea fertilization caused an equal increase of the NO₃⁻-N level of around 40 mg kg⁻¹ in all the treatments. However, the NO₃⁻-N content of 10CZ soil was extremely high both with and without the addition of urea fertilizer, meaning that probably part of the N supplied with CZ has been subjected to immediate nitrification after the application to the soil. These high NO₃⁻-N levels in the short-term period needs to be taken into account for potential immediate effects on N leaching from the soil system. However, concerning NOₓ emissions, it has to be noticed that notwithstanding the excessive N level of this treatment (CZ + urea), the cumulative emissions were not significantly different from CNTR and both NZ treatments (p>0.05), thus the total amounts of NOₓ emitted per unit of N added result considerably lower (Table 3). On the other hand, 10CZ NOₓ emissions of the unfertilized soils (Fig 1d) were comparable to the fertilized one and not statistically different from the fertilized CNTR although a 3-fold N amount was applied to the same soil (Table 3). While for the other unfertilized treatments, NOₓ emissions were very low as expected. These higher emissions in 10CZ can be attributed to an immediate transformation of the N supplied by the already N-enriched CZ. In fact, the extremely high NO₃⁻-N contents suggests strongly enhanced nitrification (biotic or abiotic) in this particular treatment. Commonly, NH₄⁺-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 NO₃⁻-N content immediately after the amendment with NH₄⁺-enriched zeolites (starting from the first week). In that work, the increase in NO₃⁻-N was attributed to an exchange reaction with soil cations (which have substituted the NH₄⁺ inside the mineral lattice) and subsequently NH₄⁺ 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 (Ferretti et al., 2017; Gholamhoseini et al., 2013).

Considering the fact that without addition of urea NO$_3^-$-N levels and NO$_x$ emissions increased significantly with CZ application to the soil with respect to the CNTR, this material likely supplied an immediate available N source so that CZ should be considered as a fertilizer. However, considering the extremely higher N input from CZ (~ 3 times more than the N added from urea), the emission evolved from CZ treated soil in relation to the amount of N added were always lower with respect to an untreated soil (Table 3).

In the present study, NH$_3$ emissions evolved within the first 24 h were comparable with those observed by other authors in the literature (Bundan et al., 2011). Soil NH$_3$ emissions are known to be highly variable and dependent by many factors such as soil pH, wind speed, soil moisture and CEC (Bundan et al., 2011). Notwithstanding the soil pH was sub-alkaline in all the treatments (Table 2) and thus favourable to NH$_3$ volatilization, the relatively low NH$_3$ losses occurred in all the incubated soils may be due to the high soil CEC that, even without the addition of zeolites, can act a good retention of NH$_4^+$ ions reducing NH$_3$ volatilization phenomena (Bundan et al., 2011).

In both fertilized and unfertilized conditions, a clear reduction in NH$_3$ emissions was found (Fig. 1e-f) in NZ treatments with respect to CNTR (p<0.05). The entity of reduction after fertilization was of -24 and -55% in 5NZ and 15NZ, respectively, while in unfertilized conditions the reduction was of -40 and -60% respectively (Table 3). This behaviour is attributable to the NH$_4^+$ ions adsorption inside NZ, which can physically protect NH$_4^+$ from NH$_3$ volatilization (De Campos Bernardi et al., 2013) and it is related to the amount of NZ added in the substrate. However, 10CZ treatment showed high 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 is interesting to note 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 CNTR (Fig 1a,c), while regarding cumulative NH$_3$ emissions, those of urea-fertilized CNTR were about twice of unfertilized 10CZ (Fig 1e-f, Table 3) notwithstanding a three times lower application rate. This means that urea-N contribute much more to NH$_3$ emissions than CZ-N and that even applying a considerably higher N input, the losses by NH$_3$ volatilization can be reduced by the half.

Finally, it has to be noticed that generally, the ratio between total emissions of each gaseous species and the amounts of N added was always higher in the CNTR soil, meaning that zeolite addition (even in presence of a great excess of N from fertilization) may have important positive effects on gaseous N (and C) losses and consequently also on fertilization efficiency.
4.0 Conclusions

The outcomes of this high-resolution short-term incubation experiment highlighted the potential of zeolite application as soil amendment for the reduction of immediate gaseous N (and C) emissions. Results revealed that after 24 h of monitoring, NZ amendments seemed to reduce the immediate production of CO₂, N₂O, NOₓ, and especially NH₃ emissions after the application of urea fertilizer, constituting thus a potential valuable material for reducing short-term soil gaseous C and N losses. The amount of NZ applied (5 % or 15 % in weight) had no influences on CO₂, N₂O and NOₓ emissions, while the cumulative emissions of NH₃ were inversely related to the amount of NZ in the substrate.

On the other hand, the outcomes of this study revealed that NH₄⁺-enriched zeolites, created following the ZeoLIFE project protocol, supplied a fraction of N that was immediately subjected to gaseous losses. CZ amendment lowered CO₂ and N₂O emissions evolved during the first 24 h after fertilizer application, however, nitrification was stimulated and NOₓ 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. The emissions per unit of N added were generally higher in the CNTR soil, meaning that zeolite addition (even in presence of a great excess of N from fertilization) may play an important role in GHG and non-GHG mitigation in agricultural context and consequently also on fertilization efficiency.

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, for this reason, further long-term investigations are required.

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Climate Change 2014: Mitigation of Climate Change, Working Group III Contribution to the Fifth Assessment

Report of the Intergovernmental Panel on Climate Change.


**Figure Captions (created with SigmaPlot 12.0 and Canvas 11) ONLINE COLOR ONLY**

**Fig 1** Cumulative emissions with standard error (error bars) over the incubation of both fertilized and unfertilized treatments. Graphs “a” and “b” refers to soil CO₂ emissions, graphs “c” and “d” to soil NOₓ emissions and graphs “e” and “f” to soil NH₃ 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)
Table 1: Experimental set-up. CNTR refer to the unamended soil, 5NZ mixture is created by adding 5% in weight of natural zeolite (NZ) to the untreated soil, 15NZ mixture is created by adding 15% in weight of NZ to the untreated soil while 10CZ mixture is created adding 10% in weight of NH$_4^+$-enriched zeolites (CZ) to the untreated soil.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Zeolite amount (wt%)</th>
<th>Zeolite type</th>
<th>N input from zeolites (kg N ha$^{-1}$)</th>
<th>N input from urea (kg N ha$^{-1}$)</th>
<th>Total N input to soil (kg N ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilized with Urea</td>
<td>CNTR 0 / / 170/170</td>
<td>5NZ NZ 0/170</td>
<td>170/170</td>
<td></td>
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<tr>
<td></td>
<td>5NZ 5 NZ 0/170</td>
<td>15NZ NZ 0/170</td>
<td>170/170</td>
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<td></td>
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<tr>
<td></td>
<td>10CZ 10 CZ 497/667</td>
<td></td>
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<td></td>
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<tr>
<td>Unfertilized (no Urea added)</td>
<td>CNTR 0 / / 0/0</td>
<td>5NZ NZ 0/0</td>
<td>0/0</td>
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<tr>
<td></td>
<td>5NZ 5 NZ 0/0</td>
<td>15NZ NZ 0/0</td>
<td>0/0</td>
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<td></td>
<td>10CZ 10 CZ 497/497</td>
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Table 2: Soil pH, exchangeable NH$_4^+$-N, NO$_3^-$-N mean values (measured at the end of the incubation) and N$_2$O-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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>NH$_4^+$-N (mg kg$^{-1}$)</th>
<th>NO$_3^-$-N (mg kg$^{-1}$)</th>
<th>N$_2$O-N (μg m$^{-2}$ h$^{-1}$)</th>
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<tr>
<td><strong>Unfertilized</strong></td>
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</tr>
<tr>
<td>CNTR</td>
<td>7.73 (0.04)$^a$</td>
<td>10.1 (0.4)$^a$</td>
<td>54.3 (0.7)$^b$</td>
<td>n.m</td>
</tr>
<tr>
<td>5NZ</td>
<td>7.80 (0.04)$^a$</td>
<td>9.7 (0.3)$^a$</td>
<td>47.9 (0.4)$^a$</td>
<td>n.m</td>
</tr>
<tr>
<td>15NZ</td>
<td>7.93 (0.06)$^a$</td>
<td>9.5 (0.2)$^a$</td>
<td>53.7 (0.9)$^b$</td>
<td>n.m</td>
</tr>
<tr>
<td>10CZ</td>
<td>7.79 (0.04)$^a$</td>
<td>79.6 (6.9)$^b$</td>
<td>309 (6.4)$^c$</td>
<td>n.m</td>
</tr>
<tr>
<td><strong>Fertilized with Urea (170 kg-N ha$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNTR</td>
<td>7.94 (0.02)$^a$</td>
<td>9.7 (0.3)$^a$</td>
<td>97.4 (0.9)$^b$</td>
<td>358 (69)$^b$</td>
</tr>
<tr>
<td>5NZ</td>
<td>7.99 (0.06)$^a$</td>
<td>10.1 (0.2)$^a$</td>
<td>84.7 (0.5)$^a$</td>
<td>165 (37)$^a$</td>
</tr>
<tr>
<td>15NZ</td>
<td>8.12 (0.09)$^a$</td>
<td>9.9 (0.4)$^a$</td>
<td>83.6 (1.0)$^a$</td>
<td>281 (21)$^{ab}$</td>
</tr>
<tr>
<td>10CZ</td>
<td>8.11 (0.10)$^a$</td>
<td>81.6 (8.3)$^b$</td>
<td>347 (12)$^c$</td>
<td>155 (11)$^a$</td>
</tr>
</tbody>
</table>
Table 3: Cumulative NOₓ, NH₃ and CO₂ emissions over 24 h, % of reduction or increment with respect to the CNTR soil and ratio between cumulative emissions and N added.

For treatments with no N added see ESM 2. 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).

<table>
<thead>
<tr>
<th></th>
<th>N input from zeolites (kg N ha⁻¹)</th>
<th>N input from Urea (kg N ha⁻¹)</th>
<th>24 h cumul. CO₂-C (kg C ha⁻¹)</th>
<th>Reduction %</th>
<th>CO₂-C/N added</th>
<th>24 h cumul. NOₓ-N (kg N ha⁻¹)</th>
<th>Reduction %</th>
<th>NOₓ-N/N added</th>
<th>24 h cumul. NH₃-N (kg N ha⁻¹)</th>
<th>Reduction %</th>
<th>NH₃-N/N added</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNTR</td>
<td>0</td>
<td>170</td>
<td>425 (60)b</td>
<td>-</td>
<td>2.5</td>
<td>1.11 (0.06)b</td>
<td>-</td>
<td>0.007</td>
<td>0.42 (0.05)c</td>
<td>-</td>
<td>0.003</td>
</tr>
<tr>
<td>5NZ</td>
<td>0</td>
<td>170</td>
<td>334 (10)a</td>
<td>-21</td>
<td>2.0</td>
<td>0.77 (0.06)a</td>
<td>-31</td>
<td>0.005</td>
<td>0.32 (0.02)b</td>
<td>-24</td>
<td>0.002</td>
</tr>
<tr>
<td>15NZ</td>
<td>0</td>
<td>170</td>
<td>323 (51)a</td>
<td>-24</td>
<td>1.9</td>
<td>0.83 (0.17)a</td>
<td>-25</td>
<td>0.006</td>
<td>0.19 (0.01)a</td>
<td>-55</td>
<td>0.001</td>
</tr>
<tr>
<td>10CZ</td>
<td>497</td>
<td>0</td>
<td>457 (85)b</td>
<td>-</td>
<td>0.9</td>
<td>1.29 (0.15)b</td>
<td>-</td>
<td>0.003</td>
<td>0.18 (0.04)b</td>
<td>-57</td>
<td>0.0004</td>
</tr>
<tr>
<td>10CZ</td>
<td>497</td>
<td>170</td>
<td>298 (34)a</td>
<td>-30</td>
<td>0.4</td>
<td>1.01 (0.16)b</td>
<td>-</td>
<td>0.002</td>
<td>0.52 (0.06)b</td>
<td>+24</td>
<td>0.001</td>
</tr>
</tbody>
</table>