

# Soil type and microclimatic conditions as drivers of urea transformation kinetics in maize plots

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## Abstract

This study presents a multidisciplinary approach (hydrological, microbiological, agronomic) to determine the dynamics of transformation of synthetic urea applied as soil fertilizer during maize production. The approach consisted in field and laboratory experiments on urea hydrolysis, ammonia volatilisation and nitrification, in four soil types (named Silty-loam, Silty-clay, Peat and Sand) intensively fertilized with synthetic urea. The field plots were modelled with HYDRUS-1D to determine the fate and transport of N species in the top soils. The numerical models successfully captured the main N transformations throughout the simulated period. In addition to the field monitoring of nitrogen species, microbial C and N, and urease activity were screened in each soil. The field soil sampling highlighted that the kinetics of ammonification was consistent with the hydrolysis of urea except for the sandy soil; kinetics of ammonification decreased in the order: Silty-clay>Peat>>Silty-loam>Sand. The differences of urease activities and nitrification potential rates between soils supported the measured field nitrification rates, and were as follow in order of decreasing rate: Silty-clay>Silty-loam>Peat>Sand. The lowest nitrification rates pertained to the inherently more vulnerable sandy soil due to the scarcity of nitrifiers. The performance of volatilization was as follow in order of decreasing rate: Sand>> Silty-loam>Peat>Silty-clay; but in general very low volatilization rates were found. This was imputed to the concurrence of both elevated soil CEC that promoted ammonium sorption and to low wind speed in the monitored plots. The presented multidisciplinary approach should be employed in many other agricultural settings to obtain robust data for numerical models simulations on the fate and transport of reactive N species in agricultural lands.

**Keywords:** urea fertilizer; nitrification rate; ammonia volatilization; numerical modelling; relative humidity; cation exchange capacity.

## 1. Introduction

In the last decades, the use of nitrogen fertilizer has increased exponentially at a global scale (Vitousek, 1997; Galloway, 2008) with detrimental consequences on freshwater and marine ecosystems (Jorgensen and Richardson, 1996; Ongley, 1996), on nitrous oxide emissions and greenhouse effect (Turner, 1991; Gruber and Galloway, 2008) and on groundwater and drinking water quality. This has caused serious health problems worldwide, such as methahemoglobinemia or ‘‘Blue Child Syndrome’’ (Fan and Steinberg, 1996), congenital malformations (Dorsch et al., 1984) and different forms of cancer (Hill et al., 1973; Ward et al. 1996).

In the last three decades nitrate and ammonium based fertilizers have been progressively abandoned in favour of the use of urea (FAO-IFA, 2001; Gilbert et al., 2006). Nevertheless, only few studies have focused on urea transformations in different soil types, and the consequences on nitrogen fate in surface and groundwater pollution.

Literature reports that urea hydrolysis is rapid and usually occurs within three days after distribution (Yadav et al., 1987; Sankhayan and Shukla, 1976). Factors as timing and modalities of application (Al-Kanani et al., 1991; Khakural et al., 1995), soil moisture (Sankhayan and Shukla, 1976; Prasertsak et al., 2001; Wali et al., 2003), soil type (Sturnpe et al., 1984; Khalil et al., 2009) and air temperature (Bolado Rodríguez et al., 2005) have been evidenced to have a predictable effect on urea hydrolysis. Other relationships remain somehow controversial, as in the case of organic matter, which in some soils seems to increase urease activity (Kanchikerimath and Singh, 2001; Liang et al. 2014; Yadav et al., 1987) and in others to decrease it (Sankhayan and Shukla, 1976).

Once hydrolysis has occurred, several processes regulate nitrogen fate. Released ammonia may adsorb onto soil particles, be assimilated by crops, volatilized to the atmosphere or be nitrified to nitrate (Pan et al., 2016; Sommer and Hutchings, 2001). Particularly, ammonia volatilization is of great concern since it can be greatly influenced by micrometeorological patterns, crop and soil types (Ferrara et al., 2014; Li et al., 2017). The nitrate not assimilated by crops is likely to be lost in runoff water, leached to groundwater (Ju et al., 2006; Mastrocicco et al., 2011) and/or be denitrified to  $N_2$  and  $N_2O$  (Jahangir et al., 2012; Zhang et al., 2015). All these processes may be influenced at microscale by all above cited parameters regulating urea hydrolysis, in a series of interconnected microbiological, physical and hydrogeological processes, which should be considered as a whole to understand and predict reactive nitrogen fate, but are very difficult to tackle when working in the field. In fact, incomplete description of processes involved and the difficulty of having unambiguous input parameters remain the main limitations in describing urea and nitrogen fate in soils, as pointed out with dedicated model simulations (Marjerison et al., 2016; Michalczyk et al., 2014; Tan et al., 2015). In order to improve process based numerical models of reactive nitrogen emission and leaching, scientific literature must still gather studies with accurate and realistic data on reactive nitrogen emission and leaching mechanisms.

This paper presents the results of an interdisciplinary study on nitrogen transformations and fate achieved through field and laboratory experiments on urea hydrolysis, ammonia volatilisation and nitrification, in four soil types intensively fertilized with synthetic urea. The aim of this study is to quantify the kinetics of urea transformation observed in the field by means of a deterministic numerical model, and highlight the role of soil type and local meteorological conditions in driving the reactive nitrogen speciation in topsoils.

## 2. Materials and methods

### 2.1 Study sites

This study was conducted in the Po Plain lowlands, which are intensively farmed due to the flat topography and to the abundance of surface water for irrigation. Here, the primary land use is maize cropping. In the study area, located in Ferrara Province (Italy) at an altitude ranging from 5 to -3 m

above sea level, four sites (named Silty-clay, Silty-loam, Peat and Sand) were selected to monitor nitrogen transformations and transport in the unsaturated zone (Figure 1).

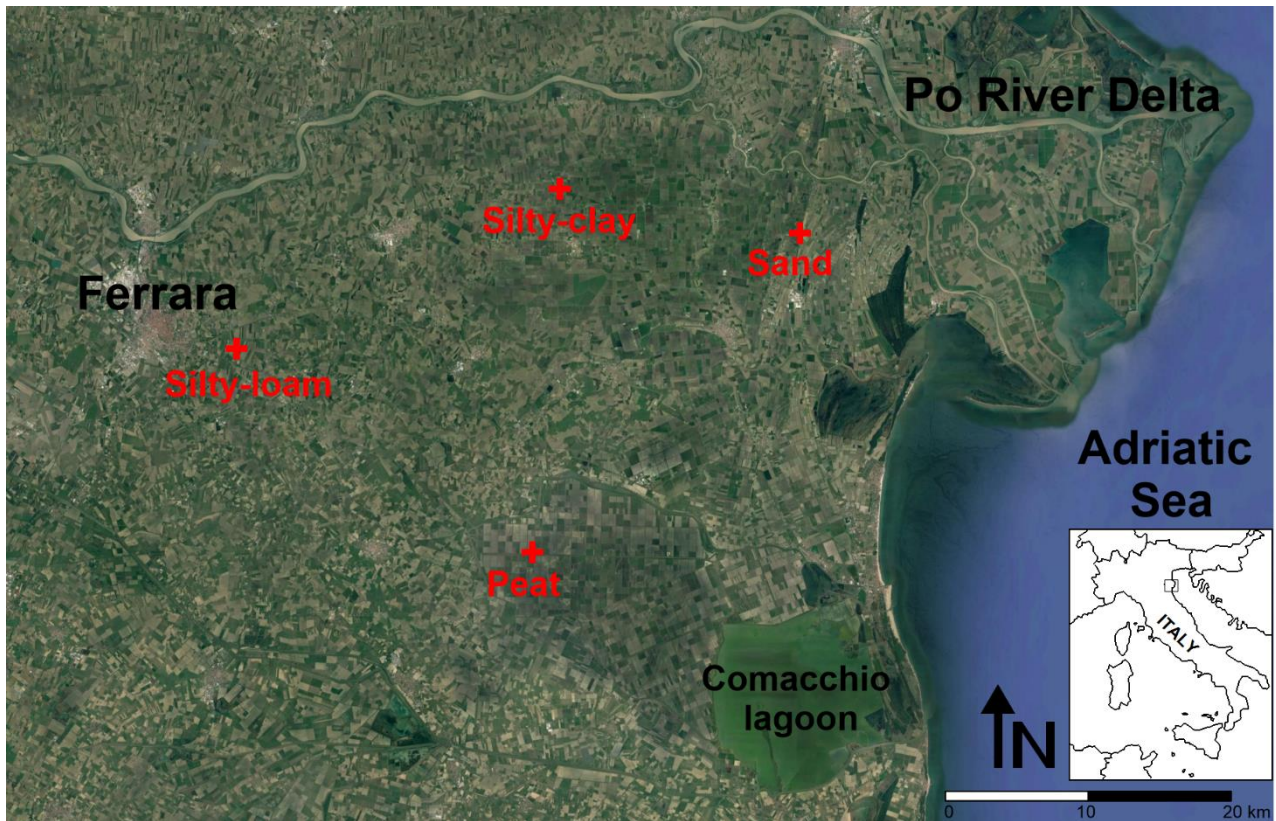


Figure 1: Sites' location within the lowland agricultural landscape of the Ferrara Province portrayed by a TerraMetrics 2016 image sourced from Google Earth.

For field site setup and characterisation, see Mastrocicco et al. (2010) and Mastrocicco et al. (2012). Meteorological stations recording rainfall, wind speed, air temperature and relative humidity every hour are located from 0.5 to 1.5 km far from the field sites and are available on-line from the meteorological regional service ([www.smr.arpa.emr.it/dext3r](http://www.smr.arpa.emr.it/dext3r)). Each field site was selected far from towns and buildings to avoid microclimatic effects induced by concrete pavements and surfaces, and to be representative of microclimatic conditions in agricultural environments. Although, sites are relatively near to each other with linear distances between 17 and 30 km (Fig. 1), the microclimates of Silty-clay and Silty-loam sites are influenced by the Po river, while the microclimates of Peat and Sand sites are influenced by the Adriatic Sea (Pavan et al., 2008). A medium maturing maize hybrid (*Zea mays* L., FAO 500, 125 days), commonly employed in this area, was selected to be grown in the four field sites, at density of 7 plants  $m^{-2}$ . Three replicate plots of 10×20 m were cultivated at each site and used to derive urea transformation and its standard deviation. The application of urea fertilizer was performed following the suggested best management practices delivered by the Emilia-Romagna Region, using the software CRITERIA that takes into account crop type, soil type, crop rotation and residual nitrogen in soil. The field experiments were conducted from the beginning of May 2008 to the end of August 2008, the urea was applied using the classical broadcast spreading, in previously ploughed fields to be representative of the local farmers' practices. The urea was applied 10 days before the maize seedling growth stage. Ploughing was performed before winter and consisted of a fall mouldboard ploughed down to 40 cm followed by disc harrow with no additional tillage operations prior to seeding and soil sampling. The applied urea-N rates were: 200 kg-N/ha for the Silty-clay, 230 kg-N/ha for the Silty-loam, 270 kg-N/ha for the Peat and 250 kg-N/ha for the Sand.

## 2.2 Field measurements

### 2.2.1 Soil characterization

From collected core samples, particle size curves were obtained using a settling tube for the sandy fraction and an X-ray Micromeritics Sedigraph 5100 for the finer one. Organic matter content (SOM) was measured by loss of ignition method, while dry bulk density and porosity were determined gravimetrically. Soil pH was determined using 1:1 soil/water ratio. CaCO<sub>3</sub> and CEC data were sourced from the soil on-line database of Emilia-Romagna region (<https://agri.regione.emilia-romagna.it/Suoli>).

### 2.2.2 C and N microbial biomass in soils

Twelve soil samples were collected (3 for each field site) in duplicates two days after fertilization, sealed in vacuum bags to maintain soil water content at field condition, stored in a cool box at 4°C and immediately transported to the laboratory. The soil samples were adjusted to field capacity according to the estimates published by Saxton and Rawls (2006), and conditioned for 7 days at 22°C. For the extraction of C and N microbial biomass ( $C_{mic}$ , and  $N_{mic}$ , respectively), two sets of soil samples, one fumigated with ethanol-free chloroform for 24 h in the dark at 25°C in a desiccator and the other non-fumigated, were treated according to the procedure proposed by Vance et al. (1987) for  $C_{mic}$  and to the procedure proposed by Horwath and Paul (1994) for  $N_{mic}$ .

The concentration of organic C in the extract was determined with a carbon analyzer (Carbon Analyzer Shimadzu TOC-V-CSM) after acidification with one drop of 2 M HCl to remove any dissolved carbonate.

$C_{mic}$  was calculated as follows (Vance et al., 1987):

$$C_{mic} = \frac{E_C}{k_{EC}} = \frac{(C_{fs}) - (C_{nfs})}{k_{EC}} \quad (1)$$

where  $C_{fs}$  is the organic C extracted from fumigated soil and  $C_{nfs}$  is the organic C extracted from non-fumigated soil, while  $k_{EC}$  (0.45) is a proportionality factor for converting the  $E_C$  (extracted carbon) value to  $C_{mic}$  (Wu et al., 1990).

The concentration of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in the extract were determined using a double beam Jasco V-550 UV/VIS spectrophotometer.

$N_{mic}$  was calculated as follows (Jenkinson, 1988):

$$N_{mic} = \frac{E_N}{k_{IN}} = \frac{(N_{fs}) - (N_{nfs})}{k_{IN}} \quad (2)$$

where  $N_{fs}$  is the flush of NH<sub>4</sub><sup>+</sup>-N due to fumigation and  $N_{nfs}$  is the NH<sub>4</sub><sup>+</sup>-N produced in the non-fumigated soil during the 10 d of incubation, while  $k_{IN}$  (0.54) is the proportionality factor for converting the  $E_N$  (extracted nitrogen) to  $N_{mic}$ .

### 2.2.3 Assay of urease activity

Subsamples for urease analysis were passed through a 2 mm sieve and kept at 4°C until the analyses were performed. Urease activity in soils was assayed by the buffer method (Tabatabai, 1994), which involves the determination of the NH<sub>4</sub><sup>+</sup>-N released when a soil sample is incubated with THAM buffer at the optimal pH (pH 9.0) with or without toluene, and urea (0.2 M) at 37°C for 2 h. When chloroform fumigation was used, urease activity was assayed within 1 h after removing the chloroform fumes by evacuation. The urease microbial activity was calculated by subtracting from the total urease activity the extracellular one.

### 2.2.4 Potential nitrification rate

Potential nitrification rate (PNR) gives an estimate on the abundance and activity of nitrifiers capable of oxidizing ammonia when oxygen and ammonium concentrations are not limiting (Mayer et al., 1995). PNR in soil samples was determined as the linear accumulation of nitrite with time due to chlorate inhibition, using the simple and sensitive azo-dye method according to the methodology proposed by Belser and Mays (1980). Calculated rates were referred to a unit of volume of measured density and expressed as potential nitrification activity of the sampled layer (0-15 cm) on a surface area of one hectare.

### **2.2.5 Field urea transformations**

Core samples (8 cm i.d.) from the top soil (0-15 cm) were taken in triplicates in each plot (10x20 m) at the four sites, at least 12 times during the entire crop cycle. Sampling was intensified to daily frequency after urea distribution. Samples for inorganic nitrogen were extracted in 2N KCl (1:10 w/v) on a rotary shaker for 1 h. Ammonium and nitrite were analysed using a double beam Jasco V-550 UV/VIS spectrophotometer, nitrate was analysed with a Technicon Autoanalyser II. All concentrations in the extracts were then expressed in kg-N/ha.

### **2.3 Laboratory experiments on ammonia volatilisation**

The effects of microclimate (wind speed, humidity and air temperature) were simulated in the laboratory: here, the wind speed was considered to be equivalent to the imposed flow rate, air humidity was controlled by the soil water content and air temperature was kept similar to field conditions. Ammonia volatilisation was estimated in laboratory batch experiments in triplicates (Bolado Rodríguez et al., 2005; Peng et al., 2015). Six hundred grams (dry weight) of each soil was placed in a 1 l incubation bottle, with a soil surface area of 86.5 cm<sup>2</sup>. The soil water content was kept at field capacity according to the estimates published by Saxton and Rawls (2006). Urea was uniformly applied on the soil surface at the amount of 420 mg N per bottle, equivalent to 240 kg-N/ha; the controls did not receive any addition. The bottles were closed with silicone rubber bungs fitted with gas inlet and outlet ports, and the circumference of the bungs were sealed with wax.

Before inlet, an air humidification system was placed while after the outlet, three tubes containing 15 ml of 0.01 N H<sub>2</sub>SO<sub>4</sub> solution were disposed in sequence.

The bottles were connected to 2 l Erlenmeyer flasks, connected to a water pump which maintained a constant depression and continuous flow of 10±0.5 ml/min, for the 14 days of the incubation. Back-flushing from the trap to the bottle was prevented by placing a sealed 20 ml empty test tube between each bottle and the tubes of the trap. Previous tests confirmed trap efficiency, with recoveries >95% of known amounts of ammonia (data not shown). All data of ammonia production in urea amended bottles were corrected for blank. Incubation temperature of 20°C was chosen from mean field temperature in the last week of May, when urea was distributed in experimental fields; while the continuous flow employed was considered representative of the very low wind speed recorded in the fields during the urea spreading and in the following week.

### **2.4 Statistical analysis**

The field dataset was analysed via multiple linear regression analysis, using Pearson correlation tests and the Ljung-Box Q statistic (LBQ) to assess the degree of correlation among variables. The Ljung-Box Q statistic indicates whether the residuals of the regression model show structural patterns. The analysis was performed using proForecaster 2.1 and Microsoft Excel 2016. One-way analysis of variance tests (ANOVA) were carried out in Microsoft Excel 2016 to investigate interrelationships between parameters. ANOVA tests determine whether there are differences in the means of two or more groups. These tests were used to investigate if there were any statistically significant differences

in parameters collected in different soils. Statistical significance was defined by a threshold of  $*P \leq 0.05$  for all statistic tests undertaken. Analyses of variance were performed on untransformed data and mean separation was accomplished using Tukey's HSD test.

## **2.5 Numerical flow model**

The daily water fluxes were calculated using the finite element model Hydrus-1D (Šimunek et al. 2008), each model domain was discretized in 101 nodes of 15 mm each, to form a regular vertical grid of 15 cm. The van Genuchten (1980) parametric functions were selected to solve Richards' equation for saturated–unsaturated water flow. The soil hydraulic parameters (Table 2) were assigned using the values found by Mastrocicco et al. (2010) and Colombani et al. (2015) except for the saturated hydraulic conductivity. The initial water content was assumed to be constant throughout the model profile. At the soil surface, variable flux and head conditions were selected to represent the atmospheric boundary. Daily reference evapotranspiration was implemented in the model using the FAO-56 recommended Penman–Monteith equation the appropriate crop coefficients for maize (Allen et al., 1998). The actual root water uptake was simulated using a S-shaped function (van Genuchten, 1985). Variable crop heights depending on the phenological state and maximum rooting depths, defined by visual inspection during core sampling, were used as input values. The lower boundary condition was set as a free drainage surface.

## **2.6 Numerical reactive transport model**

The reactive network model used to simulate the sequential transformations of four N species (from urea to ammonium, nitrite and nitrate) was the one proposed by van Genuchten (1985), coupled with linear sorption for ammonium and ammonia volatilization via a first order reaction (Šimunek et al. 2008). Denitrification was considered negligible in these environments due to aerobic conditions. The initial N species concentrations were assumed to be constant throughout the model profile. A concentration flux upper boundary condition was applied to each model, while a zero concentration gradient was applied as lower boundary condition. The linear sorption coefficients were estimated using CEC of each soil (Erskine 2000), while first order volatilization rates were estimated from laboratory experiments on ammonia volatilisation (Table 2). Diffusion values, like molecular diffusion in water and in air and Henry coefficient for ammonia were kept constant for all sites. The Marquardt-Levenberg type parameter optimization algorithm included in HYDRUS-1D was employed to calibrate the following parameters for each soil: saturated hydraulic conductivity, vertical dispersivity, urea to ammonium rate, ammonium to nitrite rate and nitrite to nitrate rate. Field concentrations of urea, ammonium, nitrite and nitrate in each site were employed as observed values. The model performance was evaluated using the modelling efficiency (EF) as proposed by Nash and Sutcliffe (1970).

## **3. Results and discussion**

### **3.1 Soil properties and meteorological parameters controlling urea dissolution**

General properties of considered soils are reported in Table 1. All soils were slightly basic or neutral, including the Peat whose pH was corrected four years before the experiment, using sugar-mill waste lime. Before synthetic urea fertilization, urea, ammonium and nitrite concentrations were low and close to the limits of detection and only nitrate presented significant concentrations that ranged from 17 to 32 kg-N/ha (Supplementary Information).

Table 1. Selected properties of investigated soils. In parenthesis, the standard deviation over 5 replicates is shown.

Parameter	Unit	Sand	Silty-loam	Silty-clay	Peat
Porosity	(-)	0.32 (0.04)	0.42 (0.05)	0.54 (0.06)	0.58 (0.03)
Dry bulk density	(g/cm <sup>3</sup> )	1.23 (0.04)	1.41 (0.16)	1.21 (0.06)	1.03 (0.02)
SOM	(%)	2.3 (0.06)	1.7 (0.01)	4.09 (0.13)	20.61 (0.25)
pH	(-)	7.5 (0.17)	7.36 (0.15)	7.53 (0.15)	7.31 (0.09)
Clay	(%)	2.0 (1.2)	25.3 (3.2)	57.8 (5.1)	12.0 (2.0)
Silt	(%)	7.9 (0.9)	63.2 (4.7)	37.2 (2.2)	70.1 (3.2)
Sand	(%)	90.1 (5.8)	11.5 (1.1)	4.0 (1.7)	17.9 (0.5)
CaCO <sub>3</sub>	(%)	2.1 (0.5)	10.3 (0.9)	5.0 (0.3)	3.9 (0.4)
CEC	(meq/100 g)	9.2 (1.0)	15.3 (2.4)	29.1 (2.3)	25.2 (4.1)

Urea granules dissolution was almost immediate and occurred overnight at the Silty-clay and Silty-loam sites. While in the other two sites dissolution occurred within two days after distribution, due to difference in soil moisture and intense humidity at night, typical of the studied area in spring. This is shown in figure 2, where wind speed, air temperature and relative humidity recorded hourly during the week of synthetic urea spreading are plotted for each site.

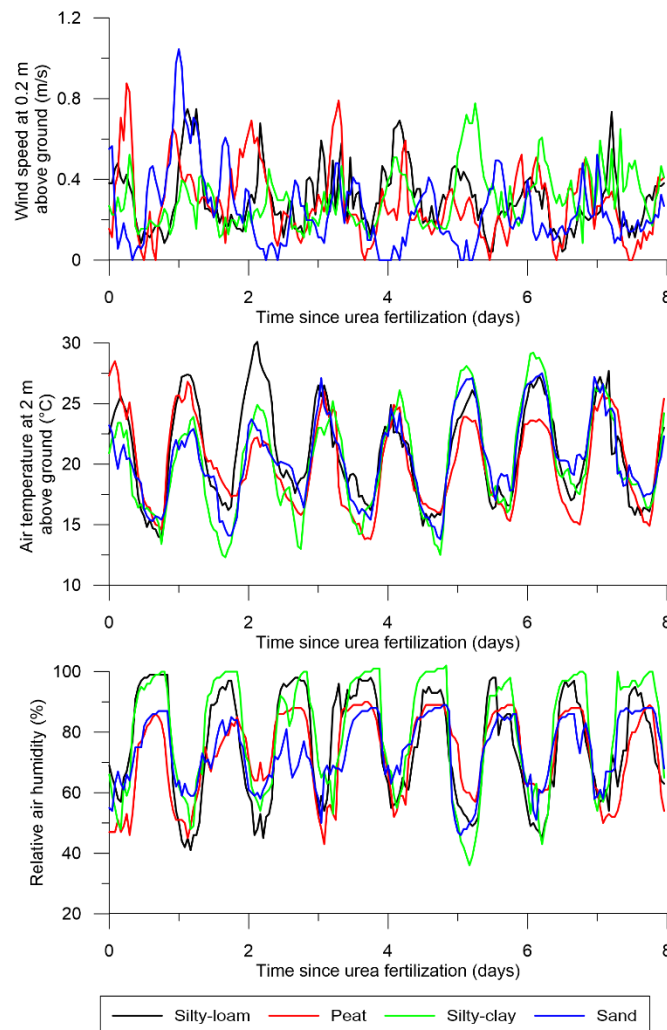


Figure 2: Wind speed at 0.2 m above ground during the week of synthetic urea spreading at each site (upper panel). Hourly air temperature recorded at 2 m above (middle panel) and relative air humidity in the same period (lower panel).

The graphs show that day-night air temperature and relative humidity fluctuations in this area are similar but not identical. However, prevailing local meteorological features, like low wind speed and high values of relative humidity, ensured that consistent night dew (no difference between air temperature and dew point) could develop only in the Silty-clay and in the Silty-loam sites. Meanwhile, in the Peat and Sand sites night dew was a less pronounced phenomenon. This produced a different dissolution rate of the urea granules applied to the soil surface, explained in detail in the following section.

### 3.2 Field monitoring of urea transformations

In all soil types, urea ammonification was rapid, in agreement with previous studies (Yadav et al., 1987; Sankhayan and Shukla, 1976), but the kinetic of this process was different from site to site. In the Silty-clay site urea ammonification was extremely fast, here in less than four days urea was completely converted to ammonium (Figure 3), which was concomitantly nitrified to nitrate without any apparent loss due to ammonia volatilization.

In fact, the reactive N amount (sum of mineral nitrogen species and urea) did not decrease during the first week after fertilizer application (Figure 3). In this case, nitrite appeared at relatively high concentrations, suggesting inhibition of nitrification reactions (Shen et al., 2003). The apparent urea ammonification rate was slightly slower in the Peat site (one week), again with concomitant nitrification to nitrate and without apparent loss due to ammonia volatilization (see Figure 4 for details). The apparent urea ammonification rate in the Silty-loam was similar to the Peat (one week), but nitrate concentrations did not increase sharply as in the Peat site. In contrast, it took more than 3 weeks to achieve complete mineralization of urea in the Sand soil, and thus exhibited the lowest ammonification and nitrification rates of all four soil types. For instance, complete nitrification took around two months to take place in Sand soil (Figure 3 and Supplementary Information). This has potentially favoured high ammonia volatilization in Sand soil due to high ammonium concentrations present for longer time in the topsoil. Table 2 reports the first order field transformation rates for each investigated soil. The rate of urea-to-ammonium conversion was in the order: Silty-clay>Peat>Silty-loam>Sand; the ammonium to nitrite rate was in the order: Silty-clay>Silty-loam>Peat>Sand; and the nitrite to nitrate rate was in the order: Silty-loam>Silty-clay>Sand>Peat. Thus the Silty-clay site appears to be the most reactive one in transforming the applied urea even more than the Peat site, while the Silty-loam and Sand sites are the less reactive ones. The observed values are in the range of literature field values using different experimental techniques (Burger and Jackson, 2003; Rosenkranz et al., 2010). In addition, table 2 reports the calibrated values of saturated hydraulic conductivity and vertical dispersivity, while the first parameter varies of more than one order of magnitude, the latter is restricted near the value of 0.02 m given the small distance travelled (0.15 m). Also these parameters values are in line with results in similar soils (Mastrocicco et al. 2010; Mastrocicco et al. 2011). Finally, the calculated modelling efficiencies are satisfactory for all the modelled sites, although the Silty-clay site performed a little worse than the others.

Hourly records of precipitation in the first week after urea distribution indicate the absence of intense events and a small amount of precipitations fallen in the Sand and Peat, enough to dissolve completely urea granules but not to generate any runoff or vertical transport (Figure 3). After 10 days since fertilization, precipitations triggered nitrate percolation in all the sites except for the Sand site, with quite different behaviours of the monitored reactive N species site by site. In the Silty-clay site, where all the urea was already converted to nitrate, leaching combined with plant uptake by roots was the driving mechanism of nitrate disappearance. Despite the elevated CEC of the Silty-clay (Table 1), ammonium was not retained by the topsoil, since it was nearly immediately converted into nitrate.



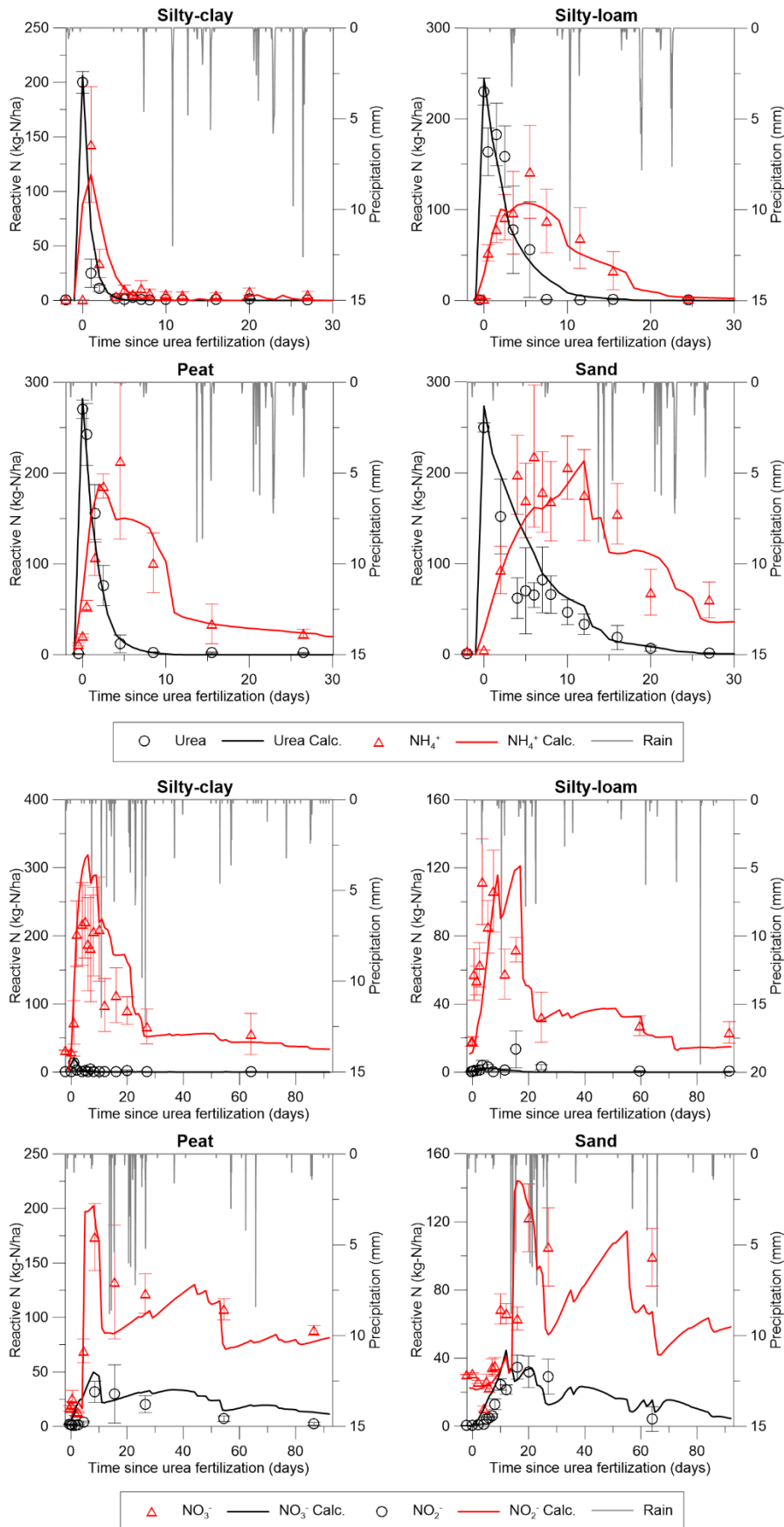


Figure 3: Major reactive N species present in the topsoil (symbols) after synthetic urea spreading at each site (right Y-axis) and modelled reactive N species (lines). Recorded precipitations in the same period are also shown (left Y-axis). Error bars represent the standard deviation of nine replicates.

Table 2. HYDRUS-1D model parameters employed to simulate the transport and transformation of reactive N species in each investigated soil. The corresponding calculated modelling efficiency values are also reported at the end.

Parameter	Unit	Sand	Silty-loam	Silty-clay	Peat
<b>Ammonia volatilization rate</b>	(1/d)	1.96	0.66	0.14	0.39
<b>Ammonium sorption coefficient</b>	(m <sup>3</sup> /kg)	1.28e <sup>-5</sup>	2.14e <sup>-5</sup>	4.04e <sup>-5</sup>	3.51e <sup>-5</sup>
<b>Saturated hydraulic conductivity*</b>	(m/d)	1.21	0.06	0.04	0.10
<b>Vertical dispersivity*</b>	(m)	0.018	0.020	0.023	0.021
<b>Urea to ammonium rate*</b>	(1/d)	0.16	0.28	1.1	0.5
<b>Ammonium to nitrite rate*</b>	(1/d)	0.03	0.12	0.8	0.05
<b>Nitrite to nitrate rate*</b>	(1/d)	0.1	5.9	5.2	0.05
<b>Modelling efficiency</b>	(-)	0.88	0.91	0.85	0.90

\*Parameters derived from the inverse calibration procedure.

This is also supported by the elevated Pearson coefficient between ammonium and nitrite ( $r=0.97$  at  $*P<0.05$ ), and LBQ ( $3.53<27.59$ ) showing a low residual autocorrelation between the two variables. In contrast, after the complete hydrolysis of urea, some ammonium was temporarily trapped in the clay interlayers, organic matter and/or iron hydroxides of the Peat, Silty-loam and Sand sites. This is consistent with the decreasing CEC of the three different soils (Table 1) and with the lower apparent ammonification rate in the same soils (Table 2). The temporary sequestration of ammonium in these soils possibly minimized the ammonia volatilization (Reynolds and Wolf, 1987). The statistical analysis showed that ammonium was positively correlated between the Peat and Silty-loam site (Table 3), with a  $r$  of 0.89  $*P<0.05$  and a low LBQ ( $6.57<27.59$ ). The similar behaviour of ammonium in these sites can be attributed to elevated CECs and very similar apparent nitrification rates (Table 2). The ammonium correlation decreased between Silty-loam and Sand (Table 3), with a  $r$  of 0.81 at  $*P<0.05$  and an elevated LBQ ( $28.92<27.59$ ) showing autocorrelation between variables; and even worst results were obtained for ammonium between Peat and Sand, with a  $r$  of only 0.73 at  $*P<0.05$  and a very high LBQ ( $46.53<27.59$ ).

Table 3. Correlation matrix showing the  $r$  values for each analysed variable; each site name was shortened using an acronym: *SC* for Silty-clay, *SL* for Silty-loam, *P* for Peat and *S* for Sand.

	Urea SC	Urea SL	Urea P	Urea S	NH4 SC	NH4 SL	NH4 P	NH4 S	NO2 SC	NO2 SL	NO2 P	NO2 S	NO3 SC	NO3 SL	NO3 P	NO3 S
Urea SC	1.00															
Urea SL	0.65	1.00														
Urea P	0.74	0.91	1.00													
Urea S	0.72	0.89	0.93	1.00												
NH4 SC	0.01	0.47	0.62	0.53	1.00											
NH4 SL	-0.28	0.15	-0.08	0.22	0.11	1.00										
NH4 P	-0.23	0.20	-0.09	0.19	0.00	0.89	1.00									
NH4 S	-0.38	-0.23	-0.38	-0.08	-0.16	0.81	0.73	1.00								
NO2 SC	-0.01	0.39	0.56	0.51	0.97	0.14	0.02	-0.11	1.00							
NO2 SL	-0.16	-0.17	-0.21	-0.24	-0.11	0.01	-0.02	0.23	-0.12	1.00						
NO2 P	-0.29	-0.62	-0.51	-0.41	-0.30	0.00	-0.18	0.48	-0.23	0.33	1.00					
NO2 S	-0.25	-0.51	-0.42	-0.47	-0.24	-0.24	-0.35	0.22	-0.22	0.55	0.84	1.00				
NO3 SC	-0.36	0.03	-0.22	0.08	-0.07	0.89	0.87	0.85	-0.05	0.05	0.16	-0.06	1.00			
NO3 SL	-0.34	-0.10	-0.24	0.06	0.03	0.84	0.84	0.91	0.09	0.22	0.22	-0.04	0.84	1.00		
NO3 P	-0.39	-0.79	-0.67	-0.54	-0.38	0.04	-0.08	0.49	-0.28	0.19	0.89	0.66	0.21	0.32	1.00	
NO3 S	-0.19	-0.52	-0.37	-0.58	-0.23	-0.66	-0.68	-0.38	-0.21	0.11	0.41	0.64	-0.51	-0.56	0.46	1.00

The decrease of ammonium correlation highlighted above is consistent with the differences in both CEC and apparent nitrification rates in the same soils.

It should be noted the large standard error for nitrate and ammonium content (see Fig. 3 and Supplementary Information), despite the elevated number of replicated cores employed in this study. This highlights the elevated spatial variability of nitrate and ammonium content in all the four topsoils here investigated, due to natural heterogeneity of soils and to the uneven distribution of urea granules. Besides, the observed variability was also due to how the urea was applied although broadcast spreading limit variability with respect to banding, a certain degree of inhomogeneity is unavoidable due to uneven urea granules redistribution above the soil surface. This also gives an idea of the limitations in estimating the ammonia losses via volatilization in field conditions. For bypassing this intrinsic limitation, a well-controlled, fully repeatable, laboratory experiment was employed to quantify ammonia volatilization.

### 3.3 Laboratory monitoring of ammonia volatilization

In all soil types the gross ammonia volatilization rate peaked after three days since urea application (Figure 4).

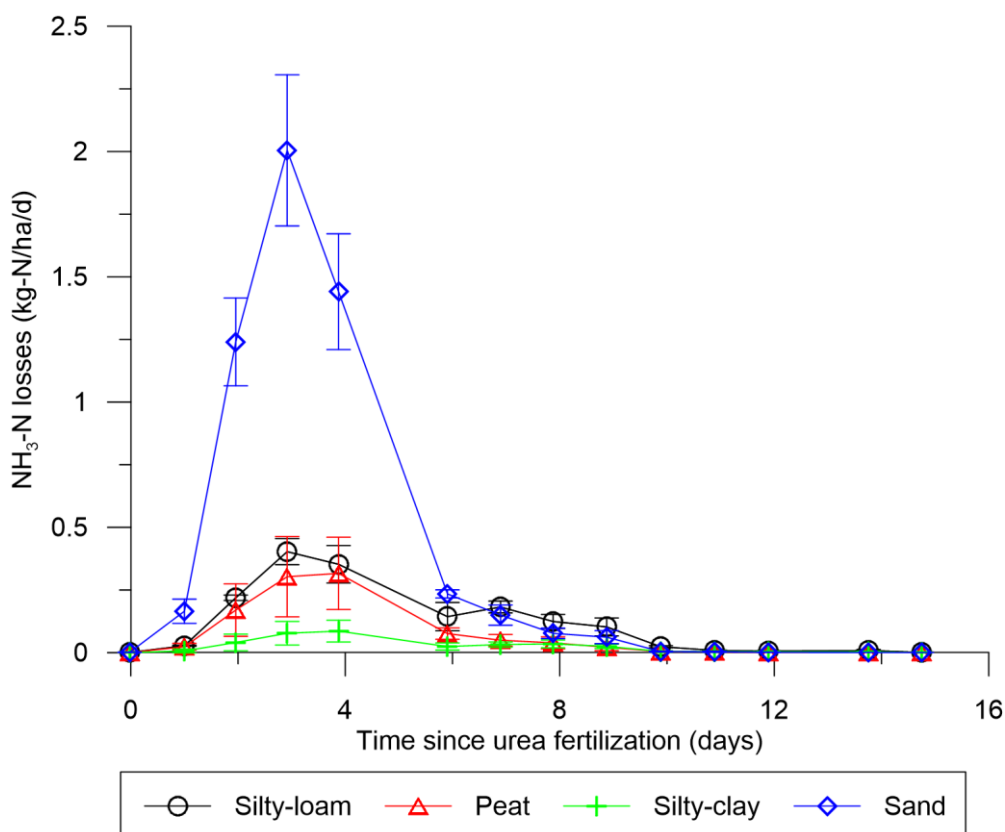


Figure 4: NH<sub>3</sub>-N volatilization from the topsoil (0-15 cm) in each soil recorded via laboratory batch experiments. Error bars represent the standard deviation of three replicates.

In general, quite low gross volatilization rates were derived, but consistent with laboratory results obtained for ploughed soils in temperate climates (Rochette et al., 2009). Gross volatilization was highest for Sand ( $4.5 \pm 0.7$  kg-N/ha) followed by Silty-loam ( $1.5 \pm 0.2$  kg-N/ha), Peat ( $1.0 \pm 0.1$  kg-N/ha) and lowest for Silty-clay ( $0.4 \pm 0.1$  kg-N/ha). The gross ammonia volatilization completely ceased after ten days since urea application in all soils. The obtained results are in the lower range of the recent literature reported in Ferrara et al. (2014). These low values may be attributed to the high CEC of the soils and to low wind speed, here simulated with a constantly vented chamber that reproduced the mean wind speed velocity observed in the field. Despite the low gross ammonia volatilization

found, neutral soil pH values and elevated soil water content should produce high ammonia emissions (Macnack et al., 2013). In fact, low ammonia emissions are usually found in dry soils with respect to wet soils (Sommer et al., 2004; Meyers et al., 2006). Thus, to explain the obtained low gross ammonia volatilization rates, the main mechanisms here postulated are the low wind speed combined with the soil CEC that possibly trapped ammonium preventing ammonia volatilization, as already hypothesized in the previous section.

### 3.4 Microbial activity assays

The microbial activity assays substantially confirmed the field and laboratory results, in fact the total and extracellular urease activity in all the four studied soils resulted to be high (Figure 5). ANOVA tests showed that the null hypothesis (equality of means) is rejected at  $*P < 0.05$  significance level for all the studied soils only for  $C_{mic}$ . This implies that the influence of soil type on this parameter is statistically representative, while for  $N_{mic}$  and urease microbial activity this effect was not so clear. The highest values of the total urease activity were found in the Silty-clay and Silty-loam with values near 300 kg-N/ha/d, followed by the Peat (277 kg-N/ha/d) and by the Sand (240 kg-N/ha/d).

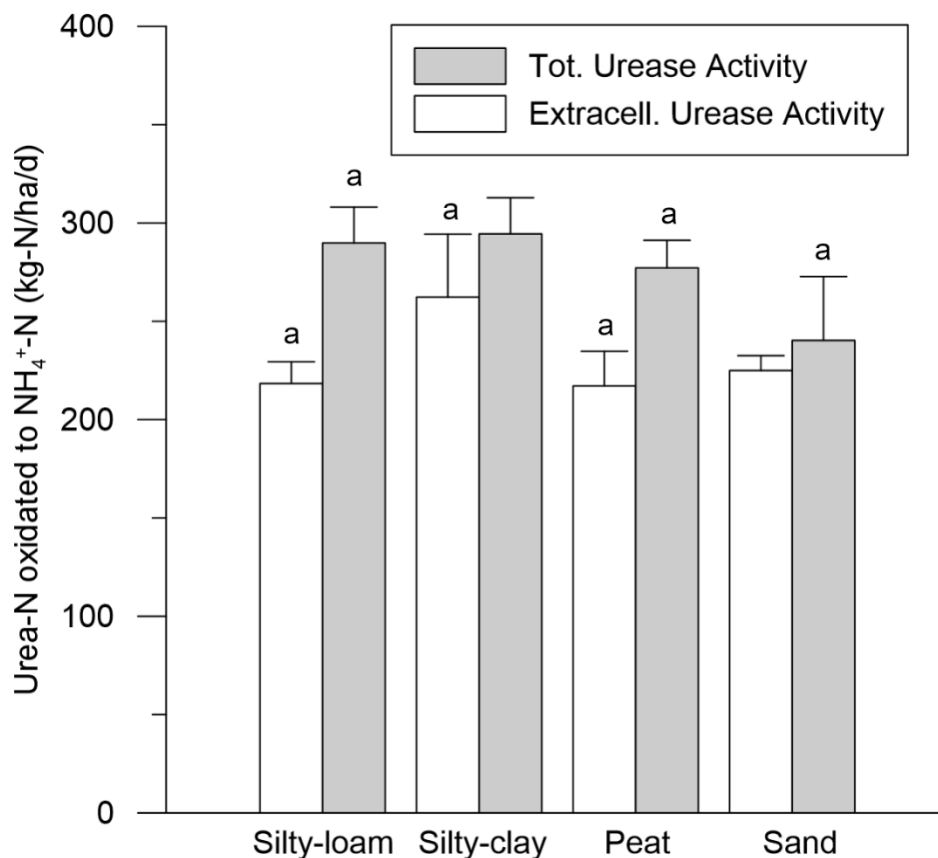


Figure 5: Total and extracellular urease activity of the topsoil (0-15 cm) in each soil. Error bars represent the standard deviation of three replicates. Mean values sharing the same superscript are not significantly different from each other (Tukey's HSD,  $*P < 0.05$ ).

The reported values are in agreement with values reported for a wide range of soils (Klose and Tabatabai, 1999) and with field monitoring (Sanz-Cobena et al., 2008). In contrast, the extracellular urease activity was two times greater than values previously reported. The elevated extracellular urease activity could be due to the elevated SOM content present in all the soils, and in particular in the topsoil, which was characterized by the abundance of labile organic substrates (Castaldelli et al., 2013). The urease microbial activity rate, is reported in Table 4.

Table 4. Microbial carbon ( $C_{mic}$ ), nitrogen ( $N_{mic}$ ) and urease activity of investigated soils. In parenthesis, the standard deviation over 5 replicates is shown. Mean values sharing the same superscript are not significantly different from each other (Tukey's HSD, \* $P < 0.05$ ).

Parameter	Unit	Sand	Silty-loam	Silty-clay	Peat
$C_{mic}$	(kg-C/ha)	153 (14.2)	609 (15.6)	348 (46.6)	413 (24.7)
$N_{mic}$	(kg-N/ha)	56.9a (7.5)	72.8b (23.4)	30.6b (18.8)	33.0ab (10.7)
<b>Urease Mic. Activity</b>	(kg $NH_4^+$ -N/ha/d)	15.4 (14.0)a	71.5 (7.2)b	32.1 (13.5)a	60.0 (3.6)b

The highest values were recorded in the Silty-loam, followed by the Peat and the Silty-clay, while the Sand exhibited the lowest values. This was only partially consistent with the field data, since the highest ammonification rate was found in the Silty-clay and not in the Silty-loam. Despite this, relatively elevated microbial urease activity rates were found in all the investigated sites. This can be explained both by the long term use of synthetic urea and by the circumneutral pH conditions suitable for ammonifier strains (Singh and Nye, 1984). In fact, all these soils have a remarkable buffering capacity due to the relatively elevated  $CaCO_3$  content (Table 1). The PNR obtained via laboratory batch experiments was coherent with the nitrification rate (FNR) obtained in the field. Clearly the PNR was in general more than 20 fold higher than the field one (Figure 6), since the PNR was obtained in non-limiting conditions (Mayer et al., 1995). This means that the PNR cannot be directly used for field based numerical modelling purposes, instead the more time and effort consuming FNR must be employed to accurately reproduce the fate of synthetic urea. ANOVA results highlighted significant differences at \* $P < 0.05$  level only for Silty-clay both PNR and FNR.

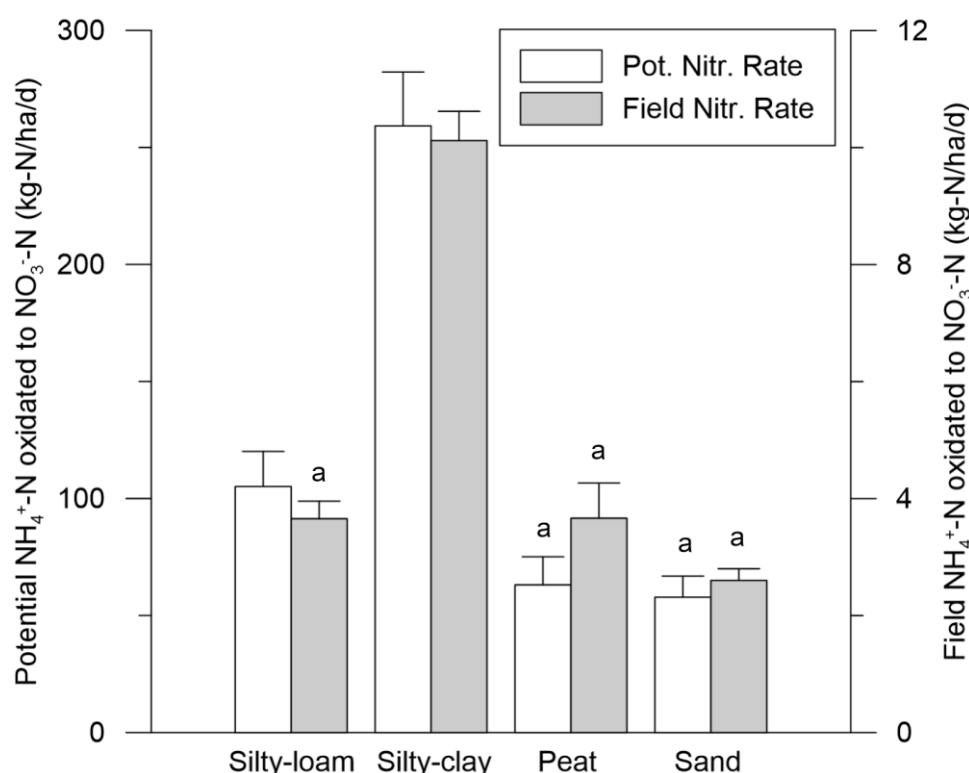


Figure 6: Potential nitrification rate and field nitrification rate. Error bars represent the standard deviation of three replicates. Mean values sharing the same superscript are not significantly different from each other (Tukey's HSD, \* $P < 0.05$ ).

Table 4 reports the  $C_{mic}$  and  $N_{mic}$  content in the topsoil, showing that the highest values were found in the Silty-loam, in accordance with the urease microbial activity, followed by the Peat and the Silty-clay. As expected, the Sand exhibited the lowest values. Indeed, the microbial assays substantially confirmed the results obtained in the field and with the laboratory batch experiments.

In general, this study showed that the kinetic of ammonification appearance in the different soils was consistent with the hydrolysis of urea and was as follow: Silty-clay, Peat, Silty-loam and Sand, while ammonium disappearance was in the order: Silty-clay, Silty-loam, Peat and Sand. The differences of urease and nitrification potential rates between soils supported the measured field evolution of concentrations, with the highest rates in the Silty-clay, intermediate rates for the Silty-loam and Peat, and the lowest rates in the Sand, which is the inherently more vulnerable soil. The laboratory experiments on urea volatilization confirm the above mentioned results, but in general very low volatilization rates were found. This was due to the concurrence of both high nitrification and soil CEC that promoted ammonium oxidation and sorption. In addition, the low wind speed in the monitored soils did not allow extensive volatilization as shown by the numerical models that were able to capture the main transformations of N species using the laboratory volatilization rates.

#### **4. Conclusions**

This paper presents the results of an interdisciplinary study on synthetic urea transformations, achieved through field and laboratory experiments in four soil types. The aim was to highlight the role of soil type and microclimatic conditions on the kinetic of urea transformations. The field plots were successfully modelled with HYDRUS-1D, capturing the main N transformations throughout the simulated period. This study shows that in soils cultivated with the same maize crops and pertaining to the same macro climatic temperate environment, very different nitrification rates can be found. This is imputable to an interplay of microclimatic conditions, abundance of nitrifiers, soil's CEC, pH and SOM. This finding highlight the inherent difficulty in obtaining robust and representative input parameters for large scale, process based numerical models of reactive N emissions and leaching. Although collecting this kind of dataset is extensively time and effort consuming, this task remains the main limitation in describing urea and reactive N fate in agricultural landscapes. Besides, the large variability of the nitrate and ammonium contents in each soil, highlights the need of large number of replicates to gain statistically consistent field data sets. The presented multidisciplinary approach could be employed in many other agricultural settings to obtain robust data sets indispensable to constrain numerical models on the fate and transport of reactive N species in agricultural landscapes.

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