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Evaluation of Different Recycling Fertilizers Regarding N Fertilizer Value and Ammonia (NH_3) Emissions in Maize (*Zea Mays)* in Climate Chamber

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Abbreviations

Physicochemical analysis or related terms

FW Fresh weight

DW Dry weight

FC Field capacity

N_{min} Mineral nitrogen

N_t Nitrogen concentration / content

RH Relative humidity

WHC Water holding capacity

Calculations, statistical terms, equations, others

C.V Coefficient of variation

ANOVA Analysis of variance

Abstract

Nitrogen (N) is one of the most important nutrients in plant nutrition due to it central role in growth and development. Despite this, it represents a major environmental hazard not only by malpractices (leaching, eutrophication, volatilization, etc.) but also by the high energy cost for production. The aim of this study was to evaluate different N-sources as alternatives to conventional mineral N by analyzing dry weight per plant (DW), fertilizer efficiency and losses by volatilization in a maize (*Zea Mays*) pot experiment under controlled climatic conditions for 7 weeks. Four nitrogen fertilizers were examined: Aurin (1:1 NH_4^+/NO_3^-, nitrified), CROP (1:14 NH_4^+/NO_3^-, nitrified), synthetic urine mimicking human urine (100% NH_3-N, raw) and urea (N_2O-N amid form). Two modes of application were used for each N fertiliser: complete and split application. All treatments received the same N amount during the experiment. No significant difference was observed between modes of application within each N fertiliser. FW and DW were higher in Aurin than other treatments, CROP and urea performed at the same level and synthetic urine was the least efficient. Total NH_3-N volatilization loss was higher in synthetic urine (11.8 and 5.2%) than in urea (2.0 and 1.2%) in complete and split application respectively; Aurin and CROP (<0.6%, both modes of application) showed the lowest N losses. This research demonstrated that nitrified urine-based fertilizers (NUF) performed similar or better than mineral and raw urine fertilizer in terms of FW, DW and fertilizer efficiency, and with significant lower NH_3 volatilization. This study confirms the NUF potential in two ways: as an alternative to reduce the mineral N dependence and as an innovative waste management option.

1. Introduction

1.1. Agricultural Challenges

The global population is growing as well as the demand for food, goods and economic growth. To be able to fulfill these challenges, it has been necessary to pay a high environmental cost. Changes in land use (forest, pastures or any other natural use) along with population growth are increasing the global carbon emissions. Ecological functions (e.g. nutrient cycling, soil conservation or watershed protection) have been disrupted due to high deforestation rates to expand the agricultural frontier (Smith et al., 1998). Instead of expanding the arable land, available land must be managed efficiently and with the implementation of new technologies to increase the yield per area.

The green revolution came to solve food security problems worldwide but also introduced other problems such as the excessive use of fertilizers in agriculture. There is a general misunderstanding in the use of mineral fertilizers. High application of inorganic fertilizers has been done to ensure high yields, leading into high greenhouse emissions, eutrophication (surface and groundwater), soil degradation and decrease the productivity potential of the land (Ding et al., 2018). Nowadays, problems of environmental degradation, reduction of resources and the increasing population, have raised new challenges such as world hunger, malnourishment, climate change, loss of biodiversity and reduction of arable land among others. Therefore, the efficiency and proper use of resources along with the maintenance of actual productive standards and quality, as well as an environmental balance, are the goals of modern agriculture and horticulture worldwide.

Many countries have implemented regulations regarding the use, type, application and dosage of fertilizers but despite this, fertilizer´s production and consumption have had a constant annual growth rate of 1.8%, variating among regions (FAO, 2015). Fertilizers are produced by high energy demanding processes (Haber-Bosch process) or come from non-renewable sources, such as phosphorus (P) mining.

Agriculture requires the use of P for food and fiber production due to the main role of this element in structural functions in nucleic acids; and during the past years, a dramatic reduction of natural P reservoirs has been reported (Marschner, 2012). Besides this, the treatment of residual P in sewage represents a problem for treatment plants, which has to incur in extra costs (Ronteltap et al., 2010). Therefore, a recycling P method would be highly recommended and an environmental path to take. The concept of "Circular Economies" is given to the production-consumption systems which are maximized by including recycled resources, renewable energy and cascading energy flows all together to create sustainable development (Korhonen et al., 2018).

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1.2. Nitrogen

Nitrogen (N), along with carbon, is the most important element in plant nutrition due to the role it has on the growth and development of plants. It has a direct impact on proteins, amino acids, chlorophyll, enzymes, and secondary metabolites among others (Marschner, 2012). It influences the quality and yield in the fields and consequently has a direct impact on food security. Despite the fact that N represents more than 78% of the atmosphere as molecular N_2, it is not available in this molecular form to be used by plants except for some adapted species (e.g. legumes). This element in combination with others (Liebig´s law) is the main limiting nutritional factor in plants. Therefore, proper supply and management are needed to accomplish an adequate plant development. Nitrogen has an intricate pathway where many different factors and processes interact with each other (**Fig. 1**) and define the distribution of this element among the different paths. Applied N can be used by the plant when certain processes occur (e.g. mineralization, nitrification) or is transformed into organic forms (e.g. immobilization) or even lost (e.g. volatilization, leaching).

Figure 1 Nitrogen cycle. Adapted from Jones et al. (2013).

The Haber-Bosch process development, enabling the production of industrialized N-based fertilizers, increasing the availability of N and the exponential increase of yield per hectare worldwide. It has been considered as one of the most important developments in human modern history (Smil, 2000). Despite the positive effects on agriculture and food security, the production of N-based fertilizers by Haber-Bosh process is energy-intensive. Pfromm (2017) calculated that the Haber-Bosch process consumes approximately 2.0% of the World´s available energy; and approximately 2.5% of the total CO_2 emissions come from

the production of fertilizers by the use of natural gas, fossil fuels or coal for the synthesis process. One of the products out of this process is urea, which is the most used N fertilizer worldwide due to it low cost and high N content, and which represents >50% of the total N use (Glibert et al., 2006). Urea can be uptaken by the plants as a non-hydrolyzed molecule although most of the applied fertilizer will be hydrolyzed in the soil creating NH_4^+ and NO_3^- molecules (Sigurdarson et al., 2018). The hydrolyzation process occurs due to the interaction of the urease enzyme (nickel depending) which catalyzes the urea to form NH_4^+ and CO_2 (Hasler et al., 2015; Marschner, 2012; Sirko & Brodzik, 2000).

1.2.1. Nitrogen uptake

Most of the N taken up by plants is ammonium (NH_4^+) and nitrate (NO_3^-). The NO_3^- form is present in higher concentrations, is more mobile and available than NH_4^+ in the soil. Marschner (2012) described 25-50 times higher NO_3^- concentrations than NH_4^+ in agricultural soils. Despite this, N assimilation varies between plant species, where some

plants prefer NH_4^+ forms rather than NO_3^- (e.g. grasses at early stages) and the other way around (e.g. legumes). Theses preferences can change depending on plant growing stages (Cui et al., 2017; Magalhes & Huber, 1989).

Nitrate is the principal N source (non-organic) worldwide. It can be reduced in the roots or shoots depending on the environmental conditions, and the reduction process consists in a two-step process at the cellular level (**Fig. 2**): first, a reduction from NO_3^- to NO_2^- in the cytoplasm, and then from NO_2^- into NH_4^+ in chloroplast or proplastids (Lea & Morot-Gaudry, 2001). The NO_2 form is toxic for plants, therefore, the NO_3^- reductase (NR) process must

be regulated to avoid accumulation. The NO_3^- reduction to NO_2^- is made by the NR which is an enzyme with two subunits, each of them containing three co-factors that participate in

the electron transfer from NADH/NADPH (Nicotinamide adenine dinucleotide phosphate).

This creates a NO_2^- molecule which is transported to the chloroplast in the leaves or to proplastids in roots or other non-green tissues (Marschner, 2012). The NO_2^- is reduced by NO_2^- reductase (NiR) in a six electron donor process, from reduced ferredoxin in photosystem I, by the ferredoxin-binding domain, an iron-sulfur cluster, and a siroheme co-

factor bound to the NiR to finally produce NH_4^+ (Marschner, 2012). The NO_3^- reduction and assimilation are energy demanding and can compete with the CO_2 assimilation under

adverse conditions (Marschner, 2012).

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Figure 2 Nitrate assimilation model via nitrate reductase and nitrite reductase processes. Adapted from Marschner (2012)

Ammonium then bounds with a glutamate molecule by the usage of ATP and the glutamine

synthetase process (**Fig. 3**). This creates a glutamine molecule that bounds with a 2-

oxoglutarate molecule, from the Krebs cycle, by catalysis from glutamate synthase using

NADPH as energy. Ammonium replaces the carbonyl group with an amino group, creating

2 molecules of glutamate. One of them for the plant and the second is retain in the system

to repeat the GS-GOGAT cycle (Marschner, 2012; Suárez et al., 2002). Theoretically, the

NH_4^+ form is preferred over NO_3^-, but NH_4^+ form can create a negative effect on plants such as toxicity (Magalhes & Huber, 1989).

Figure 3 Ammonium assimilation model via glutamine synthetase – glutamate synthase cycle (GS-GOGAT). Adapted from Marschner (2012)

Ammonia (NH_3) assimilation depends on a weak base bound, pH depending, with NH_4^+, which makes it scarce in the soil (Marschner, 2012). The NH_3 form has a minimal impact on plant nutrition. The efficient application of this N-form has to fulfill certain requirements (e.g.

pH, humidity, soil texture, organic matter, and application depth) to reduce losses by

volatilization and being available for plants (Rochette et al., 2013).

Highly efficient plants are desired due to their adeptness to metabolize and transform N into

biomass (Ding et al., 2018). Therefore the calculation of the N use efficiency (NUE) was

created to measure the ratio between N inputs (applied N) and outputs (mobilized N from

the soil to the plant) (Marschner, 2012). By nature, plants store N in different compartments

and for different purposes. This process allows plants to translocate N when needed, e.g.

activation after dormancy, rapid growth, grain formation and fulfilling, etc....(Lea & Morot-

Gaudry, 2001). Although, when high N inputs are given to low NUE plants, soil pollution,

incomplete capture, poor conversion ratios, and high greenhouse gasses emissions can

occur (Lea & Morot-Gaudry, 2001; Marschner, 2012). It is estimated that 40-50% of the

applied N is uptake by the crops, the rest is lost by leaching, volatilization, mineral fixation,

microbial transformation or any other forms (Marschner, 2012).

1.2.2. Nitrogen volatilization

Agricultural areas have higher N volatilization potential due to mineral and organic N fertilizer applications in comparison to non-applied areas (Marschner, 2012). Volatilization is affected by soil moisture, precipitation, pH, temperature, crop residues, superficial applications or N-form applied (Fenn & Hossner, 1985; Jones et al., 2013). Nitrogen losses reduce N availability for plants, therefore productivity and yield. Volatilization losses are not only by NH_3 forms. The decomposition of rich N-crops produces the degradation of N into other forms (e.g. nitrous oxide) or been precursors for microbial decomposition producing NH_3 (Nett et al., 2016). Emissions are not limited at the beginning of the crop cycle but also after harvesting when no further applications have been made.

Despite the N importance, there is a global concern about excessive N use due to its environmental impact (Recio et al., 2018). Agricultural activities (land use, livestock production, and soil and nutrient management) cause approximate 21% of total emissions of greenhouse gasses emissions worldwide and are the main source of methane and nitrous oxide (N_2O) emissions (FAO, 2017). Animal and crop production release large amounts of NH_3 into the atmosphere, been agriculture responsible for more than 90% of the NH_3 emissions. Manure management and mineral fertilizers represents approximately 80% and 20% of NH_3 agricultural emissions respectively (European Commission, 2017). Further processes can form N_2O from the N based fertilizers and manure, which has a higher global warming potential than CO_2 (298 times more). The use of fertilizers is considered the most important anthropogenic source of N_2O emissions due to the microbial processes of nitrification and denitrification (FAO, 2017; Recio et al., 2018).

1.3. Urine as a resource for N fertilizer production

Urine has been used since ancient times in Asian cultures who understood the value of "waste" and its potential source of nutrients for agriculture (King, 2004). Areas using urine as fertilizer has shown a positive effect in the soil pH and crop productivity by the use of mixed house waste (as biogas slurry), human feces/urine and ashes low fertility areas in Africa (Krause et al., 2015). Despite this, those practices are still stigmatized by social and cultural aspects (Andersson, 2015). Urine contains N (80-90%), P (50-80%) and potassium (80-90% K) of the total human nutrient excreta (Senecal & Vinnerås, 2017).

Stored urine contains N in an ammoniacal form (bound to bicarbonate); other forms as NO_3^- or

urea are found in fresh urine but is decomposed during storage due to pH changes (Kirchmann & Pettersson, 1995). Besides N, human urine contains other nutrients such as P as superphosphates ($H_2PO_4^-$ or HPO_4^{2-}), K as an ionic component (K^+), sulfate (SO_4^{2-}) and also other compounds as sodium chloride (NaCl) (Lind et al., 2001). Urine usage might entail problems such as NaCl accumulation due to its relatively high content. High amounts of salt cause plant stress, decreasing growth and productivity by affecting osmotic potential, nutrient uptake and transport, carbon harvesting, grain development and yield in sensitive crops (Farooq et al., 2015).

Nitrogen in urine is prone to higher volatilization losses compared to other mineral fertilizers (e.g. NH_4NO_3 or urea) due to the N form (Kirchmann & Pettersson, 1995). The challenge is producing sewage fertilizers which can be comparable to mineral fertilizers in nutrients content and availability (Senecal & Vinnerås, 2017). Has been shown that processed urine-based fertilizers contain high N and P with comparable absorption rates to mineral ones and a high P use efficiency (Bonvin et al., 2015; Kirchmann & Pettersson, 1995).

Urine composition variates among populations due to living conditions and food habits. Kirchmann & Pettersson (1995) determined the urine composition from closed septic tanks from two ecological villages in Sweden (**Tab. 1**), samples were collected directly from the septic tanks which contain urine with a maximum of 3 months of storage.

Table 1 Elemental urine composition, separated in toilets and retrieved from septic tanks. Adapted from Kirchmann & Pettersson (1995)

Range	N	P	K	S	Na	Cl	Ca	Mg	Mn	Fe	B	Al
	g L^{-1}						mg L^{-1}					
Low	1.79	0.20	0.12	0.18	0.94	2.20	13.34	1.50	0.00	0.16	0.44	0.19
High	2.61	0.21	0.88	0.23	0.98	2.50	15.75	1.60	0.00	0.21	0.44	0.21

Human urine has been studied to become a nutrient source for plants and reduce the agricultural dependence of chemical fertilizers (Winker et al., 2009). Although, some issues

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of using urine is the integration of it into an appropriated post-treatment process, reducing associated hazards related to human safety (e.g. pathogens, pharmaceuticals, pollutants) and thereby produce a valuable product (Simha & Ganesapillai, 2017). Despite this, some authors have found minimal or no differences between urine-based and mineral fertilizers related to human hazard (e.g. *E. coli*, *Salmonella spp., Enterococcus*) content in cabbage biomass (Pradhan et al., 2007; Pradhan et al., 2009).

1.4. Hypothesis

The term recycling is not new, it has been used for many centuries in different cultures all over the globe; it's a natural process. Waste (human excretes, animal or plant residues) are charged of nutrients which unless are recycled, they get lost and become an environmental

problem. Urine is an available nutrient source, which can be recovered and used for food or goods production. This could reduce our dependency on highly energy-dependent fertilizers and also, reduce costs in wastewater treatment plants.

Two nitrified urine-based fertilizers (Aurin, CROP), a mimic of human urine (synthetic urine) and a mineral one (urea) under two different application modes were analyzed. Tested parameters in plant material were biomass accumulation, fertilizer use efficiency and N-content; in the substrate were N-volatilization, pH, mineral N and N-content. All these results aim at comparing and answer the questions:

  i. Fertilizer treatments will not significantly differ in plant shoot N concentration (mg N

     kg^{-1} DW).

 ii. NH_3 emissions are correlated to the NH_4^+ content in Aurin (50% NH_4-N), CROP

  (<10% NH_4-N), S. urine (100% NH_3-N) and urea (CH_4N_2O) fertilizers. iii. Higher N application (complete>split) will show higher NH_3-N losses by volatilization. iv. Urea treatment will show a time delay in NH_3 emissions regarding nitrified urine-

    based fertilizers.

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2. Materials and Methods

A climate chamber experiment with Sweet Corn (*Zea Mays var. rugose*) which was conducted 20 km south of Berlin at Leibniz-Institut für Gemüse- und Zierpflanzenbau (IGZ), Großbeeren. In the present research, it was aimed to investigate the efficiency of recycled N fertilizers in a substrate-based pot experiment. There were two nitrified urine-based

fertilizers (Aurin and C.R.O.P), synthetic or non-processed urine (a mimic of human urine made artificially) and mineral control (urea) tested. A zero control (no N application) was also included. The tested parameters were biomass accumulation, fertilizer use efficiency and N-content in shoot and substrate aspects such as N-volatilization, pH, mineral N and N-content.

2.1. Experimental setup

2.1.1. Substrate

The substrate was obtained from a commercial company (ökohum GmbH, Herbertingen) without added nutrients. It contained 25% wood bark-compost and wood bark-humus, 25% wood fiber, 15% pumice and foamed clay and 35% coco peat and rice husk. Substrate was analyzed to determine physicochemical properties.

The pot size was 4L (15.7cm x 15.7cm x 23.3cm). This was selected to avoid radicular limitation, although has been described that pots of 1.5L are enough for maize development until the 7th week (Bárzana et al., 2012). Each pot was filled until approximate 2 cm from the edge and slightly compressed. In total 12 pots were weighted (out of 45) to determine the average weight of the pots. After, they were introduced in the climate chamber to adapt them to the environment.

2.1.2. Fertilizers and nutritional plan

The used fertilizers were Aurin (2.10% NH_4-N + 2.10% NO_3-N; pH<5.0, Vuna GmbH Switzerland) and CROP (0.03% NH_4^+ + 0.49% NO_3^-, pH 3.9-4.2, DLR- Deutsches Zentrum für Luft- und Raumfahrt, Bremen) as nitrified urine-based fertilizers (NUF) with ratios of NH_4^+:NO_3^- of 1:1 and 1:14 respectively. Aurin fertilizer is produced by nitrification and distillation processes (**Fig. 4**) where stored urine is used and half of the N (as NH_3 and NH_4^+) is transformed into NO_3^- by nitrification bacteria. The stabilized product then is distilled to reduce the volume to 3.0-5.0% of the initial volume (Etter & Udert, 2015). CROP is produced by a filtration process where colonized lava particles are used as media, allowing similar condition as in nature (aerobic and anaerobic zones). This process occurs inside a PVC

tube with a circulation system (Hauslage et al., 2014).

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Figure 4 Aurin mass flow though by the combined nitrification/distillation process. Adapted from Etter & Udert (2015)

Synthetic urine (100% NH_3-N, pH 8.9-9.1, adapted from Bonvin et al. (2015)) which mimics human urine was also used. One liter of synthetic urine was prepared with: 2.30 g Na_2SO_4 anhydrous, 2.10 g NaH_2PO_4 anhydrous, 3.60 g NaCl, 4.20 g KCl, 9.60 g $C_2H_7NO_2$, 21.4 g NH_4HCO_3, and 13.0 mL NH_4OH (25% NH_3) in nanopure water. The mineral control was urea (46% N, Carl Roth GmbH). Fertilization was calculated from the label of the fertilizers (**Appx. 6**), therefore the final N application varies between treatments. Further analysis by IGZ laboratory by FIA analysis and ICP-OES methodologies were made to analyze fertilizer nutrients concentration (**Tab. 2**) to determine the nutrients over/under N-application. Aurin receive +185mg N more while CROP had -34mg N in comparison to the other treatments. Therefore, fertilizer efficiency was calculated in each treatment and application mode to be comparable between them.

Table 2 Fertilizer concentration analysis by the comparison between IGZ laboratory results and the label reported concentration. Differences were multiplied by volume of fertilizers applied per pot. Values expressed as ±mg received per pot.

Element	Aurin			CROP		
	IGZ Value Mg mg L-1	Label mg L-1	mg	IGZ Value	Label	mg
NH4-N	23846	21000	+85	330	280	+12
NO3-N	24343	21000	+100	4659	4857	-46
P	3044	1750	+39	302	300	+2
K	19777	14940	+145	1320	1091	+54
Ca	376	ns	*	6005	6145	-33
Mg	80	ns	*	180	159	+5
Na	24324	17000	+220	2037	1834	+48
S	3944	3204	+22	332	331	-1
B	8	15	0	0	ns	*
Fe	0	1	0	0	ns	*
Zn	0	12	0	0	ns	*

ns Non-specified value * Not calculated

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All plants were applied theoretically the same amount of nutrients (**Tab. 3**) only differing in the application mode. The zero-control received all the nutrients except N. Complete treatments received one dose at the beginning of the experiment (1.26 g N pot^{-1}) and split treatments received two doses (0.63 g N pot^{-1} application^{-2}). The nutrient applications for the split application were made on week zero and in the third week. The nutrient requirement of the plants was calculated according to Bergmann (1993) and Bender et al. (2013). It was expected to found N content in leaves between 28.0-31.5 g kg^{-1} DW. Sodium and chloride concentrations were not controlled. Split treatments showed visual N deficiencies before the second application.

Table 3 Elemental nutrients applied pot^{-1} to fulfill nutrient requirements of maize until the 7th week of development. It was estimated a 40g shoot DW plant^{-1} according to experiments conducted in the past in IGZ. Nutritional values were adapted from Bergmann (1993) according to leaf nutrient concentration before floral induction and from Bender et al., (2013) at field experiment

Element	N	P	K	Ca	Mg	S	B	Fe	Zn	Cu	Mn
g Mg Maize requirement	1.26	0.064	0.8	0.1	0.064	0.12	0.24	11.9	0.88	0.2	1.2

The nutrient solution was applied in the surface. Aurin (26.7 mL), CROP (235 mL), synthetic urine (160mL) and urea (2.74g) for the complete application. For the split application, those values were divided by two. Each application was diluted or dissolved in 250 mL of distilled

water (both application modes).

Compound mixtures were made to fulfill nutrients that were not present in the used fertilizers. Those mixture were dissolved in 500 mL of distilled water and applied at the beginning of the experiment. The different salts were selected to avoid any N addition and to be highly soluble in water.

2.1.3. Seeds

Seeds were obtained from a commercial organic brand. The used variety was G726 Zuckermais Sugrano (AS) from the company Bingenheimer Saatgut AG - Ökologisches Saatgut. The used batch was 15902/19. Seeds were analyzed to estimate the initial N content. Seeds were weighted to obtain an average mass of the seeds and N content. The average seed weight was 118.3 mg (n=10) and 26.1 g kg^{-1} N (n=4.0) from a compound sample. It was estimated approximately 3.09 mg N per seed, which can be used as a reservoir for the plant in the first days after sprouting before starting up taking N from the substrate.

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2.1.4. Growth condition

The climate chamber had a factory nominal airflow value between 0.2 – 0.6 m/s. It was checked at pot's height with 2 airflow sensors (Air Velocity Transducer 8455-150-1, TSI Incorporated) connected to a data logger. The measurements were made every 5 minutes for 7 days. In total, 3948 values were obtained to determine air flow value of the climatic chamber (**Appx. 4**). In average, the sensors measured 0.09 ± 0.01 m/s of air flow (C.V 10.87%).

During the 7 weeks of experiment, climatic conditions were set at 19-25°C with a relative humidity (RH) of 50-70%. The light was supplied for 16 hours per day (700 μmol m^{-2} s^{-1}) with 8 hours of darkness. The parameters were adapted from Bárzana et al. (2012). Climate chamber parameters were re-established after the first week after seedling. Plants showed burned tips in the primary leaf. New parameters were 18-22°C, 50-70% RH, 600 μmol m^{-2} s^{-1} for 14 hours and 10 hours of darkness. Plants recovered almost completely, except on leaves that were highly damaged at the beginning. No further damage by climatic conditions was observed. Plants were located on cultivation tables. Soil moisture was measured by gravimetric analysis. Random pots were selected, weighted and a simple average analysis was applied to determine the water content of the pot. Distilled water was applied to the pots to avoid extra nutrient supplements. Pots were sealed with duct tape and located above a receiving tray to reduce leaching effect.

In the first week of the experiment, replicates from zero control and CROP split were lost by lack of germination. At week 3, a synthetic split sample showed growth delay until death. Aurin treatment showed tiller formation by the end of the experiment, these were included in the analysis. Plants presented male inflorescence or flower (38) and female inflorescence or fruit (5). Zero control treatment did not showed any of these developments.

2.2. Substrate

2.2.1. Field and water holding capacity

Water holding capacity (WHC) is the amount of water that a soil can hold and can be used by the crops. WHC was determined by gravimetric method described by Noggle & Wynd (1941). It was determined by the difference between initial weight (W_0), saturated weight (W_1)

and dry weight at 105°C (W_2). Changes were registered by a semi-analytical balance.

$$\%WHC = \frac{W_1 - W_0}{W_2} \times 100$$

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Field capacity (FC) is known as the amount of water that the soil can hold after saturation. The FC was adjusted in a range between 40-70% of WHC. The FC was maintained between 55-70% of WHC during the whole experiment.

2.2.2. Substrate preparation

Samples were obtained right after the experiment was finished. A soil sampler was used in three different points, were samples were taken from the pot. Then it was divided in two parts for analysis. A part of the sample was dried at 37oC in a forced-air oven for 48 hours (or until constant weight) and the plant material was removed from the sample. Samples were ground by an ultra-centrifugal mill to a <0.25mm size. The other part was stored in a fridge at 4°C until analysis (maximum 48 hours) for the mineral nitrogen analysis.

2.2.3. pH

Hydrogen ion activity (pH) was measured in calcium chloride ($CaCl_2$) at 0.01M standard method (1:7 ratio). In a 250 ml polyethylene flask, weight 10 g of the substrate and add 70 ml of 0.01M $CaCl_2$ with a dispenser. Flask was closed and located in a rotor for 60 minutes at 90 rev/min (Guvina-Hofmann GmbH, 214/12 PM1 72-35). Filtrate the solution. The extracted solution was analyzed immediately and approximated to the nearest 0.01 decimal.

2.2.4. Nitrogen content (N_t)

Samples were measured by Elemental Analysis (Vario EL Cube, Elementar Analysensysteme GmbH) for nitrogen content (g kg^{-1} DW) by dry digestion.

2.2.5. Mineral nitrogen (N_{min})

The determination of mineral nitrogen (NO_3^-/NH_4^+) was by photometric techniques by Flow Injection Analysis (FIA). Method described by LUFA-Method A 6.1.4.1 Volume 1, Bodenuntersuchung; extracting solution: $CaCl_2$ 12.5 mmol/L (1:4 ratio).

In a 250 ml polyethylene flask, weight 25 g of the substrate and add 100 ml of extracting solution. Blanks must be made. Flasks must be closed and collocate it in a rotor for 60 minutes at 80 rev/min. The solution was filtered with a funnel and filter paper (Whatman, 185mm, 602H 1⁄2). The extracted solution must be analyzed immediately or stored at -25oC until analysis.

2.3. Plant material

2.3.1. Sample preparation

Plants were divided in 5 parts: roots, leaves, stem, flower and fruits (when available). Each part was collected in individual identified bags, which were weighted before collecting the sample (empty), with the fresh and with the dried sample (60°C) to the nearest 0.01g. After dryness samples were ground to a <0.25mm size.

2.3.2. Shoot dry weight (DW)

Shoot dry weight was obtained by the sum of all the parts, except the roots. The root section was photographed and weighted (fresh and dry). It was neglected from the DW analysis due to the high adhesion between the substrate and roots.

2.3.3. Nitrogen concentration (N_t)

Same procedure as described in section 2.1.4. Nitrogen concentration was determinate individually from each plant part. The total N_t was calculated from the sum of the N-content multiplied by the DW. Results expressed as mg N per plant (mg N plant^{-1}).

2.4. NH_3 volatilization

Close chambers with an airflow system incorporated were used (Nett et al., 2016). The equipment consists of 3 pieces chamber (bottom, body and cover) with a ventilator incorporated (1.5 cm diameter) in the middle part of the body. The bottom part is secured with industrial tape to the body to avoid air leaking. The cover has a locker system (gasket seal and fasteners) and a rubber band to minimize air losses. Also, the cover had a hook for the filter papers.

Passive NH_3 filters were made using paper filters (Rotilabo®-Rundfilter, Carl Roth GmbH) type 15A, 9cm diameter (63.62cm^2). Filters were washed 2 times per 30 mins in an ultrasonic bath with deionized water, dried at 60°C and stored in a desiccator. As medium to capture the NH_3 and transform it to NH_4^+, a 2% phosphoric acid solution was used: 20ml of deionized water + 180ml methanol (>99% purity, Carl Roth GmbH) + 6.6g phosphoric acid (>98% purity, Alfa Aesar GmbH). Application of 2ml to each paper filter and dried in a desiccator with a N_2 stream (Nett et al., 2016). Filters were stored in petri dishes, sealed with Parafilm to avoid gas exchange avoidance and stored in a desiccator. High NH_3 can be capture (14 mg N per 100 cm^2) in a short time by this method (Nett et al., 2015).

Measurements were taken every 24 hours for 4 days (hour 0, 24, 48, 72) between 9 am and 3 pm. Harper et al. (1983) found volatilization picks between the 0-48 hours after urea application, then a downward trend for the following hours. It was considered urea as the reference by the long time it takes to react and produce NH_3. The extension up to 72 hours was to ensure the capture of the highest peaks. No volatilization measurements were made when the second fertilizer application was made (split application).

Nutrient solutions were applied and immediately the pot was introduced into the chamber. Three filter papers were put in the chamber´s hook and closed for one hour. During this time, the ventilator creates a homogeneous gas concentration and stable air turbulence (Nett et al., 2016). Nine pots were analyzed at the same time (one per treatment and application mode plus zero control). After the time is finished, the chamber was opened and filters collected in a 50 mL falcon tube and sealed with parafilm to avoid gas exchange. Blanks were made daily (no exposure). Tubes were stored at -20°C freezer until analysis. For analysis, were applied 40ml of mili-Q water (PureLab flex, Veola Water Solutions & Technologies) with a dispenser to the falcon tube. Then located in an ultrasonic bath for one hour. The extracted solution was analyzed for NH_4-N concentration using Flow Injection Analysis FIA (FIAmodula, MLE GmbH). Blanks were subtracted from the values of the exposed filters. Results were obtained by the formula:

mg NH_3-N pot^{-1} = $(F_{sample} - F_{blank}) \times V_{ext} \times \%N$

where F_{sample} was the filter used in the sample (mg L^{-1} NH_4-N), F_{blank} was the filter used as blank (mg L^{-1} NH_4-N), V_{ext} was the volume in which the samples were recovered (0.04L) and %N is the percentage of elemental N present in the recovered N-form
(77.65%)
The obtained results were interpolated (estimating new data points between the ranges of a known data set). These values were used to construct a model closer to reality, although does not contemplate other factors that might influences variations within analyzed points (day/night fluctuations or intermediate picks within hours). Interpolation was made by the formula:

$$y_2 = \frac{(x_2 - x_1)(y_3 - y_1)}{(x_3 - x_1)} + y_1$$

where y_2 is the desired value, x_2 is the intercept of the desired value, x_1 and x_3 are the known values for x-axis and y_1 and y_3 are the known values for y-axis.
20
The total N losses regarding the N applied (%) was calculated by the formula:

$$\% \text{ N losses} = \frac{\text{mg N} - NH_3 \; pot_{-1}}{\text{mg N applied } pot^{-1}} \times 100$$

After, NH_3 volatilization values were expressed as tons of CO_2 equivalents per hectare per fertilization cycle (tons CO_2 ha^{-1} $fertilization^{-2}$) according to IPCC (2007). This to have comparable values with other authors and provide an estimation of the Global Warming Potential (GWP) of the recycled fertilizers in comparison to mineral fertilizers. Calculations were made by the correlation hectare area and pot area. For converting NH_3 into CO_2, two factors were used: 0.002 from NH_3 into N_2O, and 298 from N_2O into CO_2 equivalents according to IPCC (2007). The obtained values are valid for this experiment under these
given conditions and circumstances.
2.5. Material Flow Analysis (MFA)
Material Flow Analysis refers to an analytical approach for understanding systems, usually complex, and thereby provide a method for natural resource management; this system describes a group of interacting processes that are defined by time and space (Krause & Rotter, 2017). In the MFA diagram were included all N-forms which interacted throughout the experiment. Those forms were defined as inputs, outputs and stock.
N inputs: seed and fertilizer
N outputs: biomass, NH_3 emissions, N_2O emissions and NO emissions
Stock (Δ): N immobilization, leaching and other non-accounted forms
Gross balance: the difference between N-inputs minus N-outputs and the changes in stock. The overall result is expected to be zero.

$$\sum N_{input} = \sum N_{outputs} \pm N_{\Delta\ stock}$$

where $\sum N_{input}$ and $\sum N_{outputs}$ are the total N in the experiment (material flow) and $N_{\Delta\ stock}$ is caused by the changes or fluctuations in the stock N concentration (Krause & Rotter, 2017). The N_2O and NO emissions were calculated from NH_3 values and following the proposed conversion factors for N_2O emissions of 1.1%, 0.8% and 0.9% in urea, NH_4NO_3 and NH_3; and NO emissions of 0.7%, 0.6% and 0.5% in urea, NH_4NO_3 and NH_3 respectively (Bouwman et al., 2002). The stock was considered as the residing mass, material or nutrient in a process or system during the balancing period or system boundaries (Baker, 2012). The stock was built from N bonded to the substrate (the difference between initial and final) 21 and the sum of non-accounted nitrogen in the experiment which have been lost by other means (e.g. leaching, further volatilization).

Diagrams and MFA were made by the program STAN (Substance Flow Analysis, Institute for Water Quality, Resource and Waste Management, Technische Universität Wien). This program allows us to make estimations and data reconciliation, showing balanced results. It was used to display and compare the different N-balances between treatments and application mode by integrating all N sources in a process diagram.

2.6. Experimental design

There were established four N fertilizers (treatment) and two modes of application (level). Also was added a zero-control group (no N-applied). All N fertilizers and application mode consisted on five repetitions to make a total of 45 observations (4 treatments x 2 application mode x 5 repetitions + 1 zero control x 5 repetitions). The complete application was identified as complete and the fractionated application was identified as split (**Appx. 5**).

The experiment consisted of two-factorial design with randomized units. The factorial designed consisted of fertilizers and N-application (treatments x application mode). Analysis of variance (ANOVA) was performed using STATISTICA (StatSoft, Inc, Tulsa, OK. USA, 2012) and graphs were performed using R-Studio Team (2016, Integrated Development for R. RStudio, Inc., Boston, MA) and Microsoft Excel (2013).

The main effects to be considered were the application rate of the treatments and the N supplied form. Means were compared using Tukey HSD (honest significant difference) with

α=0.05 (confidence level of 95%). Results were compared regarding the fertilizer, application rate and the interaction between factors to determine possible effects. The zero control was not included in the statistical analysis by not having considerable growth which leads to high variation or negative values.

3. Results

The complete application showed a higher plant growth during the first 3 weeks. After the second N dose, plants with split application recovered, reaching similar sizes as in the complete. Visual observations were made at the middle and end of the experiment (Fig. 5 & 6). All treatment comparison at **Appx. 1, 2 & 3.**

Figure 5 Visual comparison between mid- and end of the experiment, urea complete. Week 4 with approximately 60 cm height (left) and week 7 with approximately 120 cm height and masculine inflorescence formation (right). N deficiencies can be observed at the end of the experiment.

Figure 6 Visual comparison between mid- and end of the experiment, aurin split. Week 4 with approximately 60 cm height (left) and week 7 with approximately 150 cm height, masculine inflorescence and some female inflorescence formation (right). N deficiencies can be observed at the end of the experiment.

3.1. Fresh and dry weight content

Aurin treatments showed significant differences with the other treatments in FW and DW. CROP and urea had no significant differences between treatments and application mode. Synthetic urine showed the lowest values among the treatments (**Appx. 7 & 8**). No significant differences were found between application modes of the same treatment (**Tab.6).**

3.2. Fertilizer efficiency

Fertilizer efficiency was calculated from DW yield and g N-applied (g DW g N-applied^{-1}). Aurin showed the highest efficiency regarding N use, followed by CROP, urea and synthetic urine respectively (**Fig. 7**). Aurin and CROP in the complete application showed significant differences. Urea and CROP had similar N efficiency. No significant differences were found between application modes within treatments (**Tab. 6**).
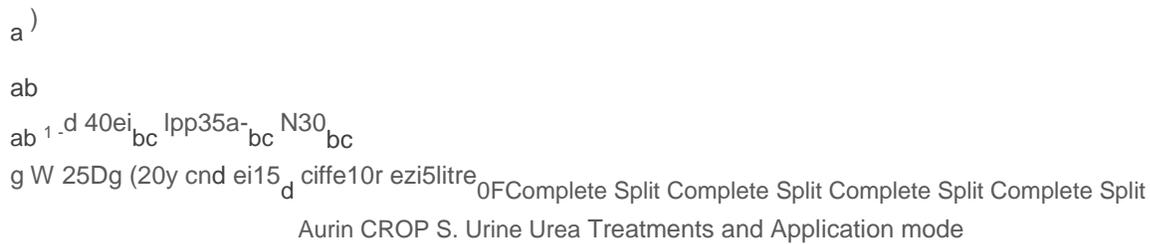
45

a $^{)}$

ab

ab ^{1} -^{d} 40ei$_{bc}$ lpp35a-$_{bc}$ N30$_{bc}$

g W 25Dg (20y cnd ei15$_{d}$ ciffe10r ezi5litre$_{0F}$Complete Split Complete Split Complete Split Complete Split

Aurin CROP S. Urine Urea Treatments and Application mode

Figure 7 Fertilizer efficiency (dry weight produced per unit of nitrogen applied). Values represented as mean ± standard error. Analysis of variance was determined with two-way ANOVA (α ≤ 0.05)

3.3. NH$_3$ Volatilization

Synthetic urine showed the highest volatilization rates among the treatments during the 72 hours of the experiment. It had values from 68.4 - 148 mg NH$_3$-N pot^{-1} in split and complete application respectively. The other treatments showed emissions of 15.3 - 25.4 for urea, 7.20 - 7.80 for Aurin and 5.00 - 5.60 for CROP expressed in mg NH$_3$-N pot^{-1} **(Tab. 5).** The zero-control treatment also emitted 4.70 ± 0.01 mg NH$_3$-N pot^{-1} despite having no N-application (data not shown).

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A four-hour timespan emission changes (in mg NH$_3$-N pot^{-1}) are shown for the total 72 hours of the experiment (**Fig. 8**)**.** Different y-axis is given to each graph for better appreciation of differences between application modes within treatments.

Synthetic urine showed high emissions since the beginning of the experiment (both application mode) and decreased immediately (next 4 hours). The highest peak was observed right after the application. Aurin treatment showed the highest peak 24 hours after application (both application modes) and decreased for the next 48 hours. All complete treatments, except Aurin complete, showed higher emissions than the split application

during the 72 hours of the experiment. Urea treatment reached the highest volatilization

peak at 48 hours after application, then a smooth decay in the following hours (48-72 hours).

This trend was also shared by CROP treatments.

Figure 8 Complete and split application comparison regarding volatilization rates in the same treatment. Values expressed in mg NH_3-N pot^{-1} for 72 hours. Dots are marked every 4 hours and correspond to interpolated values (except 0, 24, 48 and 72). No day/night or air fluctuations are contemplated in the experiment. Each graph has different y-axis to compare application modes in the same treatment.

Volatilization losses regarding applied N (in %) and the global warming potential (GWP) in

CO_2 equivalents (tones $hectare^{-1}$ $fertilization^{-2}$) were calculated (**Fig. 9**). Synthetic urine showed the highest losses and GWP among all treatments. Aurin and CROP showed

values <0.6% of the total N-applied lost by volatilization and the lowest GWP among the

treatments (**Tab. 4**). As %N losses and GWP were calculated from the volatilization experiment, significant differences were maintained between treatments and application mode (**Tab. 5**, volatilization section)

16

40

1435123010258206154102500Complete Split Complete Split Complete Split Complete Split

Aurin CROP S. Urine Urea

Figure 9 Volatilization losses regarding N application (%, bars), and the global warming potential (GWP) in CO_2 equivalents (tones hectare^{-1} fertilization^{-2}, dots) among treatments and application mode.

Table 4 Volatilization losses and GWP regarding the total nitrogen application during the 72 hours volatilization timespan experiment. Values ± standard error.

Treatments	NH₃-N lost %		CO₂ equivalents ton ha⁻¹ fertilization⁻²	
	Complete	*Split*	*Complete*	*Split*
Aurin	0.50 ± 0.03	0.54 ± 0.02	1.74 ± 0.08	1.89 ± 0.07
CROP	0.48 ± 0.03	0.43 ± 0.02	1.35 ± 0.07	1.21 ± 0.06
S. Urine	11.7 ± 0.32	5.22 ± 0.27	35.8 ± 0.96	16.5 ± 0.90
Urea	2.01 ± 0.07	1.21 ± 0.03	6.14 ± 0.20	3.70 ± 0.09

3.4. Nitrogen concentration (N_t)

Leaf N-concentration in plants showed significant differences between treatments, although no differences within application modes. Aurin split showed the highest N_t of all treatments and application mode (**Appx. 9**). The N_t in shoot showed no significant differences within application mode (**Tab. 6**). The concentration from highest to lowest was Aurin, CROP, urea and synthetic urine. Treatments CROP and urea showed no significant difference between them (**Fig. 10**).
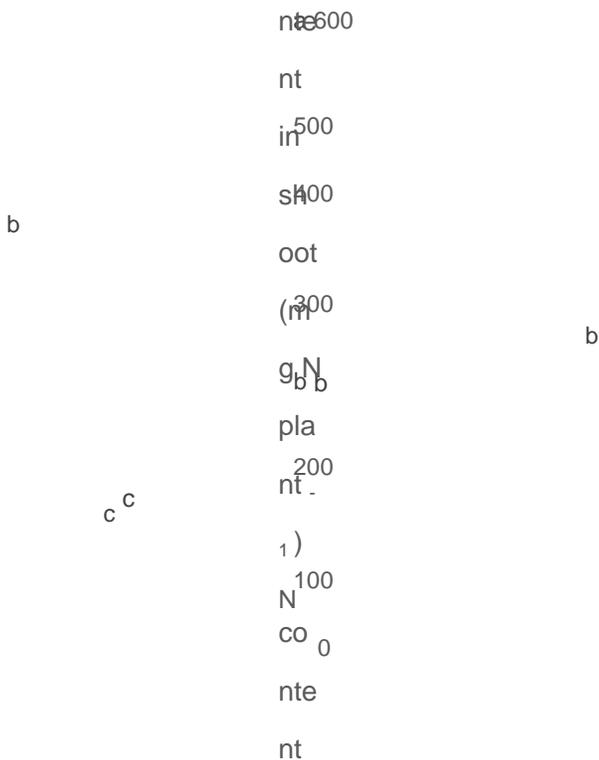
CO_2 equivalents (ton ha $_{-1}$ fertilization $_{-2}$)

% NH_3 -N pot $_{-1}$

% N losses
CO2 equivalents
26

N content in shoot (mg N plant $_{-1}$)
N content

600
500
400
300
200
100
0

b

b

c   c

b

b b

g N

pla

nt $^{-}$

$_1$)

Complete Split Complete Split Complete Split Complete Split

Aurin CROP S. Urine Urea

Treatments and Application mode

Figure 10 Total nitrogen content in shoot. Results obtained by the sum of the N content from stem, leaves, flower and fruit (when av
by each part DW. Values represented as mean ± standard error. Analysis of variance was determined with two-way ANOVA (α ≤ 0.0

3.5. Substrate Analysis

3.5.1. Nitrogen content in substrate (N_t)

The substrate had 70 mg kg^{-1} of N_{min}, 4.14 g kg^{-1} DW of N_t and a pH of 6.3 at the beginning of the experiment
Concentration and values changed but no significant differences were

found between application modes of the same treatment, although there were between

treatments (**Tab. 6**). N_{min} concentration variates from 16.4 - 76.8 mg kg^{-1} in complete application and between
mg kg^{-1} in split application; N_t variated from 4.91 - 5.98

g kg^{-1} DW; and pH from 6.28 - 6.77.

3.6. Material Flow Analysis (MFA)

The N balance was obtained by the sum of all the inputs, outputs and changes in the stock

(or delta) in the experiment. Shown results were expressed as mg N per pot during the experiment (**Fig. 11 & 12).** Aurin has a subdivision identified with a mark (*) due to the development of fruits in some replications. The data of these replicates was isolated and grouped in a different MFA scheme.

Most of the plant parts between the same treatments showed similar N concentrations. Some differences were found in Aurin complete stem and flower, Aurin split (*) flower and fruit, CROP complete flower and synthetic split leaf and stem with a difference of 11%, 23%, 50%, 16%, 18%, 35% and 38% respectively regarding their counterpart. Other parts showed changes under 10%.

Changes in the stock between application modes was 20 - 60 mg N per pot. Aurin, CROP and synthetic urine showed similar changes in stock (44 – 60 mg N) while urea showed the lowest (20 mg N). High adsorption or immobilization values were discovered in the experiment, approximately the 85% of the N-applied in synthetic urine was adsorbed to the substrate. Immobilization rates ranged between 65-85% been Aurin complete the lowest and synthetic split the highest.

Figure 11 Nitrogen balance between treatments and application modes by MFA system. Aurin treatment have a double scheme (ma feminine inflorescence formation (or fruit). The separation made by the observation of changes in the nutrient distribution. Given val in mg N per pot or productive unit (average ± standard error).

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Figure 12 Nitrogen balance between treatments and application mode by MFA system (Material Flow Analysis). Given values are ex
per pot (average ± standard error).

Table 5 Substrate analysis (Volatilization, final pH, N mineral and total N in substrate)
Treatments

Volatilization [(1)] pH N mineral Nt substrate *(mg NH₃-N pot⁻¹) (mg N kg⁻¹) (g N kg⁻¹)* Complete Split Complete Split Complete Split Com
7.2 ± 0.3 e 7.8 ± 0.3 e 6.40 ± 0.03 d 6.28 ± 0.03 e 16.4 ± 3.2 b 15.2 ± 2.6 b 4.91 ± 0.06 4.96 ± 0.09 **CROP** 5.6 ± 0.3 e 5.0 ± 0.3 e 6.77 ±
0.01 a 49.0 ± 7.8 ab 28.6 ± 1.6 ab 5.85 ± 0.21 5.96 ± 0.10 **S. Urine** 148.4 ± 4.0 a 68.4 ± 3.7 b 6.49 ± 0.01 cd 6.48 ± 0.01 cd 76.8 ± 5.7
5.89 ± 0.19 5.79 ± 0.09 **Urea** 25.4 ± 0.8 c 15.3 ± 0.4 cd 6.57 ± 0.01 bcd 6.61 ± 0.02 bc 32.5 ± 5.2 ab 42.6 ± 3.1 ab 5.79 ± 0.13 5.98 ±

given by the average ± standard error unless is specified differently. [(1)] Results are given as the sum of the results interpolation ± standa

letter in the same parameter indicates significant differences among treatments and application mode. Analysis of variance was determi

ANOVA (α ≤ 0.05)

Table 6 Plant material analysis (Shoot dry weight, nitrogen plant content and fertilizer efficiency)
Treatments

Dry Weight (DW) N concentration in shoot Fertilizer efficiency
(g plant⁻¹) (mg N plant⁻¹) (g DW g N-applied⁻¹ plant⁻²) Complete Split Complete Split Complete Split **Aurin** 54.2 ± 0.8 a 45.6 ± 1.7 ab 53
a 41.3 ± 0.5 a 35.0 ± 1.3 ab **CROP** 29.3 ± 2.0 b 37.8 ± 1.4 b 287 ± 8 b 343 ± 12 b 28.4 ± 1.9 bc 35.5 ± 1.2 ab **S. Urine** 10.4 ± 1.3 c 13.
c 143 ± 10 c 9.1 ±

Urea 26.9 ± 1.4 b 24.9 ± 1.4 b 250 ± 14 b 241 ± 7 b 23.6 ± 1.3 bc 22.1 ± 1.28 bc Values are given by the average ± standard error unles

differently. Different letter in the same parameter indicates significant differences among treatments and application mode. Analysis of v

determined with two-way ANOVA (α ≤ 0.05)

30

4. Discussion

Aurin treatment showed the highest DW yield. The combination of NH_4^+ + NO_3^- contributes to a higher N c
plant, along with higher NH_4^+ assimilation efficiency, a reduction of nitrate reductase activity and therefore a
DW yield (Barker &

Bradfield, 1963; Huffman, 1989). CROP also presented this mixture but at a lower ratio (1:1

Aurin vs 1:14 CROP). Aurin treatment achieved the expected DW yield (40 g plant⁻¹)

although the higher N input provided (+185 mg N extra compare to the other treatments)

could explain this effect. None of the treatments or application mode achieved the expected

leaf N concentration (28.0 - 31.5 g N kg⁻¹ DW). All treatments showed concentrations from

10.9 - 16.9 g N kg⁻¹ DW. Aurin treatment and CROP split showed no differences in fertilizer

efficiency despite showing differences in N concentration in shoot. This can be explained

by the dilution effect which is consider as the relationship existing between the nutrient

content and the crop response (Jarrell & Beverly, 1981). S. urine split showed similar N

concentration in leaves with Aurin (both treatments) although the DW and fertilizer efficiency

shown significant differences. Despite Aurin received more N than S. urine split (+185 mg) and showed lower N volatilization (0.6% vs 5.2%), this did not influence the N concentration. Other authors had described this as the phenome where N concentration is similar between well-fertilized plants and those under depletion or limiting nutrient supply (Lea & Morot-Gaudry, 2001).

Other factors might have influenced the N concentration in shoot. Immobilization is the process in which N is taken by soil microorganisms making it unavailable for the plants, this can be stimulated by high carbon content material in the soil (Jonhson et al., 2005). The immobilized N in the experiment was in average 77% (66 - 85%). Literature estimates values about the 19% of immobilization at field experiments (Lea & Morot-Gaudry, 2001). The substrate contained high carbon content materials (wood bark-compost, wood bark-humus, wood fiber, coco peat and rice husk) which could increase the N immobilization ratio. The highest immobilization rates were found in synthetic urine (>82%) which could explain the underperformance of this treatment during the whole experiment. By indirect methods can be inferred that 1-4% of N_t is available as N_{min} (Aranguren et al., 2018). The N_{min} analysis sho 1.30% of the applied N was found in NH_4^+ and NO_3^- form in the substrate after the experiment. By this, can I most of the N was immobilized and plant development was highly correlated to their ability to obtain nutrients from the substrate. Other factors such as salt stress was not considered, although could have a significant effect on the plant development and/or behavior during the experiment. Maize growth and development can be compromised by the exposure to high sodium and chloride concentrations, causing nutritional imbalances up to deficiency levels (Farooq et

al., 2015). Despite this, the treatments with the highest sodium or chloride concentrations (CROP and Aurin) showed better or similar results than the mineral control.

Hypothesis i: Fertilizer treatments will not significantly differ in plant shoot N concentration (mg N kg^{-1} DW). This hypothesis is rejected due to the existence of significant differences between treatments. In average, the N_t in shoot was 533, 315, 123 and 245 mg N kg^{-1} CROP, S. urine and urea respectively. Differences were

found despite the N supply was expected to be the same (1260 mg plant^{-1}) although, some

treatments receive more or less N (e.g. +185 mg for Aurin and -34 mg for CROP). No

significant differences were found between application modes within treatment.

All treatments showed NH_3-N losses by volatilization. Nitrified urine-based fertilizers showed lower volatilizati
urea and synthetic urine. This shows the importance

of the nitrification processes in comparison to the use of raw resources. This increases the

N availability and reduce losses by volatilization (Etter & Udert, 2015). The reaction time

regarding N-losses by volatilization varied between treatments. Synthetic urine showed

the highest volatilization peak right after application (hour 0), Aurin was after 24 hours and

urea and CROP after 48 hours. Non-urea based fertilizers tend to show lower volatilization

rates than urea based ones due to the lack of hydrolysis process (Pan et al., 2016). Despite

the synthetic urine is non-urea based, it showed higher volatilization rates than urea. This

could be explained by the N-form (NH_3) in this fertilizer. Urea volatilization reaction occurs approximately 2 d
application due to the hydrolysis process by the soil bacteria

(Harper et al., 1983). Urea treatment showed the expected time reaction with the highest

peak at approximately 48 hours after application. CROP treatment also showed the same

trend (48 hours peak) despite been a nitrified urine-base fertilizer with a higher rate of NO_3^- than NH_4^+. In
process, it's expected to have 2-4% losses of the total N application within the next 72 hours after superficia
soils at temperatures

between 15 - 23°C (University of Minnesota, 2018). Obtained results showed values from

1.20-2.00% in urea during the next 72 hours after application. N losses in urea treatment

was close to the ranges proposed in literature.

Hypothesis iv: Urea treatment would show a time delay in NH_3 emissions regarding nitrified urine-bas
This hypothesis is partially accepted. Urea indeed showed

the highest NH_3 volatilization peak at 48 hours after application while Aurin was after 24 hours. CROP showe
reaction time than urea, despite been a nitrified fertilizer.

Synthetic urine treatment showed the highest NH_3 volatilization peak right after application.

Ammonia volatilization was higher in treatments with a higher ratio of N present as NH_4^+. CROP treatment sh[...]
lowest volatilization rates, followed by Aurin, urea and

synthetic urine respectively. The balance between NH_3 and NH_4^+ is mainly depending on the pH of the so[...]
higher pH and temperatures promotes NH_3 formation (Purwono et al., 2017). CROP also presented the low[...]
used fertilizers. Can

be addressed that along the NH_4^+ content, the pH also has a major influence in the NH_3 formation. Synthetic u[...]
expected to present the higher volatilization values due to the

main N form (100% NH_3-N) and the pH of the solution (8.90-9.20). Urea volatilization is more related to hydrol[...]
(CH_4N_2O-N in amid form to NH_4^+ and CO_2) and the temperature. The NH_4^+ content in the fertilizer was CROP[...]
< s. urine; the

losses by volatilization followed the same pattern.

Hypothesis ii: **NH_3 emissions are correlated to the NH_4^+ content in Aurin (50% NH_4^+- N), CROP (<10% NH[...]
(100% NH_3-N) and urea (CH_4N_2O) fertilizers.** This hypothesis is accepted due to the correlation that was fou[...]
content of NH_4^+ and the losses by volatilization as NH_3. CROP had the lowest NH_4-N form, thus the lowe[...]
although it was not significantly different than Aurin treatment.

No significant differences were found in N losses by volatilization between application rates

in the same treatment in Aurin, CROP and urea. Pan et al. (2016) described that that NH_3 volatilization was n[...]
the split or complete applications, but it was related

significantly with the amount of N applied. Complete application had 50% more N than the

split application when volatilization measurements were made. Urea complete showed

higher N-losses by volatilization than split application, although no significant enough.

Ammonia volatilization in most research studies are measured in field experiments and

respond to *in-situ* conditions which can vary greatly, even between the same N-source (He

et al., 1999; Nett et al., 2016). Also, the temperature in the experiment was controlled.

Therefore, this factor might have some influence in the low N-losses by volatilization in urea.

Warm temperatures (>25°C in incubation experiments in soil) reported 2- to 3-folds higher

volatilization than temperatures below 25°C, also enhance hydrolyzation processes and

changes the chemical equilibrium, increasing the NH_3 production (He et al., 1999; IPNI, n.d.). The low NH_3 e[...]
nitrified urine-based fertilizers can be addresses to the nitrification process, in which all N sources are present[...]
or NO_3^-, therefore N transformation by soil microorganisms is minimal. Also the acidic pH in the nitrified urine[...]
based fertilizers decrease the N volatilization potential regarding alkali solutions (He et al.,

1999). The zero control also showed some NH_3 emissions, which can be explained by remaining microbia[...]
substrate, contamination from aerial NH_3 or remaining gasses in the chamber during the sample exchange.

Volatilization analysis was not performed after the second N dose. The N losses in Aurin

and CROP might have variated if the a second measure were made, increasing the losses

in the split application. Following this assumption, NH_3 losses might had no significant difference in synthetic
it might have significance in Aurin and CROP

application mode.

Hypothesis iii: Higher N application (complete>split) will show higher NH_3-N losses by volatilization.
hypothesis is partially rejected. Higher N application (or availability)

did show higher NH_3 losses in all treatments, except Aurin. Despite this, only synthetic urine showed significa
between complete and split applications. Volatilization rates

are also influenced by external factors such as pH, nitrification processes and temperature,

which could have a higher impact than the N application itself.

The material flow analysis (MFA) allowed to make an environmental impact analysis, taking

in consideration all different factors involved in agricultural production with limited

boundaries and presented in a graphical form. It was found a global warming potential

(GWP) in the treatments with values in tones CO_2 equivalents ha^{-1} fertilization^{-2} of 1.74 - 1.89 for Aurin, 1.21 -
CROP, 16.54 - 35.88 for synthetic urine and 3.70 - 6.14 for

urea. Other studies found CO_2 equivalent emissions per hectare in a ranges from 1.5 – 10.0 tones CO_2 in fertili
fields by direct measurements or estimated emission regarding the N applied to the field (approx. 2.3 tones C
ha^{-1}) by indirect measurements (Brentrup, 2008; IPCC, 2006). Aurin, CROP and urea showed lower GWP

than suggested for direct estimation (<10 tones CO_2 equivalents ha^{-1}), but urea had higher

estimations than the indirect estimation (>3.7 tones CO_2 equivalents ha^{-1}). Thanks to the MFA and the GWP
importance of nutrient recycling of available sources can

be observed. Urine is an unexploded available source of nutrients, usually lost in aquatic

ecosystems and mixed with other substances, which can cause contamination problems

(e.g. eutrophication). Urine contains a considerable amount of nutrients which are needed

by plants. Some of these nutrients come from non-renewable ones as P mining or from

highly energy demanding processes as N from Haber-Bosch process. Beside this, nitrified

urine-base fertilizers showed similar or higher performance than traditional mineral

fertilizers (Aurin/CROP vs urea) in fertilizer use efficiency and DW yield. Also showed the

importance of the nitrification process from raw urine before usage (Aurin/CROP vs

synthetic urine) regarding efficiency and losses by volatilization.

Further studies must be done regarding the feasibility of the urine recycling processes regarding recollection, transformation and energy use, besides a study regarding human safety (e.g. pharmaceutical concentration or substances bioaccumulation in eatable plant tissues).

5. Conclusions

Nitrified urine-based fertilizers (NUF) showed similar or higher accumulation of FW, DW and N efficiency than mineral fertilizer. Also, the DW accumulation was higher than expected (>40g DW). Some replications were able to develop and sustained secondary growth (tillers) and female inflorescence.

Application mode (complete and split) showed no significant differences within treatment in most of the parameters, which can be inferred that N application rates has no influence in the final results of the treatments.

Total N-concentration plant^{-1} (complete and split) showed no significant differences within treatments. A different nutrient distribution depending on the application mode was observed. The analyzed section with higher variability in the N-content was the flower. Urea was the only treatment with similar N-content values in all analyzed sections regardless of the application mode.

The NUF showed lower volatilization potential than mineral control and the human urine imitation, whereby it's possible to supply nitrified recycled nutrients to plants and reduce the N-losses by volatilization (up to 68%) compared to urea. This statement is valid for the given conditions and methods used in the experiment.

Nitrogen mixtures (NH_4^+/NO_3^-) showed better performance in all analyzed parameters regarding other N-form the transformation process from raw urine into nitrified

solutions are a suitable option to be used as a recycled N-source. Economic viability and environmental impact studies must be done to determine feasibility and sustainability in

time.

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7. Appendix

Appendix 1 Plant visual comparison at week three (3) between treatments and application mode. Aurin (first row), CROP (second), s (Third) and urea (forth). Application: Complete (left) and split (right). Each yellow line represents a 20 cm difference; pot height is app cm.

Appendix 2 Plant visual comparison at the end of the experiment (7th week) between treatments and application mode. Aurin (first ro CROP (second). Application: Complete (left) and split (right). Each yellow line represents a 20 cm difference; pot height is approxima

Appendix 3 Plant visual comparison at the end of the experiment (7th week) between treatments and application mode. Synthetic uri and urea (second). Application: Complete (left) and split (right). Each yellow line represents a 20 cm difference; pot height is approxi

Air Flow Analysis, Climate Chamber

0.120

0.1400.130

0.110
$_A$ir Flow (m/s)

0.100

0.090

0.080

0.070

0.060

3/4/2019 3/5/2019 3/6/2019 3/7/2019 3/8/2019 3/9/2019 3/10/2019 3/11/2019 3/12/2019

Appendix 4 Air flow analysis in climate chamber (7 days measurements, 5 minutes interval). Total of 3948 values analyzed with a mean m/s (10.87% C.V)

Days

Treatment Application mode Code Color ID Replicates
Aurin Complete AC Blue 1-5
Aurin Split AS Blue 1-5
CROP Complete CC Green 1-5
CROP Split CS Green 1-5

Zero control Blank B White 1-5
Synthetic urine Complete SC Yellow 1-5
Synthetic urine Split SS Yellow 1-5
Urea Complete UC Red 1-5
Urea Split US Red 1-5
Appendix 5 Treatments and application mode identification system.

Element **Aurin CROP Synthetic urine**

mmol L^{-1} mmol L^{-1} mmol L^{-1} N Nitrogen 2999 384 568 P Phosphorus 56 13 6 K Potassium 382 28 56 Ca Calcium 0 153 0 Mg Ma
Sodium 739 80 111 S Sulfur 100 10 5 Cl Chloride 874 84 118 B Boron 1.39 0.00 0.00 Fe Iron 0.02 0.00 0.00 Zn Zinc 0.18 0.00 0.00 **Ap**
composition (Aurin, CROP and synthetic urine) according to la

400

a a 360 320 280 240

b b

200
d
Complete Split Complete Split Complete Split Complete Split
Aurin CROP S. Urine Urea

Appendix 7 Shoot fresh weight (FW) plant^{-1} during 7 weeks of experiment. Value represented as mean ± standard error. Analysis of var

by two-way ANOVA (α≤0.05)
)

$_1$ .t nalpW Fb g (t $_{bc}$ hgie$_{160}$

cd w$_{120}$ h ser80 ft o40oh$_0$STreatments and Application mode

45
b 60 55$_a$ 50$_{ab}$ 4540$_b$ 35$_b$

b 30252015$_c$ c 105$_0$Complete Split Complete Split Complete Split Complete Split

Aurin CROP S. Urine Urea

S)

$_1$ .t nalpW Dg (t hgiewy rdt oohTreatments and Application mode

Appendix 8 Shoot dry weight (DW) plant^{-1} during 7 weeks of experiment. Values represented as mean ± standard error. Analysis of vari

by tow-way ANOVA (α≤0.05)
18

a)

W16$_{ab}$

D$_{ab}$ $_1$ -14$_b$

g k$_b$ b

b g 12$_b$ (s e10vael8n i6n oit4art2nec$_0$nocComplete Split Complete Split Complete Split Complete Split

N Aurin CROP S. Urine Urea Treatments and Application mode

Appendix 9 Nitrogen concentration in leaves. Values represented as mean ± standard error. Analysis of variance was determined with t

Statistical Analysis (by Statistical Software)

ANOVA (Treatment * Level ~ DW)

ANOVA (Treatment * Level ~ mg N per plant)

ANOVA (Treatment * Level ~ Yield)

ANOVA (Treatment * Level ~ N mineral)

ANOVA (Treatment * Level ~ pH final)

ANOVA (Treatment * Level ~ Nt substrate)

ANOVA (Treatment * Level ~ g/kg leaves)

ANOVA (Treatment * Level ~ FW)