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Ammonium Fixation and Microbial Immobilisation-Mineralisation Processes can Quench N Losses in a Mollisols Located in a Sugarcane Plantation in Central Venezuela

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Abstract

In a Mollisols cultivated with sugarcane (*Saccharum officinarum*) located between Farriar and Palmarejo, Edo. Yaracuy, Venezuela, with abundance of 2:1 clay it was analyzed the ammonium fixation (NH_4f) in the different horizon of the soil profile (0-200 cm), and the changes in NH_4f during two crop seasons (ratoons) of the cane plantation. The soil of the experimental plot presented a high proportion of N as fixed ammonium associated to 2:1 clay, representing 18-57 % NH_4f/total-N within the profile. Ammonium was mainly fixed in the silt fraction. There was a high positive correlation between NH_4f and silt content, correlations however decreased in the case of the association between organic matter and cation exchange capacity with ammonium fixation, on the contrary non-association was found between NH_4f and the clay content. We have found significant changes in the NH_4f contents along the growth development of the sugarcane, which are associated to the application of fertilizers and the intensity of soil microbial processes, particularly mineralisation and ammonification-nitrification. The ammonium fixed in this acts as a nitrogen-reservoir, which can be released and, in turn, be available at the stages of higher crop requirements.

Keywords: *Saccharum officinarum*; Montmorillonite; 2:1 clay; Ammonification

Introduction

In tropical, subtropical and temperate agroecosystems, nitrogen (N), phosphorus (P) and potassium (K) have merited the greatest attention due to their essential role in crop growth and plant production processes. After nitrogen fertilization, the pathways through the N of the agroecosystem is removed are multiple. A substantial proportion of the N in agricultural production systems is subtracted through harvesting, important losses can also occur through gaseous forms by burning crop residues, volatilization and denitrification processes, while also in drainage waters inorganic soluble forms of nitrate (NO_3), ammonium (NH_4) as well organic N-forms are lost [1,2]. However, the soil plays an essential regulatory role in the reactions in which the N intervenes through microbial origin processes (nitrification, ammonification, immobilization and mineralisation of N).

In many areas the mineralisation of the organic matter can supply adequate plant available N to produce maximum yields. In Florida muck soils of the Everglades Agricultural Area (EAA), the annual N mineralisation can supply well over the N requirement for sugarcane; thus, N fertilization is not recommended [3]. In muck soils of the EAA, N mineralisation rates have been reported to range between 320 to 1340 lbs N acre^{-1} yr^{-1} corresponding to 97% of the total N entering the system [4]. Another important input for N balance in sugarcane agroecosystems corresponds to the nitrogen fixation by free-living organisms associated with the rhizosphere [5]. Sugarcane has been growing in some Brazilian sites for years without fertilizer additions as one indication that N-fixing plays an important role in the nitrogen self-sufficiency of this crop [6].

However, the annual harvest of sugarcane (around 60-100 Mg ha^{-1}), together with the burning of the crop before harvesting, a common practice of management of sugarcane plantations in South America, leads to the loss of significant amounts of nutrients (N,P and K) in the agroecosystems [7,8]. In Venezuelan sugarcane plantations, the harvest is done manually, thus plantations; require previous burning, an operation which leads directly to atmospheric emissions (CO_2, N gases and generation of ozone) and the production of huge amounts of ashes. Those prescribed burning, apart from the environmental deterioration, could progressively diminish the level of soil organic matter and increase nutrient deficiency in soils [9,10], particularly in elements with gaseous biogeochemical cycles as N and S [11] and/or losses and redistribution of some elements with sedimentary cycles e.g. P and K, among others [10,11]. Moreover, the deposition of reactive nitrogen has increased in the last three decades in the northern hemisphere specifically in North America and Western Europe and it has been identified as an important factor related to global environmental change with impacts on biodiversity and human health [9,12].

On the other hand, if soil mineral materials are appropriated, a significant accumulation of ammonium can occur in the different horizons of the profile, a process known as ammonium fixation.

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Fixed ammonium (NH_4f) is defined as adsorption or absorption of ammonium ions by the mineral or organic fraction of the soil in such a way that these ions are not easily interchangeable to the solution [13-15]. Ammonium fixation is a process that occurs mainly in soils where secondary phyllosilicates of type 2:1 with interlaminar expansion such as vermiculite, illite and montmorillonite are abundant. On the contrary, clays of type 1:1 without interlaminar expansion by hydration such as kaolinite and halloysite do not fix significant amounts of ammonium, while amorphous clays (e.g. allophane) have no ammonium fixation at all. In agricultural systems must be differentiated the ammonium natively fixed from that "artificially" fixed, thereby, referring to the ammonium originally retained during soil formation processes, and an additional fixation (adsorption) product of the application of ammonium fertilizers or generated by the internal mineralisation-ammonisation processes, respectively [15-17].

For decades, the Yaracuy River valley in central Venezuela has been a sugarcane production area, yet it has experienced, like other localities in the country, a drastic reduction in the production and weight of sugarcane crop stems [18,19]. Such situation is partially attributed to the abandonment of chemical or organic fertilisation due to the elimination of subsidies by the State. The high biomass production of the cane demands huge amount of nutrients (mainly N and K), so it is customary to fertilise with significant amounts of N and K and in a lower proportion of P.

In the area is notorious the presence of expansive clays type 2:1. Clays of that nature can immobilise significant amounts of N-ammonium that can interfere with nitrogenous and potassium fertilisation of crops [20]. This contribution provides information for a sugarcane plantation about: (i) the fixation of ammonium on the different horizons of the profile (0-200 cm) of a Mollisols characterized by abundance of clays 2:1; (ii) the changes in the contents of NH_4f during two vegetative cycles (ratoons). The information obtained will be related to other processes linked to the N cycle.

Material and Methods

Study Site

The study was conducted in a sugarcane plantation near San Felipe, Yaracuy State, and Central Venezuela (10º29´44´´N and 68º31´44´´W). The experimental site is in a tropical humid climate region affected by marine aerosols with mean annual precipitation and temperature of 1479 mm and 26.8ºC, respectively. The soil of the zone is a Typic Haplaquoll with significant content of carbonates, the presence of 2:1 clay (rich in montmorillonite and muscovite) and a slightly alkaline pH, in general, has a natural high fertility as presented in Table 1.

Soil sampling

In an experimental area of 4.5 ha cultivated with *Saccharum officinarum*, four plots of 300m^2 each were selected. Two of the plots were planted with the variety Puerto Rico PR-1028 and the other two with the variety Venezuela V58-4. To study some soil processes along the experimental period, thirty soil samples (30cm depth) were randomly collected in the experimental plots. The samples were mixed up to obtain four composite samples representative of the experimental plots.

Table 1: Main physical-chemical and mineralogical characteristics of the studied soil.

Depth, (cm)	pH	CEC	%C	%N	C/N	$\%CaCO_3$	Clay type
	H_2O	cmol,kg^{-1}					
0-10 Ah_1	8.4	53	1.87	0.21	8.9	0.99	M Mu K
0-25 Ah_2	8.5	38	1.2	0.14	8.6	0.78	Mu M K
25-42 Ah_3	8.3	41	1	0.1	10	1.57	Mu K M
42-64 C1	8	16	0.6	0.1	6	1.1	Mu M K
64-105 C2	8.8	13	0.5	0.05	10	3	Mu
105-164 C3	8.8	11	0.5	0.03	17	3.6	Mu M K
164-200 2C	9.1	7	0.5	0.03	17	2.8	------

M=Montmorillonite; Mu=Muscovite; K=Kaolinite.

Table 2: Soil texture and ammonium fixation.

Depth (cm)	%Sand	%Silt	%Clay	Texture	NH_4f (mg.kg^{-1})
0-10 Ah_1	13.5	73.5	13.0	Silty loam	386
0-25 Ah_2	11.5	83	5.5	Silty	560
25-42 Ah_3	8.5	85	6.5	Silty	568
2-64 C1	28	65	7.0	Silty loam	311
64-105 C2	36	60	4.0	Silty	337
105-164 C3	29	58	13	Silty loam	275
164-2002C	52	46	2.0	Sandy loam	257

Table 3: Pearson correlation coefficients between NH_4f and other soil properties.

Soil property	CEC	OM	%Sand	%Silt	%Clay	%Clay+Silt
NH_4f	0.739**	0.519**	- 0.829**	0.936****	-0.068^{ns}	0.829**

. * and ****significant at P< 0.025. 0.01 and 0.005 respectively.

Determination of ammonium fixation

Samplings were done at the beginning of the experimental period before transplanting and fertilisation, and during the third ratoon before and after burning operations. The amount of amount fixed to 2:1 clay was determined by the method of Silva and Bremner [21].

Determination of the rate of N nitrification and mineralisation

The rate of nitrification and mineralisation were determined at field conditions in two soil depths (0-15 and 15-30 cm) during the crop season (third ratoon) according with the method proposed by Runge [22].

Statistical analysis

The relationship between NH_4f and soil properties were established using the Pearson correlation coefficient. To compare changes in NH_4f at the different stages of crop development one-way variance analyses were performed with the SP program [10]. The established confidence level was P>0.01.

Results and Discussion

Ammonium fixed in the soil

The phenomenon of ammonium fixation has been known for a century [23], and in tropical soils cultivated with sugarcane was reported as early as 1954 by Rodriguez [24]; this process is of importance in the nitrogen economy, particularly in agricultural soils, although it is restricted to the presence in the soil profile of clays with the capacity to retain ammonium. The NH_4f in the arable layer

Table 4: Nitrification and mineralisation rates in different climatic periods. Values in kg ha^{-1} day^{-1}. Different letters indicate significant differences (p<0.05) at different seasons.

	Beginning of rain	Top of rain	End rain	Dry season
Nitrification rate	1.21a	1.29a	0.49b	0.47b
Mineralisation rate	1.10a	1.37a	0.88a	0.41b

Table 5: Daily and monthly mineralisation rates during the ratoon growth. Values in kg. ha^{-1}.

Age of ratoon (days)	Daily rate	Monthly production
30	0.75	22.40
62	1.71	54.64
96	2.33	72.08
127	1.28	39.68
162	0.85	29.56
180	0.53	14.76
218	1.12	31.48
248	0.85	23.92
270	0.67	23.96
308	0.58	15.52 Cumulative values 328

of the soils has a wide range, closely related to differences in parent materials, there can also be large differences at the regional level [15,25]. In soils with high 2:1 clay contents NH$_4$f values of 2000-3000 kg N ha^{-1} have been recorded [15]. For tropical soils, Stevenson [26] stated that the amounts of nitrogen as fixed ammonium could vary between 282 and 1920 mg N kg^{-1} of soil. Therefore, the release of the fixed ammonium in agricultural soils is of special importance, since it can contribute to supply part of the necessary early N requirement of the crops.

The soil of the experimental plot presented a high nitrogen content as fixed ammonium (368-568 mg.kg^{-1}, Table 2), representing between 18-57% of NH4f/N-total in the profile (0-42 cm), and usually do not respond to N and K-fertilisation [18]. The high NH$_4$-fixation is due to the presence of montmorillonite and muscovite clays at the upper horizons of the soil (Table 1). Ammonium retention is mainly attributed to the presence of the silt fraction since the clay content in this Mollisols was not particularly high (Table 2). In the soil profile there was a slight tendency to decrease the content of NH$_4$f with depth (P<0.005) due to the lowering of the silt fraction and a concomitant increase in the sandy component (Table 2). With respect to the variation of NH$_4$f with depth, the data in the literature show contrasting results. In this regard, some authors report that the ammonium fixed increases with soil depth [25,27]; on the contrary, other studies point out a declination or that there is not a clear trend [28].

A high positive correlation (P<0.005) was found between the percentage of silt, and the percentage of silt plus clay with NH$_4$f (Table 3). Whereas, that correlation decreases with Cation Exchange Capacity (CEC) and Organic Matter (OM) content, on the contrary, no correlation was found with the percentage of clay, while the association between NH$_4$f and sand content, as expected, was negative. Those results coincide with Black and Waring data [28], who proposed that the ammonium fixed in some Australian soils is due to the components of the silt fraction, rather than the clay or cation exchange capacity parameters. Likewise, Jensen et al. [29] also found a high positive correlation between fixed ammonium and percentage of clay plus silt, not so with the percentage of clay only.

Mineralisation and net nitrification

There was an intense nitrification in this soil (Table 4). The production of nitrate (difference between incubated samples and control) varied considerably according to the different climatic periods of sampling. A maximum value was found at the top of the rainy season, and then it diminished along the dry period (Table 4). The rate of N-mineralisation (calculated as the sum of nitrate plus ammonium production) along the crop season followed a similar trend to N-nitrification (Table 5). In fact, during the 308 d which lasted the ratoon, 328kg N.ha^{-1} has been accumulated by mineralisation (Table 5), which leads, by extrapolating, to an annual mineralisation of 343kg N ha^{-1}, a value comparatively higher than those recorded in natural ecosystems [30] but a common figure in agroecosystems with a good supply of organic matter [3,31]. It is possible that this high nitrification is a consequence of the low C/N ratio (6-10) of the soil (Table 1) as well as the fertilisation with urea. It is known that in soils of low ratio C/N (<18), the process of nitrification is favored. Likewise, fertilisation with urea generates a greater production of nitrates [32].

Fixed ammonium and crop development

Changes in the NH$_4$f during certain seasons of crop growth are associated with an incorporation of NH$_4$f by crops; this form of nitrogen is enough mobile and can be considered as a type of nitrogen reserve in the soil [33]. Notable variations in the ammonium fraction fixed during crop development were recorded in the sugarcane plantation with significant increases and reductions associated with fertilizer application and the soil microbiological processes (Table 6).

To explain those differences, it is necessary to separate the two different forms of NH$_4$f that could be found in the agroecosystem. The variation in the content of NH$_4$f throughout the development of sugarcane cultivation, is possibly since the fine fraction fix and release ammonium coming from fertilisation, rainfall input and/or mineralisation [34]. In this way, a large proportion of N-ammonium "artificially fixed" with the fertiliser is released and can then follow the different steps of the nitrogen cycle: nitrification and immobilization (absorption) by plants and microorganisms. Thus, the sharp decrease observed between 2R and the beginning of 3R, when the plantation has not been yet fertilized, corresponds to a mobilisation of ammonium fixed to the soil solution (Ammonium fixed, Ammonium Interchangeable, Ammonium in solution). Some of the ammonium artificially fixed and released at the beginning of the development of the crop (30 days in the third ratoon) can be used by the crop or be nitrified. Subsequently, an increase in NH$_4$f values was observed between the beginning of 3R (30 days) and the end (309 days) which corresponds to the ammonium retained "artificially" from fertilisation and mineralisation (Table 6).

In short, it is possible to suggest that some of the nitrogen coming from the fertiliser is fixed to the fine fraction of these soils, which explains information previously published by López-Hernández et al. [35] and López-Hernández and Infante [10] of why leach losses, in

Table 6: Changes in the fixed ammonium NH_4f (means±standard deviations) during crop growth. Means followed by different letters differ significantly.

Crop age (days) at different ratoons	Crop development and agronomic management	NH_4f (mg.kg^{-1})
248 days after second ratoon (2R)	After fertilisation, near maximum biomass	544±7.5 c
30 days after third ratoon (3R)	Beginning of vegetative stage, non-fertilised	391±11.7 a
147 days after third ratoon (3R)	Mid-cycle vegetative stage, after fertilisation	474±43.0 b
309 days after third ratoon (3R)	Maximum biomass before burning	560±20.6 c

this agroecosystem, particularly ammonium losses, are so low. In this agrosystem only 2.3% of the applied fertiliser (5.31 kg.ha^{-1}.year^{-1}) is lost by leaching, mainly as nitrates [35].

On the other hand, the nitrification process was very intense in the Mollisols studied (1.24 kg. ha^{-1}.day^{-1}, Table 4). This intense nitrification is in line with a high rate of mineralisation (1.37 kg.ha^{-1}.day^{-1}, Table 4), which corresponds to a cumulative annual mineralisation of 343 kg.ha^{-1} (Table 5) [10], a value comparatively higher than that recorded in other agricultural systems [3]. In addition, urea fertilisation increases nitrate production [36]. Nommik (1981) [37] noted that the nitrification process leads to a removal of exchangeable ammonium in soils, which can lead to a release of the fixed ammonium. It has also been found that the presence of N-serve (a nitrification inhibitor) reduces the release of newly fixed ammonium, suggesting that nitrifying organisms are intermediaries in making the fraction of ammonium fixed available to crops [38].

The release of ammonium fixed to clays is of special importance, as it can contribute to supplying some of the necessary N to the cultivation [39]. In the first 30 days of crop growth, the total N content in the plantation was around 20 kg.ha^{-1}, a value like the content of N in weeds [10,35]. This indicates that by that time; the plant subsystem had incorporated approximately 40 kg.N.ha^{-1}. Considering that by this date the fertiliser had not yet been applied (Table 6), and that the amount of nitrogen available from net mineralization only reached 22.4 kg.ha^{-1} (Table 5), a deficit can be estimated in the nitrogen requirement, at that stage, of 17.6 kg.ha^{-1}. That deficit can be covered by the release of ammonium fixed in the clay component. Black and Waring [28] found that when the soil is cultivated intensely and consecutively virtually all ammonium fixed "artificially" can be available to plants; under these circumstances it can be considered that ammonium fixed in soils acts as a reservoir of nitrogen, which is released and therefore made available in the stages of greatest requirement by the crop.

Conclusion

The soil of the experimental plot did not respond to N-fertilisation, and presented a high nitrogen content as fixed ammonium (18-57% ammonium fixed relative to the N-total) in the profile. The high fixation of this mineral N is due to the presence of 2:1 clay (montmorillonite and muscovite). A high positive ratio ($P<0.005$) was found between NH_4f and the percentage of silt and the percentage of silt plus clay, that correlation decreased in the case of CEC and organic matter content; on the contrary, no correlation was found with the percentage of clay, while the association between NH_4f and the sand content, as expected, was negative. In the sugarcane plantation were recorded notable variations in the ammonium fraction fixed during crop development associated with fertiliser application and the intensity of soil microbiological processes. A significant decrease in NH_4f was found between the end of the second ratoon (2R) and the beginning of the 3R, when the plantation makes use of "artificially fixed" ammonium. In contrast, between the beginning (30 days) and the end of the third ratoon there was an increase in NH_4f related to the fertilisation practices and the mineralisation of N-organic.

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