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Is the impact of ethylammonium nitrate on basal soil respiration modified by mixing with aluminium salt to improve the performance for electrochemical uses? †

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Abstract: Interest in the possible use of EAN in electronic devices has increased in recent years. EAN could be used directly as an electrolyte, but its application can be optimized by mixing it with a metal salt, like Al. Although ionic liquids (ILs) have been considered to cause little harm because they are non-volatile, they can have toxic effects on the terrestrial environment. In this study, the impact on basal soil respiration of EAN alone, EAN combined with Al and Al was investigated by using two soils with different organic matter content. The Al did not affect soil respiration in either soil, while EAN both alone and mixed with Al affected both soils.

Keywords: Ethylammonium nitrate (EAN); EAN-aluminium salt mixture; Aluminium; Electrochemistry; Electrolyte; Soil basal respiration; Soil organic matter; Soil toxicity.

1. Introduction

Among the many applications of ionic liquids (ILs), their possible use as battery electrolytes is currently one of the most interesting given the growing demand for the development of new, more energetically efficient and environmentally friendly batteries.

ILs can be divided in two groups: protic and aprotic ionic liquids. Protic ILs can be synthesized by the transfer of a proton between a Brønsted acid and a base, and aprotic ILs synthesised in two steps, first with the formation of the cation and then by an ion exchange reaction.

Ethylammonium nitrate (EAN) is a protic ionic liquid and was the first IL to be discovered. Interest in this IL has increased considerably in recent years due to its various potential applications in the fields of chemistry, electrochemistry and biology [1]. The possible use of EAN in electronic devices is of particular interest, owing to its high electrical conductivity, good electrochemical window (even at room temperature) and high thermal stability [1]. Although EAN could be used directly as an electrolyte, its application can be optimized by mixing the IL with a metal salt [2]. So far, most studies have focused on the use of lithium, but its scarcity and high toxicity have led to the search for alternatives, one of which is aluminium [2] [3].

In addition to the thermophysical properties of ILs that make them suitable for the use for which they are being investigated, the environmental toxicity of ILs must also be investigated. Although they have been considered to cause little harm because they are non-volatile, several studies have shown that they can have toxic effects on the aquatic and terrestrial environments, and therefore this possibility must be investigated [1] [4] [5] [6]. It should also be noted that high chemical and thermal stability and low volatility, in addition to being properties that make them interesting for industrial uses, are also indications that they may present persistence problems in ecosystems [7].

Since soil is a fundamental resource of the terrestrial environment that is non-renewable, at least within the human lifespan [8], the conservation of soil quality is essential [9]. Research must anticipate the problem and verify the impact of ILs on the soil before they begin to enter the soil on a massive scale [1] [4] [5] [6]. However, of the multitude of studies available on ILs, only a small percentage deal with their potential toxicity, and few investigations propose soil as a receptor and analyse the potential impact on soil [1] [4] [5] [6]. A contaminated soil is one that has suffered an alteration in its functioning as a consequence of the entry of external substances [10]. However, unlike other resources such as air and water, for which quality standards have been established that clearly indicate when and with what intensity they degrade, for soil there are no standards, or at least none that are universally accepted [11] [12]. Biological and biochemical soil properties are the most sensitive, as they are related to soil metabolism and react very quickly to any disturbance and are therefore often considered good indicators of soil quality [13] [14]. Among the biological and biochemical properties that are useful as quality indicators, the measurement of biomass content and activity of soil micro-organisms are among the most commonly used, e.g. by means of organic matter mineralisation tests [15] [10]. On the other hand, it has been shown that soils with different characteristics, especially in relation to organic matter content, pH and clay content and type, may react differently to the presence of exogenous compounds [13], being especially important the organic matter, as it has been shown that can buffer the toxicity of both organic and inorganic pollutants. Therefore, it is necessary to establish the toxicity of ILs in soils with different characteristics, as this may affect the impact that different ILs can have on soil functioning.

In this study, the impact on basal soil respiration of EAN alone and of EAN combined with aluminium nitrate salt was investigated in order to assess whether the metal salt increases the potential toxic effects of EAN in soil. For comparative purposes, the impact of the aluminium salt on soil respiration was also studied. The study is part of the studies being performed by two research groups of the IIAG-CSIC and the University of Santiago in relation to the toxicity of several ILs with different potential applications (electrolytes in batteries, heat pumps, etc.). Two soils, already used in previous studies, were selected for the study because they are very similar in most of their general properties, but differ in their organic matter content. One of these studies [1] investigated the effect of EAN mixed with lithium nitrate salt (EAN-Li) on basal soil respiration. As also in this case the effect of EAN-Li was compared to EAN alone and the soils used in the study were the same, the EAN data are coincident in both studies.

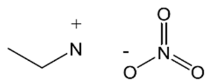
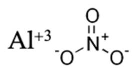
2. Materials and Methods

3.1. Chemicals

The ethylammonium nitrate (EAN) (97% pure, analytical grade) was purchased from IOLITEC (Heilbronn, Germany). The aluminium salt ($Al(NO_3)_3$; Al) was obtained from Scharlau. The main chemical and structural characteristics of these compounds are summarized in Table 1. The IL was further purified in a high vacuum device until the water content fell below 100 ppm.

A saturated solution of Al (EAN-Al) was obtained by mixing both components at room temperature for 24 to 48 h, in an ultrasound bath when needed, by increasing molality in 0.5 mol kg^{-1} intervals until saturation point was reached [2].

Table 1. Characteristics of ethylammonium nitrate and aluminium nitrate.

Name	Abbreviation [CAS number]	Structure	Mm (g mol^{-1})	Density (g mL^{-1})	Purity (%) (Brand)
Ethylamonium nitrate	EAN [22113-86-6]		108.097	1.261	>97.00 (Iolitec)
Aluminium nitrate	$Al(NO_3)_3·9H_2O$ [7784-27-2]		375.13	-	>98% (Scharlau)

3.2. Soils and soil collection procedure

Two soils were used, a maize (*Zea mays* L.) cultivation soil, collected in A Pedra (A Coruña) and a forest soil under oak (*Quercus robur* L.) vegetation, collected in Negreira (A Coruña). The forest soil was an *Umbrisol* [18], while the agricultural soil was a *Regosol* [18]. Both were developed over granite rock.

Table 2. Main physico-chemical properties of the soils used in the study. Mean values ± standard deviations.

Soil	pH KCl	pH H_2O	%Ct	%Nt	C/N	pF 2,5	%Silt	%Sand	Texture
Negreira	3.28±0.01	4.17±0.07	12.13±0.06	0.65±0.02	19	85.0%	16	71	S/L*
A Pedra (EAN)[#]	3.68±0.02	4.61±0.01	2.04±.0.21	0.17±0.01	12	34.3%	23	66	S/L*
A Pedra (EAN-Al)[#]	3.67±0.02	4.68±0.06	2.17±0.05	0.21±0.02	10	34.3%	23	66	S/L*
A Pedra (Al)[#]	3.50±0.00	4.59±0.01	2.88±0.02	0.22±0.00	13	34.3%	23	66	S/L*

*S/L: sandy-loam; [#], in parentheses the compound for which the soil was used

The A Pedra soil was sampled on three different occasions (using each of them for each of the tested compounds) while the Negreira soil was sampled on a single occasion. Between 10 and 15 subsamples of the A horizon (0-10 cm) of each soil were obtained, after removing the litter layer (forest soil) and the plant remains (agricultural soil). The subsamples were pooled in the field to produce a composite sample for each site. The soil samples were transported in isothermal bags to the laboratory where they were sieved (< 4 mm). A sub-sample of each soil was air-dried for determination of general soil properties and the remainder was stored at 4 °C until the beginning of the experiment; a portion of the air-dried sub-sample was taken and finely ground to determine total C and N contents

The two soils were acidic (with very similar KCl pH), with sandy loam texture and with very different organic matter contents. Thus, the total C content of the forest soil was 12.13 ±0.06% while the average content of the cultivated soil was 2.36±0.45%, and total N content of the forest soil was 0.65% and the average content of the cultivated soil was 0.20±0.03%. Furthermore, the C/N ratio is very different in the two soils, being 19 in the forest soil and an average of 11.7±1.5 in the arable soil. Field water capacity (or water retained at a pF of 2.5) differs, as usual, between forest and cultivated soils (as it is closely related to organic matter content), being much higher in the case of Negreira soil (85.0%) than in A Pedra (34.3%). (Table 2).

3.1. Experimental set-up

EAN-Al was synthesised in the laboratory from EAN and aluminium nitrate by diluting the aluminium salt in a given mass of EAN to obtain a 2 molal concentration of EAN-Al [2]. Stock solutions of 2 molal concentration of EAN and aluminium nitrate were prepared and then different solutions of pure (EAN) and Al-doped (EAN-Al) were prepared by dilution of these compounds in distilled water to reach final concentrations of 0, 1, 10, 25, 50, 75 and 100%, and the same dilutions were prepared for aluminium nitrate (Al). The soils were then spiked with 0.1 ml of each of the above solutions per gram of soil (equivalent to doses of 0, 1.75, 17.47, 43.68, 69.90, 104.84 and 122.32 g of compound kg^{-1} soil) and kept at 20 °C for 3 days to maximise the contact between the soil and each compound. This contact time is based on the results of previous studies [6] [17] which indicate that the main changes in the biological and biochemical properties of soil due to the presence of a potentially toxic compound occur within 72 hours after contact with such compounds. Three days after the three compounds were added to the soil, all of the EAN, EAN-Al and Al spiked soil samples were analysed to determine basal soil respiration and respiration kinetics. All the soil samples were also analysed for several soil physico-chemical properties.

3.1. Analytical methods

Total C and N contents of finely ground samples were determined in a CNH Elemental Analyser (TruPec model, LECO); soil pH was determined in a suspension in 1 M KCl (1:2.5 soil:solution ratio) as described by Guitián-Ojea and Carballas-Fernández [18]. Particle size distribution was determined using a Robinson pipette with Calgon as dispersant [18], and the textural class was assigned according to the USDA soil texture classification [19]. Field water capacity was determined as the water retained by the soil at a potential of -30.3 kPa, which was measured in undisturbed soil samples with a Richards plate-and-membrane apparatus [18]. The soil moisture content was determined after oven-drying the soil at 105 °C for 24 h.

Basal soil respiration was determined in triplicate by static incubation [17]. Briefly, moist soil samples equivalent to 25 g of oven-dried soil were incubated in tightly closed Mason jars at 80% field moisture content and at 25 °C (optimal conditions). The CO_2 produced was collected in 10 to 25 ml of a 0.1 M, 0.5 or 1M NaOH solution, which was then titrated against HCl with an automatic titrator. Two Mason jars with NaOH solution but no soil were also incubated under the same conditions to take into account the CO_2 in the jars (two jars were incubated for each of the different combinations of volume and concentration of NaOH used). To estimate the kinetics of soil respiration, the NaOH solution was collected and titrated after 1, 2, 4, 7, 10, 14 days, and every 7 days thereafter. On each occasion, the jars were left open for 30 min to allow replacement of the air before fresh NaOH solution was added. The volume and molarity of the NaOH was modified each time, depending on the evolution of the CO_2 emitted by the soils, to ensure that there was sufficient NaOH to retain the CO_2 emitted by the control and the IL-spiked soils. For the IL-contaminated soils, the incubation period had to be increased until the CO_2 emitted by soil respiration in IL-spiked soils stabilised or had reached the same level as in the control, un-spiked soil. The incubation time varied depending on the soil, the solution applied (EAN, EAN-Al or Al) and the amount of IL applied to the soil.

3. Results and Discussion

Since all three tested compounds have C (except the aluminium salt) and N atoms in their structure, different amounts of these elements were added to the soils, which varied depending on the compound (Table 3). EAN was the compound that contributed the highest amounts of C and N, followed by EAN-Al, and finally $Al(NO_3)_3$, which had no C and contributed the least. As a consequence, the final content of these elements in the soil samples would also vary, resulting in an increase of their contents.

Table 3. Total amounts of C and N added to soils with the three compounds (d.s. = air-dried soil)

Dose	Carbon (g C kg^{-1} d.s.)			Nitrogen (g N kg^{-1} d.s.)		
g kg^{-1}	EAN	EAN-Al	$Al(NO_3)_3$	EAN	EAN-Al	$Al(NO_3)_3$
1.75	0.39	0.27	0	0.45	0.34	0.20
17.47	3.88	2.72	0	4.53	3.43	1.96
43.68	9.70	6.80	0	11.32	8.56	4.89
69.90	15.52	10.88	0	18.11	13.70	7.83
104.84	23.28	16.32	0	27.17	20.55	11.74
122.32	27.16	19.04	0	31.70	23.98	13.70

The C and N contents of each of the samples added with EAN, EAN-Al and Al could not be analysed. Thus, the theoretical C and N values that would be reached in each of the soil samples were calculated (data not shown), as well as the resulting C/N ratio for all soil samples with the different amounts of EAN, EAN-Al and Al (Table 4). The addition of different proportions of C and N caused a significant variation in the C/N ratio of the soils, leading to an imbalance in the contents of the two elements, which could be observed by a sharp drop in the C/N ratio in all cases.

Table 4. C/N ratios in A Pedra and Negreira soils spiked with different amounts of ethylamonium nitrate alone (EAN), ethylamonium nitrate mixed with aluminium salt (EAN-Al) and with aluminium nitrate salt (Al).

Dose	C/N ratio A Pedra soil			C/N ratio Negreira soil		
g kg^{-1}	EAN	EAN-Al	$Al(NO_3)_3$	EAN	EAN-Al	$Al(NO_3)_3$
0.0	12	10	10	19	19	19
1.75	9	9	9	17	18	18
17.47	4	4	5	11	13	14
43.68	2	3	3	7	8	11
69.90	2	2	2	6	7	8
104.84	2	2	2	4	5	7
122.32	1	2	1	4	5	6

The reduction in the C/N ratio is greater the higher the dose of compound added to the soil and is much more important in the case of the A Pedra soil than in the cultivated soil, as the higher proportion of C in the former than in the latter (C/N 19 and 11, respectively) probably buffers the imbalance produced by the addition of EAN and EAN-Al (in both cases proportionally more N is added than C) and of Al (in the latter case only N is added to the soil). On the other hand, while in A Pedra soil no differences are observed between the modification of the C/N ratio caused by the three compounds, in Negreira soil the EAN seems to cause the highest reduction of the C/N ratio (in the sample with the highest dose of EAN the C/N ratio is 4) and Al the lowest (in the sample with the highest dose of Al the C/N ratio is 6).

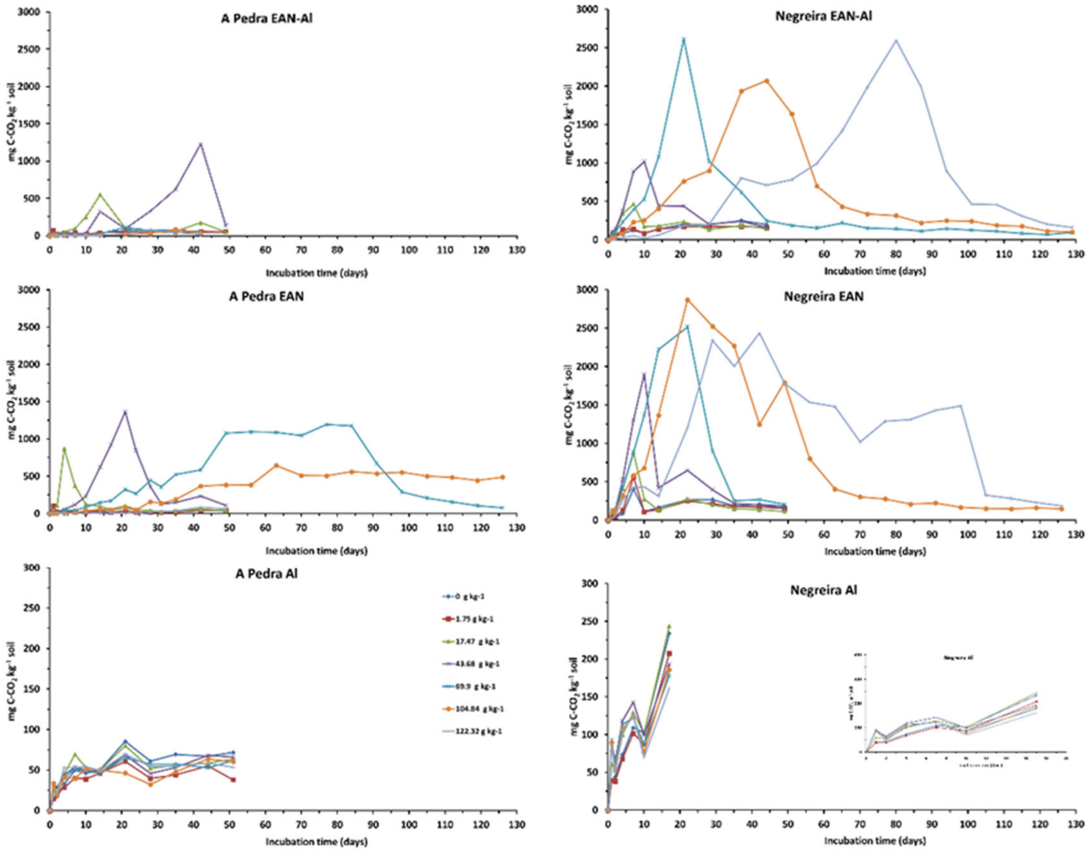


Figure 1. Kinetics of basal respiration in the un-spiked A Pedra (left) and Negreira (right) soils and in the same soils spiked with different amounts of ethylammonium nitrate (EAN-Al) mixed with aluminium nitrate salt (above) or alone (EAN) (medium) and with aluminium nitrate salt (Al) (below). Note that the limits of Y axis in A Pedra and Negreira soils spiked with aluminium salt is lower than in the other cases.

As already observed in the study with EAN-Li [1], basal soil respiration was highest in Negreira than in A Pedra soil, *i.e.* was higher in the soil with the highest organic matter content (Figure 1, Table 5).

In both Negreira and A Pedra soils, the addition of increasing amounts of aluminium nitrate did not affect soil respiration, which was practically the same for all doses. Likewise, the kinetics of carbon mineralisation in both soils, and for any of the Al doses tested, was similar to that of the un-spiked control soil. For this reason, the incubation in this soil was prolonged only the usual time in this type of incubation in our laboratory, ending after 17 days (Figure 1, Table 5). The total amounts of CO_2-C emitted by the A Pedra soil varied between 390 and 493 mg CO_2-C kg^{-1} d.s. soil without differences between samples being associated with the dose of aluminium nitrate applied to the soil; the mean value of CO_2-C emitted during the 17 days of incubation was 468 mg CO_2-C kg^{-1} d.s. soil (Table 5). In the Negreira soil, the total amount of CO_2-C emitted was, as already indicated, higher than in the A Pedra soil, varying between 595 and 708 mg CO_2-C kg^{-1} d.s. soil (mean value 632 ± mg CO_2-C kg^{-1} d.s. soil) and also in this case the differences between samples were not associated to the dose (Table 5).

As was also observed in the previous study with EAN-Li [1], both in Negreira and in A Pedra soil the application of EAN (doses above 17.47 g kg^{-1}) caused a very large increase in the total amount of CO_2-C emitted during the incubation period, and the amount of CO_2-C emitted increased with the amount of EAN added to the soil (Figure 1, Table 5). Furthermore, in addition to the changes in the total amount of CO_2-C emitted by both soils, the kinetics of the CO_2 emission was also modified by the application of EAN to the soils (Figure 1). In all cases, there was a peak in CO_2-C emission, as usually occur when the soils are incubated under optimal conditions of temperature and moisture [18]. However, while this peak occurred shortly after the beginning of the incubation (first 4 days) in un-spiked soils and in the soils spiked with the lowest amount of EAN (1.75 g kg^{-1} soil), both in Negreira and in A Pedra soil the timing of the peak was gradually delayed as the dose of EAN increased (Figure 1, Table 5) as already indicated in [1]. This delay, longer in A Pedra than in Negreira soil (Figure 1), is consistent with the delay observed in heat emission detected by microcalorimetry in the same soils spiked with EAN and EAN-Al [22], with a lag in the increase in microbial activity after glucose addition to these IL-spiked soils, similar to what was find for soils spiked with other ILs [23]. This lag was explained as reflecting the time needed by microbial communities to adapt to the new conditions, as very large amounts of new substrates were added to the soil with EAN (high amounts of C and N) and probably the soil microorganisms need some time before they start to take advantage of the new substrates available [1]. This is because even though there was a large increase of both total C and N contents (Table 3), given the much higher amount of N than of C added to the soils, there was a large decrease in the C/N ratio in the EAN spiked soils (Table 4) and therefore an imbalance in the amounts of these elements in the soils. It was hypothesized [1] that this lag could be due to a shift in the microbial populations to a fungi-dominated community, because shortly after the start of the incubation period (10-26 days), spots of fungi were visible at naked eyes on the surface of the soils spiked with the highest amounts of EAN (at concentrations of 69.90 g kg^{-1} and above). Moreover, the surface area covered by fungi increased gradually over time and the fungi also gradually appeared in the soils spiked with lowest amounts of EAN in A Pedra soil, while this did not occur in Negreira soil. This, together with the fact that the fungi were more abundant and appeared shorter after the start of the incubation in A Pedra than in Negreira soil, was considered to reflect a relatively stronger impact of the addition of new substrates to the soil with the lowest organic matter content, as in this soil the imbalance between C and N contents was stronger, then in the soil with the highest organic matter content as shown by the C/N ratio in both soils (Table 4) [1]. This different impact is clearly reflected in the very different proportion of the total C added to the soil that is mineralised in both soils, as in the A Pedra soil only up to 70% of the C added with EAN is mineralised and only up to doses of 69.90 g kg^{-1} of EAN, as for higher doses there is a decrease in the amount of CO_2-C emitted with the lower dose (this occurred for the dose of 104.84 g kg^{-1}) or even there was not an increase in the CO_2-C emission compared to the un-spiked soil (122.32 g kg^{-1} dose). However, in the soil with high organic matter content (Negreira soil), up to 74% of the added C was mineralised and there was an increase in the proportion of added C that is mineralised the higher the dose (Table 5).

In the two soils, both the amount of total CO_2-C emitted and the mineralisation kinetics are strongly modified in the soil to which EAN-Al was added with respect to the soil to which EAN was added (Figure 1, Table 5). Mixing EAN with Al causes a strong decrease in both the total amount of CO_2-C emitted and the proportion of added C that is mineralised during incubation compared to soil to which EAN was added alone. This effect is more pronounced in the poorer soil than in the soil richer in organic matter (Table 5). Likewise, the kinetics of mineralisation are also modified, so that in the Negreira soil (rich in organic matter) the delay period is longer in the soil with EAN-Al than in the soil with EAN (Figure 1).

In the A Pedra soil, in addition to an increase in the lag period for doses above 69.90 g kg^{-1} of EAN-Al, the C added with the EAN-Al is not mineralised and even the amount of CO_2-C emitted and, therefore, the mineralisation of C is reduced with respect to the un-spiked soil. In other words, and contrary to what was observed when EAN was doped with lithium [1], the doping of EAN with Al aggravates the negative effect on microbial activity caused when EAN is added to the soil alone. This negative impact of Al doping is stronger in the A Pedra soil than in the Negreira soil, confirming the protective role of the organic matter content already observed with EAN and EAN-Li (Figure 1, Table 5) [1].

Table 5. Cumulative CO_2-C emitted during the incubation by un-spiked A Pedra and Negreira soils and the same soils spiked with different amounts of ethylammonium nitrate (EAN) alone or mixed with aluminium nitrate salt (EAN-Al) and with aluminium nitrate salt alone. Mean values ± standard deviations. Note that for some of the samples (*) the duration of the incubation (126 days for EAN-spiked and 129 days for EAN-Al-spiked samples) was much longer than for the other samples (42-44 days), while for Negreira soil with aluminium nitrate alone the duration of the incubation was only 17 days.

Dose	CO_2-C emitted (A Pedra soil)			CO_2-C emitted (Negreira soil)		
g kg^{-1}	EAN	EAN-Al	$Al(NO_3)_3$	EAN	EAN-Al	$Al(NO_3)_3$
0.0	337±134	343±43	493±14	1790±155	1346±57	602±18
1.75	433±17	429±45	390±12	1856±0	1271±58	542±10
17.47	2024±77	1402±92	519±11	2663±119	2082±139	689±17
43.68	5183±149	2734±277	489±25	5886±318	4021±139	708±16
69.90	11191±166	375±33	473±25	9096±164	8509±139*	654±15
104.84	7030±147	216±35	424±27	16884±866*	11424±550*	637±21
122.32	273±70	186±38	490±20	21874±688*	1326±737*	595±11

Several possible causes could be considered as responsible for the increased negative impact on soil microbial activity when EAN is doped with Al. For example, it could be thought that Al is toxic to soil micro-organisms. However, since the addition of Al alone had no effect on soil microbial activity (Figure 1, Table 5), the reason for the negative effect observed with EAN-Al would not be the Al-toxicity per se. Another possible cause could be a strong pH change; however, although EAN-Al solutions have an extraordinarily low pH, the same is true for EAN and Al solutions and, moreover, the pH in KCl does not change significantly after the addition of EAN-Al [24], so the negative effect of this compound on microbial activity cannot be attributed to an influence on soil pH. Another possible explanation for the observed reduction in microbial activity is that the presence of aluminium blocks, by some unknown mechanism, the mineralisation of EAN. With the available data, it is difficult to know both the mechanism that produces this blockage, why it is more intense in the soil with a low organic matter content than in the soil with a high organic matter content, or why this effect becomes more intense as the amount of EAN-Al added to the soil increases, even inhibiting the mineralisation of organic C for the highest doses in the case of soil with the lowest organic matter content (A Pedra soil). It is possible that the presence of aluminium somehow affects the action of some of the enzymes involved in the mineralisation of EAN-Al, but the studies carried out so far do not allow confirming this hypothesis, because although the activity of various hydro-lases and oxidoreductases has been determined in the same soils after the addition of EAN-Al, these studies focused on investigating the immediate effect of the application of EAN-Al and it would be necessary to investigate the longer term effect [24].

4. Conclusions

Mixing aluminium salt with ethylammonium nitrate aggravates the effect on microbial activity of this latter. Possibly the negative effect of EAN is mainly due to the nutritional imbalance caused by the addition of large amounts of C and N to the soil, which are proportionally higher in N than in C. This results in a strong reduction of the soil C/N ratio, higher the highest the amount of EAN added to the soil and higher in the soil poorer in organic matter than in the richer soil. This more intense imbalance in the poorer soil in organic matter also means that the impact of the addition of EAN and EAN-Al is greater in this soil than in the soil rich in organic matter. The addition of different amounts of aluminium nitrate to the soil did not affect microbial activity, as shown by the similar mineralisation kinetics and similar amounts of total CO2-C emitted by the un-spiked soil and the soil spiked with different amounts of Al.

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