



Proceeding Paper

Is the Impact of Ethylammonium Nitrate on Soil Basal Respiration Modified by Addition of Aluminium Salt to Improve the Performance in Electrochemical Applications? †

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† Presented at the 25th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2021; Available online: <https://ecsoc-25.sciforum.net/>.

Abstract: Interest in the possible use of EAN in electronic devices has increased in recent years. EAN can be used directly as an electrolyte, but its application could potentially be optimized by mixing with a metal salt such as $\text{Al}(\text{NO}_3)_3$. 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 soil basal respiration of EAN alone, a mixture of EAN and $\text{Al}(\text{NO}_3)_3$, and $\text{Al}(\text{NO}_3)_3$ alone was investigated using two soils with different organic matter contents. The $\text{Al}(\text{NO}_3)_3$ did not affect soil respiration in either soil, while EAN, either alone or mixed with $\text{Al}(\text{NO}_3)_3$ affected both soils.

Keywords: ethylammonium nitrate (EAN); EAN-aluminium salt mixture; aluminium; electrochemistry; electrolyte; soil basal respiration; soil organic matter; soil toxicity



Citation: Sixto, T.; Priano, M.E.; Parajó, J.J.; Trasar-Cepeda, C. Is the Impact of Ethylammonium Nitrate on Soil Basal Respiration Modified by Addition of Aluminium Salt to Improve the Performance in Electrochemical Applications? *Chem. Proc.* **2022**, *8*, 98. <https://doi.org/10.3390/ecsoc-25-11789>

Academic Editor: Julio A. Seijas

Published: 14 November 2021

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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 are synthesised in two steps, first with the formation of the cation and then by an ion exchange reaction [1].

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 it with a metal salt [2]. So far, most studies have focused on the use of lithium, but the scarcity and high toxicity of this metal 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 these compounds must also be investigated. Although ILs have been considered to cause little harm because they are non-volatile [4], several studies have shown that they can have toxic effects on aquatic and terrestrial environments, and therefore, this possibility must be investigated further [4–6]. It should also be noted that the properties of ILs that are of interest in relation to industrial use, i.e., high chemical and thermal stability and low volatility, may also lead to persistence of the compounds in ecosystems [7].

Soil is a fundamental resource, but is non-renewable, at least within the human lifespan [8], and the conservation of soil quality is, therefore, essential [9]. Research must anticipate any problems and verify the impact of ILs on the soil before they begin to enter the soil on a massive scale [4–6]. However, of the multitude of studies available on ILs, only a small percentage deal with the potential toxicity of these compounds, and very few studies have considered the potential impacts on soil [4–6]. A contaminated soil is one that has suffered an alteration in its functioning as a consequence of the entry of external substances [9,10]. However, unlike other resources, such as air and water, for which quality standards have been established that clearly indicate the timing and intensity of degradation, there are no such standards for soil, or at least none that are universally accepted [11,12]. Biological and biochemical soil properties are particularly 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, biomass content and activity of soil microorganisms, measured by means of organic matter mineralisation and soil basal respiration tests [15,16], are among the most commonly used. On the other hand, it has been shown that soils with different characteristics, especially regarding organic matter content, pH, and clay content and type, may react differently to the presence of exogenous compounds [13], with organic matter being especially important, as it has been shown to buffer the toxicity of both organic and inorganic pollutants. Therefore, the toxicity of ILs must be determined in soils with different characteristics, as these may affect the impact of the ILs can have on soil functioning.

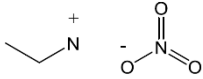
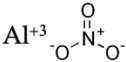
In this study, the impact on soil basal respiration of EAN alone and of EAN combined with aluminium nitrate salt was investigated in order to assess whether the metal salt increases the potential toxic effects of EAN in soil. For comparative purposes, the impact of the aluminium salt on soil basal respiration was also studied. This study is part of a larger research project conducted by two research groups in the MBG sede Santiago-CSIC and the University of Santiago in relation to the toxicity to soil microorganisms of several ILs with various potential applications (electrolytes in batteries, heat pumps, etc.). Two soils, already used in previous studies, were selected for the study because, although very similar in most of their general properties, they differ in their organic matter content. One of the previous studies [17] investigated the effect of EAN mixed with lithium nitrate salt (EAN-Li) on soil basal respiration. As in this case, the effect of EAN-Li was also compared with that of EAN alone, and since the same soils were used as in the present study, the **same** EAN data were used in both studies.

2. Materials and Methods

2.1. Chemicals

The ethylammonium nitrate (EAN) (97% pure, analytical grade) was purchased from IOLITEC (Heilbronn, Germany). The aluminium salt ($\text{Al}(\text{NO}_3)_3$) 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.

Table 1. Characteristics of ethylammonium nitrate and aluminium nitrate.

Name	Abbreviation [CAS Number]	Structure	Mw (g mol ⁻¹)	Density (g mL ⁻¹)	Purity (%) (Brand)
Ethylammonium nitrate	EAN [22113-86-6]		108.097	1.261	>97.00 (Iolitec)
Aluminium nitrate	Al(NO ₃) ₃ [7784-27-2]		375.13	-	>98% (Scharlau)

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

2.2. Soils and Soil Collection Procedure

Two soils were used in the study: a soil under a maize (*Zea mays* L.) crop, in A Pedra (A Coruña), and a forest soil under oak (*Quercus robur* L.) vegetation, 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.

The use of fresh soil is recommended for the determination of soil biological properties, including basal respiration, as drying affects the soil microbiota and its activity. For the A Pedra soil samples, the incubations were carried out on three different occasions (one for each compound) separated by long intervals of time. The fresh soil samples were obtained immediately before each incubation. By contrast, for the Negreira soil samples the incubations were conducted within a short period of time, and as the same soil was thus able to be used, the Negreira soil was sampled on a single occasion. Accordingly, the general properties of the A Pedra soil were measured three times (once for each of the compounds) while those of the Negreira were measured only once (Table 2). For soil sample collection, 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 incubation experiment. A portion of the air-dried sub-sample was finely ground for determination of the total C and N contents.

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

Soil	pH KCl	pH H ₂ O	%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; # the compound in parentheses was added to the soil.

Both soils were acidic (with very similar KCl pH), with a sandy loam texture and very different organic matter (OM) 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%. Total N content of the forest soil was 0.65% and the average content of the cultivated soil was 0.20 ± 0.03%. The C/N ratios were also very different in the two soils: 19 in the forest soil and 11.7 ± 1.5 in the arable soil. The field water capacity (water retained at a pF of 2.5) is

always different in forest and cultivated soils, as it is closely related to OM content, and it was (as expected) much higher in the Negreira soil (85.0%) than in the A Pedra soil (34.3%) (Table 2).

2.3. Experimental Set-Up

The EAN-Al solution was produced in the laboratory by diluting the aluminium salt in a given mass of EAN [2]. Stock solutions (2 M) of EAN and EAN-Al were prepared, and different dilutions of each were then made by addition of distilled water to final concentrations of 0, 1, 10, 25, 50, 75 and 100%. The aluminium nitrate ($\text{Al}(\text{NO}_3)_3$) was also diluted to the same concentrations. The moisture content of sieved soils was pre-adjusted to the required level of soil moisture, so that after addition of the solutions containing each of the three compounds, the moisture content was 80% of the soil field water capacity (Table 2). 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} d.s. soil) and held 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 h of contact with the compound. Three days after the compounds were added to the soil, all of the EAN, EAN-Al, and $\text{Al}(\text{NO}_3)_3$ -spiked soil samples were analysed to determine soil basal respiration and respiration kinetics. The soil samples were also analysed to determine several soil physico-chemical properties.

2.4. Analytical Methods

Total C and N contents of finely ground samples were determined in a CNH Elemental Analyser (TruSpec model, LECO); soil pH was determined in suspension, in 1 M KCl (1:2.5 soil:solution ratio) as described by Guitián-Ojea and Carballas-Fernández [19]. Particle size distribution was determined using a Robinson pipette with Calgon as dispersant, after removal of OM with H_2O_2 [19], and the textural class was assigned according to the USDA soil texture classification [20]. Soil field water capacity was determined as the water retained by the soil at a potential of -33 kPa (pF 2.5), which was measured in undisturbed soil samples with a Richards plate and membrane apparatus [19]. The soil moisture content was determined after oven-drying the soil at 105 °C for 24 h.

Soil basal respiration was determined in triplicate by static incubation [19]. 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, [21]). The CO_2 produced was collected in 10 to 25 mL of a 0.1, 0.5, or 1 M 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 control for the amount of CO_2 contained in the jars (two jars were incubated for each of the different combinations of volume and concentration of NaOH used). To estimate the soil respiration kinetics, the NaOH solution was collected and titrated after 1, 2, 4, 7, 10, and 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 changes in 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-spiked 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 unspiked control soil. The incubation time varied depending on the soil, the solution applied (EAN, EAN-Al, or $\text{Al}(\text{NO}_3)_3$), and the amount of compound applied to the soil.

3. Results and Discussion

As all three tested compounds include C (except the aluminium salt) and N atoms in their structure, different amounts of these elements were added to the soils depending on the compound being tested (Table 3). EAN contributed the greatest amounts of C and N, followed by EAN-Al, and finally, $\text{Al}(\text{NO}_3)_3$, which does not contain C, and contributed the lowest amount of N. As a consequence, the increase in the final content of these elements in the soil samples was also variable.

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

Dose g kg^{-1}	Carbon (g C kg^{-1} d.s.)			Nitrogen (g N kg^{-1} d.s.)		
	EAN	EAN-Al	$\text{Al}(\text{NO}_3)_3$	EAN	EAN-Al	$\text{Al}(\text{NO}_3)_3$
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 $\text{Al}(\text{NO}_3)_3$ were not able to be determined. Thus, the theoretical C and N values that would be reached in each of the soil samples were calculated (data not shown), along with the resulting C/N ratio for all soil samples with the different amounts of EAN, EAN-Al, and $\text{Al}(\text{NO}_3)_3$ (Table 4). As relatively more N than C was always added, there was an imbalance in the contents of these elements. This was observed as a sharp decrease in the C/N ratio in the soils spiked with EAN, EAN-Al, and $\text{Al}(\text{NO}_3)_3$, and the decrease became more marked as the dose of compound added increased. In addition, the higher organic matter content of the Negreira soil buffered the reduction of the C/N ratio in this soil, caused by the addition of the three compounds, in contrast to the A Pedra soil. Therefore, the reduction in the C/N ratio was greater when higher doses of the compounds were added to the soil and was much more important in the A Pedra soil than in the forest soil, as the higher proportion of C in the former than in the latter (C/N 19 and 11.7, respectively) probably buffered the imbalance produced by the addition of EAN and EAN-Al (in both cases proportionally more N is added than C) and $\text{Al}(\text{NO}_3)_3$ (which only contributed N to the soil). On the other hand, although in A Pedra soil there were no differences in the changes in the C/N ratio caused by the three compounds, in the Negreira soil, EAN seemed to cause the greatest reduction in the C/N ratio (in the sample spiked with the highest dose of EAN, the C/N ratio was 4) and $\text{Al}(\text{NO}_3)_3$ the lowest (in the sample with the highest dose of $\text{Al}(\text{NO}_3)_3$ the C/N ratio was 6). Furthermore, as none of the compounds contain P, spiking the soil with any of the three compounds caused a strong imbalance in the nutrient content of the soil, which was particularly severe after addition of high doses of the compounds.

As already observed in a study with EAN-Li conducted in our laboratory [16], basal respiration was higher in the Negreira than in A Pedra soil, i.e., it was highest 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 almost the same for all doses. Likewise, the kinetics of carbon mineralisation in both soils, and for all of the doses of $\text{Al}(\text{NO}_3)_3$ tested, were similar to those in the unspiked control soil. For this reason, incubation of this soil was prolonged (relative to the usual incubation time used in such studies 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 $\text{mg CO}_2\text{-C kg}^{-1}$ d.s. soil, and none of the differences between samples were 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

$\text{CO}_2\text{-C kg}^{-1}$ d.s. soil (Table 5). In the Negreira soil, the total amount of $\text{CO}_2\text{-C}$ emitted was, as already indicated, higher than in the A Pedra soil, varying between 595 and 708 $\text{mg CO}_2\text{-C kg}^{-1}$ d.s. soil (mean value $632 \pm 58 \text{ mg CO}_2\text{-C kg}^{-1}$ d.s. soil). Again, the differences between samples were not associated with the dose of aluminium nitrate (Table 5).

Table 4. C/N ratios in A Pedra and Negreira soils spiked with different doses of ethylammonium nitrate (EAN), ethylammonium nitrate plus aluminium salt (EAN-Al), or aluminium nitrate ($\text{Al}(\text{NO}_3)_3$).

Dose g kg^{-1}	C/N Ratio A Pedra Soil			C/N Ratio Negreira Soil		
	EAN	EAN-Al	$\text{Al}(\text{NO}_3)_3$	EAN	EAN-Al	$\text{Al}(\text{NO}_3)_3$
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

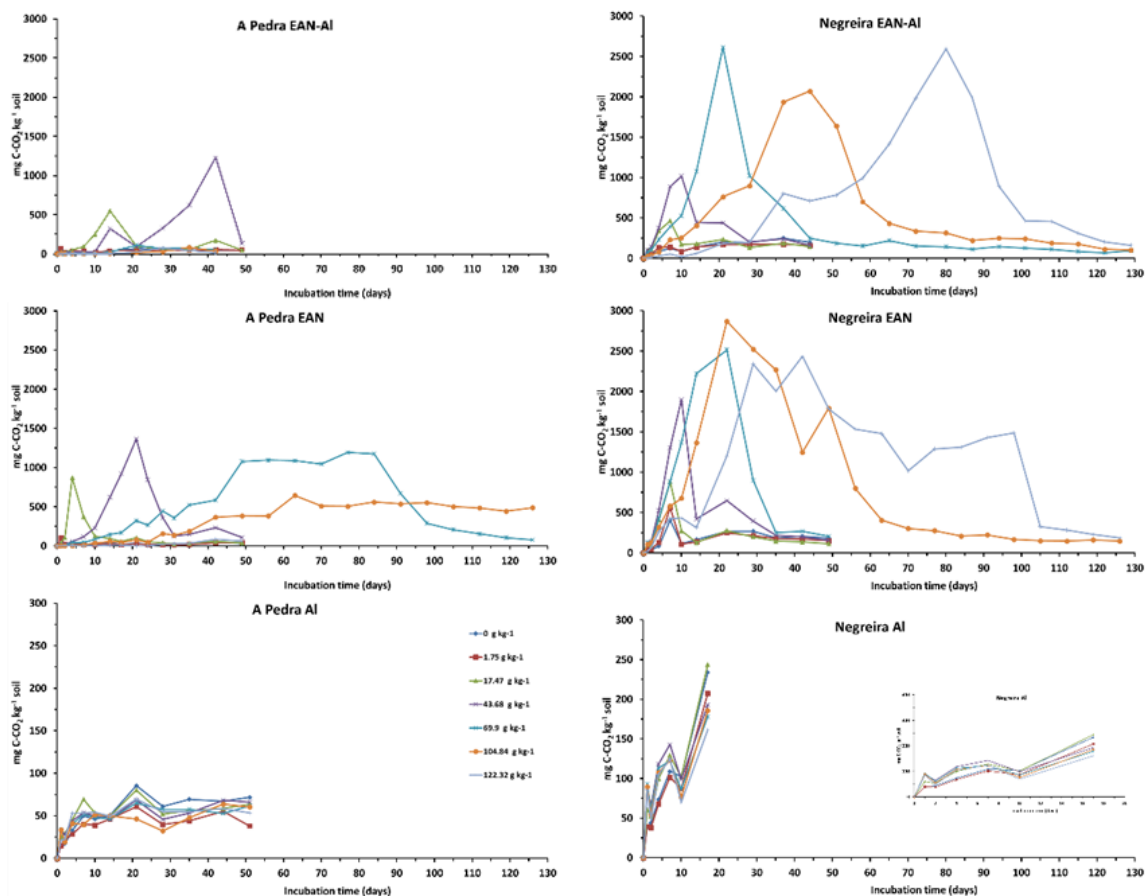


Figure 1. Kinetics of basal respiration in the unspiked A Pedra (left) and Negreira (right) soils and in the same soils spiked with different doses of the saturated mixture of ethylammonium nitrate and aluminium nitrate (EAN-Al) (upper graphs), ethylammonium nitrate (EAN) (middle), or aluminium nitrate ($\text{Al}(\text{NO}_3)_3$) (lower graphs). Note that the limits of the Y axis in A Pedra and Negreira soils spiked with the aluminium salt were lower than in the other cases.

Table 5. Cumulative CO₂-C emitted during the incubation by unspiked A Pedra and Negreira soils and the same soils spiked with different amounts of ethylammonium nitrate (EAN), EAN plus aluminium nitrate (EAN-Al) or aluminium nitrate alone (Al(NO₃)₃). 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 the Negreira soil spiked with aluminium nitrate alone, the duration of the incubation was only 17 days.

Dose g kg ⁻¹	CO ₂ -C Emitted (A Pedra Soil)			CO ₂ -C Emitted (Negreira Soil)		
	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	11,191 ± 166	375 ± 33	473 ± 25	9096 ± 164	8509 ± 139 *	654 ± 15
104.84	7030 ± 147	216 ± 35	424 ± 27	16,884 ± 866 *	11,424 ± 550 *	637 ± 21
122.32	273 ± 70	186 ± 38	490 ± 20	21,874 ± 688 *	1326 ± 737 *	595 ± 11

As also observed in a previous study with EAN-Li [16], in both the Negreira soil and the A Pedra soil, the addition of EAN (at doses above 17.47 g kg⁻¹) caused a very large increase in the total amount of CO₂-C emitted during the incubation period, and the amount 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 CO₂-C emitted by both soils, the kinetics of the CO₂ emission were also modified by the application of EAN to the soils (Figure 1). In all cases, there was a peak in CO₂-C emission, as usually occurs when soils are incubated under optimal conditions of temperature and moisture [19]. However, although this peak occurred shortly after the beginning of the incubation period (first 4 days) in the unspiked soils and in the soils spiked with the lowest amount of EAN (1.75 g kg⁻¹ soil), in both the Negreira and the A Pedra soils there was a lag period, and the peak appeared later, as the dose of EAN increased (Figure 1, Table 5), consistent with previous findings [16]. This lag period, which was longer in A Pedra than in Negreira soil (Figure 1), is consistent with the observed delay in heat emission detected by microcalorimetry in the same soils spiked with EAN and EAN-Al [22], in which a dose-dependent lag in the increase in microbial activity was observed after addition of glucose to the IL-spiked soils. A similar pattern was also observed for soils spiked with diverse 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 the soil microorganisms may not have been immediately able to take advantage of the new substrates available [16]. As indicated above, although there was a large increase in both total C and N contents (Table 3), there was also a large decrease in the C/N ratio in the EAN-spiked soils due to the much higher amount of N than of C added to the soils (Table 4). Together with the fact that no P was added to the soil, this caused an imbalance in the amounts of these elements in the soils. In a previous study [16], it was hypothesized that the lag may 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), fungal colonies were clearly visible on the surface of the soils spiked with the highest concentrations of EAN (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 the lowest amounts of EAN in A Pedra soil, while this did not occur in the Negreira soil. This, together with the fact that the fungi were more abundant and appeared more quickly after the start of the incubation in the A Pedra than in the Negreira soil [16], was considered to reflect a relatively stronger impact of the addition of new substrates to the soil with the lowest OM content (A Pedra soil). There was a greater imbalance between C and N contents in this soil than in the OM-rich Negreira soil, as indicated by the C/N ratio in both soils (Table 4). The different impact was clearly reflected in the very different proportions of total C added to the soil and by the fact that the C was mineralised in both soils. In the A Pedra soil, only up

to 70% of the C added with EAN was mineralised and only for doses of up to 69.90 g kg^{-1} of EAN. As with higher doses, there was a decrease in the amount of $\text{CO}_2\text{-C}$ emitted with the lower dose (this occurred for the dose of 104.84 g kg^{-1}) or there was even no increase in the $\text{CO}_2\text{-C}$ emission relative to the unspiked soil (122.32 g kg^{-1} dose). However, in the OM-rich Negreira soil, up to 74% of the C added was mineralised and there was an increase in the proportion of C added that was mineralised at higher doses (Table 5).

In both soils, the amount of total $\text{CO}_2\text{-C}$ emitted and the mineralisation kinetics were strongly modified in the soil spiked with EAN-Al, relative to the soil spiked with EAN (Figure 1, Table 5). The Al-saturated EAN caused a large decrease in both the total amount of $\text{CO}_2\text{-C}$ emitted and the proportion of C added that was mineralised during the incubation relative to soil to which EAN alone was added. The effect was more pronounced in the OM-poor soil than in the OM-rich soil (Table 5). Likewise, the kinetics of carbon mineralisation were also modified, so that in the Negreira soil (OM rich) the lag period was longer in the soil spiked with EAN-Al than in the soil spiked with EAN (Figure 1).

In the A Pedra soil, in addition to an increase in the lag period for doses of EAN-Al above 69.90 g kg^{-1} , the C added with the EAN-Al was not mineralised, and the amounts of $\text{CO}_2\text{-C}$ emitted and, therefore, of C mineralised were lower than in the unspiked soil. In other words, and contrary to observations for the EAN-Li solution [16], the addition of Al to EAN aggravated the negative effect on microbial activity caused by the addition of EAN alone. This negative impact of Al was stronger in the A Pedra soil than in the Negreira soil (Figure 1, Table 5), confirming the protective role of the organic matter content previously observed in a study with EAN and EAN-Li [16].

There are several possible explanations for the increased negative impact of EAN on soil microbial activity when this IL is mixed with $\text{Al}(\text{NO}_3)_3$. For example, it is possible that Al is toxic to soil microorganisms. However, as the addition of $\text{Al}(\text{NO}_3)_3$ alone had no effect on soil basal respiration, and therefore on soil microbial activity (Figure 1, Table 5), the negative effect of EAN-Al cannot be due to the Al-toxicity *per se*. Another possible cause is a large difference in pH; however, although the pH of the EAN-Al mixture was extraordinarily low, the same is also true for EAN and $\text{Al}(\text{NO}_3)_3$ solutions. Moreover, as the pH in KCl does not change significantly after the addition of EAN-Al [24], the negative effect of this compound on microbial activity cannot be attributed to its influence on soil pH. It is also possible that the presence of aluminium may block the mineralisation of EAN by some unknown mechanism. With the available data, it is difficult to determine the mechanism that produces this blockage, or 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 increases with the amount of EAN-Al added to the soil, even inhibiting the mineralisation of organic C at the highest doses in the OM-poor 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. However, the studies carried out so far by our research group do not allow confirmation of this hypothesis. Thus, although the activities of various hydrolases and oxidoreductases were determined in the same soils after the addition of EAN-Al, these studies focused on the immediate effects of the application of EAN-Al and therefore, there are no long-term data available. Clarification of the mechanisms responsible for the increased toxicity of EAN when mixed with aluminium salt would require such long-term data [24].

4. Conclusions

Adding $\text{Al}(\text{NO}_3)_3$ to EAN aggravates the toxic effects of the IL on soil microorganisms and the negative effects on the microbial activity. The negative effects of EAN are probably mainly due to the nutritional imbalance caused by the addition of large amounts of C, and particularly of N, to the soil. This nutrient imbalance was aggravated by the fact that P was not added to the soil with any of the compounds, which probably exacerbated the already existing P limitation in the soils in the region [25]. The C/N ratio decreased as the amount of EAN added to the soil increased and was greater in the OM-poor soil than in

the OM-rich soil. This greater imbalance in the OM-poor soil also indicates that the impact of the addition of EAN and EAN-Al was greater in this soil than in the OM-rich soil. The addition of different amounts of $\text{Al}(\text{NO}_3)_3$ to the soil did not affect microbial activity, as shown by the similar mineralisation kinetics and similar amounts of total $\text{CO}_2\text{-C}$ emitted by the unspiked soil and the soils spiked with different amounts of $\text{Al}(\text{NO}_3)_3$.

Author Contributions: Conceptualization, methodology, formal analysis, writing—original draft preparation, and writing—review and editing, T.S., M.E.P., J.J.P. and C.T.-C.; funding acquisition, C.T.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Spanish MICINN through project CGL2015-66857-C2-1-R co-financed by the ERDF, and by the Xunta de Galicia through the RCG project IN607A 2017/06, the Contrato-Programa IAG-Xunta 2021-2022, and the Galician Network project ED431D 2017/06. J. J. Parajó wishes to thank the I2C postdoctoral program of the Xunta de Galicia for support in funding the study. E. Priano is grateful for funding support from the Enhancing Mobility Between Latin-American and Caribbean Countries and Europe Program (Argentina-España, 2018).

Acknowledgments: The authors acknowledge technical assistance from A. Iglesias Tojo and D. Portela.

Conflicts of Interest: The authors declare no conflict of interest.

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