Effects of Ascophyllum Nodosum and Soil Amendments on the Development of Maize Seedlings Cultivated Under Acid Oxisol

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Abstract

Introduction

Maize (*Zea mays* L.) plays a fundamental role in Brazilian agriculture, and it is mostly cultivated in Brazilian Savannah areas, under high Al content and acidic pH.

Aims

The aim of this work was to evaluate the effects of soil amendments and *A. nodosum* extract on the initial development of the root system and nitrogen use efficiency of maize under aluminum toxicity.

Methods

The effects of lime and gypsum in combination with seed treatment + foliar application of *Ascophyllum nodosum* on shoot and root growth, plant nutrition and photosynthesis of 14-day-old maize seedlings were tested in two experiments. The experimental design was randomized 4x2 factorial, with the following treatments: lime (L), gypsum (G), lime + gypsum (LG) and control (C), cultivated with and without seed treatment and foliar application of the *Ascophyllum nodosum*.

Results

The treatments with *A. nodosum* extract led to an increase in the root area in the treatments with lime, and to a smaller transport of aluminum to the shoot of the plants. The lime and gypsum treatments were efficient in minimizing the deleterious effects of aluminum toxicity, even with their short-term application in sandy clay loam medium textured soil, providing better photochemical activity, better use efficiency of nutrients, better rooting and, consequently, higher dry weight of shoot and root.

Conclusions

The results show that the use of *A. nodosum* and soil amendments in acidic soils lead to promising physiological and morphological improvement, and by consequence, may influence in crop production.

Introduction

Tropical soils, especially those under Brazilian Savannah areas, are typically acid, highly weathered, with low nutrient availability and high exchangeable acidity (Al3+) (Fageria, 2001; Gonçalves Jr. et al., 2014; Bojórquez-Quintal et al., 2017; Esper Neto et al., 2019). Therefore, to mantain high yield and grain quality, a routine of soil fertilization and amendment for mitigation of the aluminum toxicity and low pH is crucial (Fageria et al., 2014). With the rise in environmental awareness over the risks of soil contamination due to the excess of mineral fertilizers (Brito et al., 2020; Hou et al., 2020), new sustainable nutrient sources, such as algae extracts, have been increasing in the market.

The use of seaweed in agriculture as a source of organic matter or soil amendment is quite old, but its biostimulant effect has only been studied in the past few decades (Du Jardin, 2015; Elansary et al., 2017; Mukherjee & Patel, 2020). Recent studies point that the agricultural use of algae extract can lead to increased plant growth, higher chlorophyll

content, higher flowering, germination and production, increased in vitro propagation, and increased resistance to pathogens, pests and abiotic stresses (Sharma et al., 2014; Elansary et al., 2017; Mukherjee & Patel, 2020). In addition, they also increase nutrient absorption, as they affect both soil processes, such as pore structure and micronutrient solubility, and plant physiology, through changes in root morphology and increased root colonization by arbuscular mycorrhizal fungi (Halpern et al., 2015; Rouphael & Colla, 2020).

Among the algae used as biostimulant in agriculture, is the extract of Ascophyllum nodosum, a natural source of macro and micronutrients such as N, P, K, Ca, Mg, S, B, Fe, Mn, Cu and Zn, amino acids such as alanine, aspartic and glutamic acid, glycine, isoleucine, leucine, among others, and cytokinins, auxins and abscisic acid (Ugarte, et al., 2006; Gõni et al., 2018; Ali et al., 2019; De Saeger et al., 2019; Carmody et al., 2020). These substances are efficient when applied in small concentrations and favor the high performance of the vital processes of the plants, thus, obtaining a higher production. It can also, in adverse environmental conditions, guarantee their performance, preventing yield losses (Gõni et al., 2018; Carmody et al., 2020).

The effects of using A. nodosum extract on crops are not commnly studied in acid soils, especially those from the Brazilian Savannah, where greater rooting, drought resistance and better nutrition are necessary for the good conduction of the season, especially in crops such as maize (Zea mays L.), which plays a fundamental role in Brazilian agriculture, given that the country has a yield above 100 million tons per year (CONAB, 2020). With the economic importance of the crop, it is necessary to study new sustainable fertilization practices that promote better rooting and greater productivity in low fertility soils. It is especially important to assess its effects when applied together with consolidated, common practice soil ammendment techniques, such as lime and gypsum, which help promoting a better root environment by precipitating Al3+, increasing soil pH and providing Ca and Mg for the crop (Fageria, 2001; Fageria et al., 2014; Bonfim-Silva et al., 2019; Esper Neto et al., 2019).

Therefore, the aim of this work was to evaluate the effects of A. nodosum extract combined with gypsum and lime soil amendment treatments on the initial development of the root system, nutrition and photosynthesis of maize seedlings in an acid Ferralsol from Goiás' Brazilian Savannah.

Materials And Methods

This work involves two trials that were simultaneously conducted in different locations: one at a climatized growth chamber at the University of Nottingham campus at Sutton Bonington, Leicestershire, U.K. (Experiment I), and the other at a glasshouse in Rio Verde, Brazil (Experiment II). Both trials were conducted using the same sandy loam soil, with high aluminum saturation, sampled at a Brazilian Savannah area in the state of Goiás, classified as Ferralsol (Anjos et al. 2015; Santos et al. 2018), with 51% of Al saturation (m) (Table 1). This aluminum concentration in soils is considered toxic for maize (Sobral & Guimarães, 1992). The soil texture was a sandy clay loam (25% clay, 4% silt and 71% sand) and the chemical properties of the B horizon of the soil are in Table 1. The chemical characterization of the soil was performed according to methods described by Silva (2006) (Table 1). The pH was determined in $CaCl_2.2H_2O$ 0.01 mol L^{-1} in the ratio soil:solution (1:2.5); the extraction of exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminum (Al^{3+}) was performed with KCl 1 mol L^{-1} and determination of Ca^{2+} and Mg^{2+} in atomic absorption spectrometry (AAS) and Al^{3+} by titration with NaOH 0.025 mol L^{-1}; the extraction of available phosphorus (P) and exchangeable potassium (K+) was in double acid solution by Mehlich I method, and determination of phosphorus was in colorimetry with the development of blue color by the formation of the phosphorus molybdenum complex in acid medium with ascorbic acid and determination of K^+ was in flame photometer; sulfate (SO_4^{2-}-S) extraction was in solution of $Ca(H_2PO_4)_2.H_2O$ solubilized with acetic acid and the determination was by turbodimetry with $BaCl_2.2H_2O$;

and the extraction and determination of soil organic matter (SOM) was by the Walkley-Black method. The saturationof aluminum (m) corresponds to the percentage content of Al^{3+} of the total exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Al^{3+}).

Table 1
Chemical and granulometric characterization of the B horizon of the Ferralsol used in Experiments I and II.

pH	SOM	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	K^{+}	P	$S\text{-}SO_4^{2}$	m
	g dm^{-3}	$cmol_c$ dm^{-3}				mg dm^{-3}			%
4,0	18,7	0,32	0,13	0,50	4,63	12,0	0,34	2,70	51

Experiment I

Experimental units were prepared in pvc tube columns of 6.8 cm diameter and 20 cm long before being placed in a climate-controlled growth chamber at the Hounsfield Facility at the University of Nottingham, Sutton Bonington, UK. The experimental design was randomized 4x2 factorial, with the following treatments: lime (L), gypsum (G), lime + gypsum (LG) and control (C), cultivated with and without seed treatment and foliar application of the *Ascophyllum nodosum* (An) “Shropshire seaweed”, manufactured by Sea-chem Limited, in four replicates. In the soil amendment treatments, the “lime” used was calcium carbonate ($CaCO_3$), calculated to increase soil base saturation (V%) to 70%, the recommended percentage for Brazilian Savannah soils according to Caires (2016). The “gypsum” used was calcium sulfate ($CaSO_4.2H_2O$), calculated according to the method created by Souza and Lobato (2004). After the application of the soil treatments, the soil was transferred to the pvc columns at 1.2 g cm^{-3} bulk density, afterwards, deionized water was applied in the volume corresponding to a water filled pore space of 60% which was maintained daily for 15 days.

The maize cultivar Arcade, by Barenbrug UK, was used due to its requirement for fertile soils (BSPB/NIAB, 2019), which makes it susceptible to the effects of aluminum. The seeds were germinated in petri dishes lined with germination paper, and, after four days, they were selected by uniformity and transferred to the columns. In the treatments with *A. nodosum* extract (An), the seeds were treated with a 2 ml extract per litre of water, according to the manufacturer’s recommendations, and then sown at 2cm depth. Each column had one plant. After complete expansion of the second leaf, a 5% *A. nodosum* solution was applied via leaf in a dose equivalent to 75 L ha^{-1}, according to the manufacturer’s recommendations. To maintain plant nutrition, 15 mL of Hoagland solution No. 2, from Sigma, H2395 series, at 25% concentration was applied throughout the planting period.

After planting the seeds, the growth chamber was maintained at a temperature of approximately 25º C during the day, and 18º C at night, with a 14 hour photoperiod for 14 days. After this period, the relative chlorophyll content (Chl) was determined with SPAD-502, the chlorophyll fluorescence with Fluorpen FP-110, and the photosynthetic activity of the plants was measured with the aid of the device LI-COR LI-6400.

The chlorophyll fluorescence device Fluorpen FP-110 is equipped with a LED (OPTOSUPPLY OSB56L5111Y) centered at ~ 470 nm, and its detector is a PIN photodiode with bandpass filters in the 667—750 nm range. The intensity of light was set at 100%, ~ 3,000 µmol (photons) m^{-2} s^{-1}. Before measuring the experimental signals, plants were kept in dark for at least 30 min. The evaluations were performed at the adaxial surface of the last fully developed leaf, with light emission and measurement of parameters within the OJIP curve, that can separated into three physiological phases: O-J phase, related to the reduction of the first quinone (Q_A) of the electron acceptor side of photosystem II (PSII); J-I phase, which involves the reduction of inter-system electron transporters such as secondary quinone electron acceptor (Q_B), plastoquinone pool (PQ), cytochrome (Cyt) and plastocyanin (PC); and lastly, the I-P phase, which reflects the

reduction of final electron receptors, on the acceptor side of photosystem I (PSI), that is, ferredoxin (Fd), other intermediates, and NADP (Yusuf et al. 2010).

The photosynthesis evaluations were carried out with photosynthetically active radiation of 2000 μmol m^{-2} s^{-1} and CO_2 ambient partial pressure of 39,6 ± 0,7 Pa. Regarding gas exchange, the variables analysed were: CO_2 assimilation (A), transpiration (E), stomatal conductance (gS), water use efficiency (WUE), and vapor deficit pressure (VDP).

At the end of the cultivation period (14 days), analysis of root system development and growth were carried out, using a Phoenix V|TOME|X M 240 high-resolution X-ray Computed Tomography (CT) system (GE Sensing and Inspection Technologies, Wunstorf, Germany). The scanning parameters were optimized to allow a balance between a large field of view and high-resolution. Each sample was scanned with a voltage and current of 170kV and 230μA, respectively, at a voxel size resolution of 50 μm, with the specimen stage rotating 360 degrees over a period of approximately 1 hour and 15 minutes (per sample). A total of 1880 projection images were obtained by averaging 5 frames with an exposure of 131 ms each, at every rotation step. Due to the height of the cylinder (20 cm), 3 separate scans were required to image the entire sample. Each separate scan was then reconstructed using DatosRec software (GE Sensing and Inspection Technologies, Wunstorf, Germany) and then manually combined in VGStudio MAX v2.2 (Volume Graphics GmbH, Heidelberg, Germany) and exported as a single 3D volumetric dataset. To distinguish the phases of the root system from the soil material, image processing techniques were applied by segmenting the reconstructed CT data using a region-growing method in VGStudio MAX v2.2. Through the processed images, the root surface (mm^2) and root volume (mm^3) were determined using the software VGStudio MAX v2.2.

After CT scanning, plant height and leaf number were determined. The plants were harvested, washed and dried in a forced convection oven at 65–70 ºC until constant weight. Subsequently, the material was weighed for shoot (SDW) and root (RDW) dry weight determination. The dry samples were milled, and part of them was used for C and N analysis using an organic elemental analyser Flash 2000 (Thermo Scientific, EUA), the remaining material was digested with nitric acid in a microwave Multiwave 5000 (Anton Paar GmbH, Áustria), for Ca, Mg, K, P, S and Al analysis using ICP-MS iCAP Q (Thermo Scientific, EUA). Based on the values of root and shoot dry mass, the nutrient transportation and carbon partitioning were calculated.

Data were analyzed using an ANOVA, with a p-value of ≤ 0.05 using the software SISVAR (Ferreira, 2014). When significant effects of treatments were found, multiple means comparison was carried out using Fisher's LSD analysis with a 95% confidence interval.

Experiment II

Experiment II was conducted in a glasshouse at the Goiano Federal Institute, in Rio Verde, Brazil. The plants were sown in glass rhizotrons of 60 cm of height, 40 cm width and 3 cm depth. They were filled with the same soil as Experiment I: a sandy loam Ferralsol (Anjos et al. 2015; Santos et al. 2018), with 51% of Al saturation (m) at 1.2 g cm^{-3} density.

The experimental design was randomized 4x2 factorial, with the following treatments: lime (L), gypsum (G), lime + gypsum (LG) and control (C), cultivated with and without seed treatment and foliar application of the *Ascophyllum nodosum* "Shropshire seaweed", manufactured by Sea-chem Limited, in six replicates. For the soil amendment treatments, the "lime" used was calcium oxide (CaO), calculated to increase soil base saturation (V%) to 70%, the recommended percentage for Brazilian Savannah soils according to Caires (2016), and the "gypsum" used was calcium sulfate ($CaSO_4.2H_2O$), calculated according to Souza and Lobato (2004).

In the treatments with lime application, the rhizotrons were filled with untreated soil up to 20 cm, and from there, with the limed soil up to the surface. This was undertaken as a means to replicate field conditions, where most farmers

apply lime on the surface and allow it percolate to the subsurface (~ 20 cm depth) over around 8 weeks before sowing. It was not undertaken on Experiment I due to the limitations in pot size associated with CT imaging. After the rhizotrons were filled, deionized water was applied to achieve 60% water-filled pore spaces and maintained daily for 15 days for the treatment's reaction within the soil.

In the treatments with *A. nodosum* extract (An), aluminum sensitive cultivars (P3754PWU, Pioneer) were treated with a 5% *A. nodosum* extract solution, and then sown at 10 cm depth and 20 cm apart. Each rhizotron had two plants. After complete expansion of the second leaf, a 5% *A. nodosum* solution was applied via leaf in a dose equivalent to 75 L ha^{-1}, according to the manufacturer's recommendations. During plant development, the rhizotrons' surfaces were covered with a black film, keeping only the soil surface exposed to light. They were also positioned in a 30° inclination, in order to keep the root growth close to the glass surface.

The experimental evaluations were carried out fifteen days after plant emergence. The glass surface of the rhizotrons was photographed, where the inclination provided the contact of the root system with the surface. The images were processed in the QUANT 1.0.1 program (Vale et al., 2001) to estimate the root surface in 2400 cm^2 of rhizotron area.

The root depth was determined, and subsequently, the rhizotrons were disassembled and the plants harvested. The material was separated into shoot and root, then washed and dried in a forced convection oven at 65–70ºC until constant weight. Subsequently, the material was weighed for shoot (SDW) and root (RDW) dry weight determination. The dry samples were milled and sent for N analysis according to the method described by Malavolta (1997), using sulphuric digestion in a digestion block, followed by micro-Kjeldahl distillation and then titration with hydrochloridric acid 0,01 mol L^{-1}. Based on the dry mass values of each tissue and the N contents, the accumulation of N in shoots and roots was calculated, as well as its transport to the shoots.

To assess the location of aluminum in the root apices, samples of approximately 1 cm were fixed in FAA 70% for 24 hours. After that, the material was pre-washed in phosphate-buffered saline and dehydration ethanol series (30–100%), pre-infiltrated and infiltrated in HistoResin (Leica, Germany), according to manufacturer's directions. Subsequently, the samples were sectioned transversely at 5 µm depth in a rotary microtome (Model 1508R, Logen Scientific, China), the cuts dyed in chrome azurol S and kept in the dyeing solution for 60 minutes (Kukachka & Miller, 1980). Images were projected on an Olympus microscope (BX61, Tokyo, Japan) coupled with a DP-72 camera using the brightfield and fluorescence option.

Data were analyzed using an ANOVA, with a p-value of $\leq 0{,}05$ using the software SISVAR (Ferreira, 2014). When significant effects of treatments were found, multiple means comparison was carried out using Fisher's LSD analysis with a 95% confidence interval.

Results

Experiment I

Plant Growth

Plant height and root and shoot dry mass varied only in soil amendment treatments (Table 2). The highest values of shoot and root dry mass (SDW and RDW, respectively) and plant height were obtained with the isolated application of limestone (L) and gypsum (G), (Fig. 1A). The joint application of limestone and gypsum (LG) obtained intermediate values, and the lowest growth were obtained in the control treatment (C) (Fig. 1A).

Table 2

ANOVA summary (means square) of the growth variables: shoot (SDW) (g plant^{-1}) and root dry weight (RDW) (g plant^{-1}), height (cm), leaf quantity (LQ), diameter (mm), root surface (mm^2) and root volume (mm^3).

Variables	Ammendment (A)	*A. nodosun* (An)	A*An	Error	VC (%)	$\bar{x}$
SDW	0,0440*	0,0019^{ns}	0,0094^{ns}	0,0104	42,34	0,2403
RDW	0,0214*	0,0048^{ns}	0,0036^{ns}	0,0045	26,66	0,2522
Height	315,7809*	0,0331^{ns}	111,7659^{ns}	69,5500	22,59	36,9115
LQ	1,0312*	1,5312*	0,2812^{ns}	0,3437	16,31	3,5900
Diameter	1,0743^{ns}	0,0604^{ns}	0,4777^{ns}	0,5384	18,49	3,9690
Root Surface	8,3x10^7*	5,6x10^{6ns}	4,5x10^{7ns}	2,9x10^7	28,93	1,8x10^4
Root Volume	2,3x10^{5ns}	1,4x10^{5ns}	3,5x10^{5ns}	3,4x10^5	27,38	2,1x10^3

*, ** and ^{ns}Significance levels 1%, 5% and not significant, respectively.

The number of leaves per plant (LQ) changed according to soil amendment and the application of *A. nodosum* (Table 2, Fig. 1B), where, similarly to the other biometric variables, the plants that received the isolated application of L and G obtained the highest values, followed by the joint application of LG, and the lowest values obtained by the control treatment (C) (Fig. 1B). The stem diameter of the plants did not vary according to the treatments, with a general average of 3.96 mm (Table 2).

The root surface (RS) varied according to the soil treatments (Table 2), where the application of isolated gypsum (G) and limestone (L) obtained the highest values, followed by the joint application of limestone plus gypsum (LG) and control (C) (Fig. 1C).

Through the root images obtained by X-ray CT, it was observed that in treatments with the isolated application of lime and gypsum, the growth was greater when compared to the joint application of the two soil amendments (Fig. 2). Regarding the application of *A. nodosum* extract, a greater number of secondary roots was observed when associated with G and LG, and in the control, without any soil amendments (Figs. 2B, F and H). However, the application of *A. nodosum* extract associated only with liming had an inverse effect, when applied with lime, there was a noticeable reduction in the secondary maize roots.

Nutrition

The nutrient accumulation of maize plants at 14 days after germination varied with the treatments. C, K, Ca and S accumulation in the shoot varied according to the soil amendment, and the accumulation of shoot Mg and Al varied as in function of the application of *A. nodosum* extract (Table 3). There was no statistical difference in the accumulation of N in the shoot in function of the treatments. At the root, the accumulation of C, K, Ca, Mg, S and Al happened due to the soil amendment and N and P due to the *A. nodosum* application (Table 3).

Table 3 ANOVA summary (means square) of the nutritional variables: N, C, Mg, P, S, Ca, K and Al accumulation (mg plant^{-1}) in shoot and root of maize plants at 14 days after germination (V2 stage) under ammendment tratments, and

its interactions with *Ascophyllum nodosum* (An) extract application.

Variables	Section	Ammendment (A)	*A.nodosun* (An)	A**An*	Error	VC(%)	$\bar{x}$
C	SHOOT	$8{,}2x10^{3}$**	$1{,}5x103^{ns}$	$5{,}8x102^{ns}$	$1{,}5x10^{3}$	37,25	102,6571
	ROOT	$2{,}2x10^{3}$*	$7{,}4x10^{2ns}$	$3{,}9x10^{2ns}$	$6{,}2x10^{2}$	28,55	87,4200
N	SHOOT	$10{,}0064^{ns}$	$8{,}0723^{ns}$	$1{,}8291^{ns}$	4,2179	30,60	6,7100
	ROOT	$1{,}6400^{ns}$	7,5415*	$1{,}4527^{ns}$	1,2780	26,53	4,2606
P	SHOOT	$3{,}97x10^{-2ns}$	$6{,}48x10^{-2ns}$	$3{,}89x10^{-2ns}$	$2{,}33x10^{-2}$	40,86	0,3733
	ROOT	$1{,}3x10^{-3ns}$	$24{,}3x10^{-3}$*	$2{,}13x10^{-3ns}$	$4{,}41x10^{-3}$	26,66	0,2491
K	SHOOT	24,1015**	$8{,}79x10^{-2ns}$	$2{,}8073^{ns}$	4,8854	33,68	6,5624
	ROOT	6,3901**	$0{,}3013^{ns}$	$1{,}1272^{ns}$	1,1807	29,54	3,6789
Ca	SHOOT	15,716**	$0{,}1646^{ns}$	$0{,}5571^{ns}$	1,8195	43,53	3,0987
	ROOT	16,0125**	$0{,}7024^{ns}$	$0{,}9919^{ns}$	0,5124	27,48	2,6045
Mg	SHOOT	$0{,}1082^{ns}$	0,2048*	$0{,}664^{ns}$	$4{,}48x10^{-2}$	37,35	0,5668
	ROOT	$6{,}37x10^{-2}$*	$4{,}41\ x10^{-2ns}$	$1{,}49\ x10^{-2ns}$	$1{,}54\ x10^{-2}$	37,96	0,3272
S	SHOOT	0,4821**	$2{,}16x10^{-2ns}$	$4{,}38x10^{-2ns}$	$6{,}23x10^{-2}$	45,32	0,5510
	ROOT	5,25**	$8{,}43x10^{-2ns}$	$2{,}84x10^{-2ns}$	$9{,}97x10^{-2}$	28,53	1,1068
Al	SHOOT	$3{,}26x10^{-4ns}$	$3{,}528x10^{-3}$*	$1{,}83x10^{-4ns}$	$3{,}59x10^{-4}$	86,86	0,0218
	ROOT	17,3690**	$3{,}78\ x10^{-4ns}$	$2{,}046^{ns}$	2,3097	37,21	4,0840

*, ** and ^{ns}Significance levels 1%, 5% and not significant, respectively.

The biggest accumulation of C in shoots and roots occurred with the application of soil amendments (Fig. 3A). The shoot accumulation of N did not vary with treatments and the general average was 6,71 mg plant^{-1} (Table 3). However, in the roots, the greatest accumulation of N was obtained in plants without the application of *A. nodosum* (Fig. 3B). The *A. nodosum* did not change the biomass production of the plants (Table 2), however there was an alteration in the N accumulation. Thus, the lower N accumulation with the application of *A. nodosum* producing the same biomass as the plants without the algae application (Table 2), means that there was an increase in the use efficiency of the applied N, with the production of the same biomass, compared to plants not treated with the algae extract, but with a smaller amount of accumulated N.

On a similar effect, the lowest Mg shoot accumulation was obtained in plants treated with *A. nodosum* (Fig. 3C). Thus, indicating that there was also an increase in the use efficiency of Mg. In the roots, the largest accumulations of Mg were obtained with the soil amendment treatments (Fig. 3C). The shoot accumulation of P was not altered by the treatments,

with an average of 0.37 mg plant^{-1} (Table 3). In the roots, however, the effect was similar to N (Fig. 3B) and Mg (Fig. 3C), with lower P accumulation in the presence of *A. nodosum* (Fig. 3D).

The largest accumulation of S in the shoot and root was obtained in treatments with G application, reflecting the presence of sulfate (SO_4^{2-}) in the treatment (Fig. 3E). Similarly to S, the greatest accumulation of Ca in shoots and roots was obtained in treatments with the application of L and G (Fig. 3F). This element is a primary constituent of both soil amendments. Potassium (K) was more accumulated in shoots with the application of L and G, followed by LG and C (Fig. 3G). In the root, the greatest accumulation was obtained without the application of soil amendments (C).

Plants with *A. nodosum* application accumulated less Al in their leaves (Fig. 3H). The root Al accumulation response varied according to the soil amendment treatments (Table 3), being higher in plants under the influence of L and G (Fig. 3H).

The C partition in the plants varied with the soil treatments (Table 4), where the control had the highest C partition towards the root system (Fig. 4A), and the lowest shoot dry weight (SDW) (Fig. 1A) among the treatments, a characteristic effect of Al stress (Silva et al. 2010).

The transport of N, K, Ca and S to the shoot changed as a result of the soil amendment treatments, and Al changed only in function of *A. nodosum* application (Table 4). The transport of P and Mg remained unchanged with the treatments, with a general average of 58 and 62,4%, respectively (Table 4). There was greater transport of N and K to the shoot in treatments with soil amendment (Figs. 4B and C, respectively).

The highest contents of S were obtained in the roots in the treatments with G (Fig. 3E), and the Ca was also more accumulated in the roots, with the application of G and/or L (Fig. 3F). That effect is a reflection of the high amounts of SO_4^{2-} and Ca supplied in the treatments with the soil amendments. Being concentrated in the soil, the highest percentage accumulation of SO_4^{2-} and Ca was in the roots, resulting in low percentage values of transport of these nutrients to the shoot (Figs. 4B and C, respectively).

Table 4
ANOVA summary (means square) of the variables: C partitioning in shoot and root (%), and C, N, P, K, Ca, Mg, S and Al transport (%) in maize plants at 14 days after germination (V2 stage) under ammendment tratments, and its interactions with *Ascophyllum nodosum* (An) extract application.

Variables	Ammendment (A)	*A. nodosun* (An)	A*An	Error	VC(%)	$\varvec{x}$
C Shoot	231,7391**	3,3593^{ns}	34,7704^{ns}	32,9393	10,86	52,8252
C Root	231,7391**	3,3593^{ns}	34,7704^{ns}	32,9393	12,17	47,1748
N	236,3583**	22,1637^{ns}	61,7061^{ns}	35,2836	9,80	60,6410
P	253,6774^{ns}	9,9348^{ns}	391,3083^{ns}	143,2282	20,66	57,9345
K	640,7906*	183,6823^{ns}	180,6035^{ns}	180,7683	21,66	62,0600
Ca	376,0949**	171,2935^{ns}	246,5796^{ns}	105,8789	18,67	55,1145
Mg	262,5400^{ns}	0,4943^{ns}	281,3996^{ns}	151,9849	19,74	62,4415
S	1558,0103**	123,2050^{ns}	43,8347^{ns}	63,1997	20,84	38,1414
Al	0,1180^{ns}	2,1004**	0,0294^{ns}	0,1478	69,17	0,5558

*, ** and ^{ns}Significance levels 1%, 5% and not significant, respectively.

Aluminum (Al) was less transported to the shoot of the plants treated with *A. nodosum* (Fig. 4D). These results, showing less Al transport to the shoots and greater accumulation in the roots with the application soil amendments (Fig. 3H) demonstrate the possibility that some physiological mechanism was activated with the application of *A. nodosum*, reducing the transport of Al to the shoots of the plants. The greater accumulation in the roots with the application of G and/or L, may be due to precipitation in the intercellular spaces of Al in different hydroxide ionic species [$Al(OH)_n^{n+/-}$] and/or aluminum hydroxysulfate ($AlSO_4OH$).

Photosynthesis

Regarding relative content of chlorophyll (Relative Chl), there was variation with the application of soil amendment (Table 5), in which plants in the control treatment had the lowest chlorophyll indexes among the others (Fig. 5A). Variable fluorescence changed according to the soil treatments (Table 5). Vj was higher in the treatments C and L, and Vi in C and LG (Fig. 5B). This variation is a reflection of stress in the photosystem of plants with higher fluorescence, mainly noticed in the control treatment.

Mo, Ss, Ψ_o and Φ_{Eo} also varied according to soil treatments (Table 5). Mo reflects the closure rate of the PSII reaction centers, and the lower its value, the better the structure of the photosystem, therefore, it is an indirect index that reflects the structural organization of the chloroplast (Strasser et al., 2000). Thus, similarly to variable fluorescence data (Fig. 5B), the control treatment is the one that demonstrates the greatest disruption of PSII (Fig. 5C).

The Ss index evaluates the amount of energy needed to promote the simple turn over of Quinone a, and by requiring more light energy to initiate photosynthetic activity, the plant protects itself from the photooxidation of chloroplasts. Ss values were higher in all plants grown in amended soils, indicating, again, a possible disruption of the photosynthetic apparatus on the control plants (Fig. 5C).

The photochemical efficiency (Ψ_o) is the probability of the excitation energy moving an electron after Quinone a, and is intrinsically linked to the plant's ability to keep the photosynthetic process active. Φ_{Eo} is directly linked to Ψ_o, referring to the probability of the energy of an absorbed photon moving an electron after Quinone a, so the higher the value of Φ_{Eo}, the greater the probability of the electron chain remaining active, providing energy to the photosynthetic process. The lowest values of Ψ_o and Φ_{Eo} were obtained in the plants of treatment C, followed by soil amendment with L (Fig. 5C), which means that, especially the control plants, were more likely to interrupt the photosynthetic activity.

Table 5

ANOVA summary (means square) of relative chlorophyll, determined with SPAD, fluorescence indices, determined with Fluorpen FP110 and photosynthesis indices determined with LiCor (LI 6400-XT) in maize plants at 14 days after germination (V2 stage) under ammendment tratments, and its interactions with *Ascophyllum nodosum* (An) extract application.

Variables	Ammendment (A)	*A.nodosun* (An)	A*An	Error	VC(%)	$\bar{x}$
Chl	628,11**	25,8481^{ns}	74,4527^{ns}	69,6366	28,28	29,5056
Fo	231.679,84^{ns}	358281,1250^{ns}	63345,5841^{ns}	312833,6806	12,01	4658,75
Fj	6.299.215,53^{ns}	3262096,53^{ns}	1066908,28^{ns}	2701384,89	13,30	12360,343
Fi	663.022,97^{ns}	2363138,00^{ns}	1052212,80^{ns}	4753714,91	13,36	16322,875
Fm	1.290.577,71^{ns}	2632365,25^{ns}	2106917,71^{ns}	7092612,23	13,79	19319,188
Fv	896.253,25^{ns}	1048352,00^{ns}	1509186,88^{ns}	4794318,35	14,94	14660,438
Vj	$1{,}93x10^{-2}$**	$1{,}14x10^{-3}$^{ns}	$2{,}19x10^{-3}$^{ns}	$2{,}69x10^{-3}$	9,84	0,5272
Vi	$1{,}09x10^{-3}$*	$4{,}80x10^{-5}$^{ns}	$5{,}91x10^{-4}$^{ns}	$3{,}54x10^{-4}$	2,36	0,7964
Fm/Fo	0,1018^{ns}	0,0158^{ns}	0,0143^{ns}	0,0692	6,34	4.1518
Fv/Fo	0,1018^{ns}	0,0158^{ns}	0,0143^{ns}	0,0692	8,35	3,1518
Fv/Fm	$3{,}36x10^{-4}$^{ns}	$5{,}5x10^{-5}$^{ns}	$6{,}1x10^{-5}$^{ns}	$2{,}31x10^{-4}$	2,00	0,7579
Mo	0,1996**	$3{,}04x10^{-2}$^{ns}	$7{,}8x10^{-3}$^{ns}	$1{,}29x10^{-2}$	12,02	0,9435
AREA	$2{,}0835x10^{13}$^{ns}	$3{,}3498x10^{10}$^{ns}	$9{,}1483x10^{11}$^{ns}	$3{,}1054x10^{13}$	85,76	$6{,}4980x10^{6}$
FIX AREA	$1{,}2244x10^{12}$^{ns}	$2{,}4925x10^{12}$^{ns}	$2{,}1227x10^{12}$^{ns}	$6{,}8285x10^{12}$	13,76	$1{,}8994x10^{8}$
Sm	130294,6754^{ns}	322,5292^{ns}	665,1949^{ns}	180566,6279	93,92	452,4166
Ss	$1{,}5226x10^{-2}$**	$5{,}512x10^{-3}$^{ns}	$5{,}94x10^{-4}$^{ns}	$1{,}899x10^{-3}$	7,69	0,5669
N	682103,8991^{ns}	2787,2818^{ns}	8729,6392^{ns}	684837,9255	101,15	818,1586
Φ_{Po}	$3{,}36x10^{-4}$^{ns}	$5{,}5x10^{-5}$^{ns}	$6{,}1x10^{-5}$^{ns}	$2{,}31x10^{-4}$^{ns}	2,00	0,7579
Ψo	$1{,}9406x10^{-2}$**	$1{,}116x10^{-3}$^{ns}	$2{,}212x10^{-3}$^{ns}	$2{,}69x10^{-3}$	10,97	0,4729
Φ_{Eo}	$1{,}2899x10^{-2}$**	$8{,}61x10^{-4}$^{ns}	$1{,}352x10^{-3}$^{ns}	$1{,}897x10^{-3}$	12,13	0.3590625
Φ_{Do}	$3{,}31x10^{-4}$^{ns}	$6{,}1x10^{-5}$^{ns}	$6{,}5x10^{-5}$^{ns}	$2{,}31\ x10^{-4}$	6,27	0,2421
Φ_{Pav}	244,4028^{ns}	6,4333^{ns}	43,8778^{ns}	239,1458	1,63	950,5152
Π_{ABS}	1,0920**	0,1308^{ns}	0,1299^{ns}	0,1175	26,58	1,2900
ABS/RC	0,3221**	$9{,}1592x10^{-2}$^{ns}	$6{,}219x10^{-3}$^{ns}	$2{,}9802x10^{-2}$	7,34	2,3524

Variables	Ammendment (A)	*A.nodosun* (An)	A*An	Error	VC(%)	$\bar{x}$
TRo/RC	0,1545**	$4{,}3808x10^{-2ns}$	$3{,}242x10^{-3ns}$	$1{,}5593x10^{-2}$	7,01	1,7816
ETo/RC	$1{,}6759x10^{-2ns}$	$1{,}164x10^{-3ns}$	$8{,}135x10^{-3ns}$	$1{,}3256x10^{-2}$	13,74	0,8382
Dio/RC	$3{,}1311x10^{-2}$**	$8{,}878x10^{-3ns}$	$1{,}090x10^{-3ns}$	$3{,}966x10^{-3}$	11,03	0,5709
A	42,0109**	$0{,}037592^{ns}$	$4{,}4259^{ns}$	8,1955	27,70	10,3332
gS	$1{,}69x10^{-4}$**	$1{,}7x10^{-5ns}$	$0{,}9x10^{-5ns}$	$4{,}7x10^{-5}$	33,94	0,0203
E	$8{,}6342x10^{-8}$**	$5{,}3232x10^{-9ns}$	$3{,}9447x10^{-9ns}$	$2{,}1191x10^{-8}$	34,19	0,0004
DPV	$4{,}213x10^{-3}$*	$2{,}02x10^{-4ns}$	$2{,}499\ x10^{-3ns}$	$1{,}102x10^{-3}$	1,97	1,6889
WUE	$0{,}6190^{ns}$	$0{,}1416^{ns}$	$4{,}8187x10^{-2ns}$	0,2262	18,78	2,5322

*, ** and ^{ns}Significance levels 1%, 5% and not significant, respectively.

The π_{ABS}, ABS/RC, TRo/RC and Dio/RC indices were influenced by soil amendments (Table 5) and reflect the overall performance of photosystem II. These indices reflected a possible disruption of the chloroplasts of the control plants, which had a lower performance index (π_{ABS}), as the higher flux of absorption (ABS/RC), capture (TRo/RC) and electron transport (Dio/RC), did not result in an increase in photochemical efficiency (Ψ_o), but in energy loss in the form of fluorescence (Fig. 4D), demonstrating low use of the electrons generated in the water photolysis process, at the beginning of the electron transport chain.

The photosynthetic rate (A), stomatal conductance (gS), transpiration (E) and water vapor deficit (VPD) also only varied in function of soil amendment treatments (Table 5). The lowest values of A, gS, E and VPD were obtained in control plants, that is, in plants under abiotic stress due to soil acidity and aluminum toxicity (Figs. 6A, B, C and D). These results are a consequence of the worst fluorescence indices (Fig. 5B, C and D) and reflect the low carbon partition in the aerial part of the control plants (Fig. 4A).

Experiment II

In the rhizotron experiment, the variables shoot (SDW) and root dry weight (RDW), root surface, N accumulation in shoot (NCS) and N transport (NT) were significant (Table 6). The root depth and N accumulation in root (NCR) did not change with the treatments, and their means were 35,85 cm and 33,69 mg plant^{-1}, respectively (Table 6).

Table 6

ANOVA summary (means square) of the growth variables: shoot (SDW) (g plant^{-1}) and root dry weight (RDW) (g plant^{-1}), root surface (cm^2) and root depth (cm), and the N accumulation in shoot (NCS) and root (NCR) (mg plant^{-1}) and N transport (%) (NT) in maize plants at 15 days after germination (V2 stage) under ammendment tratments, and its interactions with *Ascophyllum nodosum* (An) extract application.

Variables	Ammendment (A)	*A.nodosum* (An)	A*An	Error	VC (%)	$\overline{\text{varvec}x}$
SDW	0,2010**	0,0157^{ns}	0,0459^{ns}	0,0370	32,84	0,5863
RDW	2,7772**	0,0609^{ns}	0,4709^{ns}	0,6523	37,41	2,1591
Root Depth	53,4591^{ns}	5,0052 ^{ns}	125,5541 ^{ns}	55,3509	19,66	37,8479
Root Surface	1847,5469**	263,4282 ^{ns}	570,8867**	130,1198	30,74	37,1052
NCS	890,8675 **	6,9901^{ns}	99,9974*	33,7636	37,51	15,4929
NCR	328,5625 ^{ns}	56,1192 ^{ns}	37,2272 ^{ns}	163,5340	37,95	33,6945
NT	714,0858**	417,7621**	610,2377**	60,3482	25,89	30,0054

*, ** and ^{ns}Significance levels 1%, 5% and not significant, respectively.

The plants that had higher shoot and root dry weight were the ones under L and LG treatments, while those with the lowest dry weight were those of the control treatment (Fig. 7A).

The root surface was under the interaction between soil treatments with and without the application of *A. nodosum*, (Table 6). The soil amendment treatments that provided the largest root surface with *A. nodosum* were L and LG, and in the absence of the algae extract, LG, L and G were the ones with the largest root surface (Fig. 7B). The positive correlation between L and LG and algae application demonstrates that, in these treatments, with the application of *A. nodosum* the plant developed its root system better, having a larger area and increasing its contact surface with the soil, which is essential for greater absorption of water and nutrients, and greater tolerance to abiotic stress conditions.

The interaction of soil amendment treatments with the application of *A. nodosum* also altered N accumulation in shoots (Table 6). The greatest accumulation of N occurred in plants treated with LG x An (Fig. 7C). In plants without the application of algae extract, the greatest accumulation of N was obtained in treatments with application of L and LG, and also in the control (C) (Fig. 7C). For N (NT) transport, there was also an interaction between soil treatments and the application of algae extract (Table 6). The highest N transport occurred in plants grown with the application of LG x An and G x An, and in the absence of the algae extract, the highest NT was with the application of L and LG, and also in C (Fig. 7D).

Through the images obtained from the rhizotron surface (Fig. 8) it was possible to notice the increase in the number of root hairs and secondary roots in treatments with higher amounts of calcium, such as LG x An and LG (Fig. 8A and B), with emphasis on the association of lime, gypsum and *Ascophyllum nodosum* extract (LG x An), which obtained greater root surface between treatments (Fig. 7B), with an increase of 42% compared to the same treatment without the algae extract. A similar effect was also observed in the L x An treatment, with a visible increase in the root surface and root hairs with the application of *A. nodosum* (Figs. 7B and 8E).

It is worth noting that, in treatments only with gypsum (Figs. 8C and D), the predominance of roots in the upper third of the rhizotron reflected its application only on the soil surface, a common practice of farmers in the field. Possibly, in the time period of the experiment (15 days), there was not enough time for the calcium sulfate to percolate down the soil. The same limitations imposed by surface application of gypsum can be observed in the other variables evaluated (Figs. 7A, B, C and D), where the gypsum treatments had a similar performance to the control treatment, while the ones with lime, which was incorporated down to 20 cm, had the best performance.

Despite the limitations, in terms of N accumulation and transport (Figs. 7C and D), the treatment with gypsum alone was the most responsive to the application of algae extract, with an increase of 328,7% in the transport of N to the shoot when compared to gypsum without *A. nodosum*.

Discussion

Soil amendment, regardless of the treatment in both experiments, led to plants with greater dry mass, greater root surface and greater C accumulation, results similar to those observed by Tiecher et al., (2018) and Schenfert et al., (2019). This is due to the improvement of the root environment through liming, which increases the soil pH and precipitates the Al^{3+} cations into aluminum hydroxide, while also providing Ca for the crop; and the gypsum application, which despite not amending soil acidity, provides Ca and S at greater depths, while also promoting Al precipitation (Caires et al., 2011).

The application of lime or gypsum, alone or combined, increased the accumulation of Ca, Mg and S. Potassium (K) was more accumulated in treatments with isolated amendment, possibly because it was displaced in the soil in the LG treatment, due to the large amount of Ca^{2+} (Soares et al., 2018). Even for the nutrients where accumulation in the shoot and root was not significant, the amendment altered their partition in the plants, resulting in more N and K transported to the shoot than retained in the root.

That said, through Al^{3+} precipitation and the reduction of soil aluminum toxicity, treatments with lime and gypsum increased the relative content of chlorophyll, the photosynthetic activity of the plants, and also reduced fluorescence losses. The decrease in chlorophyll a and b levels is one of the consequences of aluminum toxicity (Peixoto et al., 2002), and it works as a defense mechanism, since in addition to decreasing light absorption by reducing the concentration of chlorophyll, the presence of Al increases the closure of reaction centers, as a way of protecting the plant against damage from light stress (Jiang et al., 2008), at the same time, leading to a reduction in the photosynthetic efficiency.

The reduction of Ψ_o, and consequently, Φ_{Eo} in plants under aluminum stress was also observed by Jiang et al. (2008) working with *Citrus grandis* (L.). The results demonstrate that aluminum toxicity reduced the performance index (πABS), absorption flux (ABS/RC) and electron transport (Dio/RC), in addition to reducing CO_2 assimilation (A). Jiang et al. (2008) found similar results, where the reduction in the electron transport capacity, accompanied by the lack of reducing equivalents seems to be the main factors that contribute to the decrease in CO_2 assimilation in plants under Al stress.

The plants in the control treatment generally had the worst performance in shoot and root growth, nutrient uptake and photosynthetic activity, due to these plants being grown in an environment under Al toxicity. When the exchangeable aluminum content is above the critical level, it restricts root growth and consequently lessens the plant's nutrient uptake efficiency (Yadesa et al., 2019). It is evident in the control plants, that the carbon partition (Fig. 4A) favored the roots, with the plants under stress deferring the emission of new leaves, possibly in an attempt to decrease transpiration losses (Silva et al., 2010), and mobilizing carbon to the roots in search of a less toxic environment, with greater possibility of nutrient absorption. Nonetheless, plants cultivated in untreated soil (Control) had less root surface, which can be seen in the root images (Figs. 2G and H, and Figs. 8G and H) .

Many works over the years (Wright, 1989; Ryan et al., 1992; Ryan & Kochian, 1993; Wang et al., 2004; Poschenrieder et al., 2008; Trachsel et al., 2010; Silva, 2012; Furlan et al., 2019) report the inhibition of root growth under Al toxicity, which was also observed in this work through the X-ray CT images. All the control plants' roots were irregularly curved and presented thick and stunted tips, reduced root hairs and thin laterals starting near the tip of the axial roots - typical effects of aluminum toxicity, according to Wright (1989) and Čiamporová (2002).

In the rhizotron assay (Experiment II), there was an interaction between the soil treatments and the application of *A. nodosum* extract. In treatments with the application of LG, the highest root surface value was obtained when associated with algae extract, as well as the greatest accumulation of N. In this trial, all lime treatments, when in the presence of algae extract, had an increase in the root surface and nitrogen accumulation, which may suggest greater effects of *A. nodosum* in the presence of lime and/or a pH close to neutrality. This result is similar to those obtained for the mung bean crop using the extract of the algae *Ecklonia maxima* (Arthur et al., 2013) and the grape crop under *A. nodosum* (Sabir et al., 2014). Arthur et al. (2013) also reported an increase in the levels of photosynthetic pigments (ChlA, ChlB and carotenoids) when the seaweed extract was associated with a supply of calcium and soil with pH 6,5.

The increase in root growth as a result of the association between algae extract and liming occurs due to the presence of phytohormones in the *A. nodosum* extract, mainly cytoplasmic auxin, which is transported between plant tissues when bound to Ca^{2+}. Auxin moves acropetally through vascular tissue, and basipetally to the outer cortex and epidermis of the roots, through specific inflow and outflow carriers to facilitate movement between cells. The auxin gradient is fundamental to regulate the root meristem organization and its activity. The extra supply of auxin by seaweed extract reduces cytosolic pH and increases Ca^{2+} concentrations. This change in pH can cause fluctuations in cell membrane potential, increasing proton excretion (Shishova & Lindberg, 2004; Lanteri et al., 2006; Arthur et al., 2013; Vanneste & Friml, 2013).

Another effect observed with the application of algae extract was the reduction of Al accumulation in the leaves, when compared to plants without algae application. Reducing the accumulation of foliar Al is extremely important, since the presence of large amounts of the metal in the leaves can lead to the production of reactive oxygen species (ROS), with consequent peroxidation of plasmalemma lipids, causing damage to cellular components (Panda et al., 2009).

The results of root Al accumulation suggest that it may have been retained in the apoplast of the roots, especially those under soil amendments and algae extract. It is possible to visualize this accumulation through microscopy images (Figs. 9 and 10), with the presence of large amounts of Al in the apoplast of the roots, mainly under lime and lime + *A. nodosum* (Figs. 9D and E, respectively). It is also noticeable that in the control treatments there is the presence of aluminum inside the cells (Fig. 10A and B), which corroborates with the other deleterious effects on growth and photosynthesis observed in the control plants.

The high accumulation of Al in the root tissue and the low concentration of the metal in the leaves suggest that it may have been precipitated by organic acids or different ionic species of hydroxide and/or aluminum hydroxysulfate in the apoplast of the roots, difficulting its transport to the symplast and consequently for the aerial part. The release of Al-complexing solutes, particularly anions of organic acids such as malate and citrate, into the Al-sensitive apical root zone is the most effective way to reduce Al's impact on apoplastic functions, through the formation of non-toxic Al complexes, reducing its deleterious effects on plants (Horst et al., 2010; Kopittke et al., 2017).

Although there are few studies on the effects of *A. nodosum* on plants grown under Al stress in the soil, several authors report that the application of the algae extract can lead to an increase in root exudates, including flavonoids (Lola-Luz et al., 2013; Elansary et al., 2016; Jithesh et al., 2018; and Shukla et al. 2019), secondary metabolites from the group of

phenolic compounds, with high Al chelation ability, and their exudation by roots can potentially reduce Al activity in the apoplast, preventing it from being transported to the shoot (Kidd et al. 2001; Panda et al., 2009).

In general, the positive result of *A. nodosum* leading to an increase in the nutritional use efficiency in plants under soil Al stress is unprecedented, and can assist in the development of new technologies. Further studies are needed to evaluate the hypothesis of *A. nodosum* influencing root exudates that can complex aluminum in the apoplast, and reduce its transport to the shoot, lessening its toxic effects on plants.

Conclusions

The seed treatment with *A. nodosum* extract and its application on maize seedlings led to an increase in the root surface in treatments with lime, and to a lower aluminum transport to the aerial part of the plants. The treatments with lime and gypsum were efficient in minimizing the harmful effects of aluminum toxicity, even with its short-term application in medium textured soil, leading to better photochemical activity, better use and efficiency of nutrients, better rooting and, consequently, higher dry weight of shoots and roots.

More studies focusing on the effects of *A. nodosum* in plants under aluminum toxicity and acid soils are needed, especially with a focus on root growth, rhizosphere effects and root exudates, for a better understanding of the mechanisms reducing the deleterious effects of the Al toxicity.

Declarations

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DECLARATIONS

The authors declare that there are no relevant financial or non-financial competing interest to report.

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Figures

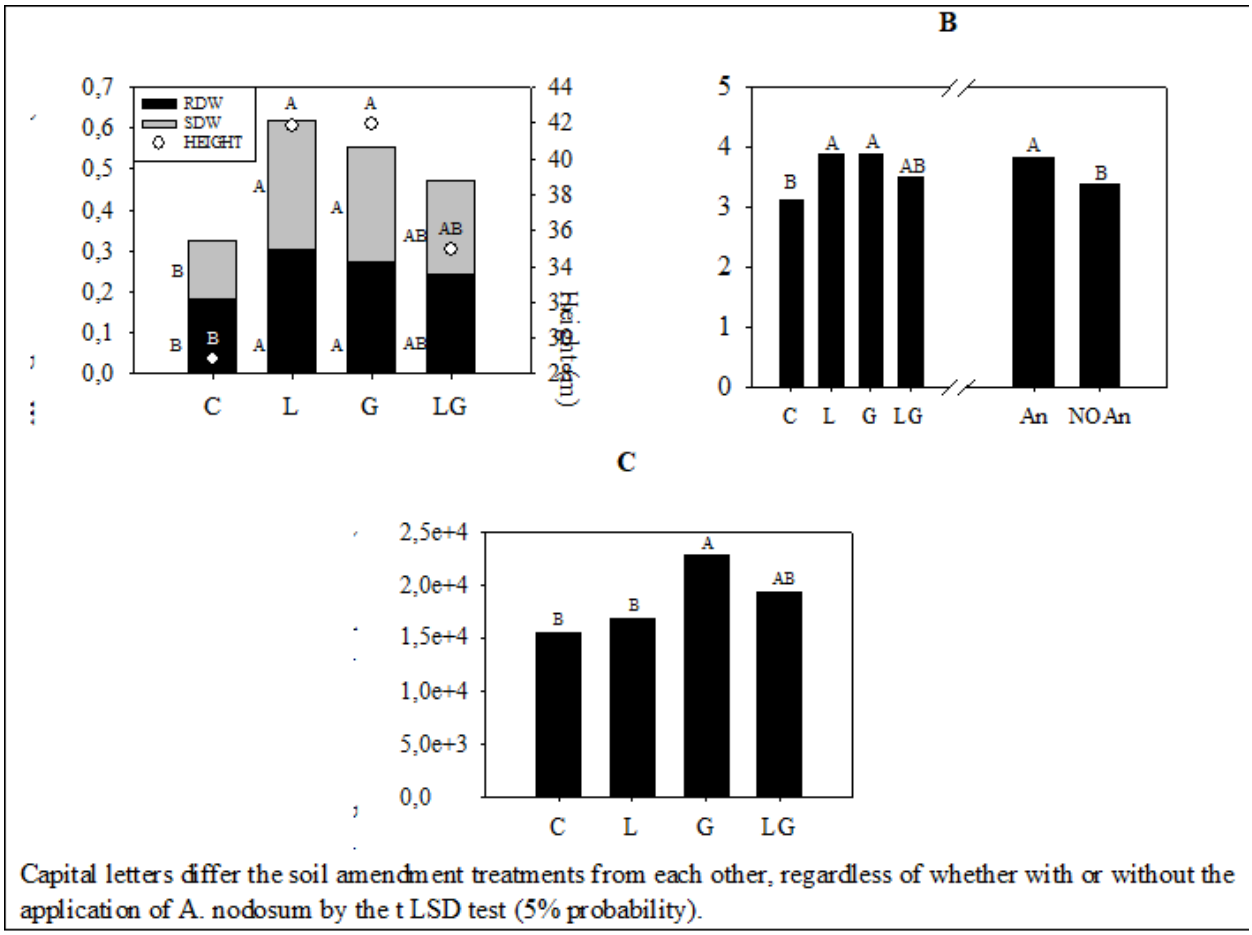


Figure 1

Initial maize growth: (A) shoot (SDW) and root dry weight (RDW) (g plant^{-1}) and height (cm); (B) leaf quantity; (C) root surface (mm²), varying according to soil amendment treatments and *A. nodosum* application. Nottingham, 2019.

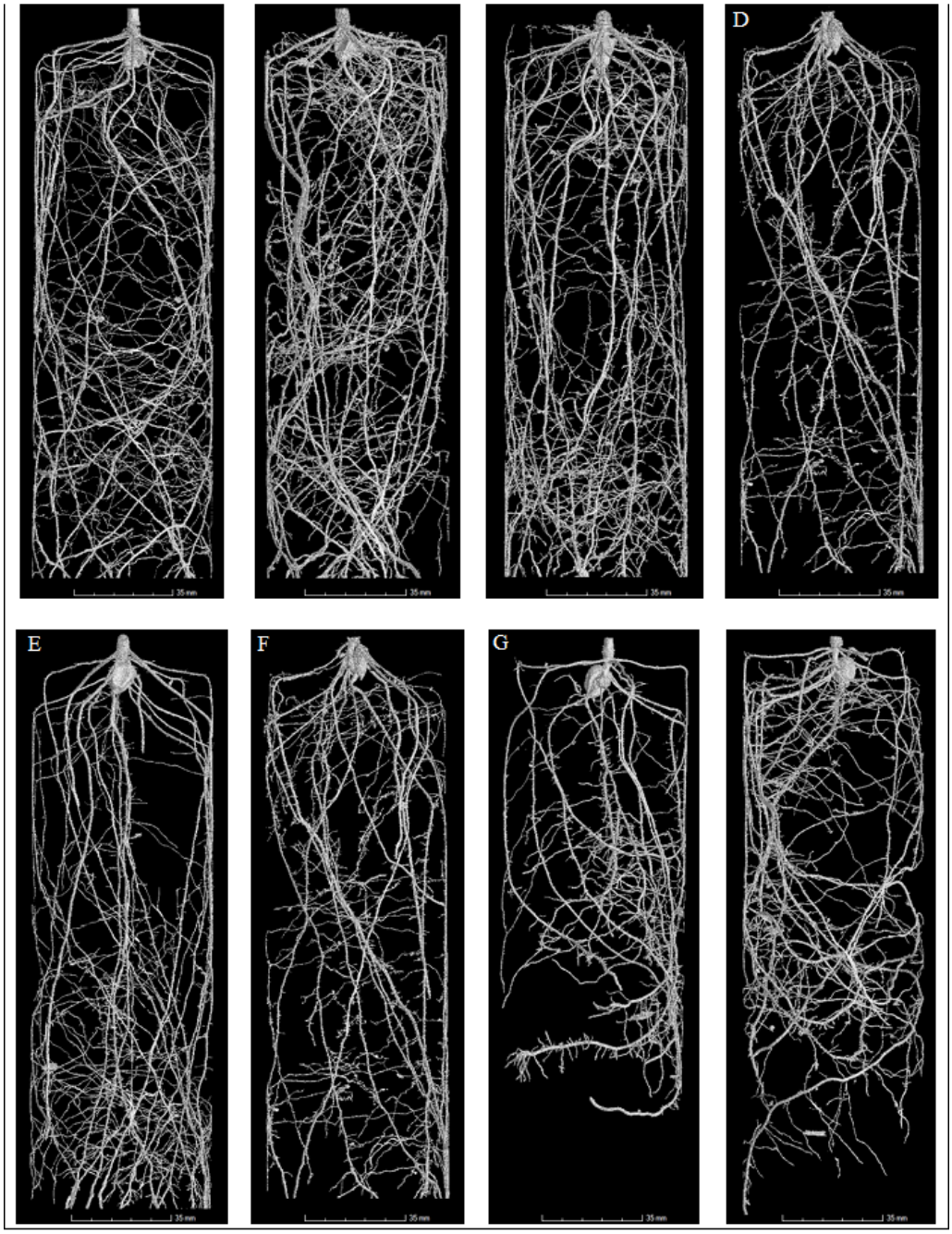


Figure 2

Root images of maize plants at 14 days after germination (V2 stage), obtained through 3D X-ray CT. (A) Gypsum; (B) Gypsum and *A. nodosum*; (C) Liming; (D) Liming and *A. nodosum*; (E) Liming and Gypsum; (F) Liming, Gypsum and *A. nodosum*; (G) Control; (H) *A. nodosum*. Notthingham, 2019.

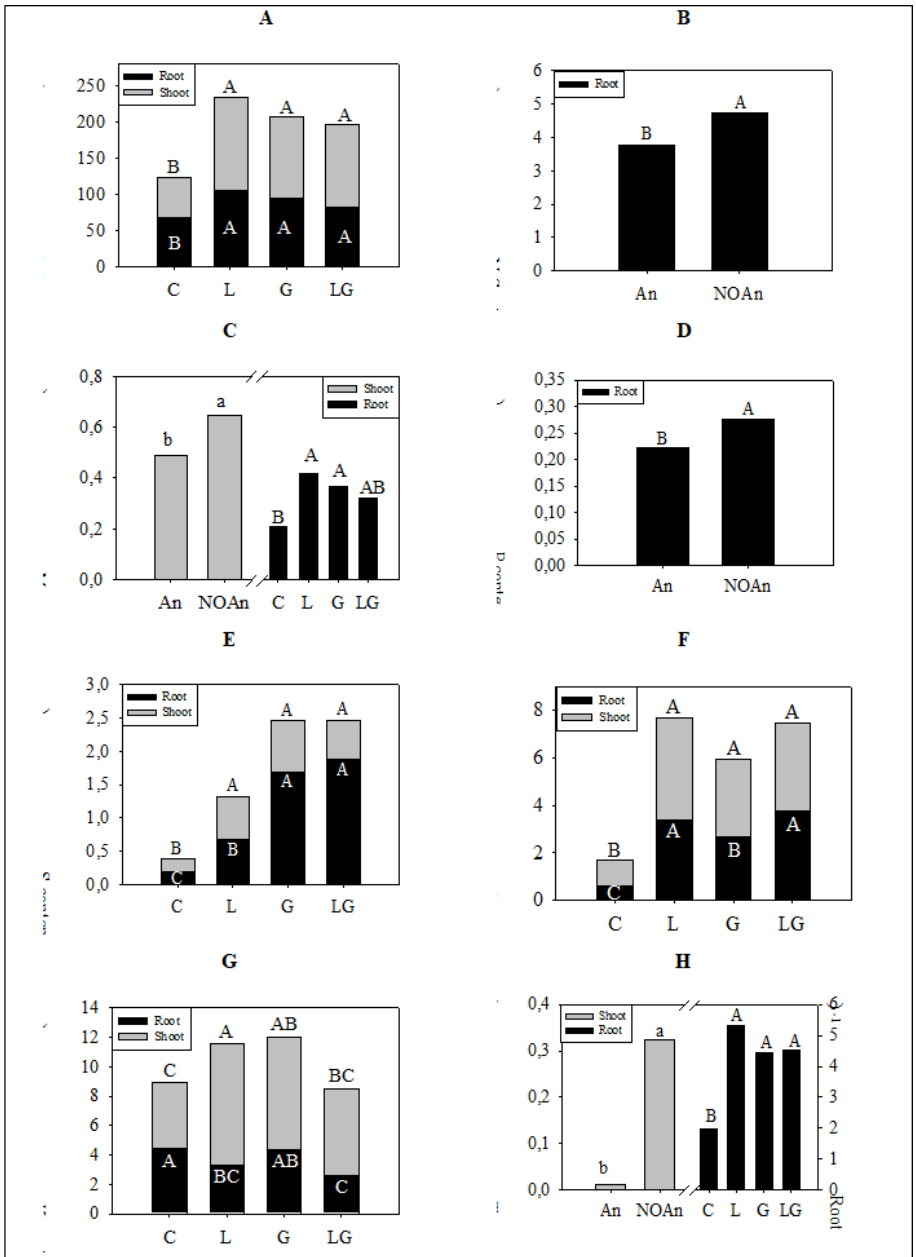
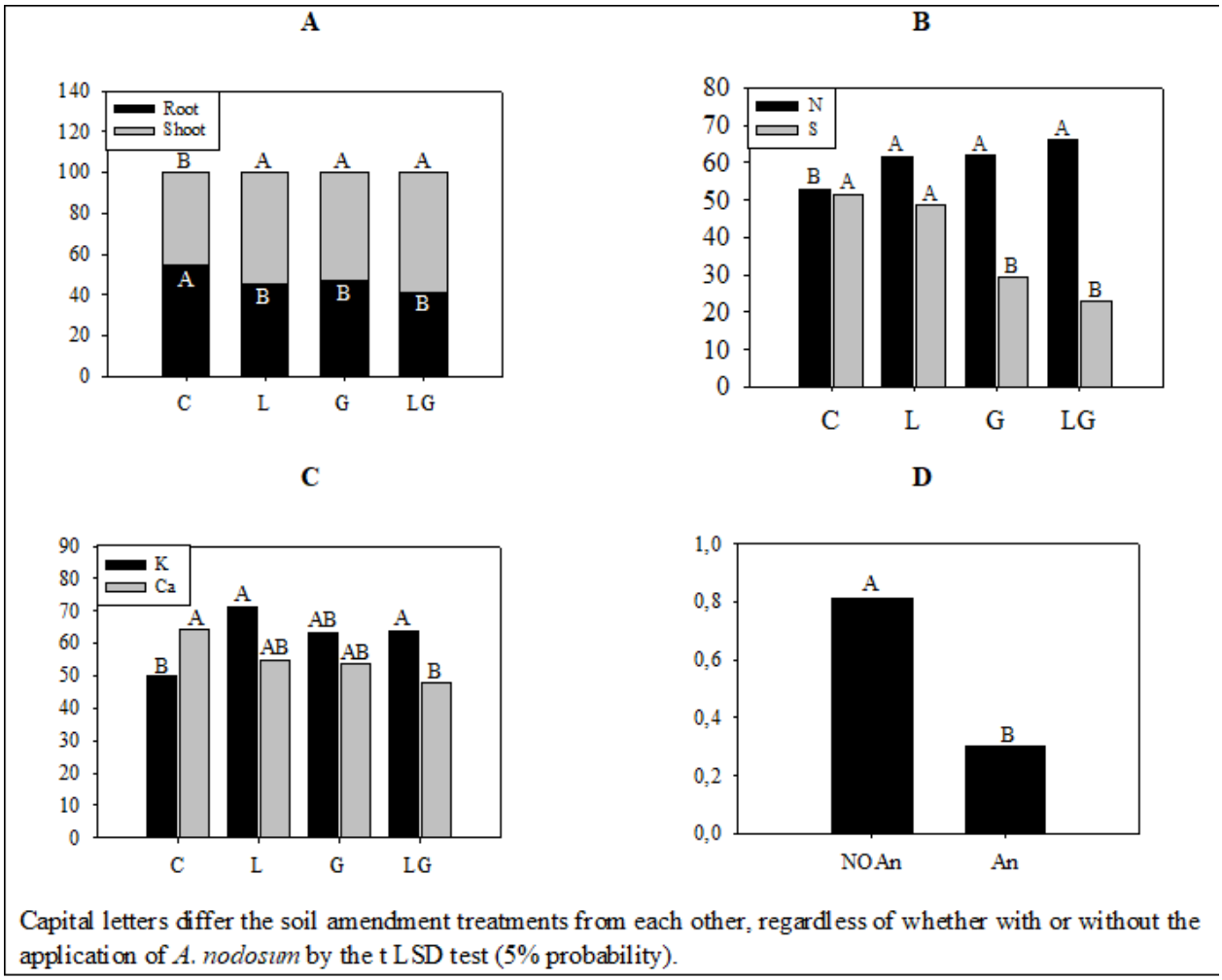


Figure 3

Accumulation of macronutrients in shoots and roots of plants in function of soil amendment treatments and application of *A. nodosum*. Nottingham, 2019.



Capital letters differ the soil amendment treatments from each other, regardless of whether with or without the application of *A. nodosum* by the t LSD test (5% probability).

Figure 4

(A) C partitioning (%); and transport (%) of (B) N and S; (C) K and Ca; and (D) Al transport (%) de N e S (B), K e Ca (C) e Al (D) in function of soil amendment treatments and application of *A. nodosum* in maize plants at 14 days after germination (V2 stage). Nottingham, 2019.

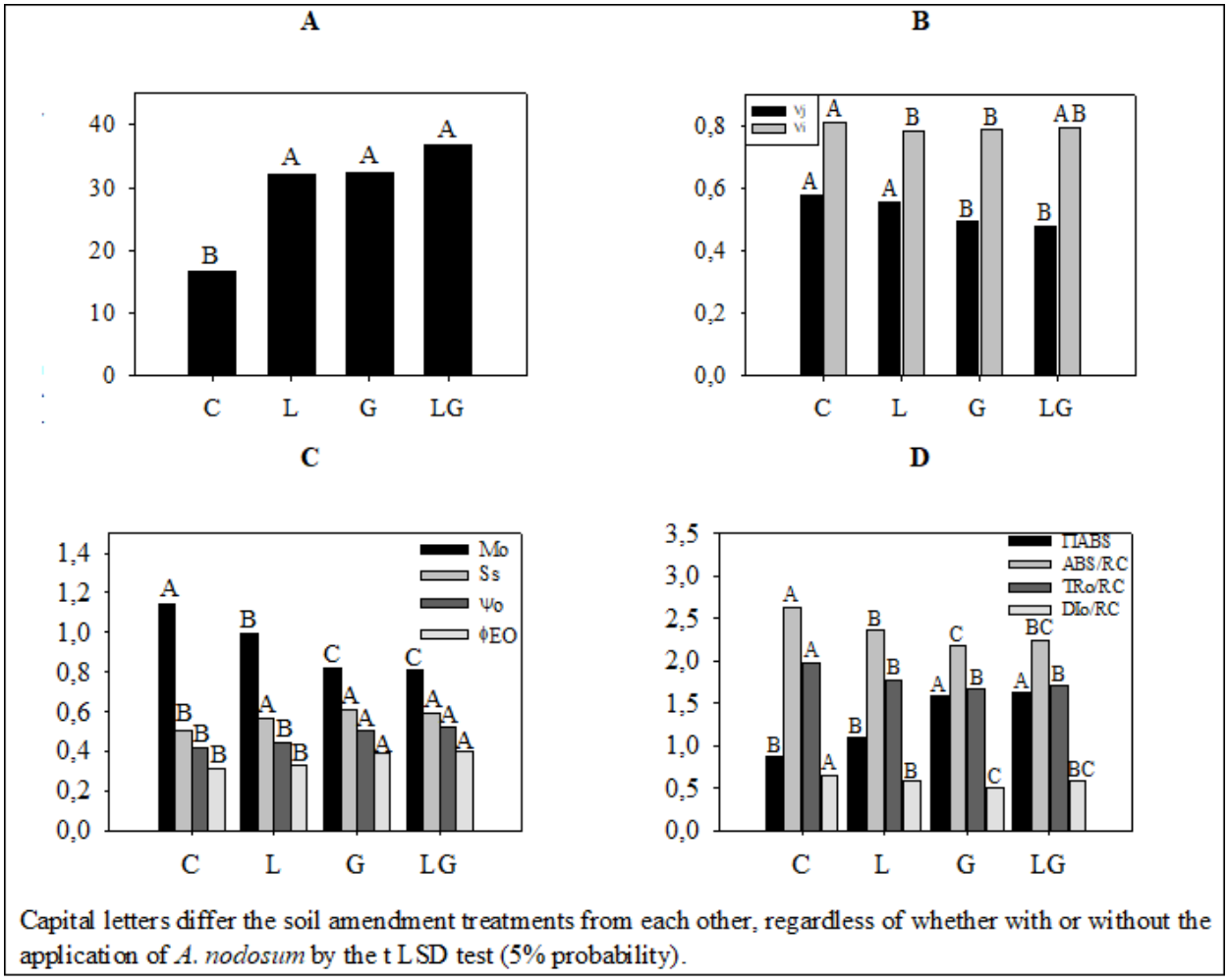


Figure 5

(A) Relative chlorophyll content; (B) Variable fluorescence indices (Vi and Vj); (C) Closure rate of the PSII reaction centers (Mo), energy needed for the simple turn over of Qa (Ss), photochemical efficiency (Ψo), probability of the energy of an absorbed photon moving an electron after Qa (Eo); (D) Performance index (πABS), flux of absorption (ABS/RC), capture (TRo/RC) and electron transport (Dio/RC) in maize plants at 14 days after germination (V2 stage) under ammendment tratments, and its interactions with *Ascophyllum nodosum* (An) extract application. Nottingham, 2019.

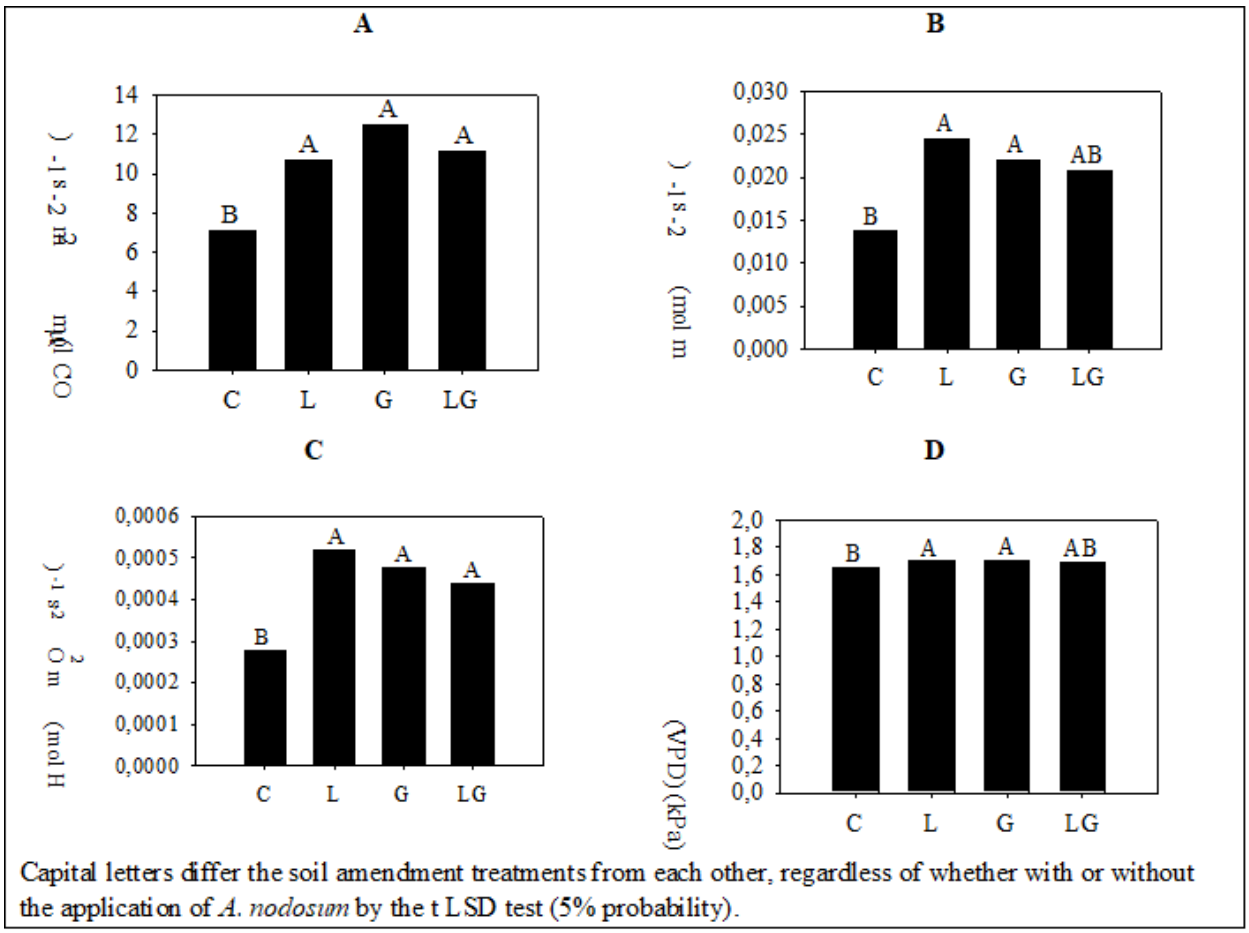
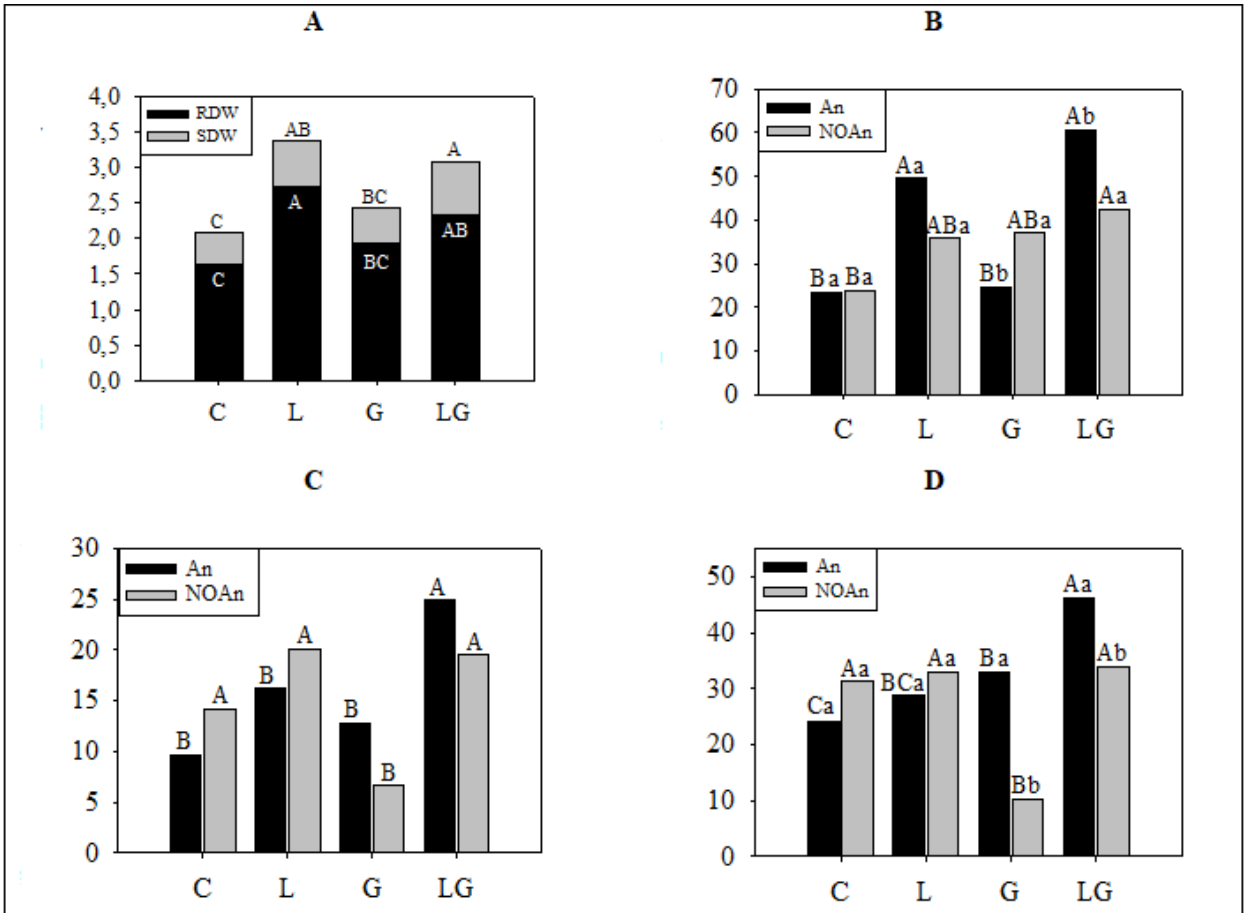


Figure 6

(A) Photossyntetic rate; (B) Stomatal conductance (gS), (C) transpiration (E) and (D) vapor pressure deficit (VPD in maize plants at 14 days after germination (V2 stage) under ammendment tratments, and its interactions with *Ascophyllum nodosum* (An) extract application. Nottingham, 2019.



Capital letters differ the soil amendment treatments from each other, regardless of whether with or without the application of *A. nodosum*, and lowercase letters differ the treatments with *A. nodosum*, regardless of corrective treatments, by the t LSD test (5% probability).

Figure 7

Initial maize growth: (A) shoot (SDW) and root dry weight (RDW) (g plant^{-1}) and height (cm); (B) Root surface (cm²); (C) N content in shoot (mg plant^{-1}) and (D) N transport to shoot (%) varying according to soil amendment treatments and *A. nodosum* application. Rio Verde, 2019.

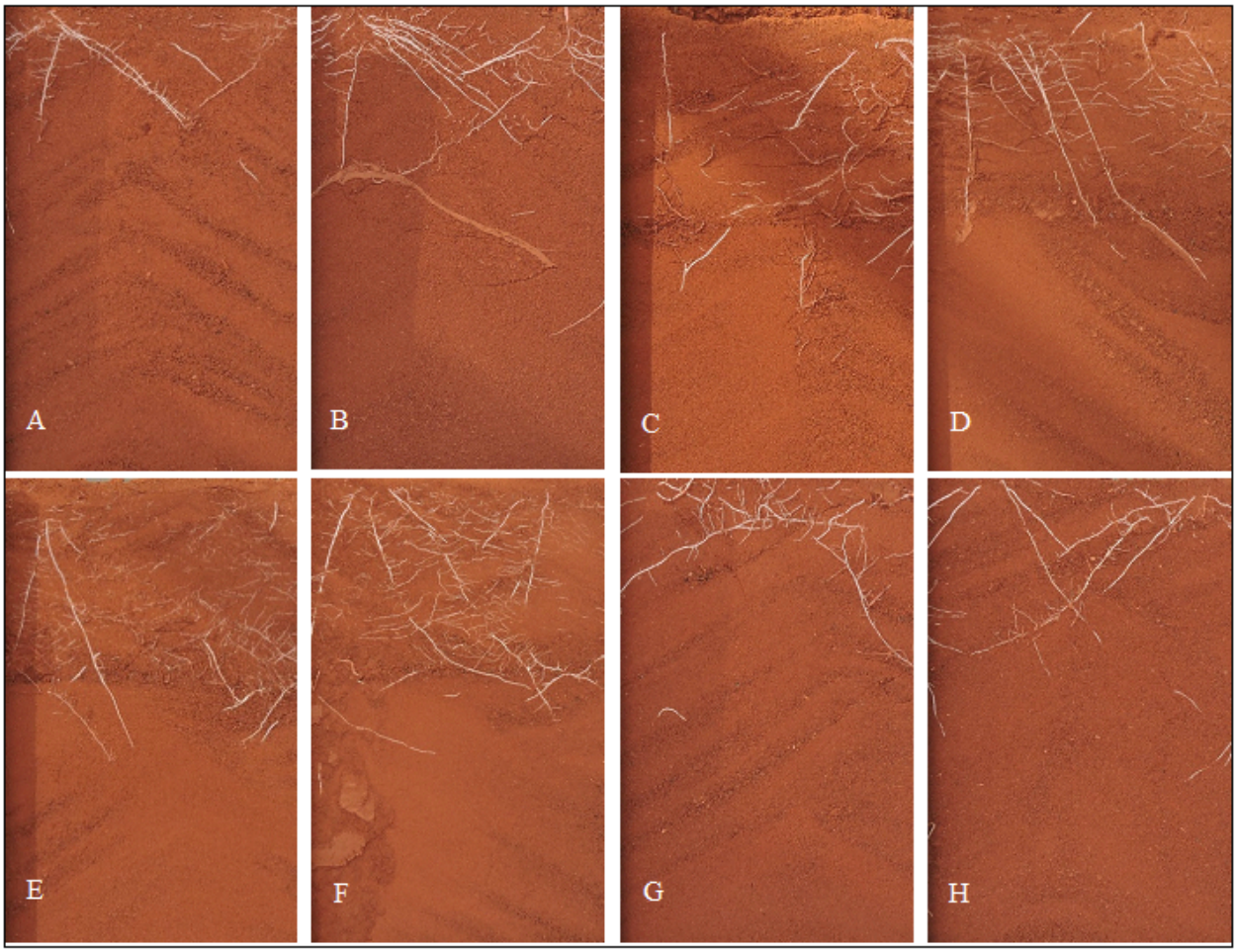


Figure 8

Root images of maize plants at 15 days after germination (V2 stage) in a 60cm depth rhizotron, (A) Gypsum; (B) Gypsum and *A. nodosum*; (C) Liming; (D) Liming and *A. nodosum*; (E) Liming and Gypsum; (F) Liming and Gypsum and *A. nodosum*; (G) Control; (H) *A. nodosum*. Rio Verde, 2019.

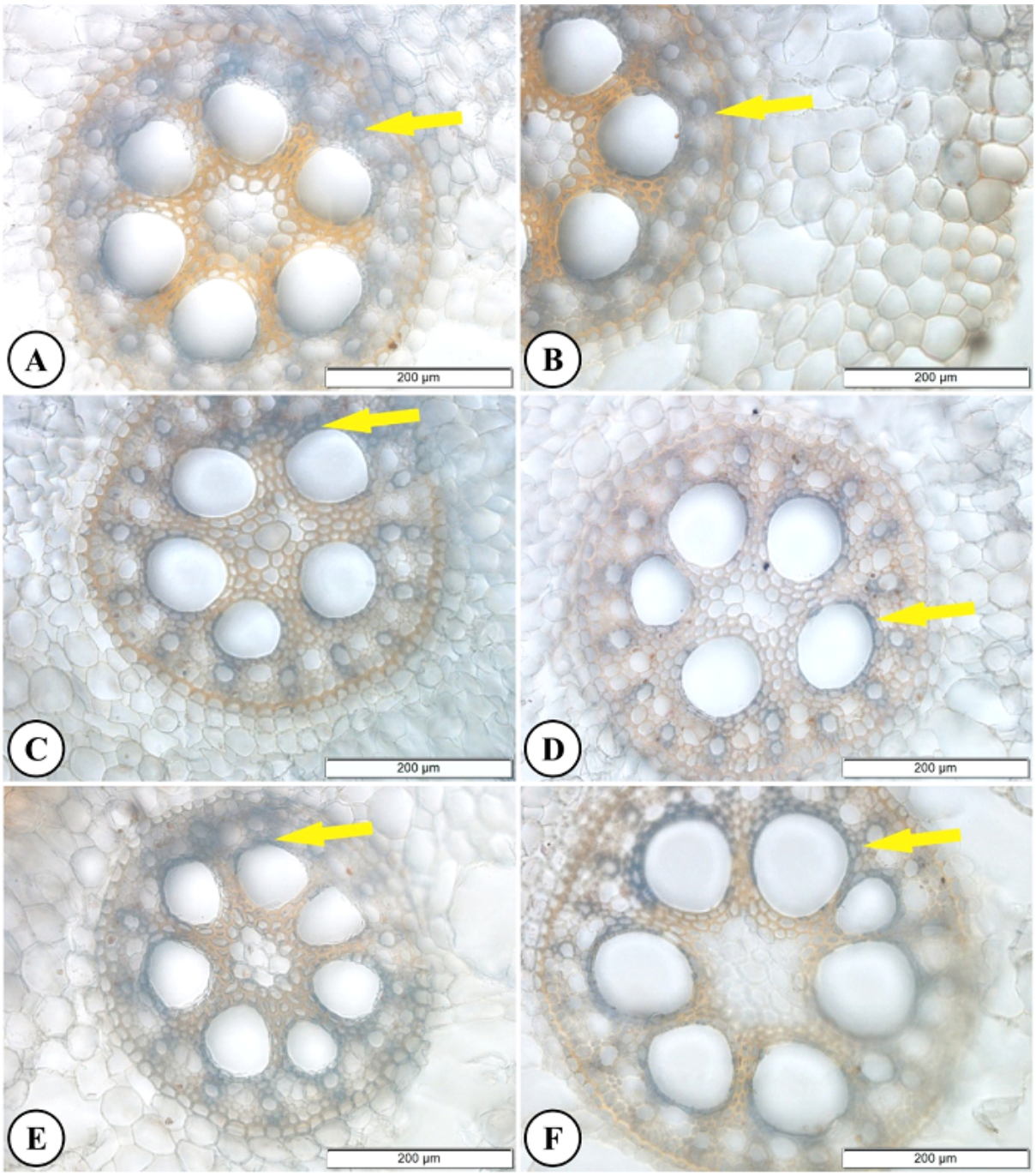


Figure 9

Root anatomical characteristics of maize, dyed with chrome azurol 15 days after germination (V2 stage) where: (A-B) Control; (C-D) *Ascophyllum nodosum*; (E) Liming and *A. nodosum*; (F) Liming. Bar 200 µm. Yellow arrows point to Al accumulation, all images are scaled to the same size. Rio Verde, 2019.

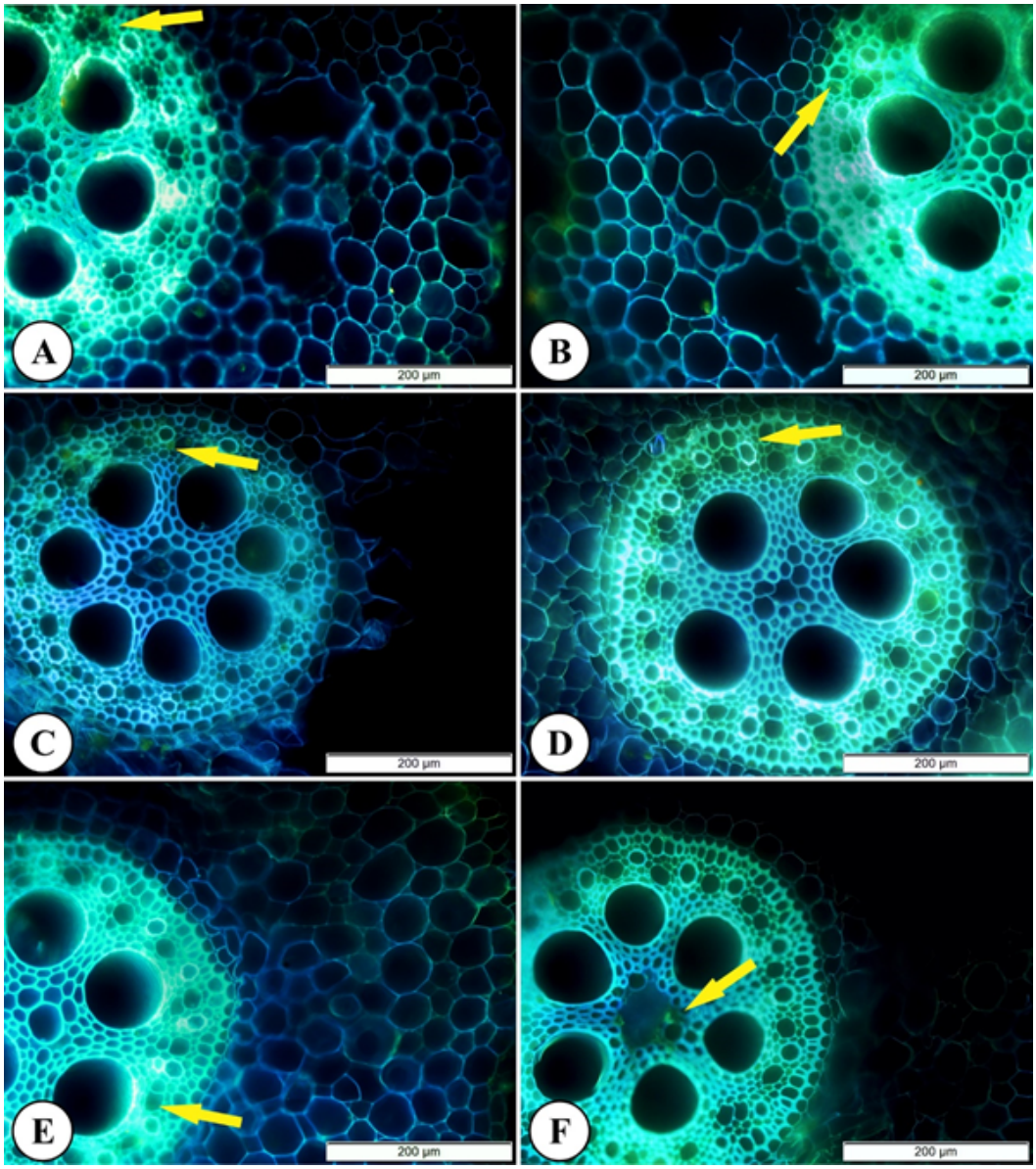


Figure 10

Fluorescence image of maize root anatomical characteristics, treated with morin fluorochrome15 days after germination (V2 stage) where: (A-B) Control; (C-D) *Ascophyllum nodosum*; (E) Liming and *A. nodosum*; (F) Liming. Bar 200 µm. Yellow arrows point to Al accumulation, all images are scaled to the same size. Rio Verde, 2019.