





Article

# Effect of *Casuarina* Plantations Inoculated with Arbuscular Mycorrhizal Fungi and *Frankia* on the Diversity of Herbaceous Vegetation in Saline Environments in Senegal

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**Abstract:** Land salinization is a major constraint for the practice of agriculture in the world. Considering the extent of this phenomenon, the rehabilitation of ecosystems degraded by salinization has become a priority to guarantee food security in semi-arid environments. The mechanical and chemical approaches for rehabilitating salt-affected soils being expensive, an alternative approach is to develop and utilize biological systems utilizing salt-tolerant plant species. *Casuarina* species are naturally halotolerant, but this tolerance has been shown to be improved when they are inoculated with arbuscular mycorrhizal fungi (AMF) and/or nitrogen-fixing bacteria (*Frankia*). Furthermore, *Casuarina* plantations have been proposed to promote the development of plant diversity. Thus, the aim of the current study was to evaluate the impact of a plantation comprising the species *Casuarina* inoculated with AMF and *Frankia* on the diversity of the sub-canopy and adjacent vegetation. Work was conducted on a plantation comprising *Casuarina equisetifolia* and *C. glauca* variously inoculated with *Frankia* and *Rhizophagus fasciculatus* prior to field planting. The experimental area of 2500 m<sup>2</sup> was divided into randomized blocks and vegetation sampling was conducted below and outside of the *Casuarina* canopy in 32 m<sup>2</sup> plots. A total of 48 samples were taken annually over 3 years, with 24 taken from below the *Casuarina* canopy and 24 from outside the canopy. The results obtained show that co-inoculation with *Frankia* and *Rhizophagus fasciculatus* improves the height and survival rate of both species. After 4–5 years, there was greater species diversity and plant biomass in the sub-canopy

environment compared with that of the adjacent environments. Our results suggest that inoculation of beneficial microbes can improve growth of *Casuarina* species and that planting of such species can improve the diversity of herbaceous vegetation in saline environments.

**Keywords:** *Casuarina*; salinization; diversity; rehabilitation; herbaceous vegetation

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## 1. Introduction

Land salinization is characterized by high plant mortality, loss of biodiversity, and loss of soil fertility [1]. Acidic and hyper-saline halomorphic soils occupy more than 85% of the land in Palmarin (Fatick, Senegal) [2].

In recent years, an increase in salinity combined with the impacts of climate change has led to the decrease of agricultural yields. In Senegal, the area of Fatick is one of the regions most affected by salinization, and in the village of Palmarin, the agricultural production systems are threatened by the salinization of the land. Rice cultivation, which was practiced in Palmarin in the 1960s, has been abandoned because of the lack of rainfall and the increasing salinity of the land.

The increasing decline in arable land needed to provide food security for this population is exacerbated by the increasing population in this area. Official estimates taken between 1988 [3] and 2015 [4] suggest an annual growth rate of 2.72%. The lack of potential income from this area has furthermore increased the phenomenon of rural exodus, especially among the local youth [5].

It thus becomes essential to implement low-cost, ecological strategies to rehabilitate ecosystems and improve ecosystem benefits (e.g., firewood, fodder, and timber production). The use of salt-tolerant species of the Casuarinaceae family in association with symbiotic microorganisms could be a bio-remediation strategy [6,7]. Casuarinaceae form root nodules in association with the nitrogen-fixing actinobacteria *Frankia*, which enables survival in nutrient-poor soils. Furthermore, they can develop a symbiotic association with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EM), which enhance plant water absorption and promote the uptake of nutrients, particularly, phosphorous and nitrogen [8–10]. Inoculation with salt-tolerant AMF and bacteria can improve plant salinity tolerance [11] and enhance nutrient acquisition, plant growth, and yield [12,13]. Economically, Casuarinaceae species are valued for the density of their wood and for facilitating nitrogen fertilization of soils through their symbiotic association with the soil bacteria *Frankia* [14].

What is also of importance is the regeneration of natural herbaceous vegetation in salt-affected areas, so as not to ultimately lose the natural diversity in this region. This in turn could be of reciprocal benefit. The positive effect of herbaceous vegetation on tree growth under saline conditions has been described for *Prosopis juliflora* and *Vachellia seyal* seedlings [15]. Thus, the hypothesis of our study is that salt-tolerant *Casuarina* species could have a positive effect on herbaceous diversity and reduce soil salt concentrations, thereby collectively improving growth.

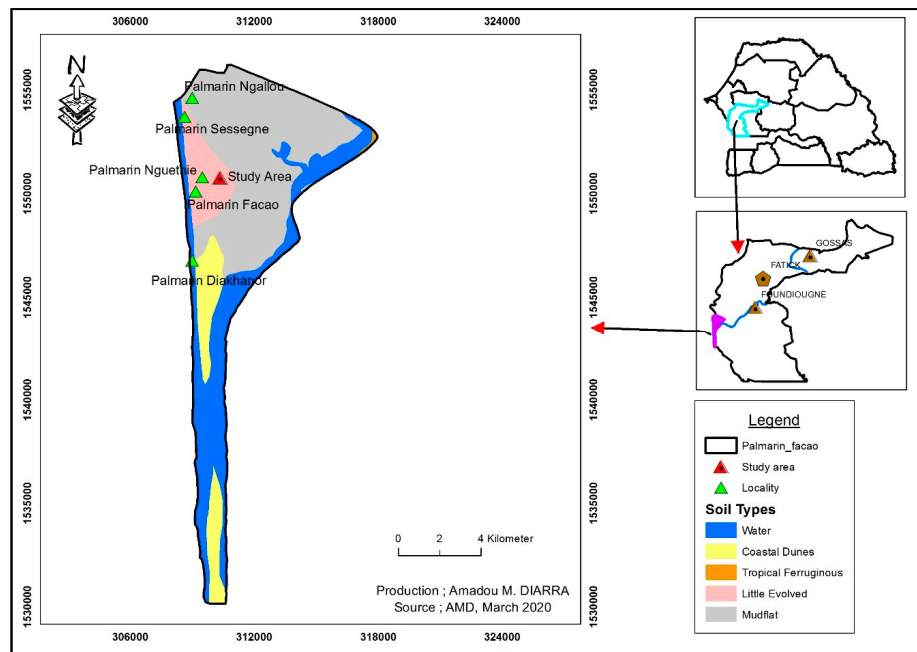
To test this hypothesis, field plantations of *C. equisetifolia* and *C. glauca*, species which have been tested under controlled conditions for their ability to tolerate salt stress [7], were established in 2013. Subsequent to this, we studied (i) the effect of inoculation (*Rhizophagus fasciculatus*/*Frankia* strain CeD) on the growth of *C. equisetifolia* and *C. glauca* under saline environments and (ii) the impact of planting on herbaceous vegetation diversity after four years of planting in saline conditions.

Our data showed a beneficial effect of *Casuarina* on the diversity of herbaceous vegetation and that inoculation has a positive effect on wood production. We thus propose that the establishment of the vegetation layer through these programs could improve biogeochemical cycles, carbon fluxes that significantly influence the soil microbial community [16,17].

## 2. Materials and Methods

### 2.1. Field Study Site

The experimental plantation where the work was conducted is located in the Fatick region (Senegal) in the municipality of Palmarin, 14°01'14 N and 16°45'23 W (Figure 1). The plantation has a surface area of 2500 m<sup>2</sup> and the soil has a sandy loamy texture (analysis performed by the INP “National Institute of Pedology”). The annual rainfall is between 400 mm and 900 mm isohyets (Figure A1) and the study site is subject to periodic waterlogging during the rainy season. Soil salinity levels vary from 40 to 500 mM in the dry season. Due to its geographical location, which gives it the character of a peninsula, the temperature in the municipality of Palmarin varies between 16 °C in January and 38 °C in June.



**Figure 1.** Map of the study site in Palmarin (Fatick, Senegal).

### 2.2. Experimentation in the Field

The *Casuarina* plantation was established in 2013 and is composed of *Casuarina glauca* and *Casuarina equisetifolia* which were planted according the experimental designed shown in Figure 2. Prior to field establishment, plants were inoculated with an AMF (*Rhizophagus fasciculatus* (Thaxt.) C. Walker & A. Schüßler strain DAOM227130 isolated in Quebec) according to Djighaly et al. [7] and *Frankia* (CeD) strain isolated by Diem et al. [18] from *C. equisetifolia* as described in Ngom et al. [19]. The AMF (*R. fasciculatus*) and *Frankia* strain CeD were selected on the basis of previous studies showing their ability to enhance the growth of *C. equisetifolia* and *C. glauca* under salt conditions [7,20]. The bacterial inoculum for growth of *Frankia* was prepared by culture under propionate medium (BAP) [16] for 4 days to obtain a phase of exponential bacterial growth. The plants were inoculated with 5 mL of this liquid culture (absorbance = 0.02) and the AMF inoculum containing 32 spores/g and 80% mycorrhized roots. Plants were inoculated 21 days after seeding with the following combinations: (1) plants inoculated with with *R. fasciculatus* (Rf) only, (2) plants inoculated with *Frankia* (CeD) only, and (3) plants co-inoculated with Rf and *Frankia*. Non-inoculated plants were used as controls. After 4 months in the nursery, the seedlings were transferred to the field. The experimental design included 4 blocks (each row of 8 2 × 10 subplots in Figure 2), each treatment was replicated 4 times (1 replicate per block), and in each replicate 10 plants were planted. A spacing of 2 m × 2 m between the plants was left (Figure 2). A total of 320 plants were transferred to the plot.

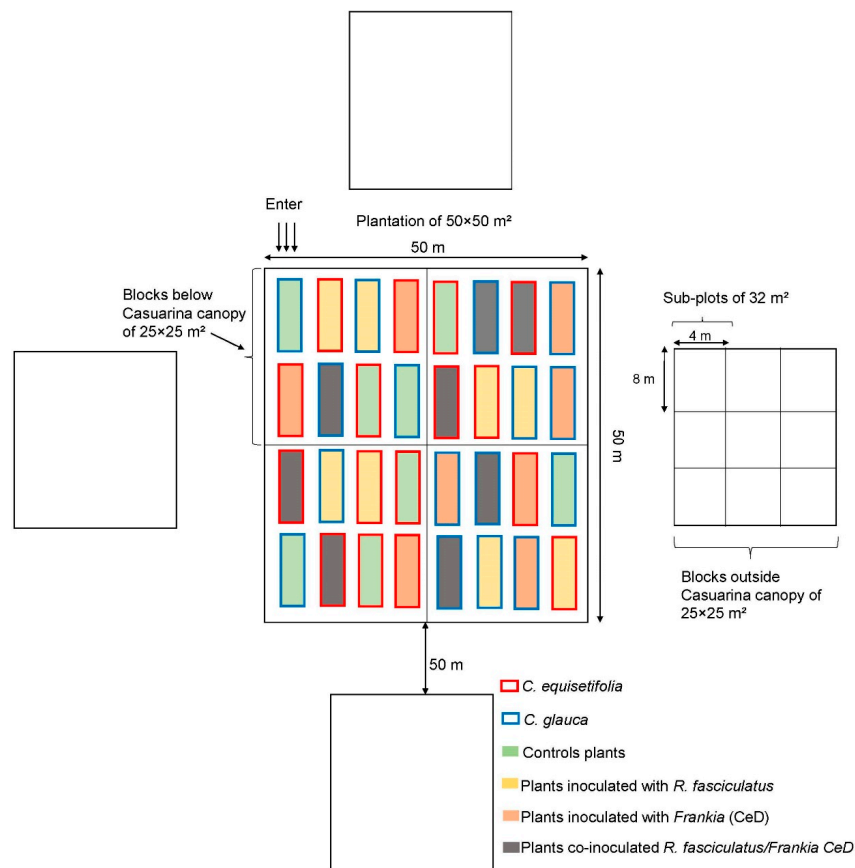


Figure 2. Experimentation design.

### 2.3. Physicochemical Characterization of the Site

The physical and chemical characteristics of the soil were determined at the sites before planting. Four soil samples were taken at depths of between 0–20 cm from the east, west, north, and south directions. At least 3 samples were pooled per replicate from each sampling point. Soil chemical parameters such as pH, salinity, N, C, and P were analyzed at the LAMA (“Laboratoire des Moyens Analytiques”), certified ISO 9001 version 2000.

The height and collar diameter of the plants were measured using a tape measure. This was performed after 6 months, 2 years, and 3 years to assess plant growth rates. Plant survival rate was determined by counting using the formula:

$$\text{Survival rate (\%)} = (\text{number of surviving plants}) / (\text{total number of plants}) \times 100 \quad (1)$$

### 2.4. Diversity of Herbaceous Vegetation

An inventory of plants growing in the 2500 m<sup>2</sup> plantation was undertaken 3 years after planting to limit the disturbances linked to the transplanting of the *Casuarina* trees, but also to allow the planted *Casuarina* to reach a size big enough to have an impact on the fertility of the soil and the diversity of the environment. A comparison was made between the plots below the *Casuarina* canopy and outside the *Casuarina* canopy. To do this, the plantation was divided into four blocks of 25 × 25 m<sup>2</sup> (Figure 2). In each block, 32 m<sup>2</sup> sub-plots were delimited, which is the minimum area with saline land at Palmarin. The surveys were carried out using the Braun–Blanquet [20] phytosociological method. Each species inventoried was given an abundance-dominance coefficient (+ to 5), which was transformed into percentages using the method of Gillet [21].

A total of 48 vegetation surveys were carried out, including 24 below the *Casuarina* canopy, randomly distributed in each block (25 × 25 m<sup>2</sup>) to take into account the heterogeneity of the

environment. A comparison was made with 24 surveys outside the *Casuarina* canopy distributed into four blocks in eastern, western, northern, and southern directions. Botanical samples were identified in the field or in the laboratory using the flora of Senegal [22]. The species names were updated on the basis of the list of flowering plants in tropical Africa from Lebrun and Stork [23,24]. The diversity of herbaceous vegetation below and outside the *Casuarina* canopy was evaluated after 3 years (2016), 4 years (2017), and 5 years (2018) of planting.

## 2.5. Diversity and Regularity Indices

### 2.5.1. Shannon–Weaver Index

The Shannon index ( $H'$ ) is used to calculate the level of species diversity in a given environment. It takes into account not only the number of species, but also the distribution of individuals within these species. It is often between 0 and 4.5, rarely higher [25]. It is calculated using the following equation:

$$H' = -\sum (n_i/N) \log_2 (n_i/N) \quad (2)$$

where  $n_i$  is the abundance of species  $i$ ;  $N$  is the total abundance.

### 2.5.2. Evenness Measure

Evenness expresses the distribution of species within the association. It varies between 0 and 1, is without units, and corresponds to the ratio of Shannon index ( $H'$ ) and maximum value of Shannon index ( $H' \text{ max}$ ):

Evenness is represented as follows:

$$E = (H')/(H' \text{ max}) \quad (3)$$

where  $H' \text{ max} = \log_2 (n_i/N)$ .

### 2.5.3. Jaccard's Similarity Index

The Jaccard similarity index allows a comparison between two sites, as it assesses the similarity between two surveys by relating the species common to both surveys to those specific to each survey. Jaccard's similarity index is represented as follows:

$$J = a/(a + b + c) \quad (4)$$

where  $a$  is the number of species present in both samples (joint occurrences),  $b$  is the number of species present in sample one, and  $c$  is the number of species present in sample two.

### 2.5.4. Specific Presence Contribution (S.P.C.)

It is the ratio expressed as a percentage between the centesimal frequency of this species and the sum of the centesimal frequencies of all species; it reflects the participation of the species in covering the soil surface [26].

$$Csi (\%) = (Fsi/\Sigma Fsi) \times 100 \quad (5)$$

where  $Fsi$  is the specific frequency of species  $i$ ;  $\Sigma Fsi$  is the sum of the frequency of all species; and  $Csi$  is the specific contribution of species  $i$ .

## 2.6. Biomass

The above-ground biomass of the sub-canopy and adjacent herbaceous vegetation was estimated within the 1 m<sup>2</sup> blocks by the integral harvesting method [27]. These plots were randomly distributed

within the 32 m<sup>2</sup> plots. A total of 48 plots of 1 m<sup>2</sup> were used to evaluate the plant mass, 24 of which were below the *Casuarina* canopy and 24 others outside the *Casuarina* canopy.

The harvesting method consisted of harvesting the entire plant and subsequent separation into tissue types. The fresh mass of tissues was determined in the field using a spring scale. The dry mass was obtained after drying in an oven at 70 °C until a constant weight was obtained. Production is expressed in tonnes of dry biomass per hectare (t DB/ha).

### Specific Contribution Biomass (Csib)

This measure reflects the participation of a species in above-ground plant biomass and is calculated as

$$Csib (\%) = (Wsib/\Sigma Wsib) \times 100$$

where *Wsib* is the weight of dry biomass of the species *ib*;  $\Sigma Wsib$  is the total weight of dry biomass of all species; and *Csib* is the specific contribution of biomass *ib* [26].

### 2.7. Statistical Analysis

Data collected were processed using R software version 3.4.2. Normality of all data sets was assessed using the Shapiro–Wilk test and the equality of variances by the Levene test. For data following normal laws, a two-way ANOVA was performed to evaluate the effects of inoculation and species on survival rate, height, and collar diameter. Chemical parameters, coverage, specific richness, Shannon index, and dry biomass were also analyzed with Bonferonni test with a significance threshold set at 0.05. We analyzed species composition below and outside *Casuarina* canopy as a function of years using non-metric multidimensional scaling (NMDS).

## 3. Results

### 3.1. Physicochemical Characteristics of Soil

Four composite soil samples were taken at horizons of 0–20 cm before planting and 3 years after planting. The 2013 planting site had salt concentrations of approximately 0.53‰ before planting. This concentration decreased significantly 3 years after planting to 0.28‰ at the end of the dry season. The soil had an average carbon content of 2.15 g/kg, nitrogen content of 155.75 mg/kg, and phosphorus content of 42.25 before planting. This content increased to 3.3 g/kg, 275 mg/kg, and 170 mg/kg respectively for carbon, nitrogen, and phosphorus 3 years after planting (Table 1). The pH of the site was acidic; approximately 5.3 and 5.95 before planting and 3 years after planting, respectively (Table 1).

**Table 1.** Chemical characteristics of soil before planting in 2013 and 3 years after planting. Lower-case letters (a–b) indicate significant differences between before planting and 3 years after planting.

Analysis	pH	Salinity ‰	Nitrogen Kjeldahl mg/kg	Carbon Organic g/kg	Phosphorus Total mg/kg
Before planting	5.3 a	0.45 a	155.75 b	2.15 b	42.25 b
3 years after planting (below <i>Casuarina</i> canopy)	5.95 a	0.28 b	275 a	3.3 a	170 a

### 3.2. Effect of Inoculation with AMF/*Frankia* on the Growth of *C. equisetifolia* and *C. glauca* Planted in the More Saline Area in Palmarin

The effects of inoculation of these species in terms of survival and growth rates are given in Table 2. After 6 months of planting, in *C. equisetifolia*, all plants that were co-inoculated had survived, with those inoculated by *Frankia* or Rf only showing 80% and 77% survival, respectively. In *C. glauca*, higher survival rates occurred in plants inoculated with *Frankia* (93.34%) compared to those inoculated with Rf only (90%) or co-inoculated (87%). Two years after planting, a decrease in the survival rate was

observed in both species. The highest survival rate of 67.5% was obtained in co-inoculated and *Frankia* only inoculated *C. glauca*. Compared to control plants, co-inoculation improved survival rates by 20% and 12.5% in *C. equisetifolia* and *C. glauca*, respectively (Table 2). In *C. equisetifolia*, the lowest survival rates were observed in the control plants, with 30% of the plants surviving. Of the 320 plants initially transplanted, 53% survived after 3 years (Table 2). Inoculation did not improve survival in *C. glauca* 3 years after planting. However, it did improve the survival rate of *C. equisetifolia*.

**Table 2.** Effect of inoculation with *R. fasciculatus* and *Frankia* on the survival rate and height of *C. equisetifolia* and *C. glauca* plants under saline stress conditions 6 months, 2 years, and 3 years after planting. Control, Rf: *R. fasciculatus*, Frankia: *Frankia* strain CeD, and Rf + Frankia: AMF/*Frankia* co-inoculation. Lower-case letters (a–e) indicate significant differences between control and inoculated plants for each time (6 months, 2 years, and 3 years).

	Species	Treatments	Survival Rate (%)	Height (cm)	Collar Diameter (cm)
6 months after planting	<i>C. equisetifolia</i>	Control	85 b	65.14 de	5.45 a
		<i>Frankia</i>	80 b	68.94 cde	6.13 a
		Rf	76.67 b	60.51 e	6.05 a
		Rf + <i>Frankia</i>	100 a	73.67 bcd	5.15 a
	<i>C. glauca</i>	Control	73.4 b	78.51 abc	5.75 a
		<i>Frankia</i>	93.34 a	79.10 abc	6.02 a
		Rf	90 a	81.98 ab	5.51 a
		Rf + <i>Frankia</i>	86.67 ab	88.00 a	6.21 a
		Species	.	***	ns
		Inoculation	**	**	ns
		Species*Inoculation	ns	ns	ns
	2 years after	<i>C. equisetifolia</i>	Control	30 c	107.81 c
<i>Frankia</i>			55 b	111.77 c	13.87 a
Rf			47.5 b	168.13 ab	13.45 a
Rf + <i>Frankia</i>			50 b	172.26 ab	13.49 a
<i>C. glauca</i>		Control	55 b	144.40 b	11.30 a
		<i>Frankia</i>	67.5 a	141.29 b	12.31 a
		Rf	45 b	181.05 a	15.00 a
		Rf + <i>Frankia</i>	67.5 a	166.98 ab	12.30 a
		Species	*	*	ns
		Inoculation	**	***	ns
		Species*Inoculation	.	.	ns
3 years after		<i>C. equisetifolia</i>	Control	30 b	125.63 c
	<i>Frankia</i>		55 a	127.45 c	14.07 a
	Rf		47.5 a	180.13 ab	14.86 a
	Rf + <i>Frankia</i>		50 a	186.88 ab	14.63 a
	<i>C. glauca</i>	Control	55 a	152.77 bc	12.97 a
		<i>Frankia</i>	57 a	163.29 abc	15.33 a
		Rf	45 a	196.50 a	16.52 a
		Rf + <i>Frankia</i>	57.5 a	192.38 a	15.40 a
		Species	.	*	ns
		Inoculation	*	***	ns
		Species*Inoculation	ns	.	ns

ns, no significant difference; \*, \*\* and \*\*\* indicate significant difference at  $p < 0.1$ , 0.05, 0.01, and 0.001, respectively.

In terms of the effect of inoculations on plant growth, there was no significant increase in the height of the *C. equisetifolia* plants compared to control plants 6 months after planting. However, significantly greater heights were observed in *C. glauca* that were co-inoculated compared to the control plants. After 3 years, plants of both species that were inoculated with Rf and co-inoculated had greater heights than control plants. However, inoculation did not significantly increase collar diameter compared to control plants during 3 years of planting.

### 3.3. Effect of the Casuarina Plantation on Species Diversity and Specific Contribution below and outside Their Canopy

The herbaceous vegetation of the site comprises 37 species from 28 genera and 14 families (Table 3). Below the *Casuarina* canopy, 24, 29, and 33 species were inventoried in 2016, 2017, and 2018, respectively. From outside the canopy, 21, 22, and 24 species were inventoried in 2016, 2017, and 2018, respectively. Of the 37 species inventoried, 14 were common in all areas. The species specifically inventoried below the *Casuarina* canopy in 2018 were *Ipomoea sinuata* Ort., *Corchorus tridens* L., and *Melochia corchorifolia* (L.).

**Table 3.** Presence/absence and specific contribution presence below the *Casuarina* canopy (BCC) and outside the *Casuarina* canopy (OCC).

Family	Genera	Species	BCC			OCC		
			2016	2017	2018	2016	2017	2018
Poaceae	<i>Dactyloctenium</i>	<i>Dactyloctenium aegyptium</i> (L.) Willd	0.64	2.96	2.12	6.03	3.40	3.87
	<i>Schizachyrium</i>	<i>Schizachyrium compressa</i> (K. Schum.)	3.21	2.59	5.30	-	-	-
		<i>Schizachyrium rupestre</i> (Stapf.) Stapf	-	-	-	0.86	2.04	1.66
	<i>Chloris</i>	<i>Chloris prierurii</i> Kunth	3.85	3.33	3.53	3.45	3.40	5.52
		<i>Chloris barbata</i> (L.) Sw.	-	2.96	2.12	-	-	-
	<i>Pennisetum</i>	<i>Pennisetum polystachion</i> (L.) Schul.	-	3.70	3.53	2.59	4.76	4.97
	<i>Eragrostis</i>	<i>Eragrostis tenella</i> (L.) Beauv.	5.13	2.96	1.41	-	-	-
		<i>Eragrostis tremula</i> Hochst. Ex Steud.	3.85	4.07	2.12	5.17	4.76	4.97
		<i>Eragrostis aspera</i> (Jacq.) Nees.	3.21	3.70	1.77	-	-	-
	<i>Paspalum</i>	<i>Paspalum vaginatum</i> (L.)	4.49	3.33	3.53	-	-	-
	<i>Aristida</i>	<i>Aristida funiculata</i> Trin. & Rupr.	-	2.96	3.53	5.17	5.44	1.10
		<i>Aristida mutabilis</i> Trin. & Rupr.	-	1.11	2.47	-	-	-
	<i>Digitaria</i>	<i>Digitaria horizontalis</i> Willd.	3.85	2.96	2.47	1.72	6.12	6.08
	<i>Brachiaria</i>	<i>Brachiaria lata</i> C.E. Hubb.	-	0.37	0.71	-	-	-
<i>Sporobolus</i>	<i>Sporobolus robustus</i> Kunth	5.77	8.15	7.07	3.45	6.80	3.87	
Malvaceae	<i>Hibiscus</i>	<i>Hibiscus rostellatus</i> (Guill. & Perr.)	0.64	1.48	1.41	-	-	-
		<i>Hibiscus asper</i> Hook.	3.21	3.33	7.42	3.45	2.72	-
	<i>Corchorus</i>	<i>Corchorus tridens</i> L.	-	-	0.71	-	-	-
	<i>Sida</i>	<i>Sida alba</i> L.	4.49	-	-	3.45	5.44	3.87
Cyperaceae	<i>Fuirena</i>	<i>Fuirena ciliaris</i> (L.) Roxb.	2.56	-	-	0.86	0.68	5.52
	<i>Cyperus</i>	<i>Cyperus esculentus</i> L.	1.92	4.07	2.47	-	4.76	3.31
		<i>Cyperus bulbosus</i> Vahl.	3.21	2.22	1.06	5.17	4.08	2.76
Amaranthaceae	<i>Amaranthus</i>	<i>Amaranthus gracizans</i> L.	0.64	3.70	3.89	6.03	2.04	2.21
	<i>Phloxeris</i>	<i>Phloxeris vermicularis</i> (L.) Sm.	8.33	3.33	8.13	17.24	16.33	12.71
Convolvulaceae	<i>Ipomoea</i>	<i>Ipomoea coptica</i> Willd.	1.28	0.74	1.77	5.17	2.04	3.87
		<i>Ipomoea sinuata</i> Ort.	-	-	3.18	-	-	-



Table 3. Cont.

Family	Genera	Species	BCC			OCC		
Fabaceae	Indigofera	<i>Indigofera berhautiana</i> JB. Gillett.	-	-	1.06	-	-	1.66
		<i>Indigofera linifolia</i> (Lf) Retz.	-	-	1.06	-	-	2.21
	Alysicarpus	<i>Alysicarpus ovalifolius</i> Schum.& Thonn.	1.92	3.33	1.77	-	-	2.21
Amaryllidaceae	<i>Pancreatium</i>	<i>Pancreatium trianthum</i> Herb.	-	1.48	3.18	-	-	-
Nyctaginaceae	<i>Boerhavia</i>	<i>Boerhavia repens</i> L.	3.21	4.07	3.53	3.45	3.40	3.87
Plantaginaceae	<i>Scoparia</i>	<i>Scoparia dulcis</i> L.	-	3.70	-	5.17	0.68	1.10
Asteraceae	<i>Sphaeranthus</i>	<i>Sphaeranthus senegalensis</i> DC.	7.05	4.44	1.77	2.59	5.44	3.87
Aizoaceae	<i>Sesuvium</i>	<i>Sesuvium portulacastrum</i> (L.) L.	14.10	8.89	3.53	7.76	9.52	7.18
Acanthaceae	<i>Hygrophila</i>	<i>Hygrophila senegalensis</i> (Nees) T.A	8.33	7.78	7.77	5.17	5.44	6.63
Rubiaceae	<i>Spermacoce</i>	<i>Spermacoce verticillata</i> L.	5.13	2.22	3.18	6.03	0.68	4.97
Sterculiaceae	<i>Melochia</i>	<i>Melochia corchorifolia</i> L.	-	-	0.71	-	-	-

The specific presence contribution reflects the participation of the species in the spatial occupation of the plantation. In 2016, the larger contribution towards spatial occupation below the *Casuarina* canopy was provided by the species *Sesuvium portulacastrum* (L.) L. (14.10%), *Philoxerus vermicularis* (L.) Sm. (8.33%), *Hygrophila senegalensis* (Nees) T. Anderson (8.33%), and *Sphaeranthus senegalensis* DC. (7.05%). Outside of the *Casuarina* canopy, *Philoxerus vermicularis* (17.24%), *Sesuvium portulacastrum* (7.76%), *Spermacoce verticillata* L. (6.03%), and *Amaranthus greacizans* L. (6.03%) occupied the greatest space. In 2017, below canopy domination, the species *Sesuvium portulacastrum* (L.) L. (8.89%), *Hygrophila senegalensis* (7.78%), *Sphaeranthus senegalensis* (4.44%), *Boerhavia repens* L. (4.07%), and *Cyperus esculentus* L. (4.07%) were more significant, whereas outside the *Casuarina* canopy, *Philoxerus vermicularis* (16.33%), *Sesuvium portulacastrum* (9.52%), *Melochia corchorifolia* L. (5.44%), and *Sphaeranthus senegalensis* (5.44%) were more dominant. Similarly, in 2018, greater spatial occupation below the canopy was found in the species *Philoxerus vermicularis* (8.13%), *Hygrophila senegalensis* (7.77%), *Corchorus tridens* L. (7.42%), and *Sporobolus robustus* Kunth. (7.07%), whereas outside the canopy *Philoxerus vermicularis* (12.71%), *Sesuvium portulacastrum* (7.18%), *Hygrophila senegalensis* (6.63%), and *Digitaria horizontalis* Willd. (6.08%) were more prevalent (Table 4).

**Table 4.** Inter-annual variation of coverage, specific richness, and diversity index below the *Casuarina* canopy (BCC) and outside the *Casuarina* canopy (OCC). Lower-case letters (a–c) indicate significant differences according to the two-way ANOVA between conditions and years.

Years	BCC			OCC		
	2016	2017	2018	2016	2017	2018
Coverage (%)	48.28 b	67.48 a	71.95 a	37.57 b	43.26 b	45.03 b
Specific richness	24 b	29 ab	33 a	21 b	22 b	24 b
Shannon index	1.9 b	2.26 a	2.39 a	1.67 c	1.76 c	2.05 b
Evenness	0.41	0.46	0.47	0.38	0.39	0.44

### 3.4. Coverage, Specific Richness, and Diversity Index below and outside the *Casuarina* Canopy

The coverage of the herbaceous vegetation below the *Casuarina* canopy was 48.28% in 2016, 67.48% in 2017, and 71.95% in 2018. Outside the canopy vegetation coverage was of 37.57% in 2016, 43.26% in 2017, and 45.03% in 2018.

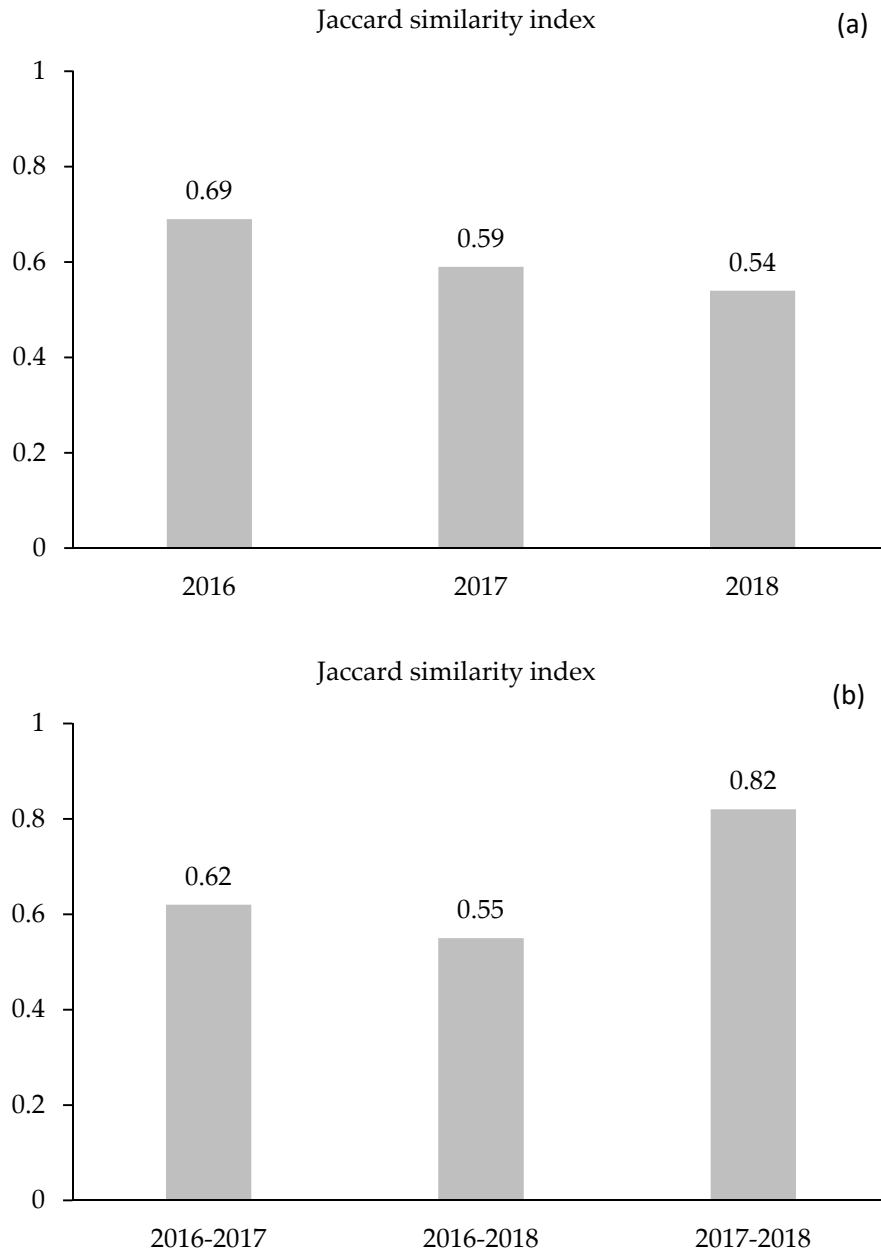
Below the *Casuarina* canopy, the specific richness was 24, 29, and 33 species in 2016, 2017, and 2018, respectively. Outside of the canopy, the specific richness was 21, 22, and 24 species in 2016, 2017, and 2018, respectively (Table 5). In 2016, the below-canopy Shannon index ( $H'$ ) and evenness

(E) were of 1.9 and 0.41, respectively. In 2017, an increase of Shannon index and evenness of 2.26 and 0.46, respectively, was observed, whereas outside of the *Casuarina* canopy, they were 1.67 and 0.38, respectively. In 2018, a further increase of Shannon index and evenness was also observed (2.39 and 0.47) with the outside canopy indices being 2.05 and 0.44, respectively.

**Table 5.** Specific contribution dry biomass (Csib) below (BCC) and outside the *Casuarina* canopy (OCC).

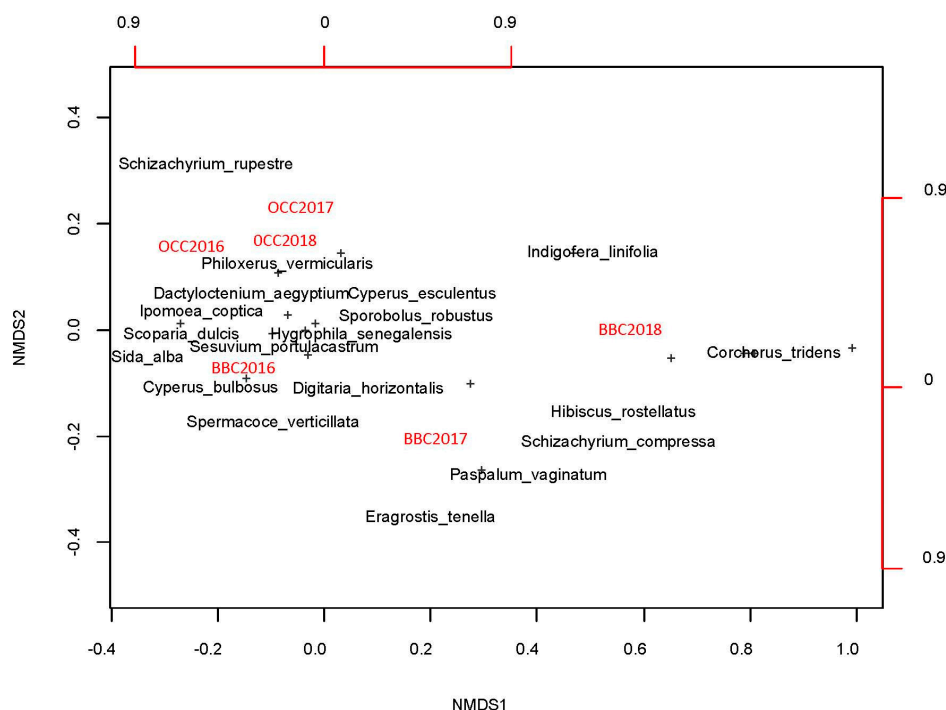
Species	BCC			OCC		
	2016	2017	2018	2016	2017	2018
<i>Dactyloctenium aegyptium</i> (L.) Willd	3.12	1.44	1.97	2.46	2.56	1.90
<i>Schizachyrium compressa</i> (K. Schum.) Stapf	1.78	1.65	3.63	-	-	-
<i>Schizachyrium rupestre</i> (Stapf) Stapf	-	-	-	1.87	1.21	0.56
<i>Chloris prieurii</i> Kunt	10.79	8.21	1.14	5.32	4.56	4.64
<i>Chloris barbata</i> (L.) Sw.	-	10.29	5.43	-	-	-
<i>Pennisetum polystachion</i> (L.) Schul.	-	2.30	5.99	3.97	4.75	3.08
<i>Eragrostis tenella</i> (L.) Beauv.	4.24	2.01	3.41	-	-	-
<i>Eragrostis tremula</i> Hochst. Ex Steud.	5.53	2.73	2.68	3.77	2.41	5.84
<i>Eragrostis aspera</i> (Jacq.) Nees.	3.48	4.44	3.30	-	-	-
<i>Paspalum vaginatum</i> (L.)	1.22	5.64	2.79	-	-	-
<i>Aristida funiculata</i> Trin. & Rupr.	-	2.19	1.83	5.24	3.05	3.27
<i>Aristida mutabilis</i> Trin. & Rupr.	-	1.22	4.04	-	-	-
<i>Digitaria horizontalis</i> Willd.	2.59	2.48	1.43	3.10	3.84	3.70
<i>Brachiaria lata</i> (Schumach.) C.E. Hubb.	-	2.80	2.38	-	-	-
<i>Sporobolus robustus</i> Kunth.	12.97	4.40	7.80	4.45	8.07	4.21
<i>Hibiscus rostellatus</i> (Guill. & Perr.)	2.28	0.61	4.65	-	-	-
<i>Hibiscus asper</i> Hook.	-	-	0.83	-	-	-
<i>Corchorus tridens</i> L.	0.58	1.13	2.88	2.82	3.17	-
<i>Sida alba</i> L.	0.89	-	-	4.17	3.35	4.96
<i>Fuirena ciliaris</i> (L.) Roxb.	0.53	-	-	3.34	3.77	3.10
<i>Cyperus esculentus</i> L.	2.01	2.16	2.39	-	2.68	3.38
<i>Cyperus bulbosus</i> Vahl.	2.23	1.72	2.65	2.30	3.09	1.85
<i>Amaranthus greacizans</i> L.	0.94	1.69	1.20	3.89	3.77	2.68
<i>Philoxerus vermicularis</i> (L.) Sm.	9.31	9.59	4.36	8.42	8.82	5.46
<i>Alysicarpus ovalifolius</i> Schum. & Thonn.	1.35	0.74	0.43	-	-	1.42
<i>Pancratium trianthum</i> Herb.	-	2.17	3.80	-	-	-
<i>Boerhavia repens</i> L.	0.91	0.43	-	1.43	2.15	0.91
<i>Scoparia dulcis</i> L.	-	1.37	0.97	3.97	4.67	3.91
<i>Sphaeranthus senegalensis</i> DC.	2.18	1.44	-	3.02	2.26	3.11
<i>Sesuvium portulacastrum</i> (L.) L.	13.35	12.22	6.57	16.44	11.99	7.61
<i>Hygrophila senegalensis</i> (Nees) T. Anderson	14.67	9.18	6.54	12.91	10.06	7.88
<i>Spermacoce verticillata</i> L.	1.24	2.23	4.01	2.66	2.00	2.20
<i>Ipomoea coptica</i> Willd.	1.80	1.51	2.35	4.45	7.76	2.98
<i>Ipomoea sinuata</i> Ort.	-	-	2.39	-	-	-
<i>Indigofera berhautiana</i> JB. Gillett.	-	-	1.44	-	-	5.01
<i>Indigofera linifolia</i> (Lf) Retz.	-	-	1.23	-	-	5.01
<i>Melochia corchorifolia</i> L.	-	-	1.10	-	-	-

The Jaccard similarity index between below and outside the *Casuarina* canopy surveys was 0.69 in 2016. A decrease of this index to 0.59 and 0.54 in 2017 and 2018, respectively, was observed (Figure 3a). Below the *Casuarina* canopy, the Jaccard similarity index varied from 0.62 between 2016 and 2017, to 0.55 between 2016 and 2018 and 0.82 between 2017 and 2018 (Figure 3b).



**Figure 3.** Jaccard similarity index (a) between below and outside the *Casuarina* canopy surveys in 2016, 2017, and 2018 (b) Jaccard similarity index below *Casuarina* canopy between the years 2016–2017, 2016–2018, and 2017–2018.

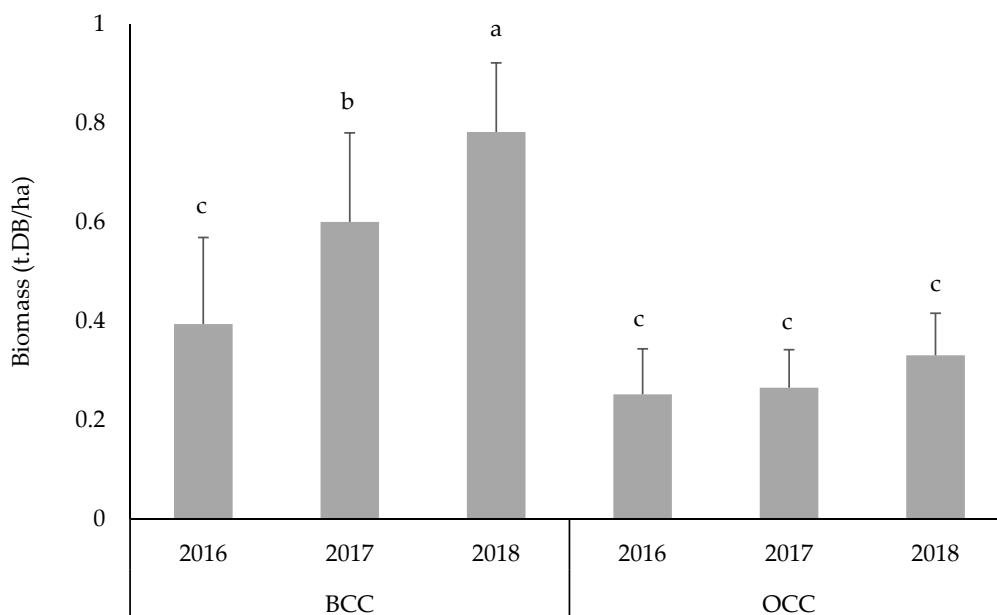
NMDS was used to explore variation among species below and outside *Casuarina* canopy as a function of the years. Floristic differences were observed between the surveys conducted on below-canopy species in 2017–2018 as well as outside *Casuarina* canopy in 2016–2017 and 2018 (Figure 4).



**Figure 4.** Non-metric multidimensional scaling (NMDS) ordination of species composition below *Casuarina* canopy (BBC) and outside *Casuarina* canopy (OCC) in 2016, 2017, and 2018.

### 3.5. Effect of *Casuarina* Species on Herbaceous Biomass in Saline Conditions

The results obtained showed a significant difference in biomass production below the *Casuarina* canopy in 2017 and 2018 ( $0.60 \pm 0.18$  t.MS/ha and  $0.78 \pm 0.14$  t.MS/ha, respectively) compared to 2016 ( $0.39 \pm 0.17$  t.MS/ha). There is no significant difference in the biomass production outside the *Casuarina* canopy between 2016, 2017, and 2018 (Figure 5). Below the *Casuarina* canopy, biomass was significantly higher compared to outside the *Casuarina* canopy biomass in 2017 and 2018.



**Figure 5.** Inter-annual variation of biomass (BCC) below the *Casuarina* canopy (OCC) and outside the *Casuarina* canopy. Lower-case letters (a–c) indicate significant differences between the years 2016, 2017, and 2018 for each condition.

### 3.6. Specific Contribution of Dry Biomass below and outside the *Casuarina* Canopy

In 2016, the predominant species in terms of dry biomass production were *Hygrophila senegalensis* (14.64%), *Sesuvium portulacastrum* (13.35%), and *Sporobolus robustus* (12.97%). Outside the *Casuarina* canopy, the largest contributions towards dry biomass came from *Sesuvium portulacastrum* (16.44%) and *Hygrophila senegalensis* (12.91%). In 2017, the greatest biomass production was provided by below-canopy species *Sesuvium portulacastrum* (12.22%), *Chloris barbata* (10.29%), and *Hygrophila senegalensis* (9.18%). Outside the *Casuarina* canopy, the species *Sesuvium portulacastrum* (11.99%) and *Hygrophila senegalensis* (10.06%) provided the greatest dry biomass. By 2018, the largest biomass producers below the *Casuarina* canopy were *Sporobolus robustus* (7.80%), *Sesuvium portulacastrum* (6.57%), *Hygrophila senegalensis* (6.54%), and *Pennisetum polystachion* (5.99%). Outside of the *Casuarina* canopy, biomass was greatest from *Sesuvium portulacastrum* (7.88), *Hygrophila senegalensis* (7.61%), and *Eragrostis tremula* (5.84%) (Table 5).

## 4. Discussion

Data provided in this study confirm that both *C. glauca* and *C. equisetifolia* can grow in saline soils. A soil is considered to be salty if it contains a minimum of 40 mM NaCl [28]. However, in our study area, the salt concentration of the soil can be 500 mM or higher during the dry season. It has been shown that *Casuarina glauca*, *Casuarina obesa*, and *Casuarina equisetifolia* will survive up to 500 mM NaCl if grown in a hydroponic medium supplemented with adequate nitrogen [19]. However, under field conditions, other factors such as drought, temperature, and the presence of other toxic ions, such as  $Mg^{2+}$ ,  $SO_4^{2-}$ ,  $HCO_3^{-}$ , and  $CO_3^{2-}$ , can have negative effects on plant growth and survival [1]. In our study, the highest survival rates in both *C. glauca* and *C. equisetifolia* occurred in plants that had been inoculated with either *Frankia* or and co-inoculated with *Frankia* and Rf. These results show the importance of inoculation with AMF (*R. fasciculatus*) and/or nitrogen-fixing bacteria (*Frankia*) in improving *Casuarina* salt tolerance, especially in reducing post-transplant stress [7,17] and wood production. We did note, however, a decline in survival rate of *C. glauca* in the third year of this study. This could be linked to competition between the inoculum with native strains [29]. However, significant plant mortality could be related to the extreme sodium levels in the dry periods and periodic waterlogging observed at certain points in the plantation.

The effects of co-inoculation (AMF/*Frankia*) on plant growth under salt stress conditions under field conditions are poorly documented. However, similar results have been obtained under greenhouse conditions by the work of Soliman et al. [30], Ashrafi et al. [31], and Ren et al. [32] on the positive effect of co-inoculation with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi on the development of *Acacia saligna*, *Medicago sativa*, and *Sesbania cannabina* plants at 250, 180, and 200 mM, respectively.

The increased height of plants co-inoculated with *Frankia* and arbuscular mycorrhizal fungi (AMF) could be explained by their facilitation in absorption of and control in levels of mineral elements. For example, selective absorption of ions, such as phosphorus, nitrogen, and magnesium and reduced absorption of  $Na^+$  ions [33]. This selective ionic uptake reduces ionic imbalances and the antagonistic effects between ions [32] that often lead to nutritional problems caused by the saline conditions exacerbated by water deficit stress.

In terms of the effect of *C. glauca* and *C. equisetifolia* on biodiversity of herbaceous vegetation in the area, this was evident only four years (2017) after planting. There were no significant differences between the two *Casuarina* species with respect to the biodiversity in this vegetation (Table A1). The Shannon diversity indexes were 1.9, 2.26, and 2.39 bits in 2016, 2017, and 2018, respectively, below the *Casuarina* canopy and 1.67, 1.76, and 2.05 bits outside the *Casuarina* canopy. This inter-annual increase of the diversity could be explained by the presence of the tree, which gradually improves soil porosity, promotes good drainage [34], maintains better activity by the microbial community, and consequently increases the diversity of herbaceous vegetation. However, there is little data on the effect of trees on the diversity of herbaceous vegetation in saline conditions. Studies by Trites and Baley [35] showed a decrease in the richness of the plant community as a function of the salinity gradient and pH.

Our opposite results could be related to the heterogeneity of the electrical conductivity in the soil due to periodic waterlogging at some points of the plantation during rainy season.

Phosphorus (P) and nitrogen (N) are considered the most important elements in plant growth, and paucity thereof has been suggested to be the greatest limitation to plant growth in most ecosystems [36]. Here, we show that the presence of *Casuarina* species results in increased phosphorus, nitrogen, and carbon contents (Table 1). These results can be explained by the decomposition of *Casuarina* litter that can improve soil fertility. The decomposition of *Casuarina* litter under semi-arid conditions results in the release of many nutrients in the following order: Ca > N > K > Mg > Na > P > Fe > Zn > Cu > Cr [37]. The work of Hata et al. [38] suggests that decomposition of *C. equisetifolia* litter can alter the total N and N cycle in invaded forest ecosystems.

A decrease in the Jaccard similarity index between the populations below and outside *Casuarina* canopy over the successive years of this study (Figure 3) suggests differences in biological diversity among the environments. The NMDS analysis also showed a difference in the floristic composition between the surveys below *Casuarina* canopy in 2017–2018 and outside *Casuarina* canopy in 2016–2017 and 2018. This difference could be related to the beneficial effects of *Casuarina* species, which improve soil fertility through nitrogen inputs [39] and create a microclimate favorable to the establishment of herbaceous vegetation. These results can also be explained by the inter-annual presence of perennial herbaceous plants capable of improving the physicochemical properties of the soil.

The beneficial effect of herbaceous plants on decreasing soil salinity has been observed by several authors [40,41]. These herbaceous plants can improve leaching and interactions between soil chemical properties supposedly restoring soil fertility. Our results confirm this proposal with significant increases in carbon, nitrogen, and phosphorus levels occurring 3 years after planting.

A better herbaceous vegetation coverage was obtained below the *Casuarina* canopy (67.48% and 71.95% in 2017 and 2018, respectively) compared to that outside the canopy (43.26% and 45.03 in 2017 and 2018, respectively). This positive effect could be due to the presence of certain herbaceous plants capable of symbiosis with nitrogen-fixing endophytes. This has been reported for *Sporobolus robustus* with *Rhizobium* bacteria [15] and *Leptochloa fusca* with *Azoarcus* bacteria [42]. In turn, these can be considered pioneering species that improve soil fertility and encourage the establishment and growth of other herbaceous species.

The species that contributed most to the coverage of the plantation were *S. portulacastrum* and *S. robustus*, while outside the *Casuarina* canopy, *S. portulacastrum* and *Phloxerus vermicularis* were dominant. Ravindran et al. [43] have shown that *S. portulacastrum* and *S. maritima* accumulate salt in their tissues with a corresponding reduction thereof in the soil. It is estimated that these two halophytes could remove 474 and 504 kg of NaCl, respectively, per ha over a 4 month period. Our results similarly showed a decrease in soil salt concentration 3 years after establishment of the plantation. This in turn suggests that at least some of these herbaceous plants are able to reduce NaCl in the environment and make conditions more favorable for growth of *Casuarina* species by inter alia improving soil water availability. Grouzis and Akpo [44] have shown that in arid environments, the presence of trees improves biomass of other plants in their vicinity. The same could be true under saline environments. We have shown an increase in herbaceous biomass in association with the presence of *Casuarina* species, which in turn could promote the incorporation of organic biomass and facilitate salt leaching, especially Na<sup>+</sup> by rain.

## 5. Conclusions

*C. equisetifolia* and *C. glauca* species can be grown in saline conditions, albeit with some limitations related to the increase in salt concentration in the dry season and waterlogging conditions in the rainy seasons. Inoculation with AMF and *Frankia* increased plant growth during 3 years of planting. The *Casuarina* plantation had a positive effect on the diversity of herbaceous vegetation in salty conditions after 5 years of planting. Salt-grasses that have a greater contribution to coverage can be used to improve soil drainage and reduce salt concentration in future rehabilitation programs.

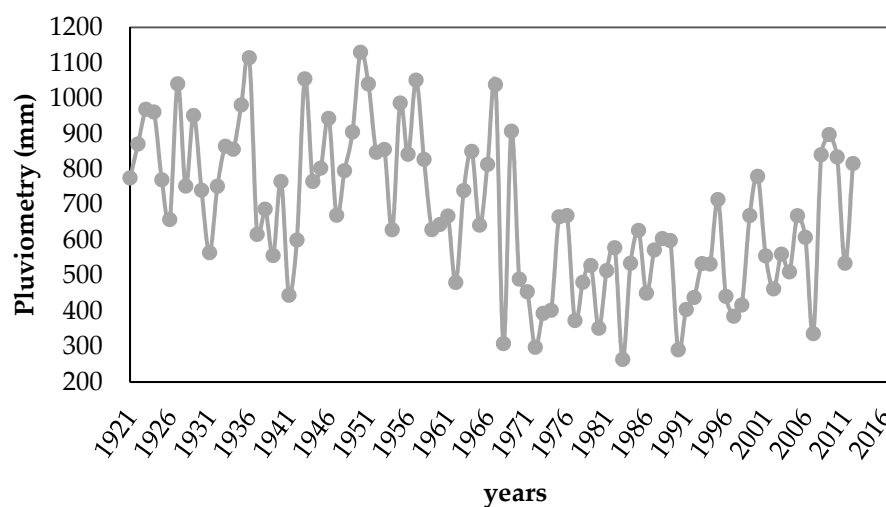
**Author Contributions:** P.I.D., D.N. and N.D. did the experimental work and analysis thereof and wrote the manuscript; V.H., D.F., D.D., M.N., L.L. A.C., and S.S. contributed in designing, supervision and interpretation of the results. J.M.F. edited the final manuscript. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare that they have no conflict of interest.

## Appendix A



**Figure A1.** Rainfall data from the Fatick region (Senegal) over the period 1921–2016.

**Table A1.** Two-way ANOVA analysis of plant species, inoculation, and their interactions with height, collar diameter, survival rate, specific contribution presence, and dry biomass.

	Height	Collar Diameter	Survival Rate	Specific Contribution Presence	Dry Biomass
Species	**	ns	*	ns	ns
Inoculation	***	ns	**	ns	ns
Species× Inoculation	ns	ns	ns	ns	ns

ns, no significant difference; \*, \*\* and \*\*\* indicate significant difference at  $p < 0.1$ , 0.05, 0.01, and 0.001, respectively.

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