Biodegradation (2012) 23:907–916
DOI 10.1007/s10532-012-9583-4

ORIGINAL PAPER

Influence of hybrid giant Napier grass on salt and nutrient distributions with depth in a saline soil

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Received: 6 May 2012 / Accepted: 3 August 2012 / Published online: 17 August 2012

Abstract Cultivation of the biofuel plant, hybrid giant Napier grass (HGN), in saline soil was investigated in a greenhouse study. The results show that HGN is a salt tolerant plant which can flourish in saline soil and product a large amount of biomass. The extensively developed fibrous root system of HGN plays a significant role in the uptake of sodium from saline soil so that both soil salinity and pH are reduced. Fibrous roots of HGN are well distributed in the soil below the surface, where the metabolism of the root system produces a gradient at the depth between 10 and 20 cm in soil salinity, pH and organic content. The degradation of the HGN by the biota within the soil results in an increase in nutrients and improved soil quality. The experimental results suggest that HGN adapts to saline soil, which is promising for phytoremediation of such soils. Additional advantages of HGN include the large biomass produced which can be used for renewable energy generation.

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Keywords Hybrid giant Napier (HGN) · Energy crop · Fibrous root · Saline soil · Spatial distribution · Nutrient

Introduction

Soil salinity is a worldwide problem that poses significant threat to the sustainable development of agriculture. Much arable land gradually becomes salinized due to either natural causes or improper water and land management practices. Seven percent of the world's arable land is impacted by salinity which greatly reduces agricultural output and threatens global food supply (Szabolcs 1994). Nearly eight percent of the total land area in Australia and nearly three percent in China consist of salinized soil (Salinity 2010; Zhang et al. 2010). Excess salinity in soil results in decreased water availability, resulting in plants becoming stressed and starved of water (Munns 2002). Excess sodium ions entering plant cells inhibit metabolic processes resulting in stunted growth of plants and reduced crop yields (Munns and Termaat 1986; Tester and Davenport 2003; Saqib et al. 2006). Furthermore, salinity in soil also reduces soil water conduction and blocks water transportation, potentially affecting plant health (Rengasamy and Olsson 1991). Desalination of the land can increase agricultural output and value.

Table 1 Characteristics of soil chemistry of saline soil and control soil

Soil	pH	Soluble salt (%)	EC (ds/cm)	Na (g/kg)	Total organic C (g/kg)	Total N (%)	Extractable N (mg/kg)	Extractable P (mg/kg)	Extractable K (mg/kg)
Saline	9.29 a	0.99 a	2.94 a	3.55 a	7.57 a	0.06 a	124.37 a	14.74 a	91.99 a
Control	4.75 b	0.02 b	0.46 b	0.01 b	5.38 b	0.05 a	237.54 a	10.44 a	58.85 b

Different letters represent significant difference ($p < 0.05$)

Among several methods for combating soil salinity, phytoremediation is a cost effect technique to grow salt tolerant plants in saline soil to phytoremediate soil salinity (Flowers 2004; Qadir et al. 2005; Qadir et al. 2007). Some salt tolerant plants can survive excess salinity in soil or even thrive under high salinity (Akhter et al. 2004). Phytoremediation is possible when biomass is harvested, along with excess salt, which bring about an improvement in soil chemical and biological conditions in the rhizosphere. Effects of remediation can be observed by monitoring changes in soil characteristics and growth condition of the plant selected during the period of phytoremediation. One promising candidate plant species for phytoremediation of saline soils is hybrid giant Napier grass (HGN, *Pennisetum hybridum*). HGN is a fast-growing perennial *Gramineace* crop with strong adaptability and tillering ability in soil and has potential as biomass for production of biofuel (Magcale-Macandog et al. 1998; Xiao et al. 2010). With its highly developed fibrous shallow root system and strong resistance to water stress, HGN is tolerant to poor soil quality and grows well on barren land, degraded sandy soil and on steep slopes subject to erosion (Angima et al. 2002; Xiao et al. 2010). As HGN is capable of improving soil structure and increasing the fertility of soil after a period of cultivation it is widely used for phytoremediating barren or degraded land and for soil and water conservation (Angima et al. 2002; Mutegi et al. 2008; Tsai 2009).

The aim of the study is to determine the potential of HGN for phytoremediation of saline soil. The extent of phytoremediation on soil salinity in a glasshouse experiment was examined over a two year period to investigate its effects on soil properties, including pH, content of soluble salt and nutrients. In particular, soil chemical variation near the rhizosphere of the HGN in relation to depth of soil was closely monitored to determine its potential for improving the root zone. Biomass production generated during the two year study was compared with those grown in local common soil and determined the potential of HGN to produce biomass in saline soils.

Materials and methods

Plant material and greenhouse study

HGN was propagated by cutting the middle stem of a two-year old healthy HGN plants. The cuttings from the mature plant were about 8 cm long with well developed lateral buds. The cuttings were immersed in water immediately after cutting.

Saline soil used in this experiment was a meadow podzolic soil from Lianyungang, Shandong, China (34° 65′ N; 119° 44′ E). The control soil was non-saline oxisols collected locally (Shaoguan, Guangdong, China, 24° 46′ N, 113° 39′ E), a common type of acidic soil in southern China. The collected soil had been dried, sieved, evenly mixed and stored before the experiment. The characteristics of both soils listed in Table 1 indicated that the control soil was acidic soil and the saline soil was alkaline. The high electric conductivity in saline soil suggests a large amount of soluble ionic species existed in the soil, which is consistent with high contents in sodium and soluble salt found in the saline soil. This soil is normally classified as a moderate saline soil (Watling 2007). In contrast, only traces of sodium and soluble salts were detected in the control soil. Furthermore, the saline soil contained more nutrients than those in the control soil except nitrogen.

A pot study of HGN was conducted in a greenhouse from Aug. 2009 to Aug. 2011. The pots were made from plastic with no holes in the bottom to prevent nutrients and salt in the soil from leaching out. The result of a preliminary study for HGN grown in control soil during the two year field trial listed in Fig. 1 indicates that over 80 % fibrous roots were located in

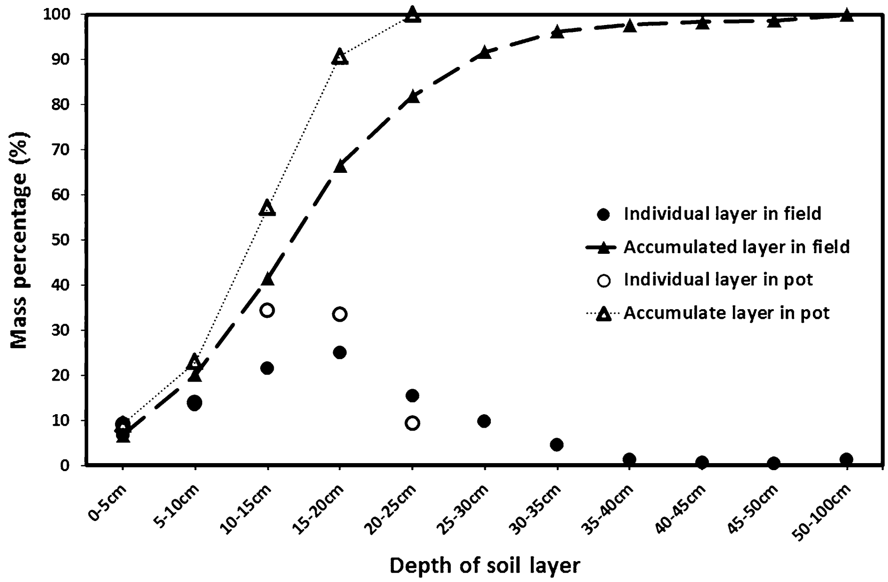

Fig. 1 A comparison of distribution of HGN roots related to soil depth between field trial and pot study

25 cm depth from the surface. The diameter of the pots' opening was 20 cm at the top and 15 cm at the bottom. The pots were 25 cm tall. Each pot was filled with 2.5 kg saline soil or control soil near to the top of the pot. The cutting segment of HGN with lateral buds was laid horizontally over the top of the partly filled pots and then covered with 2 cm of soil without addition of fertiliser. Triplicates of the samples were taken for any measurement. In total 12 pots of each soil prepared, six of them were used for the first and second year harvests. The remaining pots were used for observation of HGN growth. After planting, the moisture of the soil with HGN was maintained at 60 % of field capacity in the first week and added 1L per pot weekly afterwards.

The HGN above ground biomass was harvested in August of each year. Three pots of the HGN in each experimental were randomly selected and the above-ground portion of the HGN from the soil horizon was cut. The height, number of tillers and fresh weights of the HGN were recorded. The spatial distribution with depth of salts and nutrients in the pots of soil were investigated after each harvest by removing soil and all roots from each pot, separating them from depths of 0–5, 5–10, 10–15, 15–20, and 20–25 cm and storing them in separate plastic bags. The pH, soluble sodium, salt contents and some nutrients were determined at each depth in air-dried samples. Tap water was used to remove remaining soil attached to the fibrous root system and was followed by rinsing with Milli-Q water. The rinsed roots were carefully wiped with paper towels and the roots dried in air for 10 min before the weighing. The fresh weight of the roots and the distribution of the roots in each layer were recorded.

Soil chemistry

Soil pH was measured in 1:5 soil:water suspensions using a pH electrode. Total N was determined using the micro-Kjeldahl method with 2.0 g soil being digested in H_2SO_4 and H_2O_2 solution before measurement (Stuart 1936). Extractable N was extracted with 2 M KCl using a 1:10 soil:extractant ratio and a 1 h end-over-end shake followed by filtration, which was determined colorimetrically (Blakemore et al. 1987). Total C and soil organic matter were determined using $K_2Cr_2O_7$–H_2SO_4 digestion (Walkely and Black 1934; Nelson and Sommers 1996). P was extracted using 0.5 M $NaHCO_3$ and determined spectrometrically (Schollenberger and Simon 1945). K and Na were extracted using 1.0 M NH_4OAC and determined spectrometrically (Shainberg et al. 1987). Total soluble salt was determined using both dissolved solid matter and electrical conductivity methods (Pansu and Gautheyrou 2003).

Data analysis

The mean value of different elements from three soil samples from the replicate treatments was used to

Table 2 A comparison of HGN growth in saline soil and control soil

Harvest after	Height of plant (cm)		Tillers (No.)		Weight of plant (g/plant)	
	Control soil	Saline soil	Control soil	Saline soil	Control soil	Saline soil
Year one	156 ± 11 b	180 ± 12 a	3 ± 1 b	6 ± 1 a	462 ± 21 b	785 ± 26 a
Year two	177 ± 13 a	194 ± 18 a	6 ± 2 a	10 ± 2 a	733 ± 29 b	1258 ± 35 a

Different letters represent significant difference ($p < 0.05$)

compare the differences in soil nutrients and soluble salt in both saline and control soils. Triplicate samples of biomass were selected and analysed statistically on the different sample pots. Statistical analyses were performed using SPSS (version 14.0) and Excel. Analysis of variance (ANOVA) was used to compare the differences in characters between saline soil and control soil. Multiple sample comparison of HGN was conducted by Duncan's new multiple range test (Duncan 1955).

Results

Growth of HGN in saline soil

HGN cultivated in pots in the glasshouse thrived in saline soil with strong growth and healthy foliage of dark green colour. The general growth characteristics including the height and weight of the plants in the saline soil during the two-year period are 15 and 70 % more than those in the non-saline acidic control soil (Table 2). Although the emergence of new shoots from the HGN cuttings was generally slower in saline soil than in the control soil, the new shoots emerging from saline grew rapidly and exceeded the height of those planted at the same time in the control soil after 15 days. The number of tillers of HGN in the saline soil after the first year was also double those in the control soil ($p < 0.05$). During the second year of growth the height and tillering of the HGN in the saline soil were 10 and 67 % more than those in the control soil but there was no significant differences shown. Leaves of the plants turned slightly yellower than those grown in same saline soil in the first year, but remained darker than those grown in control soil. During two years of the experiment the fresh weight of the HGN in saline soil harvested each year was more than 70 % greater than those in the control soil ($p < 0.05$). This indicates that HGN is able to grow healthily in moderately saline soil with high nutrient contents and produce substantial amount of biomass.

Spatial distribution of root system of HGN in soil

The main characteristics of the roots of HGN are those highly developed with a widespread fibrous root system. The amount of fibrous root systems of the HGN grown in saline soil was more than 30 % greater than those grown in the control soil. The mean fresh weight of harvested roots of HGN in saline soil after the first and second year was 34.5 and 60.6 %, respectively, greater than those in the control soil ($p < 0.05$) (Table 3). The vertical mass distribution of roots of HGN in both soils, displayed in Fig. 2, indicates that a large portion of the rooting system grows in 10–15 cm layers below the surface horizon, which accounts for more than a third of the mass of the total root. Another 30 % of the root occurred in the layer between 15 and 20 cm under the surface horizon. The root system distributed in both layers between 10 and 20 cm accounts for approximately 65 % of mass in total fresh root. Furthermore, over 80 % of the root system occurred between 5 and 20 cm below the surface if the root in the layers between 5 and 10 cm is also included. The mass of the HGN root distribution pattern in the pot study was similar to those in the field trial in Fig. 1 except that the layer with maximum mass in the field trial was observed between 15 and 20 cm. It is worth mentioning that when the roots were pulled out from the pots and removed from the attached soil a large amount of tiny holes and channels were clearly visible due to the spreading root systems. It indicates well developed root systems of HGN in both soils that not only can take up nutrient effectively but also create many new soil pores and channels to increase porosity, aeration and water infiltration.

Table 3 Spatial distribution of fibrous roots of HGN with depth in saline soil and control soil

Depth below surface horizon (cm)	Fresh fibrous root in year one (g)		Fresh firbrous root in year two (g)	
	Control soil	Saline soil	Control soil	Saline soil
0–5	1.14 ± 0.12	1.45 ± 0.11	1.32 ± 0.11	2.07 ± 0.16
5–10	1.73 ± 0.15	2.37 ± 0.16	2.26 ± 0.13	3.13 ± 0.22
10–15	3.72 ± 0.31	4.92 ± 0.24	4.85 ± 0.24	7.78 ± 0.51
15–20	3.33 ± 0.22	4.58 ± 0.23	4.43 ± 0.18	7.59 ± 0.62
20–25	1.01 ± 0.05	1.38 ± 0.12	1.26 ± 0.07	2.11 ± 0.18
Total	10.93 ± 0.85 c	14.70 ± 0.85 b	14.12 ± 0.85 b	22.68 ± 1.37 a

Different letters represent significant difference between columns ($p < 0.05$)

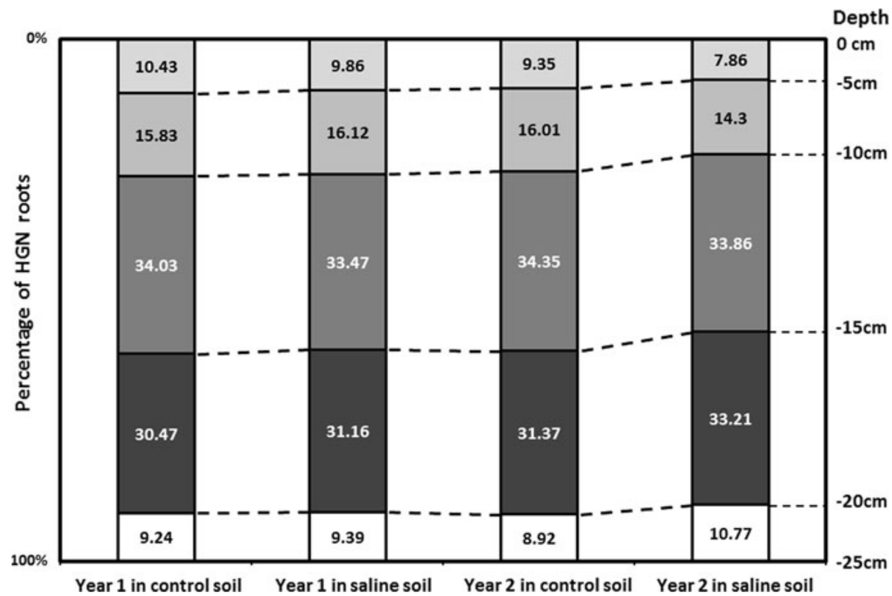

Fig. 2 Distribution of fibrous root system of HGN below the surface horizon

Spatial improvement in soil chemistry

Phytoremediation of saline soil

Phytoremediation of soil salinity by cultivation of HGN was observed. As the soil used for this study had been evenly mixed before plant growth, it is assumed that the soil was uniform in depth at commencement. The vertical spatial distribution of soluble salt, sodium concentration and soil pH are shown in Fig. 3. It is evident that after the first year of growth of HGN, pH, total amount of soluble salts and content of sodium in the saline soil declined most at 10–20 cm depths, where most roots were distributed (33.5 % of total root at 10–15 cm depth and 31.2 % at 15–20 cm depth respectively). The maximum reduction in soil pH, soluble salt and sodium are 1.5 pH unit, 57.6 and 49.3 % respectively. During the second year HGN growth the salinity of the soil was further phytoremediated due to further development in fibrous root system of the plant. The maximum reduction compared with the original saline soil is 1.7 pH unit, 50.5 and 44.5 % respectively. The reduction in total soluble salt and sodium in the second year was slightly less than in the first year, although the pH dropped further. The reduction of soluble salt and sodium concentration during HGN plantation can be attributed to their uptake by the fibrous root system into the biomass of HGN during growth. In fact, after two years' HGN growth, electrical conductivity of the soil, i.e., soil

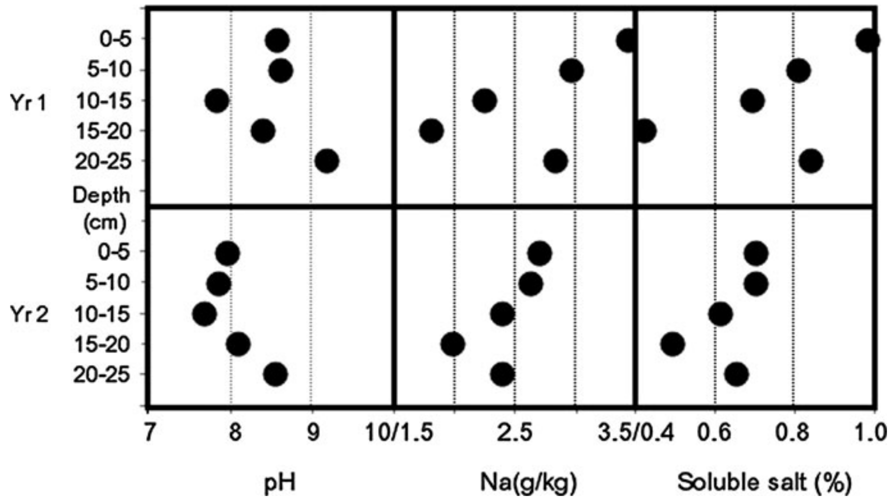

Fig. 3 Spatial distribution of pH and salinity below the surface of horizon during two year HGN plantation in saline soil (pH = 9.29, soluble salt = 0.99 %, C_{Na} = 3.55 g/kg in original soil)

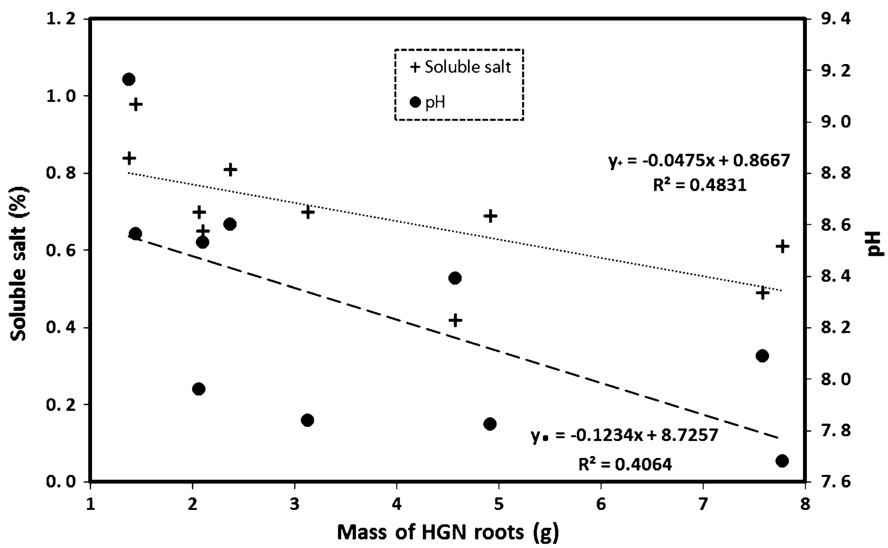

Fig. 4 Mass of HGN roots in relation to soil pH and salinity during two year growth study

salinity decreased from 2.94 to 2.38 ds/cm, suggesting substantial reduction in the amount of mobile salts. The correlation between distribution of HGN root grown and change in soil salinity is displayed in Fig. 4. Some broad correlations between mass of HGN root and the amount of soluble salt ($R^2 = 0.4831$) and between mass of HGN roots and soil pH ($R^2 = 0.4064$) in this figure suggests that an increase in the amount of HGN roots is beneficial in reducing the amount of soluble salt and soil salinity. The maximum reduction in soluble salt and sodium in saline soil during the two year HGN pot study was found in the layer between 15–20 cm below the surface horizon, whereas the lowest value of pH was in the layer of 10–15 cm. Furthermore, the soil pH in the layers over 20 cm below surface horizon still remained high during the two year HGN plantation.

Nutrient spatial distribution in saline soil

The soil nutrient distribution with depth from 2 years of HGN growth is shown in Fig. 5. Changes in layers below the surface horizon in saline soil were observed, with average contents of total organic C and total N showing increases annually, while extractable P, K and N showed declines. Furthermore, the trend of such

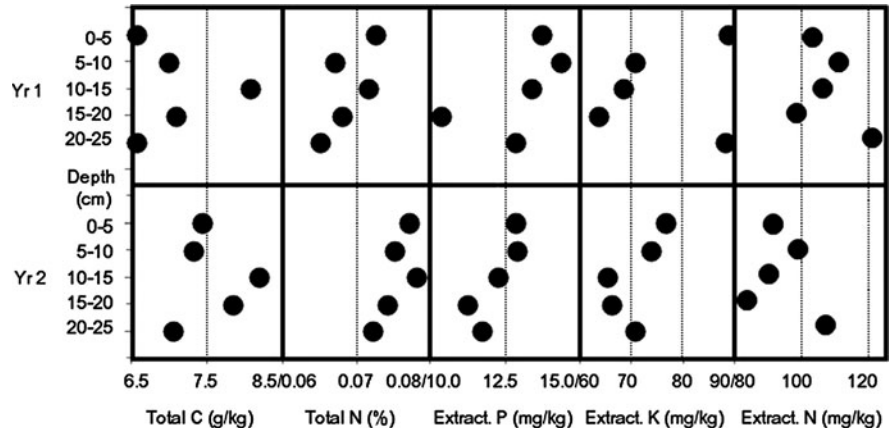

Fig. 5 Spatial distribution of nutrient below the surface of horizon during two year HGN plantation in saline soil ($C_{C(T)} = 7.57$ g/kg, $C_{N(T)} = 0.99$ %, $C_{P(ext)} = 14.74$ mg/kg, $C_{K(ext)} = 91.99$ mg/kg, $C_{N(ext)} = 124.37$ mg/kg in original soil)

variation generally increased with the period of growth. The maximum amount of total organic carbon (8.07 g/kg at year one and 8.19 g/kg at year two) and total nitrogen (0.072 % at year one and 0.078 % at year two) accumulated was observed in the range between 10 and 15 cm below the surface horizon. In contrast, the maximum reduction in extractable phosphorus (10.35 mg/kg at year one and 11.24 mg/kg at year two), potassium (63.48 mg/kg at year one and 65.98 mg/kg at year two) and nitrogen (98.84 mg/kg at year one and 84.10 mg/kg at year two) was detected mostly in the layer below surface horizon 15-20 cm range during 2 year growth period.

The results in Fig. 5 also indicate that the contents of total organic C and N in saline soil during the second year not only increased further, from 8.07 to 8.19 g/kg for C and from 0.072 to 0.078 % for N respectively, but were also distributed much more evenly than in the first year, ranging from 6.56-8.07 g/kg in year one to 7.05–8.19 g/kg in year two for C and ranging from 0.065–0.072 % in year one to 0.072–0.078 % in year two for N. The correlation between mass of HGN root and total C and N in soil is displayed in Fig. 6. Simple linear regression between mass of HGN root and total C results in coefficient of determination $R^2 = 0.7017$ indicating that the increase in C content in the saline soil is mainly attributed to soil biometabolic action, which converts dead fibrous root and litters in soil into organic substances. In contrast, a very poor coefficient of determination $R^2 = 0.1883$ for total N implies that accumulation of N in the soil may not be totally related to the mass of HGN root but some other factors. The extent of reductions in extractable nutrients P, K and N during the second year of HGN cultivation was less than those in the first year. The distribution of all nutrients among all layers below the surface horizon in the second year is more homogeneous than those in the first year and the difference among various layers between P and K is relatively insignificant. It is probably that P cannot be obtained from soil metabolic digestion of litter while K, a typical soluble salt, is taken up by fibrous roots. Such an even distribution trend in all elements can be attributed to the fibrous root system movement and water transportation within the soil. The correlation between mass of HGN roots and individual available nutrients shown in Fig. 7 indicates that, though low in coefficient of determination among three nutrients, general trends of nutrient reduction were related to the mass of HGN roots, probably due to strong demands on nutrient by rapid growth of HGN.

Discussion

The results from this study suggest that HGN can grow well in saline soil and even better than when cultivated in a non-saline acidic red earth. This shows that HGN thrives in moderate saline soil. According to the results from this study, fibrous root system of HGN was the densest in soil layers 10–20 cm below the surface. Due to the highly developed fibrous root system the vertical content of soluble salt including sodium and potassium varied substantially to form a concentration gradient with the lowest value at 10–20 cm depth. Furthermore, due to the same reason, decomposition, humification and nitrification of root

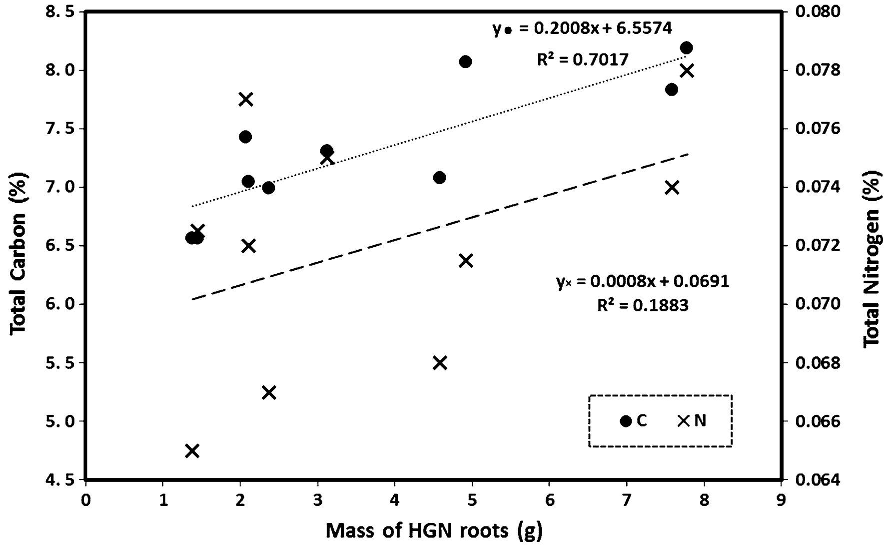

Fig. 6 Mass of HGN roots in relation to accumulation of total C and N during two year growth study

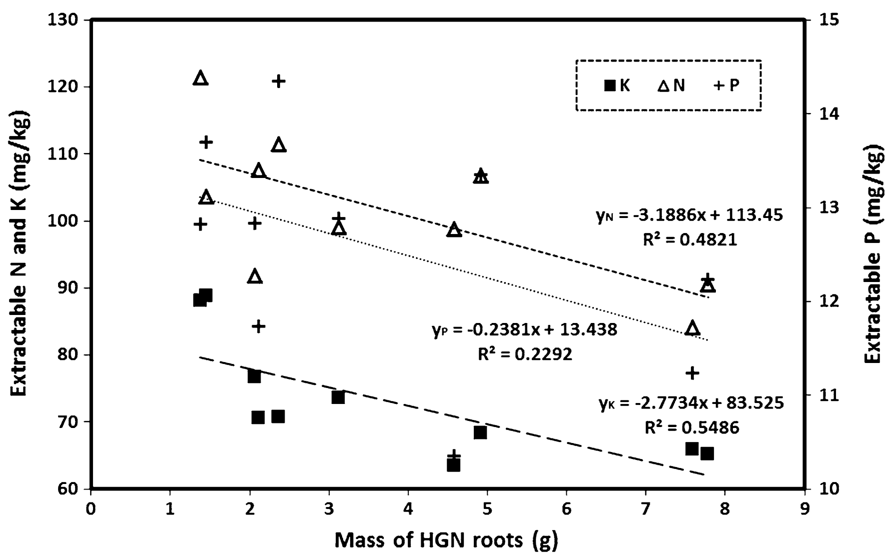

Fig. 7 Mass of HGN roots in relation to extractable nutrients during two year growth study

and other organic matter increased organic C and N in this range. Meanwhile, absorption and utilization of extractable P, K and N by fibrous root for growth were also evident. During the first year of HGN growth the most significant variation of chemical substances in the soil was observed in the layer between 10 and 20 cm below the surface, where the majority of the fibrous roots occurred. However, the amount of nutrients with depth of soil became relatively homogeneously distributed during the second year of growth. The reduction of concentration gradient in soluble salt and nutrients during the second year HGN cultivation can most likely be attributed to some of the elements being gradually transferred and exchanged with neighbouring areas of rhizosphere, therefore resulting in further declining soil salinity and pH reduction (Greenway and Ungar 2001). Large amounts of tiny holes and channels observable in the soil grown with HGN, indicates a fibrous root system of HGN creating micro/macro pores in the soil and to improve hydraulic conductivity in the soil structure, and provide the available water for HGN to establish and grow (Tejada et al. 2006; Hayashi et al. 2006; Jozefaciuk et al. 2006).

There is a broad correlation between the amount of fibrous roots distributed and nutrient accumulation/

salinity reduction in the soil. The fibrous roots distributed in the soil promotes adsorption, transportation and biometabolic action of ionic species in soil which results in the accumulation of nutrient and uptake of soluble salts. The results in Fig. 2 indicate more fibrous root growth in the layer at the 10–15 cm range than in any other layers in the soil, and more C and N are accumulated in this range. Biometabolic action of dead roots in soil can produce organic acid which results in the lowest soil pH among all soil layers. Accessibility of oxygen may also play an important role to promote such a process. For example, the reduction in pH at top soil layers 0–5, 5–10, 10–15 was higher than those at 15–20 and 20–25. In particular, amount of fibrous roots found in either 0–5 or 20–25 layer were very close, the pH drop in the top was much greater than that in bottom, probably due to more available air in the top layer. However, the high nutrient contents and low soil pH may not be beneficial for sorption and the transportation process of ionic species so that the maximum reduction of soluble salts including Na and K was not observed in this range, but rather in the layer below where the second highest amount of fibrous roots of HGN were found.

HGN is a C4 plant with a rapid growth rate and a high photosynthetic efficiency. Continuous increase in organic carbon in soil suggests that highly developed fibrous root system endows a strong capacity of absorbing and fixing C, even in saline soil. Cultivation of HGN in saline soil results in improved soil quality with production of biomass as a bonus for energy generation. However, contents of extractable P, K and N in soil decline annually with the growth of HGN, suggesting that HGN requires a high demand for all extractable nutrients, especially K. Therefore, attention should be paid to supply phosphate and potash fertilizers and promote vigorous growth in cultivation process of HGN. This study suggests that halophyte HGN is of great potential to the recovery of saline wasteland and energy production (Magcale-Macandog et al. 1998; Akhter et al. 2004).

The results from this study indicate that HGN is a potential candidate to phytoremediate saline soil. It must be acknowledged that there is a real gap between pot study and field work. Although the field trial on HGN in control soil over a two year period displayed rooting patterns as a function of soil depth was close to those in the pot study (Fig. 1), HGN rooting systems in the pots were restricted to spreading vertically which can potentially impact the healthy growth of HGN, as the height of HGN in the field trial can reach over four meters with much more tillers multiplied. HGN rooting systems play a very important role in the conversion and uptake of nutrients, in reducing salt content and improving soil quality. Due to this fact, more HGN rooting system could be developed under the field condition, which is most likely to enhance the phytoremediation outcome of salinity by HGN. However, as this study is conducted under one set of climatic variables and one type of saline soil, more studies are needed on a wider range of saline soil under variety of climates before the potential use of HGN as a remediation tool globally.

Conclusion

HGN is a good potential candidate to be an effective salt tolerant plant that can grow well in saline soil, and reduce pH of saline soil. With highly developed root system, HGN in saline soil is capable of taking up both nutrients and soluble salts in soil so that the salinity of the soil is reduced. Well-developed root systems of HGN are primarily distributed in soil layers 10–20 cm below the surface, where the chemical metabolism take place resulting in accumulation of nutrients and phytoremediation of soil salinity. Furthermore, to cultivate HGN in saline soil not only reduces soil salinity but also produces considerable amount of biomass, which can be used for renewable energy generation.

Acknowledgments Support from Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) and Shaoguan Haiyue Agricultural Development Co., Ltd., are gratefully acknowledged.

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