# The physiological and biochemical changes associated with the growth of bioenergy grasses in soils with optimal and deficient nutrient conditions

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## Abstract

Four potential energy grasses viz. *Saccharum arundinaceum,* Hybrid *Napier* var. CO-3 (HNC)*, Saccharum spontaneum* and *Arundo donax* were grown in nutrient optimal (NO) soils and nutrient deficient (ND) soils. NO soils had organic carbon, P and K content much higher than ND soils. Various physiological and biochemical parameters viz. MDA content, proline and total sugar accumulation, antioxidant enzyme activity, chlorophyll *a* fluorescence and photosystem I and II activity of grasses grown in both soil conditions were measured. *S. spontaneum* and *A. donax* growing in ND soils maintained a better osmotic status by accumulating compatible solutes such as proline. Both these grasses showed a less reduction in sugar content than *S. arundinaceum* and HNC in ND soils. *S. spontaneum* and *A. donax* growing in ND soils showed an efficient free radical scavenging potential as evidenced by low MDA level and increase in peroxidase activity. *S. arundinaceum* and HNC growing in ND soils recorded a reduction in quantum yield of PS II ($F_\sqrt{}/F_m$), photosystem I and II activities as compared to the same species growing in NO soil and
this decrease was to higher extend in these two species as compared to *S. spontaneum* and *A. donax.* The better performance exhibited by *S. spontaneum* and *A. donax* in ND soils makes them appropriate choice for marginal lands characterized by nutrient deficient status.

**Keywords**: antioxidants, bioethanol, marginal land, osmotic potential, photosystems

## Introduction

Biofuels are renewable source of energy and can be used as alternative source for fossil fuels. For the production of first-generation bioethanol, food crops are being utilized which may lead to food shortage (Smith *et al.,* 2013). Therefore, there is increased attention for enhancing the production of second generation bioethanol. In this context, high biomass accumulating grasses (bioenergy grasses) are getting greater attention nowadays As arable land is falling short even for the cultivation of food crops, marginal lands characterized by low nutrient status, turns out to be an alternative for growing energy grasses. But the hope remains with some energy grasses which were already tested for their fitness to the marginal lands. *Miscanthus* and *Arundo donax* were found to survive in a wide range of soil types with varying nutrient status (Chung and Kim, 2012; Nassi *et al.*, 2013).

Marginal lands are deficient of many important mineral nutrients such as N (nitrogen), P (phosphorus) and K (potassium). The deficiency of N leads to the impairment of water and nutrient uptake, protein metabolism, photosynthesis, carbon partitioning, enzyme and plant hormonal activities (Ahmad *et al.*, 2014). Phosphorus is the most limiting nutrient for crops after nitrogen (Vance *et al.*, 2000). Phosphorus is one of the most unavailable and inaccessible macronutrient required by plants and it plays a key role in an array of plant processes (Vance *et al.*, 2003). Potassium plays a major role in the basic functions of plant growth and development. In addition, K is also involved in numerous physiological functions related to plant health and resistance to biotic and abiotic stresses (Oosterhuis *et al.*, 2013) and its deficiency leads to the impairment of photosynthetic process and stimulation of reactive oxygen production.

In our earlier study we had identified four potential grass species with biomass accumulation potential (Chandran *et al.*, 2010). In the present study the growth performance of these four grass species were analyzed in nutrient deficient (ND) soils (a characteristic feature of marginal lands) in terms of various physiological and biochemical features and the same was compared with the grasses growing in nutrient optimal (NO) soils.

## Materials and Methods Plant

## Materials

Four grass species viz. *Arundo donax* L.*, Saccharum arundinaceum* Retz. *Saccharum spontaneum* L. and hybrid *Napier* var. CO-3 (*Pennisetum purpureum* X *Pennisetum americarnum*) were selected for the present investigation. Stem cuttings were collected from different areas of Calicut, Malappuram and Kannur districts of Kerala, India. Healthy stem cuttings of 20-30 cm long were selected and planted in polybags of size 30x28 cm containing 5 kg of soil. The top layer soil was collected from two different locations of Malappuram district, one being an area where there was rich vegetation and other area was with scanty vegetation and showing all characters of marginal land. One set (12 plants each) was grown directly in the former type of soil and the other set was grown in the latter type of soil by planting the stem cuttings. The plants were acclimatized to the respective soils by growing them for four weeks in green house at relative humidity of 60±5%, light intensity 300 μmol/m$^2$/sec and temperature of 28±2°C. Studies were initiated to analyze various parameters at the end of four weeks growth period.

## Analysis of Soil Physicochemical Properties and Physiological Indexes

Available phosphorus was estimated by Brays method No. 1 (Bray and Kurtz 1945) and available potassium in the soil was estimated using flame photometer (Bhattacharjee and Sharma 2011). Organic carbon (OC) content and electrical conductivity (EC) of the soil was determined as described by Datta *et al.* (1962) and Corwin and Lesch (2005), respectively. The Ψs of leaf was determined using a vapour pressure osmometer (Wescor 5520, USA). The accumulation of osmolytes such as proline and total soluble sugars were carried out according to Bates *et al.* (1973) and Montgomerry (1957). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content as described by Heath and Packer (1968). The activity of

antioxidant enzyme peroxidase (POD, EC 1.11.1.7) was estimated according to Gasper *et al.* (1975). Chl *a* fluorescence emission was measured by a Handy PEA (Plant Efficiency Analyser) (Hansatech, Norfolk, UK) (Mirshad *et al.*, 2014). Photochemical activities of isolated thylakoids were assayed polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech, Norflok, UK) (Puthur, 2000).

## Results and Discussion

## Soil Characteristics

The result of the soil analysis revealed that the nutrient optimal (NO) soil had a neutral pH (7.0) and the EC, OC, P and K were 0.01%, 1.25%, 88.2 and 350 Kg/ha respectively. The nutrient deficient (ND) soil had an acidic pH (4.8), high EC (0.08), OC, P and K content was 0.73%, 2.58kg/ha and 34.72 kg/ha, respectively (Table 1). As evident from the results, the ND soils selected for the study had a lower OC, P and K content than that of NO soils. Marginal lands generally contained low organic matter and were highly eroded and poor in mineral nutrients especially nitrogen and phosphorus (Malab, 1992). The pH of marginal lands was more to an acidic range (4.5 – 5.5) and in some cases extreme acid range (<4.5) was also recorded (Liu *et al.*, 2011).

## Osmolytes and Ψs

The grasses grown in ND soils showed a decrease in total sugar content as compared to plants growing in NO soils. The decrease was less in *A. donax* and *S. spontaneum* (34 and 39%, respectively) and in *S. arundinaceum* and HNC, the reduction was 51 and 58% respectively as compared to plants grown in NO soils (Fig. 1A). *A. donax* and *S. spontaneum* growing in ND soils accumulated 262% more proline and HNC accumulated 180% more proline than those growing in NO soils (Fig. 1B). The odd factors in the ND soil including the deficiency of various minerals may be negatively affecting the synthesis of metabolites as reflected in the case of low sugar content of plants growing in ND soils. The phosphorous deficiency in soil could be a major factor for the high rate accumulation of proline in *Oryza sativa* as reported by Tian-rong *et al.* (2012).

Plants increase their Ψs by accumulating more osmolytes, such as proline to maintain better osmotic balance. The Ψs in *A. donax* and *S. spontaneum* growing in ND soil showed 42 and 38% increase over that growing in NO soils,

whereas the increase recorded in *S. arundinaceum* and HNC was only 24% lesser (Fig. 1C). The highly reduced Ψs in *A. donax* and *S. spontaneum*

was due to the high accumulation rate of

compatible solutes than other two grasses, which considerably contributed towards the lowering the Ψs. As proline was found to increase ~ 4 fold higher in plants of ND soils as compared to NO soils it can be presumed that this iminoacid would be contributing in major towards the lowering of Ψs other than contributing towards enhancing the Ψs, proline stabilizes subcellular structures (membranes and proteins) by scavenging free radicals and also buffers cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Among the grass species studied, the least reduction in Ψs was observed in *S. arundinaceum* and HNC that grows in ND soil and it was very well coinciding with the least accumulation of proline in them. Osmolytes are generally accumulated in the cytoplasm to protect the cellular components from dehydration during osmotic stress by the adjustment of Ψs (Seki *et al.*, 2007).

## Free Radical Generation and Scavenging Mechanism

It was observed that the maximum malondialdehyde (MDA) accumulation was observed in *S. arundinaceum* and HNC (87 and 122%, respectively) and least was recorded in *A. donax* and *S. spontaneum* (57 and 66%) growing in ND soils as compared to that growing in NO soils (Fig. 2A). Among the grasses studied nutrient deficient condition affects more negatively on HNC as resulted by enhanced lipid peroxidation followed by *S. arundinaceum*. It was earlier observed that nitrogen deficiency leads to an enhanced production of MDA content in rice leaves which is a direct indicator of lipid peroxidation (Huang *et al.*, 2004). The low level of free radical generation recorded in *A. donax* and *S. spontaneum* was mainly because of the enhanced activity of antioxidant enzymes such as guaiacol peroxidase which partially protects these plants from oxidative damage. In the present study, the highest guaiacol peroxidase activity was recorded in *A. donax* and *S. spontaneum*, i.e. 258 and 210% and least activity was recorded in *S. arundinaceum* (152%) and HNC (124%) growing in ND soils (Fig. 2B). It was earlier reported that the deficiency of potassium in the soil leads to the production of reactive oxygen species and simultaneous increase in antioxidant enzyme peroxidase in *Solanum lycopersicum* (Hernandeza *et al.*, 2012).

## Chlorophyll a Fluorescence Analysis

According to Debez *et al.* (2010) in non-stressed plants the $F_v/F_m$ ratio falls between 0.75-0.85 and the ratio below this value indicate that plants are under stressed condition. Among the grasses studied, all the grasses growing in ND soils showed a decline in Fv/Fm and the maximum reduction was shown by HNC and *S. arundinaceum* (0.21 and 0.26, respectively) followed by *S. spontaneum* and *A. donax* (0.34 and 0.51, respectively). Coinciding with these results the initial fluorescence (Fo) was higher in all the grasses growing in ND soil and it was maximum in *S. arundinaceum* and HNC (1074 and 1034, respectively) (Table 2). According to Jia *et al.* (2008) PSII, photochemical efficiency (Fv/Fm), electron transport rate of PSII and photochemical quenching decreased under low-K treatment. All the grasses growing in ND soils exhibited a reduction in Fv/Fo (maximum potential for primary photochemistry), and the maximum reduction was recorded in *S. arundinaceum* and HNC and the reduction was lesser in *A. donax* and *S. spontaneum*. The decrease in Fv/Fo or the increase in Fo are closely related to the structural damage to the thylakoid membranes (Pereira *et al.*, 2000).

ETo/RC representing the electron transport per reaction center decreased in all the grasses growing in ND soil as compared to that growing in NO soil. At the same time DIo/RC, which represents the ratio of the total dissipation of untrapped excitation energy from the reaction centres in the form of heat, fluorescence and energy transfer (Strasser *et al.*, 2004) increased in all the grasses growing in ND soil as compared to that growing in NO soil (Table 2). The decrease in ETo/RC and increase in DIo/RC was higher in *S. arundinaceum* and HNC as compared to *A. donax* and *S. spontaneum*.

## Photosystem I and 11 Activities

All the grass species studied exhibited a decrease in PSI and PSII activity when grown in nutrient deficient condition. The PS II activity in *S. arundinaceum* and HNC was 59 and 70%, respectively lower than those growing in NO soils. Whereas in *A. donax* and *S. spontaneum* growing in ND soils, the reduction of PS II activity was only 45 and 49%, respectively (Fig. 3A). Similarly the activity of PSI was lesser in *A. donax* and *S. spontaneum* (31% and 35%, respectively) as compared to those growing in NO soils. Whereas in *S. arundinaceum* and HNC the reduction was 40

and 57% respectively (Fig. 3B). The reduction in PS I and II activities are mainly due to the disruption of electron transport process, and structural changes in the reaction centres, which ultimately reduce the photosystem activities. According to Antal *et al.* (2010) the deficiency of nitrogen in *Phaseolus vulgaris* reduces the electron transfer from QA to plastoquinone pool in PSII. The oxidative stress in cucumber induced by the action of herbicide (Blazer) damages the structure and function of both PSI and PSII reaction centers resulting in the impairment of the photosynthetic apparatus. (Tripathy *et al.*, 2007).

In conclusion, among the grasses studied, *A. donax* and *S. spontaneum* were better adapted to nutrient deficient soil than *S. arundinaceum* and HNC. Therefore, these varieties are recommended for the reclamation of marginal lands which are affected by low soil nutrient status. Growing these perennial grasses in marginal lands can help to utilize vast area of unproductive land and can be further exploited for the production of biomass.

**Table-1**

The various soil parameters in nutrient deficient (ND) soil and nutrient optimal soil (NO). The data is an average of recordings from three independent experiments each with three replicates (i.e. n=9). The data represent mean+SE.

| Samples | pH | ES(S/m) | OC (%) | P (Kg/ha) | K (Kg/ha) |
|---|---|---|---|---|---|
| Nutrient deficient (ND) soil | 4.80±0.24 | 0.08±0.01 | 0.73±0.04 | 2.58±0.12 | 34.72±1.73 |
| Nutrient optimal (NO) soil | 7.0±0.34 | 0.01±.001 | 1.25±.008 | 88.23±4.4 | 350±12.3 |

**Table-2**

Photosynthetic performance of four different energy grasses evaluated by the chlorophyll a flourescence-test of four different energy grasses (AD - A. donax, SS - S. spontaneum, SA – S. arundinaceum, HNC Hybrid Napier var. CO-3, NOS – nutrient optimal soil, NDS – nutreint deficient soil).
The data is an average of recordings from three independent experiments each with three replicates (i.e. n=9). The data represent mean+SE.

| Chl *a* flourescence parameters | AD (NOS) | AD (NDS) | SS(NOS) | SS(NDS) | SA(NOS) | SA(NDS) | HNC(NOS) | HNC(NDS) |
|---|---|---|---|---|---|---|---|---|
| Fo | 425±21.25 | 153±7.65 | 426±20.23 | 614±30.7 | 382±19.1 | 1074±53.7 | 774±38.7 | 1034±53.5 |
| Fv/Fo | 2.86±0.14 | 1.08±0.05 | 2.82±0.12 | 1.09±0.05 | 2.63±0.13 | 0.20±0.01 | 0.53±0.02 | 0.21±0.1 |
| Fv/Fm | 0.78±0.03 | 0.516±0.02 | 0.78±0.03 | 0.34±0.01 | 0.76±0.03 | 0.26±0.01 | 0.74±0.04 | 0.21±0.2 |
| $ET_0/RC$ | 1.23±0.06 | 0.91±0.04 | 1.21±0.05 | 0.97±0.04 | 1.36±0.06 | 0.43±0.02 | 0.80±0.05 | 0.17±0.008 |
| $DI_0/RC$ | 0.74±0.06 | 2.40±0.12 | 0.76±0.08 | 2.21±0.11 | 0.88±0.04 | 16.24±0.08 | 5.42±0.8 | 15.82±0.85 |
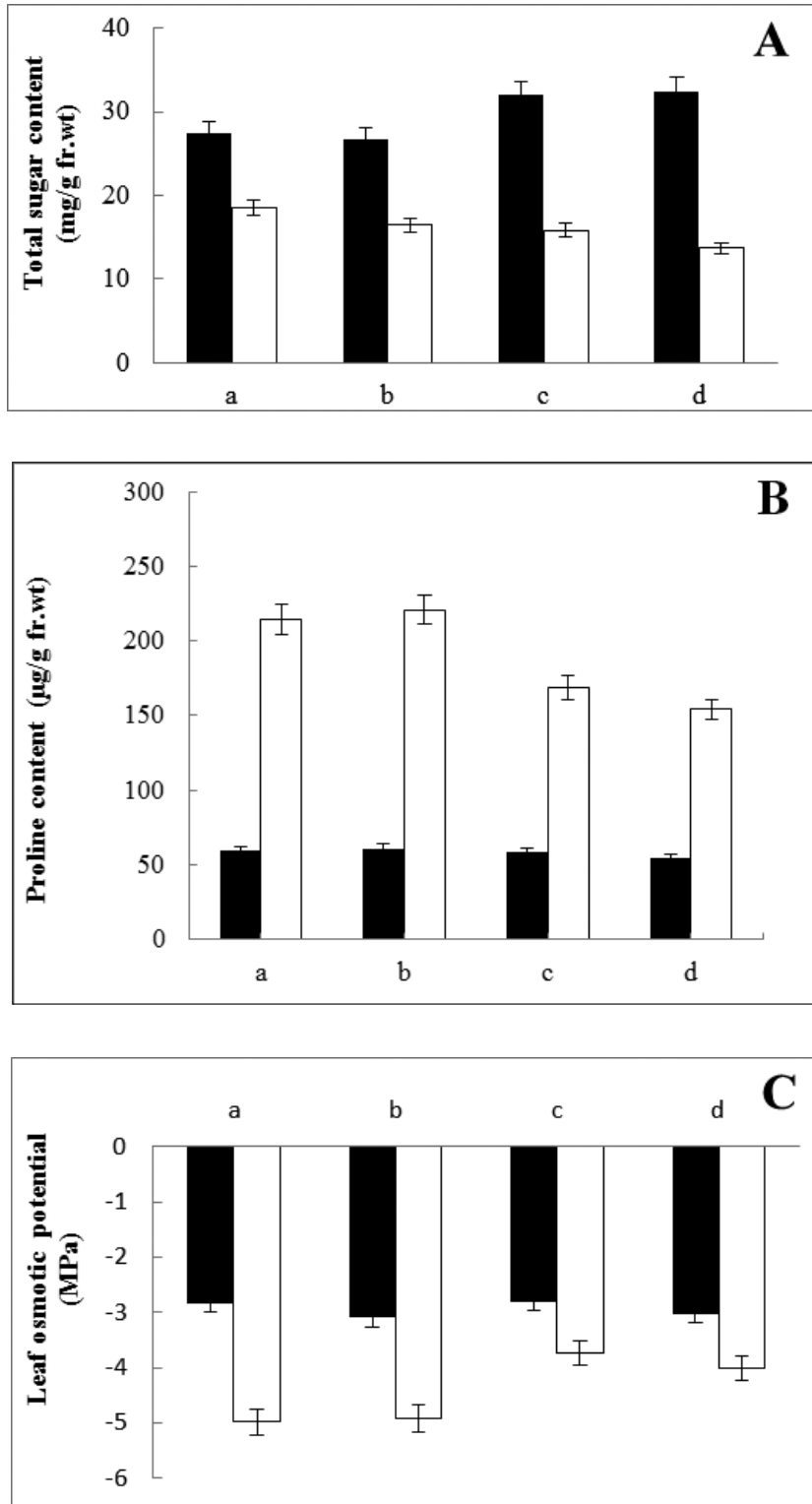
Fig 1. Total sugar (A), proline content (B), and leaf Ψs (C) in four different energy grasses (a - A. donax, b - S. spontaneum, c - S. arundinaceum, d - HNC) grown in nutrient optimal (black bars) and nutrient deficient (white bars) soil. The vertical bars represent SE of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates
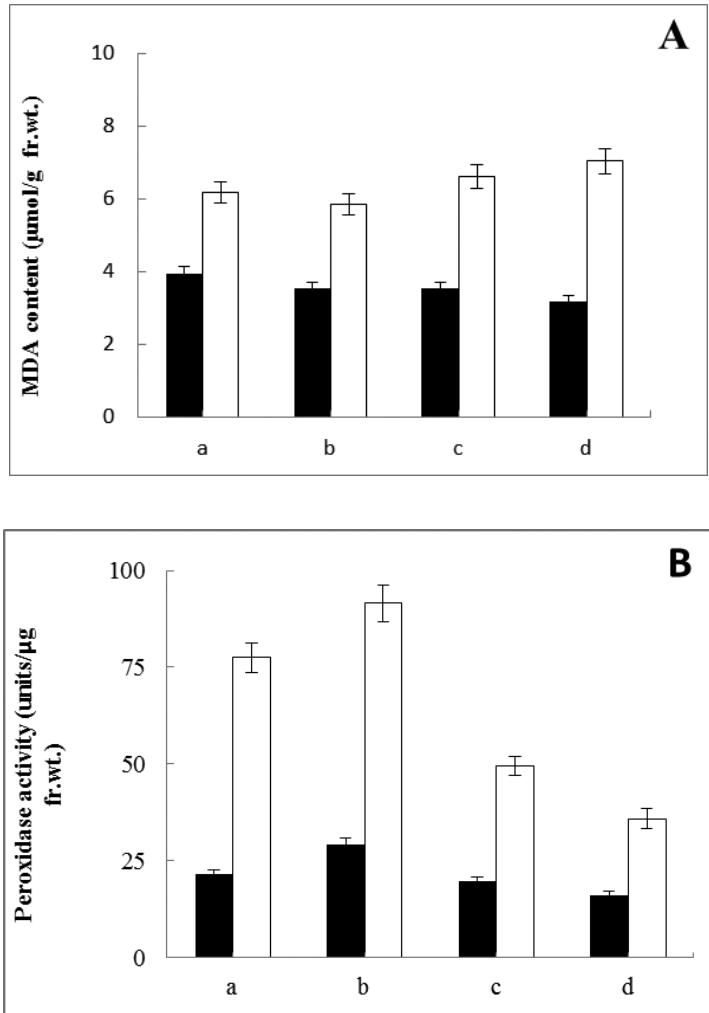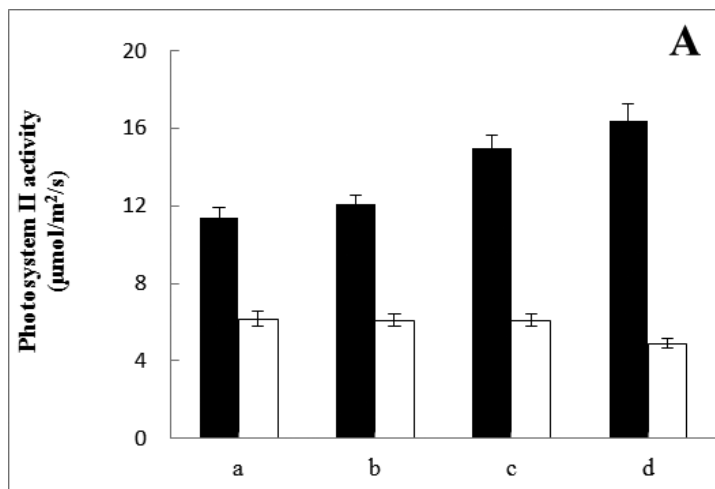
Fig 2. MDA content (A), Peroxidase activity (B) in four different energy grasses
(a - A. donax, b - S. spontaneum, c - S. arundinaceum, d - HNC) grown in nutrient optimal (black bars) and
nutrient deficient (white bars) soil. The vertical bars represent SE of the mean value of recordings from
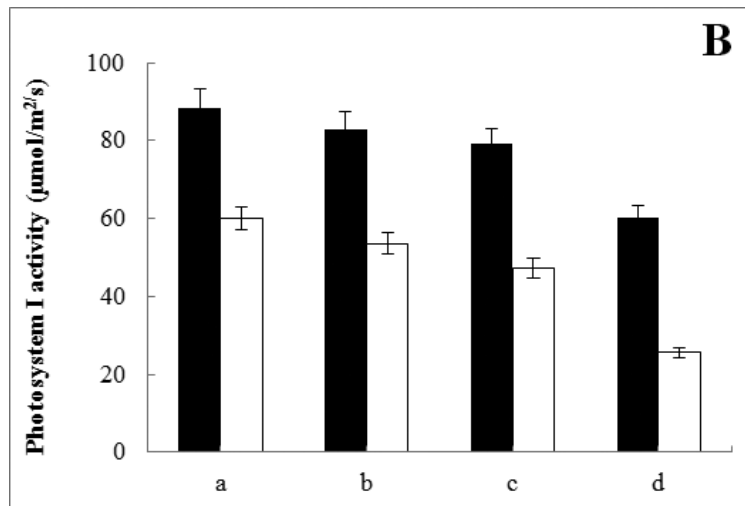3 independent experiments each with a minimum of 3 replicates.

Fig 3. Photosystem II (A) and photosystem I (B) activity in four different energy grasses (a - A. donax, b - S. spontaneum, c - S. arundinaceum, d - HNC) grown in nutrient optimal (black bars) and nutrient deficient (white bars) soil. The vertical bars represent SE of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates.

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