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Original Article

Interactive effects of O_2 level and Fe supply on growth, morphology, and mineral allocation of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1)

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Abstract

Plants that grow on wastewater are affected not only by low O_2 but also by the presence of iron leading to low efficiency for nutrient removing from the wastewater treatment systems. Currently, hybrid Napier grass (*Pennisetum purpureum* Schumach × *P. americanum* (L.) Leeke cv. Pakchong 1) was introduced for use in wastewater treatment systems. However, the ecophysiology of this species has rarely been studied. Therefore, this research investigated the effects of O_2 levels and Fe supply on growth, morphology, nitrogen uptake, and mineral allocation of hybrid Napier grass. The plants were grown on nutrient solutions with a NH_4^+:NO_3^- ratio of 75:25 at 500 µM-N under three different levels of O_2 (anoxic, hypoxic, and normoxic) with and without Fe supply (40 mg L^{-1}). Plant growth rate dramatically decreased in anoxic conditions, especially in Fe supply treatment. The root number, root length, and leaf number were negatively affected by O_2 deficiency. Both NH_4^+ and NO_3^- uptake rates were lowest in the anoxic treatment, and were also low in the presence of Fe. Under anoxic conditions, mineral concentrations of N, P, K, Mg, and Fe also decreased particularly in the roots. However, the plants responded to low O_2 by producing adventitious roots and increasing aerenchyma porosity that could support plant growth under low O_2. We concluded that this species could still grow well at low O_2 supply. Therefore, this species can be used in constructing wetlands to treat wastewater. Moreover, high nitrogen in the plant tissue makes it appropriate for animal feed or green manure.

Keywords: aerenchyma, Fe toxicity, N uptake rates, Pakchong 1, O_2 stress

1. Introduction

Water pollution is a worldwide problem affecting aquatic ecosystems and consequently human life. Wastewater from human activities, especially from non-point sources, alters the physical and chemical factors in the water columns. The major mass of wastewater flows into natural water sources, which in turn becomes contaminated with nitrogen in the forms of ammonium (NH_4^+) and nitrate (NO_3^-) (Breisha, 2010). Both NH_4^+ and NO_3^- are essential nutrients required for plants (Britto & Kronzucker, 2013). However, the excessive amount of nitrogen benefits not only aquatic plants, but also accelerates the growth of phytoplankton which can limit light penetration to the bottom of the water due to their high density (Dugdale, Wilkerson, Hogue, & Marchi, 2007; Durand, Breuer, & Johnes, 2011). The result of this limitation consequently affects O_2 dissolved in the water, which can lead to anoxic conditions. Under low O_2, most aquatic macrophytes

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exhibit decreased growth, leaf chlorosis, and decreased yields (Arslan Ashraf, 2012; Jampeetong & Brix, 2009; Maryam & Nasreen, 2012). Minerals in the plant tissue such as N, P, K, Ca, and Mg are also low when plants grow under anoxic conditions (Akhtar & Nazir, 2013). Furthermore, under anoxic conditions, ferric iron (Fe^{3+}) is reduced into ferrous iron (Fe^{2+}), which is more soluble. Excess Fe^{2+} taken up by plants causes Fe toxicity (Becker & Asch 2005). A study by Hochmuth (2011) found that rice (*Oryza sativa* L.) grown on waterlogged systems of wetland showed bronzing of the leaves. A similar result was found in a study by Takehisa and Sato (2007) in rice exposed to high external Fe (550 ppm). Concentrations of P, K, and Mg also decreased in plants supplied with high Fe concentration (Dobermann & Fairhurst, 2000; Suresh, 2005).

Wastewater runoff into water bodies needs to be treated prior to discharge. Phytoremediation using constructed wetlands (CWs) is an alternative option. The benefits include being economically and environmentally friendly (Brix & Schierup, 1989; Ghosh & Singh, 2005; Padmavathiamma & Li, 2007). Recently, the number of plant species used for wastewater treatment has increased. Survey results from 43 countries showed 150 species of plants were used in CWs. The most common genera were *Eleocharis*, *Juncus*, *Phragmites*, *Scirpus*, and *Typha* (Vymazal & Svehla, 2013).

The *Pennisetum* species is native to Africa. It is mainly used as animal feed, biofuel feedstock, and fertilizer (Jones, Devonshire, Holman, & Ajanga, 2004; Nagasuga, 2007; Singh, B.P. Singh, H.P., & Obeng, 2013). In order to develop high biomass production and quality for livestock, cross breeding of the *Pennisetum* is already being implemented. In Thailand, the hybrid Napier grass (*Pennisetum purpureum* x *P. americanum* cv. Pakchong 1) has been suggested and widely used, especially for cattle feeding. This cultivar has several advantages, including high productivity and dry matter, high protein content, and suitability for animal fodder with an average production rate of 5-8 kg $plant^{-1}cut^{-1}$ or 250-350 t ha^{-1} yr^{-1} (Premaratne & Premala, 2006). Currently, various cultivars of hybrid Napier grass are used in CWs in many countries such as India, Uganda, and Egypt. It was found that the grass was highly effective in removing nitrates, ammonium, and phosphates (Abdel-Shafy & Dewedar, 2012; Dhulap & Patil, 2014; Jaya & Vijayan, 2013). However, there is little research on the ecophysiology of the hybrid Napier grass (*Pennisetum purpureum* x *P. americanum* cv. Pakchong 1) regarding the various conditions found in wastewater. Hence, the objective of this study was to assess the growth, morphology, nitrogen uptake, and mineral concentrations of the hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) under various levels of O_2 and Fe supply. It is expected that results from this study may provide useful information for the selection of plants used for wastewater treatment in tropical constructed wetlands.

2. Materials and Methods

2.1 Plant cultivation and experimental design

Plant stocks of the hybrid Napier grass (*Pennisetum purpureum* x *P. americanum* cv. Pakchong 1) were collected from the Department of Animal and Aquatic Science, Faculty of Agriculture, Chiang Mai University, Thailand. Stems were cut from mother plants, (approximately 250-300 mm stem sections). They were subsequently immersed in shallow water in plastic trays (27x54x5 cm) until new shoots with fibrous roots emerged. Then, the new shoots were grown on a full strength standard N and P free growth medium prepared according to Smart and Barko (1985), to which micronutrients (TROPICA, Denmark), 100 µmol L^{-1} of KH_2PO_4 and 500 µM of NH_4NO_3 (NH_4^+:NO_3^- ratio of 75:25) were added. The pH was adjusted to 6.5. All plants were cultivated for 45 days in the greenhouse at the Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The light regime in the greenhouse was 12.54 h light/11.06 h dark and the temperature was 25-31 ºC by day and 18-22 ºC by night.

Plants (n=5) with similar size were selected, measured for fresh weight, and dried in a hot-air oven at 60 °C. Then, the dry weight was measured and the dry weight: fresh weight ratio (DW/FW ratio) was calculated. For the experiment, 24 similar-sized plants (approximately 43.0-90.0 g fresh weight and 520-780 mm tall) were selected. These were separated into six treatments (n=4) combining three levels of O_2 (normoxia, hypoxia, and anoxia) and two levels of Fe availability (0 and 40 mg L^{-1} Fe prepared from $FeSO_4.7H_2O$). The three O_2 levels were set up according to Jampeetong and Brix (2009): (i) normoxic condition by continuous bubbling of atmospheric air; (ii) hypoxic condition by continuous bubbling of N_2 gas; and (iii) anoxic condition by continuous bubbling with N_2 gas. $Na_2S_2O_4$ (0.1 g L^{-1} growth medium) was added as a reducing reagent. For the anoxic treatment, the top of the water surface was covered by polyethylene sheets to prevent the diffusion of air into the water. In all treatments, the O_2 concentrations in the water were checked daily using an O_2 electrode (Table 1). All plants were grown hydroponically in 5 L plastic containers with diameters of 0.25 m and height of 0.22 m. Full strength standard N and P free growth medium was prepared according to Smart and Barko (1985) to which micronutrients (TROPICA, Denmark), 100 µmol L^{-1} of KH_2PO_4 and 500 µM of NH_4NO_3 (NH_4^+:NO_3^- ratio were 3:1) were added. The pH was adjusted to 6.5. The growth medium was changed every day to maintain the O_2 conditions.

Table 1. Concentrations of O_2 dissolved in the water (DO) in 6 treatments combining three levels of O_2 (normoxia, hypoxia and anoxia) and two levels of Fe availability (0 and 40 mg L^{-1}).

	Anoxia		Hypoxia		Normoxia	
	Fe supply	Without Fe	Fe supply	Without Fe	Fe supply	Without Fe
DO (mg L^{-1})	0.00-0.21	0.00-0.20	2.38-2.57	2.45-2.53	4.22-5.20	4.41-5.18

2.2 Growth study and morphology

On day 15, plant morphology was recorded, including total plant height, root numbers, root length, leaf number, and average leaf area. Then, all plants were cleaned and harvested. The plants were separated into 3 segments (roots, stems, and leaves). All plant parts were then freeze-dried and the dry weights were measured. The relative growth rate (d^{-1}) was calculated using the formula RGR = (ln final dry mass – ln initial dry mass)/(day_t-day_0) where day_t was the final day and day_0 was the initial day of the experiment and the shoot elongation rate (SER, mm d^{-1}) was calculated from the increase of total shoot length throughout the period of the experiment divided by the number of days.

2.3 NO_3^- and NH_4^+ uptake rate

Before plant harvest, the NO_3^- and NH_4^+ uptake rates were studied on day 11 and day 13, respectively. All plants of each treatment were pre-incubated in filtered water for at least 18 h in the same growth condition. The plants were then placed in glass jars containing 2 L of 150 µM of NH_4^+ or NO_3^-. Samples were collected once an hour during a 6-h period. The NH_4^+ concentration was measured using the salicylate method (Jampeetong & Brix, 2009). The NO_3^- concentration was measured following the method of Cedergreen and Madsen (2003). The nitrogen uptake rate was calculated from the linear regression curve related to volume and root DW.

2.4 Chlorophylls

Chlorophyll concentrations (Chl *a*, Chl *b*, and total Chl *a* + *b*) were analyzed and calculated according to Lichtenthaler (1987). The freeze-dried leaves were cut into small pieces, and 5 mg sub-samples were soaked in 96% ethanol and placed in dark conditions at room temperature for 24 h. Subsequently, absorbance of the extractions was measured at 648.6 nm and 664.2 nm using a UV-VIS spectrophotometer.

2.5 Mineral elements

One hundred and fifty to 180 milligrams of subsample of dried leaves, roots and stems were cut into fine pieces and digested in a mixture of acid solution (conc. H_2SO_4, Na_2SO_4 100 g and selenium 1 g) at 100-380 °C. Nitrogen (N) in the plant tissue was analyzed using the Kjeldahl method (Hanlon, Gonzales, & Bartos, 1994) and phosphorus (P), potassium (K), magnesium (Mg), and iron (Fe) were analyzed according to Chapman and Pratt (1978).

2.6 Plant anatomy

The root cross section was conducted by free-hand section technique. The mature roots (n=4) were cut at the center of the root. The sections were then stained with 0.05% Safranin O. Arenchyma development was recorded and areas of air space in the roots were observed under a 40× light transmission-microscope.

2.7 Statistics

All statistical analyses were performed using SPSS statistics, version 17.0. (SPSS Inc., Chicago, IL, USA). The data were tested for the normal distribution and both one-way and two-way analysis of variance (ANOVA). The differences between treatments were identified by the Tukey's HSD test at a 5% significance level.

3. Results

3.1 Growth and morphology

Generally, growth of the Napier grass was significantly affected by both O_2 levels and Fe supply. The relative growth rate (RGR) of the plants grown in hypoxic conditions did not significantly differ from the plants grown under normoxic conditions. But the plants showed significantly decreased RGR when they grew in anoxic condition with Fe addition, and there was no interaction between the O_2 level and Fe supply (Figure 1a, Table 2). Similarly, the shoot elongation rates (SERs) of the plants grown in anoxic conditions were significantly decreased in both the Fe-supplied plants and non Fe-supplied plants (Figure 1b). Similarly, O_2 levels had significant effects on the total leaf and stem dry weight and a significant interaction between Fe supply and O_2 level on total root and leaf dry weight was observed (Table 2). Without Fe supply, the total root and leaf dry weights were significantly decreased under both anoxic and hypoxic conditions compared with normoxic conditions. In contrast, the total root number and leaf dry weight of the Fe

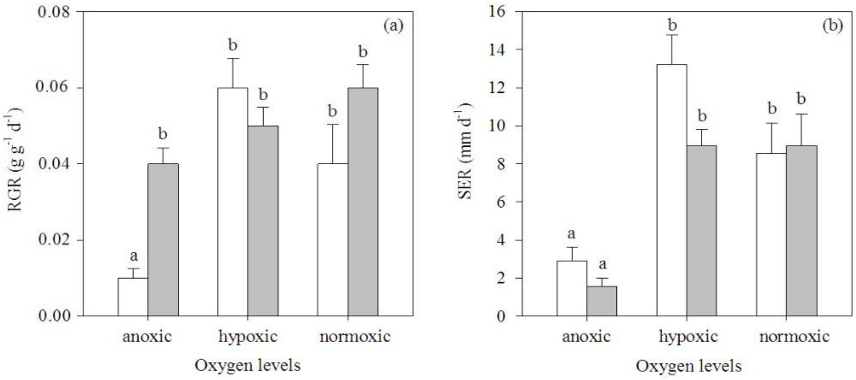

Figure 1. Relative growth rate, RGR (a) and shoot elongation rate, SER (b) of hybrid Napier grass (*Pennisetum purpureum* ×*P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply. Different letters above columns indicate significant differences between treatments.

Table 2. Results of a two-way ANOVA (F-ratio) showing the effects of O_2 levels (anoxia, hypoxia and normoxia) and Fe supply and their interaction on growth, morphological characteristics, total dry weight (DW) of leaves, stems and roots, shoot: root ratio, NH_4^+ and NO_3^- uptake rate, chlorophyll and mineral concentrations of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1).

	Main effects		Interaction
	O_2 level	Fe supply	O_2 level x Fe supply
Relative growth rate (RGR) (g g^{-1} d^{-1})	2.54	3.32	1.37
Shoot elongation rate (SER) (mm d^{-1})	7.11**	1.53	0.79
Root number	8.82**	5.12*	0.95
Root length (cm)	14.86***	0.00	0.13
Leaf number	13.20***	0.03	0.35
Average leaf area (cm^2)	2.04	0.09	1.59
Total root dry weight (g)	2.27	0.46	8.40***
Total leaf dry weight (g)	4.20*	0.03	3.81*
Total stem dry weight (g)	4.09*	0.34	2.57
Shoot:root ratio	3.70*	0.18	0.81
NH_4^+ uptake rate (µmol NH_4^+ g^{-1} root DW h^{-1})	22.62***	36.00***	0.63
NO_3^- uptake rate (µmol NO_3^- g^{-1} root DW h^{-1})	45.61***	2.37	3.95*
Chl *a* (mg g^{-1} DW)	5.80*	25.74***	1.00
Chl *b* (mg g^{-1} DW)	1.44	1.97	0.08
Total Chl *a* + *b* (mg g^{-1} DW)	13.06***	41.99***	0.98
Lysigenous air space of the roots (cm^2)	1.70	0.61	0.79
N (mg g^{-1} DW)			
Leaves	6.27**	2.69	5.53*
Stems	0.23	0.11	0.41
Roots	16.37***	0.19	0.73
P (mg g^{-1} DW)			
Leaves	7.59**	272.42***	8.98**
Stems	5.03*	851.50***	5.32*
Roots	7.18**	130.81***	6.51**
K (mg g^{-1} DW)			
Leaves	2.90	137.15***	3.78*
Stems	4.83*	61.26***	6.15**
Roots	72.69***	132.14***	35.31***
Mg (mg g^{-1} DW)			
Leaves	9.02**	6.17*	1.94
Stems	5.76*	1.00	0.18
Roots	16.04***	5.81*	2.67
Fe (mg g^{-1} DW)			
Leaves	3.37	0.15	0.85
Stems	1.33	15.67**	3.59*
Roots	4.53*	17.29**	0.86

*P<0.05, **P<0.01, ***P<0.001

supplied plants increased in the hypoxic treatment. However, in anoxic conditions with Fe supply, there was still a reduction of total leaf dry weight. Plant morphology, root number and length, and leaf number were affected by the O_2 level, whereas the Fe supply did not influence the root and leaf morphology. Under anoxic conditions, new root and leaf production was reduced compared with plants grown in hypoxic and normoxic conditions (Figure 2a, c). These plants also showed decreased root length (Figure 2b). However, the average leaf areas of the plants in each treatment were not significantly different (Figure 2d).

3.2 N uptake rate

Both NH_4^+ and NO_3^- uptake rates decreased when the O_2 supply to the roots decreased and a dramatic reduction in N uptake rates was found in the Fe supplied plants. Under anoxic conditions, the plants that were grown without Fe-supply had a higher uptake rate for NH_4^+ than NO_3^- and the plants also had higher NH_4^+ uptake rate than the plants grown with Fe-supply (Figure 3).

3.3 Chlorophyll concentrations

High concentrations of Chl *a* were found in the plants grown with and without Fe supply, and there was no significant difference between hypoxic and normoxic conditions. The plants supplied with Fe had a very low concentration of Chl *a* when grown in anoxic conditions (Table 3). However, no differences in Chl *b* concentrations among treatments were found.

3.4 The mineral elements

Mineral elements in the plant tissue were affected by O_2 levels and Fe supply. However, the Fe concentrations in

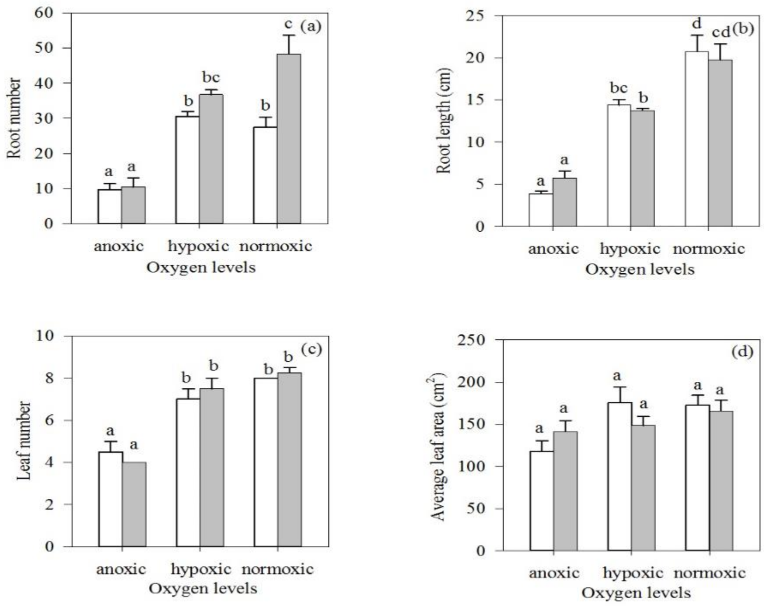

Figure 2. Root number (a), root length (b), leaf number (c) and average leaf area (d) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply. Different letters above co-lumns indicate significant differences between treatments.

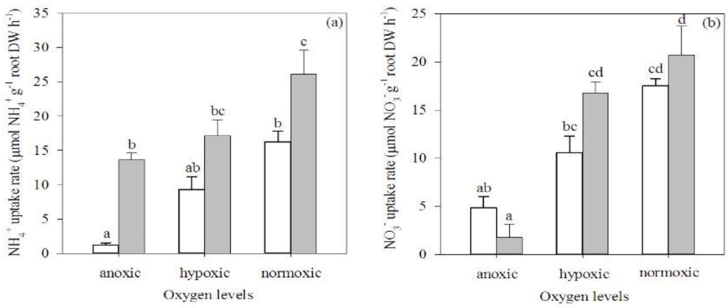

Figure 3. NH_4^+ uptake rate (a) and NO_3^- uptake rate (b) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply. Different letters above columns indicate significant differences between treatments.

Table 3. Concentrations of chlorophylls in leaves (mean±SE) of hybrid Napier grass (*Pennisetum purpureum* x *P. americanum* cv. Pakchong 1) grown with different O_2 levels (anoxia, hypoxia and normoxia) and with or without Fe supply. Different letter superscripts between columns indicate significant differences between treatments.

	anoxia		hypoxia		normoxia		*F-ratio*
	Fe supply	without Fe	Fe supply	without Fe	Fe supply	without Fe	
Chl *a* (mg g^{-1} DW)	$0.04±0.01^a$	$0.18±0.07^{ab}$	$0.15±0.04^a$	$0.41±0.04^b$	$0.13±0.03^a$	$0.42±0.09^b$	7.87***
Chl *b* (mg g^{-1} DW)	0.13±0.04	0.20±0.03	0.20±0.07	0.29±0.04	0.23±0.07	0.28±0.08	1.00
Total Chl *a* + *b* (mg g^{-1} DW)	$0.17±0.04^a$	$0.38±0.07^a$	$0.34±0.05^a$	$0.70±0.04^b$	$0.37±0.08^a$	$0.70±0.04^b$	14.01***

***P<0.001

the leaves and N in the stems were not affected by either factor (Table 2). However, the concentrations of N, K, Mg, and Fe in the leaves decreased in the plants grown under anoxic conditions but not significantly different from the other treatments (Figure 4). In the roots, the concentrations of N, K, Mg, and Fe of the plants grown under anoxic conditions decreased and were significantly different compared with the hypoxic and normoxic conditions (Figure 5). In the stem, the P and K concentrations also decreased under anoxic conditions, and especially P decreased in the plants supplied with Fe (Figure 6).

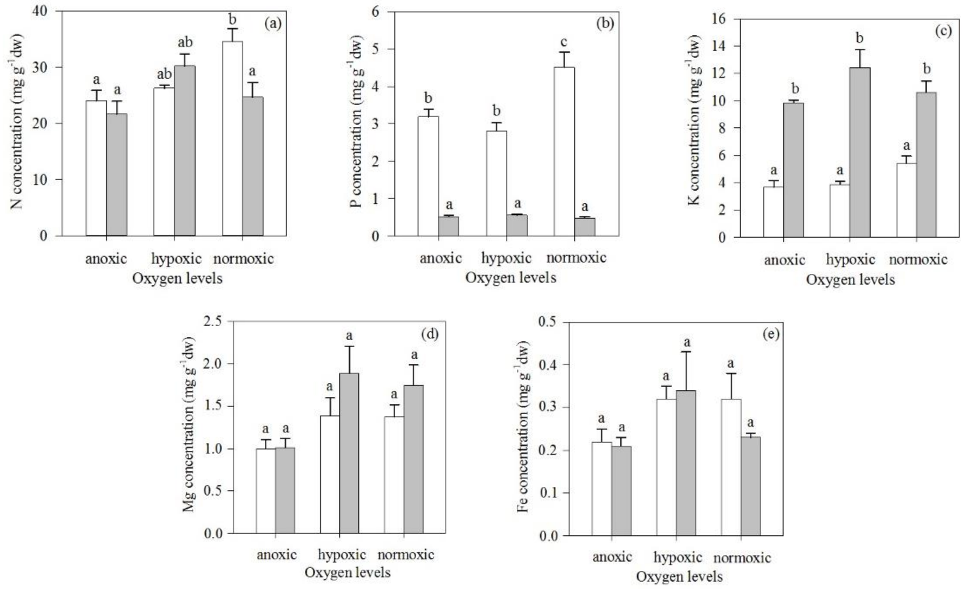

Figure 4. Concentrations of N (a), P (b), K (c), Mg (d) and Fe (e) in leaves of hybrid Napier grass (*Pennisetum purpureum* ×*P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply. Different letters above columns indicate significant differences between treatments.

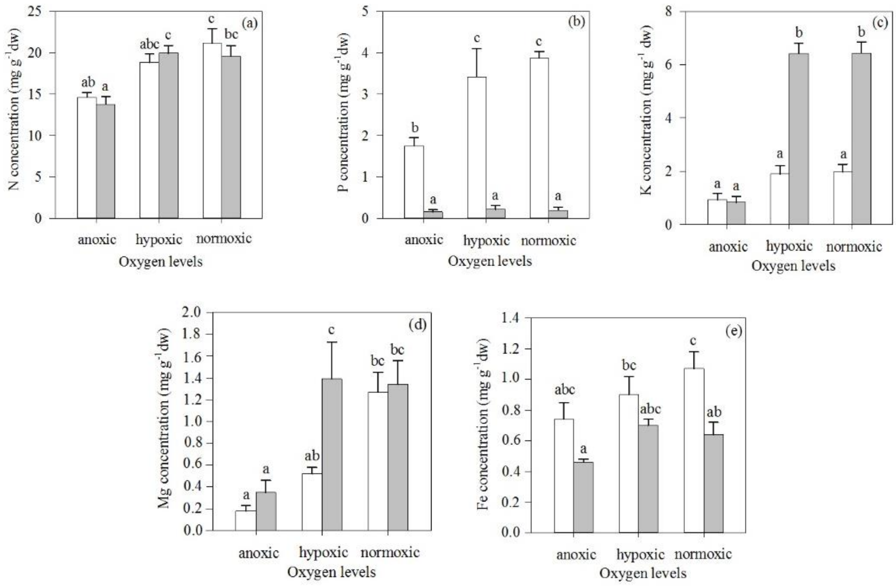

Figure 5. Concentrations N (a), P (b), K (c), Mg (d) and Fe (e) in roots of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply. Different letters above columns indicate significant differences between treatments.

3.5 Aerenchyma formation

The lysigenous air space of the roots was not affected by O_2 levels and Fe supply. The average area of the air spaces trended to increase when the plants were grown under anoxic conditions, particularly in the Fe-supplied plants (Figure 7).

4. Discussion

This study showed that O_2 levels had a stronger effect on the hybrid Napier grass cv. Pakchong 1 than Fe addition. Under anoxic conditions, the plants showed a drastic decrease in RGRs, in particular the plants supplied with Fe. Our previous studies showed similar results when this species

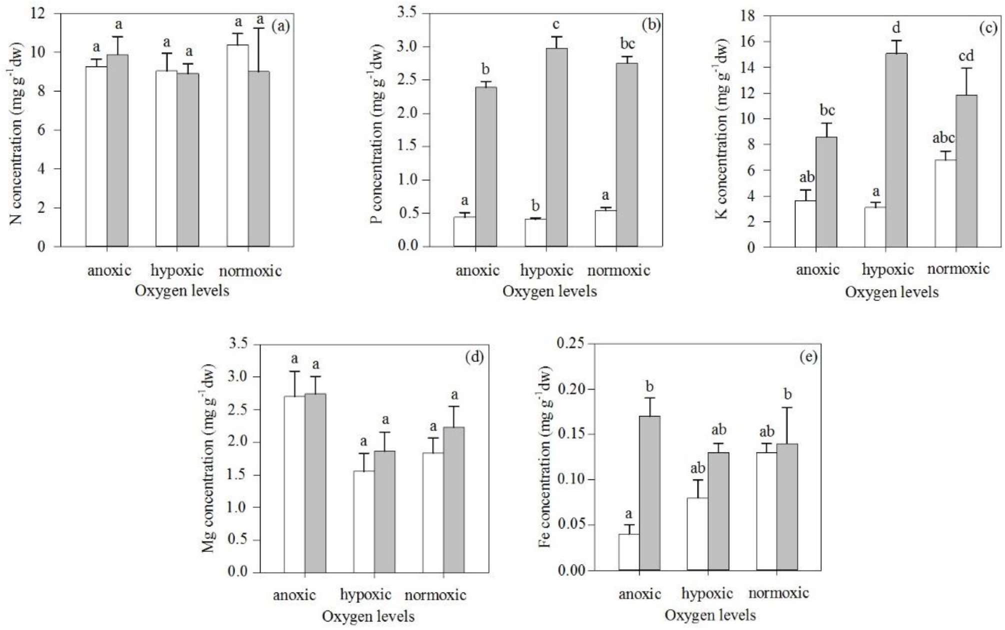

Figure 6. Concentrations of N (a), P (b), K (c), Mg (d) and Fe (e) in stems of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply. Different letters above columns indicate significant differences between treatments.

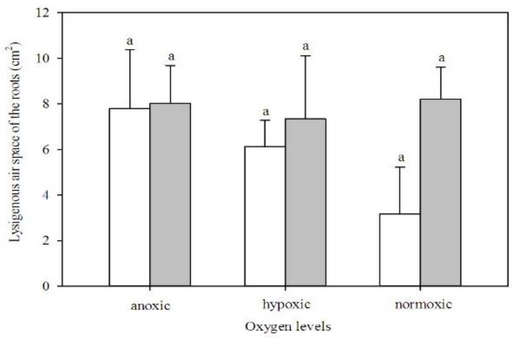

Figure 7. Lysigenous air space of the roots of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) grown at different O_2 levels (anoxic, hypoxic and normoxic), with Fe (☐) and without Fe (■) supply.

was grown under deficient O_2 conditions coupled with high NH_4^+ concentrations (Jampeetong & Muenrew, 2016). In this study, the RGRs were also affected by Fe supplements. It is likely that under anoxic conditions, the ferric form of iron (Fe^{3+}) was reduced to the toxic ferrous form (Fe^{2+}). The ferrous form was subsequently absorbed by the plants and led to tissue damage as evident from the chlorosis on several leaves. The damage could be observed under anoxic conditions in which the concentration of Fe in the leaves and roots were found to be high. However, they were not significantly different from the plants grown under normoxic conditions. Other studies also found that *Oryza sativa* grown under flooded soils showed bronzing of leaves due to excess Fe^{2+} uptake (Becker & Asch, 2005). Similarly, other studies showed decreased plant growth under Fe concentration in the range of 100-250 mg L^{-1} (Pena-Olmos, Casierra-Posada, & Olmos-Cubides, 2014). However, the levels of Fe that are toxic to plants are different between species. In some species the RGRs decreased when the plant was exposed to 10-40 mg L^{-1} of Fe (Nenova, 2006). For the hybrid Napier grass in this study, the RGRs were not significantly different between the treatments in both the normoxic and hypoxic conditions. This may be due to the low Fe concentration supplied (40 mg L^{-1}), which is not toxic for this species.

The results from this study showed that the roots were sensitive to O_2 stress, especially in anoxic conditions regardless of Fe treatments. Most plants had short rotting roots, and a decreased number of roots compared with hypoxic and normoxic conditions. Hence, the deleterious effects on roots may lead to a reduction of plant leaf and stem total dry weight. The average leaf area and leaf number were also reduced in the anoxic condition. Similar results including decreased plant height, leaf area, and root length were found in the study of Promkhambut, Polthanee, Akkasaeng and Younger (2011). However, it is worth noting that plants will exhibit morphological adaptations to increase their survival rates under anoxic conditions. This is achieved by production of adventitious roots from the stem node above the water surface and increased air space in the root cortex (lysigenous aerenchyma). These root adaptations are likely to increase root porosity, which in turn facilitates O_2 transport. Similar results have been found in *Triticum aestivum* L. grown in waterlogged conditions where plants developed extensive adventitious roots and aerenchyma (Hossain & Uddin, 2011; Parent, Capelli, Berger, Crevecoeur, & Dat, 2008).

The hybrid Napier grass had higher uptake rates for NH_4^+ than NO_3^- when grown at all three levels of O_2 (normoxic, hypoxic, and anoxic). This is because NO_3^- uptake requires more energy than NH_4^+ (Konnerup & Brix, 2010). A higher uptake rate of NH_4^+ was reported in many species, such as *Phragmites australis* (Cav.) Trin. ex Steud., *Glyceria maxima* (Hartm.) Holmb., *Canna indica* L., and *Schoenoplectus validus* (Vahl) A. Love & D. Love (Munzarova, Lorenzen, Brix, & Votrubova, 2005; Zhang, Rengel, & Meney, 2009). A combination of O_2 deficiency and Fe toxicity has the effect of decreasing the uptake rates of NH_4^+ and NO_3^-. These two conditions have a high correlation to the decrease in the number and length of roots, which can be observed by a hindered ability to absorb nutrients, which in turn can be noticed from the concentration of N in the leaves and roots of the plants. Under anoxic conditions, the concentration of N was found to be lower than under hypoxic and normoxic conditions. However, the concentration of N in plant tissue was still high especially in plant leaves (13.54-34.62 mg g^{-1}DW) which indicated its high protein content. Thus, this plant can be good for animal feed after harvest.

The chlorophyll contents of Chl *a*, Chl *b*, and total Chl *a*+*b* of hybrid Napier grass were reduced due to O_2 deficiency and Fe toxicity. It is possible that these treatments caused lower uptake of nutrients, especially N and Mg, which are major components of chlorophyll (Soetan, Olaiya, & Oye wole, 2010). This hypothesis was well supported by the plants grown under anoxic conditions, in which N and Mg concentrations in leaves and roots were lower than in the plants grown under hypoxic and normoxic conditions. In other studies, it was reported that the chlorophyll content was reduced by 50% in *Iris pseudacorus* L. grown under anoxic conditions (Schluter & Crawford, 2001). In addition, a study by Xing, Huang and Liu (2009) showed that *Spirodela polyrrhiza* (L.) Schleid. had reduced Chl *a*, Chl *b*, and total Chl *a*+*b* concentrations and carotenoids due to excessive uptake of Fe. In the present study, plants grown under anoxic conditions with Fe supplement showed a similar trend in reduced concentrations of K and Mg. Similarly, it was reported that *Annona glabra* L. grown under flooded conditions in the presence of Fe were found to have decreased concentrations of K and Mg in the leaves (Ojeda, Schaffer, & Davies, 2004). Besides that, under anoxic conditions, both with and without Fe supply, it was observed that a decrease of N, P, K, Mg, and Fe accumulations in the roots was evident compared with normoxic and hypoxic conditions. In other studies, *T. aestivum* and *Hordeum vulgare* L. grown under O_2 deficiency were found to have decreased uptake of trace elements (Steffens, Hutsch, Eschholz, Losak, & Schubert, 2005; Wlodarczyk *et al.,* 2001). These results suggest that O_2 plays important roles in root respiration and nutrient uptake. However, regardless of O_2 supply, Fe addition can promote P uptake resulting in high P concentrations in leaves and roots. Similar results were found in *Phragmites australis* (Cav.) Trin ex. Steudel where the plants grown under high Fe^{2+} concentrations had increased uptake of phosphate (Batty & Younger, 2003).

In conclusion, the hybrid Napier grass cv. Pakchong 1 can grow well under normoxic and hypoxic conditions. But under anoxic conditions, the plants exhibited decreased growth rate, root number, root length, and leaf number, particularly in the Fe-supplied plants. Moreover, under anoxic conditions, the nitrogen uptake and mineral elements in the plant tissue were reduced in the Fe-supplied plants. Due to its ability to grow under low O_2 conditions, the plants can adapt to low O_2 by increasing adventitious roots and root porosity. This may enable them to maintain N uptake, both NH_4^+ and NO_3^-, resulting in high nitrogen accumulation in shoots. Therefore this species could be good for treating wastewater in CWs where hypoxic conditions are usually found as well as the presence of iron. The plants also can be used for animal feed after harvesting. However, further study of the performance of this species in real constructed wetlands is still needed.

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