CHAPTER 3

CHLOROPHYLL CONTENT AND FLUORESCENCE IN *SCIRPUS GROSSUS* L. GROWN IN DIFFERENT SOIL, FERTILIZER AND WATER DEPTH CONDITIONS

3.1.1 Photosynthetic assessment

The leaf of a plant carry out photosynthesis in organelles known as chloroplasts, where the photosynthetic apparatus are located in the thylakoid membranes and the stroma. Ultimately this important process results in the production of organic matter, initially in the form of starch and sucrose and later in the form of fatty acids, amino acids and other life supporting molecules. Photosynthesis depends on a large number of factors, externally, mainly light, temperature and carbon dioxide concentration, amongst others. Foremost, within the plant itself, is the chlorophyll content of the leaves as it is the major light harvesting pigment in the photosynthetic process. It has been reported that the rate of photosynthesis increases with increasing chlorophyll content (Whitelam and Halliday 2007). Studies have shown that the application of macro-nutrients such as NPK can lead to an increase in the levels of photosynthetic pigments and metabolites in foliar tissues of the leafy vegetable *Beta vulgaris* and subsequently its photosynthetic capacity (Singh *et al.* 2005).

In an attempt to provide a theoretical basis for monitoring the growth and development of winter wheat and for providing guidance on the application of fertilizer, Huang *et al.* (2010) reported that the leaf concentration of chlorophyll are affected by the crop growth status and can be a guide for fertilizer and irrigation management. Serrano *et al.* (2000) reported in one of their studies on the detection of nitrogen concentration in the soil and its impact on chlorophyll content, low concentrations of nitrogen caused a decrease in chlorophyll content while high concentrations of nitrogen caused an increase in chlorophyll content. Thus measuring chlorophyll content is one approach that can be used to determine the extent of N limitations in plants, as

chlorophyll constitutes a major component of N content in plants (Plaxton and McManus 2006), as each of its four pyrrole rings has a nitrogen atom.

The NPK fertilizer has often been used to enrich soil and increase crop productivity and yield. It has been well documented in the literature that nitrogen, potassium, and phosphorous are important macroelements for healthy plant growth, in addition to other macro- and microelements (Daughtry *et al.* 2000). Nitrogen is present in all the macromolecules in the cell, such as amino acids, proteins, lipids and carbohydrates. Probably more importantly, nitrogen concentration in green vegetation is often related to chlorophyll content, and therefore indirectly to one of the basic plant physiological processes, namely photosynthesis (Daughtry *et al.* 2000). Recently, Huang *et al.* (2010) has shown in a study on rice seedlings that nitrogen deficiency brought about adverse effects on the chlorophyll content of the leaves and chlorophyll fluorescence, both of which are good indicators of photosynthetic capacity. Thus nitrogen deficiency in soils will result in plants exhibiting limited growth and deficiency symptoms such as chlorosis. Many studies have shown that a significant increase in growth rate of plants will occur with the application of nitrogen (Ozer *et al.* 2003). Baki (1988) reported that additions of phosphate enhanced the rate of flowering in *S. grossus.*

It was reported that increasing of nitrogen fertilization had an effect on the leaf, stem, rhizome, and root growth and thus caused an increase in the growth of seedlings (Alderman *et al.* 2011). Similarly it has been reported in *Panicum virgatum*, there was an increase in biomass at harvesting because of the increase in nitrogen in the soil (Jung and Lai 2011). Under drought stress in 240 kg N ha^{-1} treatment reported that nitrogen limitation caused a decrease in chlorophyll and protein concentration in the leaf (Verma *et al.* 2000; Singh *et al.* 2005). In a study to evaluate nitrogen fertilizer impact on photosynthesis it was reported that an additional nitrogen fertilizer dose of 60 kg ha^{-1}

was most effective in increasing chlorophyll content in hemp leaves and improving chlorophyll *a* fluorescence parameters (Maļceva *et al.* 2011). Nitrogen deficiency decreased photosynthetic reaction rate in Leymus chinensis especially after long-term soil drought. It decreased plant biomass, leaf-biomass ratio between the green leaf and total plant biomass, net photosynthetic rate (Xu and Zhou 2006).

In *vivo* measurements of chlorophyll fluorescence (Fv/Fm) determined using non-destructive and non-invasive techniques, have been used to evaluate the physiological effects in plants undergoing different treatments, to see how it affects photosynthetic capacity. It was also correlated with leaf potassium (K) levels in Cleopatra mandarin in its grafted combination, with leaf phosphorus (P) in Cleopatra mandarin grafted on Alemow and with leaf nitrogen in Cleopatra mandarin. Results showed that chlorophyll fluorescence differed according to whether it was used alone or in combination, and it also differed from Cleopatra mandarin in different K and P concentrations (Francisco *et al.* 2011). Potassium (K) plays an important role in leaf photosynthesis, and the K^+-efficient rice genotype could maintain a significantly higher net photosynthetic rate compared to K^+ deficient rice (Yanbo *et al.* 2008). K^+ can influence the photosynthesis process in a number of ways. Studies have shown that leaf K^+ concentrations of less than <0.7–0.8% K^+ limited the leaf net CO_2 assimilation rate and the relative limitation of photosynthesis due to non-stomatal conductance (g_m) and stomatal conductance (g_s) decreased with increasing supplies of K^+. These results indicated that the photosynthetic rate is primarily limited by the biochemical processes of photosynthesis, rather than by g_m and $g_{s,}$ in K^+ deficient plants (Song *et al.* 2011). Chlorophyll fluorescence measurements have also shown that the quantum yield of photosystem II increased with K^+ addition. These results confirmed that mineral nutrient addition can enhance the photosynthetic processes (Pasquini and Santiago 2011).

3.1.2 Objectives of study

In this study leaf chlorophyll content and chlorophyll fluorescence was determined to gauge the health and photosynthetic capacity of *S. grossus* grown under different soil, fertilizer regimes and water depth conditions.

MATERIALS AND METHODS

3.2.1 Plant materials

Young ramets of *S. grossus* at 2-3-leaf stage were obtained from rice fields in Tanjung Karang, Selangor.

3.2.2 Experimental sites

Synthetic populations of *S. grossus* were established on peat soils at the Malaysian Agriculture Research Development Institute (MARDI) Research Station, Jalan Kebun, Klang (N 2.98^{o} / E 101.50^{o}), Malaysia for 24 weeks commencing on 24 February 2010 and While paddy soil in (MARDI) Research Station, Tanjung Karang, Selangor (N 3.28^{o} / E 101.08^{o}), Malaysia for 24 weeks commencing on 26 October 2010 (Fig. 2.1, Fig. 2.2 and 2.3).

3.2.3 Experimental design

Experiments were executed following a randomized complete block design (RCBD) as shown in Chapter 2 (Fig. 2.6).

3.2.4 Effect of fertilizer application experiments

Each ramet was planted in the centre of a plot measuring 2m x 2m, previously demarcated and lined with 5 cm x 5 cm grids and sub-plots. Fertilizer applications with Nitrophoska Blue Special NPK fertilizer at the rate of 100:30:30 were made one week prior to planting. A set of 3 replicated plots with fertilizer application was allocated while another 3 sets devoid of fertilizer application served as control. Watering of the plots was made twice daily, one in the morning and the other in the late afternoon using a fine rose fitted to a water hose. No weeds were allowed to grow in the plots during experimentation (Fig. 2.6).

3.2.5 Effect of water depth experiments

Each young ramet of *S. grossus* was planted in the center of the frame of 20X 40X cm in size, on paddy soil type from the MARDI Station of Tanjung Karang. A set of 3 replicates were allocated (R1, R2, R3) with 4 levels of water depth (D1 = 0 cm water depth (control); D2 = 5 cm; D3 = 10 cm; D4 = 20 cm) (Figs. 2.7 and 2.8).

3.2.6 Leaf number per ramet (plant) and phenology of ramet populations

For each replicated mother plant or plot, a set of 15 emerged aerial ramets were selected for the number of leaves that were produced per ramet was also recorded, again at weekly basis until the end of experimentation.

3.2.7 Determination of chlorophyll content

Chlorophyll content of leaves was recorded using a SPAD value meter (Minolta Japan). The SPAD value of the leaves was determined on a weekly basis. For each plot, 15 leaves were randomly selected for measurements per treatment (Fig. 3.1).

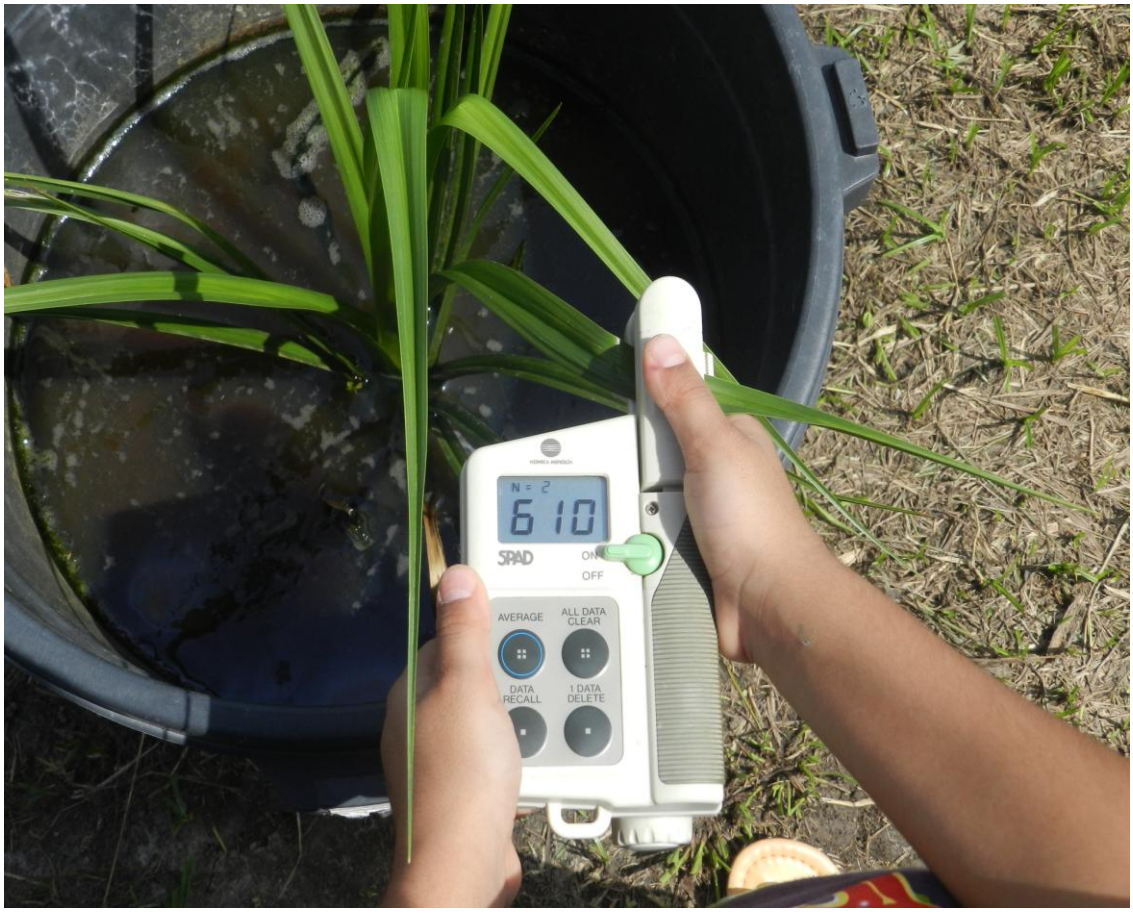

Fig. 3.1 Chlorophyll content measurements in *Scirpus grossus* were taken every month during the experimentation period in University Putra Malaysia using a Minolta chlorophyll meter.

3.2.8 Determination of chlorophyll fluorescence/quantum efficiency of photosynthesis

Chlorophyll fluorescence can provide detailed information on the saturation characteristics of electron transport, as well as the overall photosynthetic performance of a plant (Ralph and Gademann 2005). Generally, chlorophyll fluorescence is the light energy absorbed by chlorophyll molecules in a leaf that is re-emitted as light, instead of use for photosynthetic purposes and dissipated as heat. Thus, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be obtained (Maxwell and Johnson 2000).

Leaves of selected uniformed branches were used for chlorophyll fluorescence determination between 12.00 to 2.00 pm on a sunny day during the fruit developmental stage. Chlorophyll fluorescence was determined using a Plant Efficiency Analyzer (Hansatech Instruments Ltd., England, Figs. 3.2 and 3.3). A leaf clip was attached to the leaf and kept in the dark for at least 10 min minutes for dark adaptation to take place. Then the shutter plate was opened and light was applied on the leaf. The fluorescence signal was determined for 3 second and the quantum yield or photosynthetic yield determined as Fv/Fm. The maximum fluorescence (F_m) and minimum fluorescence (F_0) was read from the display pad of the Plant Efficiency Analyzer and the variable fluorescence (F_v) calculated as F_m-F_0. The quantum yield was determined as being equal to F_v/F_m.

The dark-adapted values of Fv/Fm are used as a sensitive indicator of plant photosynthetic performance, with optimal values of around 0.83 measures for most plant species. Values lower than 0.82, are normally indicative of plants undergoing stress.

Fig. 3.2 Photosynthetic efficiency analyzer (Hansatech Ltd. UK).

Fig. 3.3. Chlorophyll fluorescence measurements of *Scirpus grossus* in MARDI Research Stations, Jalan Kebun, Selangor, Malaysia.

3.2.9 Determination of biomass of leaves, stem and flower of *Scirpus grossus* plants in fertilized and unfertilized peat and paddy soils

After 24 weeks 10 samples were randomly selected for measurements per treatment for each plot. The biomass of plant parts we used weight measure. The biomass of plant parts was determined using a weighing balance. The biomass of plant parts was determined using a weighing balance

2.2.10 Pot experiment: Effect of water depth and different concentration of fertilizer experiments on the growth of *Scirpus grossus*

Paddy soil of Jawa series from the MARDI Station of Tanjung Karang were placed in the lower part of pots at a of depth of 20 cm, while the top part were covered with water at 4 different levels of water depth (D1 = 0 cm water depth (control); D2 = 5 cm; D3 = 10 cm; D4 = 20 cm) by making some holes at the required level of water depth. In the same experiment different fertilizer concentrations were applied. Four levels of NPK fertilizer concentrations (F2, F3, F4, F5), were used with F0 as control, and F2 = 50 g/500 ml; F3 = 75 g/750 ml, F4 = 100 g/1000 ml, F5 = 125 g/1250 ml. Each young ramet of *S. grossus* was planted in the center of the pots measuring 20 x 40x cm in size. A set of 3 replicates were allocated (R1, R2, R3), encompassing 60 plots in a randomized complete block design (RCBD) (Fig. 2.9).

3.2.11 Determination of physico-chemical characteristics and nutrient status of matured *Scirpus grossus* plants grown in fertilized and unfertilized peat and paddy soils

After 24 weeks of growth *S. grossus* plant samples from Jalan Kebun and Tanjung Karang, respectively, were taken for physico-chemical analysis. The plant samples were sent to a laboratory in the Malaysian Agriculture Research and Development Institute (MARDI) for analysis. The analyses focused on the major elements, moisture, total nitrogen, pH and protein content.

2.2.12 Data analysis

Data were transformed to $\log^{+1}$ or log prior to statistical analysis and subjected to one-way ANOVA and Tukey's HSD tests wherever appropriate, using the SAS Computer Programs. Further analyses were made to determine the significance of planting in fertilized soil and unfertilized soils, planting in different soils (peat and paddy soils) and weekly differences in the rate of increase or recruitment of any parameter, e.g. leaf number, rhizome length, ramet number, mortality number, plant height, flower number by regressing the recruitment values of the $\log^{+1}$ or log of transformed data against time. The relative rates of increase or recruitment for each appropriate parameter were calculated using the equation:

$$R = \log_e N_2 - \log_e N_1 / (t_2 - t_1)$$

Where R= relative rate of increase or recruitment of the values (N_1, N_2) of each parameter at t_1 and t_2, respectively.

In all experiments, ANOVA and Tukey's HSD tests were carried out to compare treatment means. In first set of experiments the results were subjected to one-way

ANOVA and Tukey's HSD tests for fertilizer application and timing factors from the 1st week to the 24th weeks. Subsequently two-way ANOVA was employed comparing fertilizer application with timing factors. In second set of experiments one-way ANOVA and Tukey's HSD tests was used for fertilizer application concentrations, water depths and timing factors from the 1st week to the 16th weeks. Two way ANOVA was used to compare fertilizer application concentrations and water depths with timing factors. Similar analyses have been used in many previous studies (Baki, 1986 &1988; Ministry of Forestry and Range, 2007; Juan *et al.*, 2010; Levi *et al.* 2011).

RESULTS AND DISCUSSION

3.3.1 Field experiments

3.3.1.1 Leaf number per ramet of *Scirpuss grossus* in grown on fertilized and unfertilized peat and paddy soils

Scirpus grossus reiterate by subterranean rhizomatous growth and branches from a single mother plant. It is through this rhizomatous growth at the nodes that aerial ramets proliferate above ground displaying phalanx (*sensu stricto* Baki 1986) growth strategy in concordat with lateral proliferation of rhizomes through soil layers or lattices in search of space for further exploration and intake of nutrients in nutrient pools.

As shown in Table 3.1, increasing in leaf number between week to week. At the end of the 24 weeks of study period, when the plots are completely filled with *S. grossus* plants, the total average leaf number of emerged ramets in fertilized soils were 16.40 leaves and 16.20 leaves in unfertilized soils, respectively, although these readings were significantly different at $p < 0.05$. (LSD tests). The values mean leaf numbers

(ramets^{-2}) per week (3.00, 3.13, 3.67, 4.40, 5.07, 5.27, 5.87, 6.27, 6.53, 7.07, 7.80, 8.27, 9.13, 9.80, 10.47, 11.73, 12.33, 12.47, 13.40, 13.80, 14.60, 15.27, 16.20, 16.20) respectively in unfertilized soils, while in fertilized (3.00, 3.13, 3.80, 4.60, 5.00, 5.20, 5.80, 6.13, 6.47, 7.00, 7.60, 8.27, 9.53, 10.00, 11.00, 11.87, 12.13, 12.40, 13.60, 13.93, 14.60, 14.93, 16.20, 16.40) respectively, significantly different at $p < 0.05$ (HSD tests).

As shown in (Table 3.2), the total average leaves number of emerged ramets in fertilized soils was 17.00 leaves and 16.80 leaves in unfertilized soils, significantly different at $p < 0.05$, (LSD tests). The values mean leaf numbers (ramets^{-2}) per week (3.13, 3.67, 4.20, 4.87, 5.27, 5.67, 6.00, 6.27, 6.80, 7.00, 8.27, 8.53, 9.87, 10.40, 10.93, 11.93, 12.60, 12.73, 14.07, 15.13, 15.47, 16.80, 16.80) respectively in unfertilized soils, while in fertilized (3.13, 3.67, 4.33, 4.93, 5.27, 5.60, 6.00, 6.33, 6.93, 7.27, 8.20, 8.53, 9.93, 10.27, 11.20, 11.93, 12.80, 12.93, 14.27, 14.53, 15.13, 15.60, 17.00,17.00) respectively, significantly different at $p < 0.05$ (HSD tests).

Mortality of leaves ramets, the numbers of dead leaves recorded were 9.47 leaves in unfertilized peat soils and 9.73 leaves in fertilized soils after 24 weeks (Table 3.1), while the net leaves population of ramets were 6.73 leaves in unfertilized soils and 6.67 leaves in fertilized soils, significantly different at $p < 0.05$, (HSD tests) (Figs. 3.4a and 3.4b). Leaves mortality set in on 3th week at 1.27 leaves in unfertilized soils. The parallel values for the ensuing weeks were 2.00, 2.53, 2.87, 3.13, 3.60, 3.93, 4.13, 4.73, 5.07, 5.67, 6.13, 6.67, 7.27, 7.47, 8.07, 8.40, 8.80, 9.07, 9.27, 9.47, 9.47 leaves, while in fertilized set in on 3th week at 1.20 leaves. The parallel values for the ensuing weeks were 2.07, 2.80, 3.00, 3.20, 3.87, 4.00, 4.53, 4.87, 5.33, 5.93, 6.33, 7.00, 7.33, 7.80, 8.13, 8.33, 8.87, 9.07, 9.33, 9.53, 9.73 leaves.

At 24th week of experimentation, the mortality number of leaves ramets recorded was 9.73 leaves in unfertilized paddy soils and 9.73 leaves in fertilized paddy soils (Table 3.2), while the net leaves population were 7.07 leaves in unfertilized soils and 7.27 leaves in fertilized soils (Figs. 3.4a and 3.4b) significantly different at 0.05%, (HSD tests). Leaves mortality set in on 3th week at 0.67 leaves in unfertilized soils. The parallel values for the ensuing weeks were 1.60, 2.60, 3.27, 3.73, 4.00, 4.47, 4.73, 5.07, 6.00, 6.13, 6.87, 7.33, 7.60, 8.07, 8.27, 8.80, 9.07, 9.33, 9.53, 9.73 leaves, while in fertilized set in on 3th week at 0.67 leaves. The parallel values for the ensuing weeks were 1.60, 2.60, 3.27, 3.80, 4.13, 4.60, 4.93, 5.27, 6.20, 6.40, 7.00, 7.60, 7.80, 8.13, 8.47, 8.93, 9.13, 9.33, 9.53, 9.73 leaves. The Anova are summarised for all results in appendixs 11 and 12 (pp. lxii and lxiii).

The results of experiments on population flux of *S. grossus* leaves in fertilized and unfertilized peat and paddy soil have shown that, although the population flux leaves were slightly higher in the fertilized soil, the differences were significant. The results of experiments on population flux of *S. grossus* leaves in fertilized and unfertilized peat and paddy soil have shown that, the differences were significant (Figs. 3.4a and 3.4b).

These observations may be due to the elemental resources present in the soil, necessary for plant growth, being enough and thus the addition of fertilizers did not bring about any significant differences, in chlorophyll content of the leaves and the photosynthetic ability of the plants. In addition, the use of the NPK fertilizer did not result in the development or growth of larger leaves, stem and flowers when grown on the same soil, although these plant parts were significantly larger when *S. grossus* was grown on paddy soil. This indicated that the paddy soil was more suitable for the growth of *S. grossus*, because it is also naturally found growing in rice fields. The

observations recorded in this study is different from what has been reported in many previous studies which have shown that the application NPK fertilizer can effect clonal growth of crop plants, such as in wheat (Ognjanovic *et al.* 1994; Biberdzic *et al.* 2011).

Table 3.1 Mean leaf and mortality numbers and net leaf population of ramets (m^{-2}) of *Scirpus grossus* plant (plural) grown on fertilized (F1) and unfertilized (F0) peat soil at MARDI Research Station, Jalan Kebun, Selangor, Malaysia[#]

	Leaf numbers/m^2		Leaf mortality number/m^2		Net leaf population/m^2	
Weeks after planting	F0	F1	F0	F1	F0	F1
1	3.00 q	3.00 o	0.00 o	0.00 o	3.00 h	3.00 h
2	3.13 q	3.13 o	0.00 o	0.00 o	3.13 gh	3.13 gh
3	3.67 p	3.80 n	1.27 n	1.20 n	2.40 gh	2.60 gh
4	4.40 o	4.60 m	2.00 m	2.07 m	2.40 fgh	2.53 fgh
5	5.07 n	5.00 m	2.53 l	2.80 l	2.47 fgh	2.20 fgh
6	5.27 n	5.20 lm	2.87 kl	3.00 kl	2.40 fgh	2.20 fgh
7	5.87 n	5.80 kl	3.13 k	3.20 k	2.73 efgh	2.60 efgh
8	6.27 m	6.13 jk	3.60 j	3.87 j	2.60 efgh	2.27 efgh
9	6.53 lm	6.47 jk	3.93 ij	4.00 ij	2.60 efgh	2.47 efgh
10	7.07 kl	7.00 ij	4.13 i	4.53 i	2.93 defgh	2.47 defgh
11	7.80 jk	7.60 hi	4.73 h	4.87 h	3.00 cdefgh	2.73 cdefgh
12	8.27 ij	8.27 h	5.07 h	5.33 h	3.20 cdefgh	2.93 cdefgh
13	9.13 hi	9.53 g	5.67 g	5.93 g	3.40 bcdefg	3.60 bcdefg
14	9.80 gh	10.00 fg	6.13 g	6.33 g	3.67 bcdefg	3.67 bcdefg
15	10.47 fg	11.00 ef	6.67 f	7.00 f	3.67 abcdef	4.00 abcdef
16	11.73 ef	11.87 e	7.27 ef	7.33 ef	4.40 abcde	4.53 abcde
17	12.33 def	12.13 de	7.47 de	7.80 de	4.80 abcd	4.33 abcd
18	12.47 cde	12.40 cde	8.07 cd	8.13 cd	4.40 abcd	4.27 abcd
19	13.40 bcde	13.60 bcd	8.40 bc	8.33 bc	4.93 abcd	5.27 abcd

Table 3.1(*continued*)

20	13.80 abcd	13.93 bc	8.80 abc	8.87 abc	5.00 abcd	5.07 abcd
21	14.60 abc	14.60 ab	9.07 ab	9.07 ab	5.53 abcd	5.53 abcd
22	15.27 abc	14.93 ab	9.27 a	9.33 a	6.00 abc	5.60 abc
23	16.20 ab	16.20 a	9.47 a	9.53 a	6.73 ab	6.67 ab
24	16.20 a	16.40 a	9.47 a	9.73 a	6.73 a	6.67 a

[#]Figures in a column with same lowercase letters are not significantly different at $p<0.05$ (HSD tests).

Table 3.2 Leaf and mortality numbers and net leaf population of ramets (m^{-2}) of *Scirpus grossus* plant (plural) grown on fertilized (F1) and unfertilized (F0) paddy soil at MARDI Research Station, Tanjung Karang, Selangor, Malaysia[*#]

Weeks after planting	Leaf numbers/m^2		Leaf mortality number/m^2		Net leaf population/m^2	
	F0	F1	F0	F1	F0	F1
1	3.13 q	3.13 q	0.00 p	0.00 p	3.13 n	3.13 n
2	3.67 pq	3.67 pq	0.00 p	0.00 p	3.67 mn	3.67 mn
3	4.20 op	4.33 op	0.67 o	0.67 o	3.60 lmn	3.67 lmn
4	4.87 no	4.93 no	1.60 n	1.60 n	3.33 lmn	3.33 lmn
5	5.27 mn	5.27 mn	2.60 m	2.67 m	2.67 lmn	2.60 lmn
6	5.67 lmn	5.60 lmn	2.80 lm	2.80 lm	2.87 klmn	2.80 klmn
7	6.00 klm	6.00 klm	3.27 kl	3.27 kl	2.80 jklmn	2.73 jklmn
8	6.27 jkl	6.33 jkl	3.73 jk	3.80 jk	2.53 ijklm	2.53 jklmn
9	6.80 jk	6.93 jk	4.00 ij	4.13 ij	2.80 ijkl	2.80 ijkl
10	7.00 j	7.27 j	4.47 hi	4.60 hi	2.60 ijkl	2.67 ijkl
11	8.27 i	8.20 i	4.73 hi	4.93 hi	3.53 ijk	3.27 ijk
12	8.53 i	8.53 i	5.07 gh	5.27 gh	3.47 hij	3.27 hij
13	9.87 h	9.93 h	6.00 fg	6.20 fg	3.93 hij	3.73 hij
14	10.40 gh	10.27 gh	6.13 f	6.40 f	4.33 ghij	3.87 ghij
15	10.93 fg	11.20 fg	6.87 ef	7.00 ef	4.00 fghi	4.20 fghi
16	11.93 ef	11.93 ef	7.33 de	7.60 de	4.60 efgh	4.33 efgh
17	12.60 e	12.80 e	7.60 cde	7.80 cde	5.00 defg	5.00 defg
18	12.73 e	12.93 e	8.07 bcd	8.13 bcd	4.67 cdef	4.80 cdef
19	14.07 d	14.27 d	8.27 abcd	8.47 abcd	5.73 cde	5.80 cde

Table 3.2(*continued*)

20	14.47 cd	14.53 cd	8.80 abc	8.93 abc	5.67 bcd	5.60 bcd
21	15.13 bc	15.13 bc	9.07 ab	9.13 ab	6.07 abcd	6.00 abcd
22	15.47 b	15.60 b	9.33 ab	9.33 ab	6.13 abc	6.27 abc
23	16.80 a	17.00 a	9.53 a	9.53 a	7.27 ab	7.47 ab
24	16.80 a	17.00 a	9.73 a	9.73 a	7.07 a	7.27 a

[#]Figures in a column with same lowercase letters are not significantly different at $p<0.05$ (HSD tests).

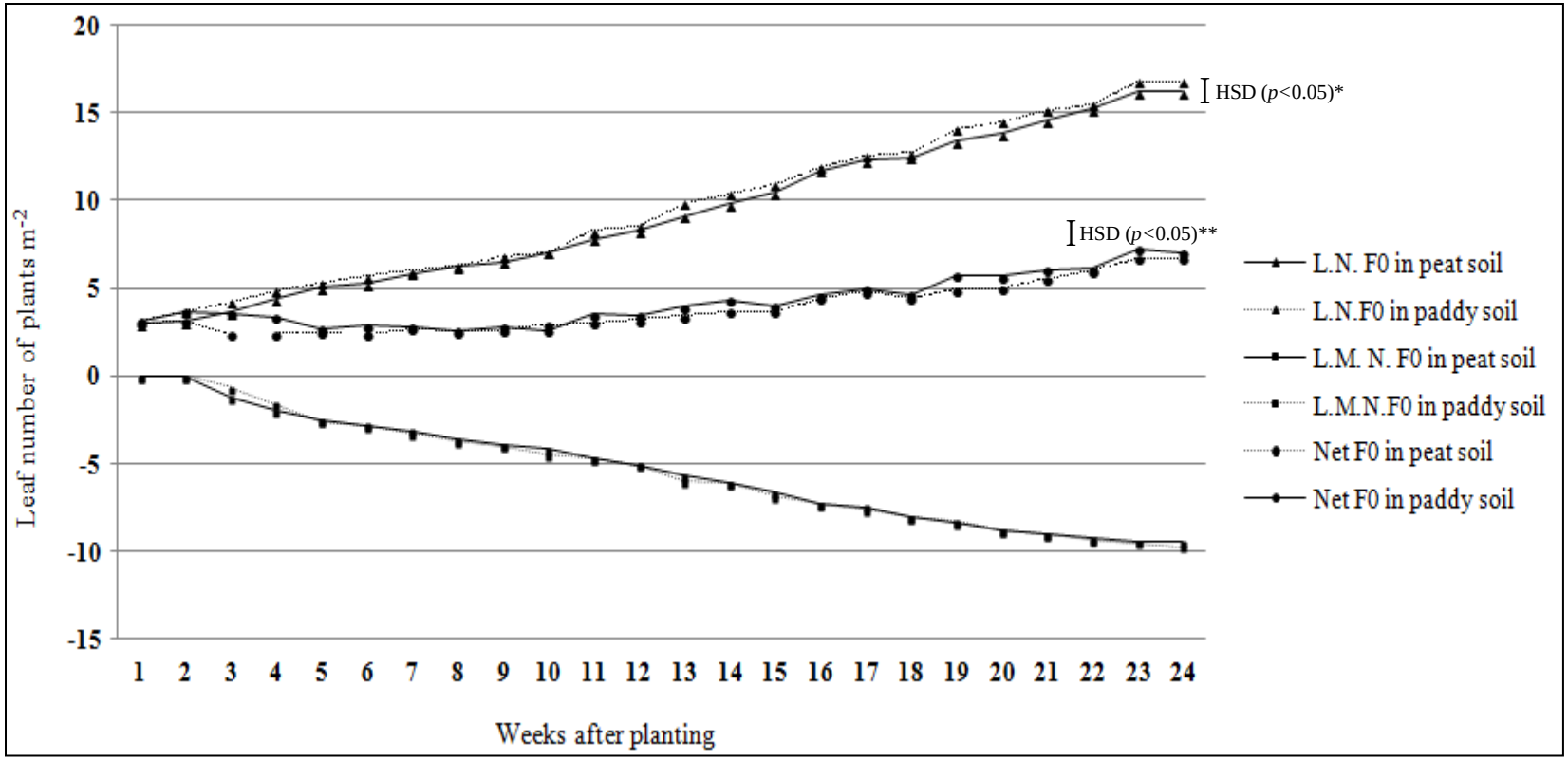

Fig. 3.4a Population fluxes of *Scirpus grossus* grown on unfertilized peat and paddy soils. Leaf number (▲), Leaf mortality number (■), Leaf net population (●).* Leaf number, ** Leaf mortality number.

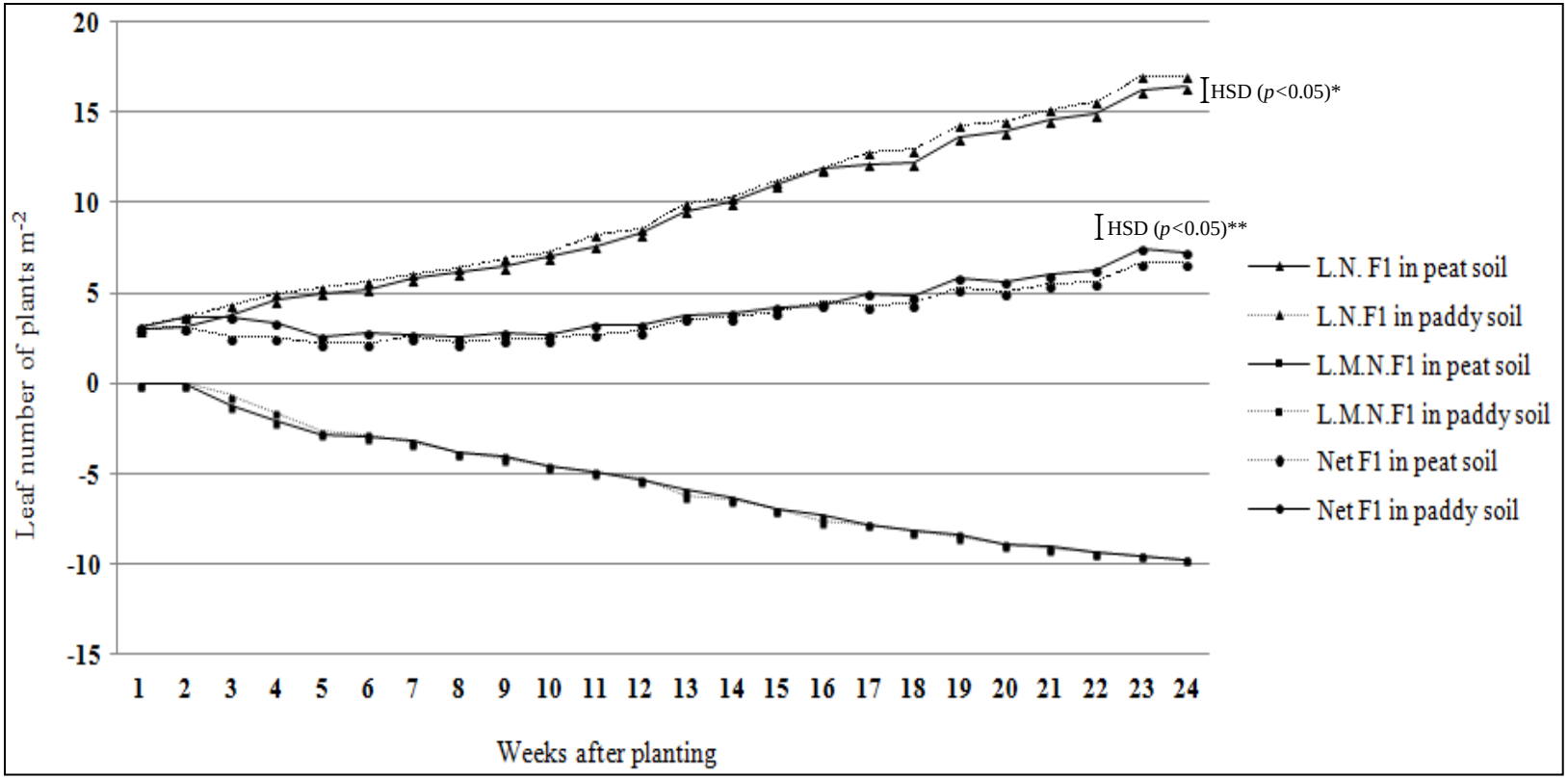

Fig. 3.4b Population fluxes of *Scirpus grossus* grown on fertilized peat and paddy soils. Leaf number (▲), Leaf mortality number (■), Leaf net population (●).* Leaf number, ** Leaf mortality number.

3.3.1.2 Chlorophyll content in leaves of *Scirpus grossus* grown on fertilized and unfertilized peat and paddy soils

Chlorophyll content in leaves has always been regarded as a measure of the health status of a plant. Fig. 3.5 shows the chlorophyll content in leaves of *S. grossus* grown in fertilized and unfertilized peat soils. As can be seen it was slightly higher (49.48) in plants that were fertilized than that recorded in leaves of the unfertilized control plants (46.51). However the difference was not significant at $p < 0.05$ (LSD tests). The Anova are summarised in Appendix 13 (pp.lxiv).

Fig. 3.6 shows the chlorophyll content in leaves of plant growing in fertilized paddy soil was slightly higher (49.56) than that recorded in leaves of the control plant (47.79). However the difference was not significant at $p < 0.05$, (LSD tests). The SPAD chlorophyll values were very similar to that recorded in plants grown in peat soil (Fig. 3.6 above). The Anova are summarised in Appendix 14 (pp.lxv).

Many studies previously have shown that the addition fertilizers can enhance growth and productivity of crops worldwide, as sufficient macro and micro elements will be made available to the plant for a wholesome growth (Johannes *et al.* 2013; Jose *et al.* 2013; Mucheru-Muna *et al.* 2013). However as can be seen from this study the effect of fertilizer treatment, on chlorophyll content, was not significant. This is probably due to the availability of the necessary macro and micronutrients for chlorophyll synthesis being already sufficient in both the peat and paddy soils used (see Tables 2.9 and 3.4).

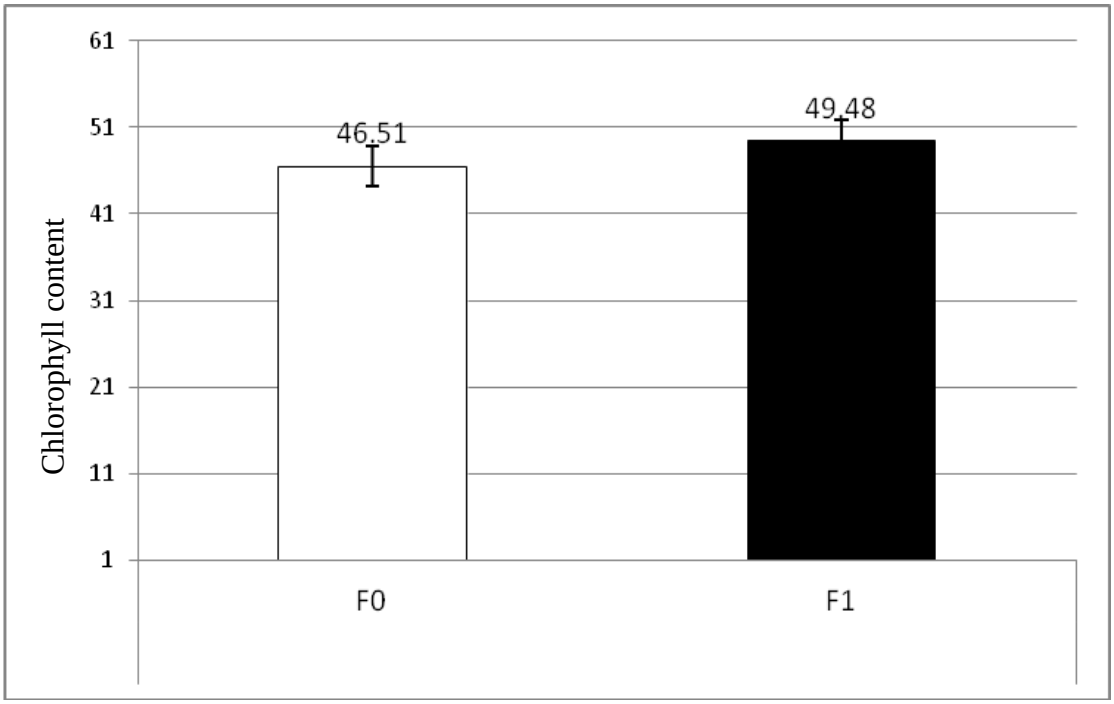

Fig. 3.5 Chlorophyll content of *Scirpus grossus* leaves grown in ■ fertilized and □ unfertilized peat soil at MARDI Research Station site, Jalan Kebun, Selangor, Malaysia, taken at week 24.

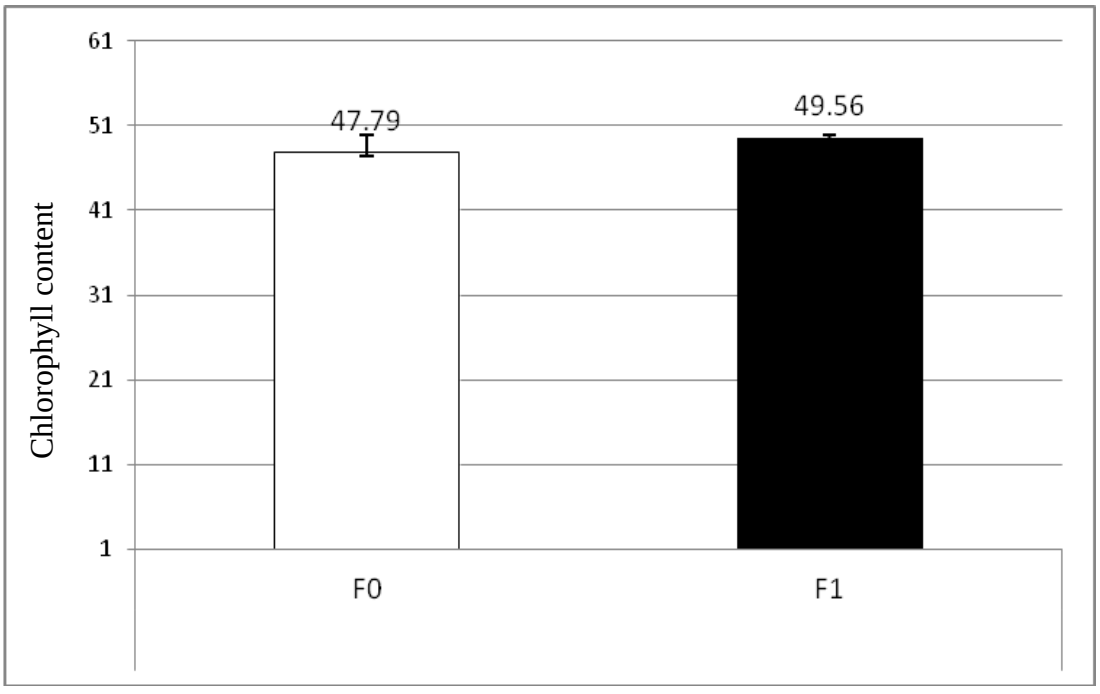

Fig. 3.6 Chlorophyll content of *Scirpus grossus* leaves grown in ■ fertilized and □ unfertilized paddy soil at MARDI Research Station site, Tanjung Karang, Selangor, Malaysia, taken at week 24.

3.3.1.3 Biomass of various plant parts of *Scirpus grossus* grown on fertilized and unfertilized peat and paddy soils

Fig. 3.7 shows the dry biomass of selected plant parts of *S. grossus* taken after harvest at 24 weeks after transplanting, displaying measurable differences according to fertilizer regimes. In unfertilized peat soils: the leaves were 6.90 g, and the stems, 7.99 g whilst the flowers were 1.92 g in weight. In fertilized peat soils these were measurably higher with 9.73 g (leaves), 10.51 g (stems) and 2.77 g for flowers. However these differences were not significant at $p < 0.05$ (LSD tests). The Anova are summarised in Appendix 13 (pp.lxiv).

Fig. 3.8 shows the dry biomass of selected plant parts of *S. grossus* taken after harvest at 24 weeks after transplanting, displaying measurable differences according to fertilizer regimes. In unfertilized paddy soils: the leaves were 12.72 g, and the stems, 17.56 g whilst the flowers were 2.38 g in weight. In fertilized paddy soils these were measurably higher with 14.84 g (leaves), 18.61 g (stems) and 3.13 g for flowers. However these differences were not significant at $p < 0.05$, (LSD tests). Nevertheless these values were significantly higher (~1.5 x) than the results when grown in peat soils (Fig. 3.9). The Anova are summarised in Appendix 14 (pp.lxv).

With regard to these parameters, it has been well documented that fertilizer treatment can bring about significant increase in plant biomass (Sabine *et al.* 2003; Magrini-Bair *et al.* 2009; Soh *et al.* 2013), although this was not observed here. These results concur with that of the chlorophyll content study above where the differences were also not significant. It probably indicates either of two things, or both, that *S. grossus* requirement for a healthy growth is not dependent on much macro and micro nutrients and/or both the soils have sufficient macro and micro nutrients to support healthy growth in *S. grossus*. (see Tables 2.9 and 3.4). It thrives in both soils without the addition of fertilizers.

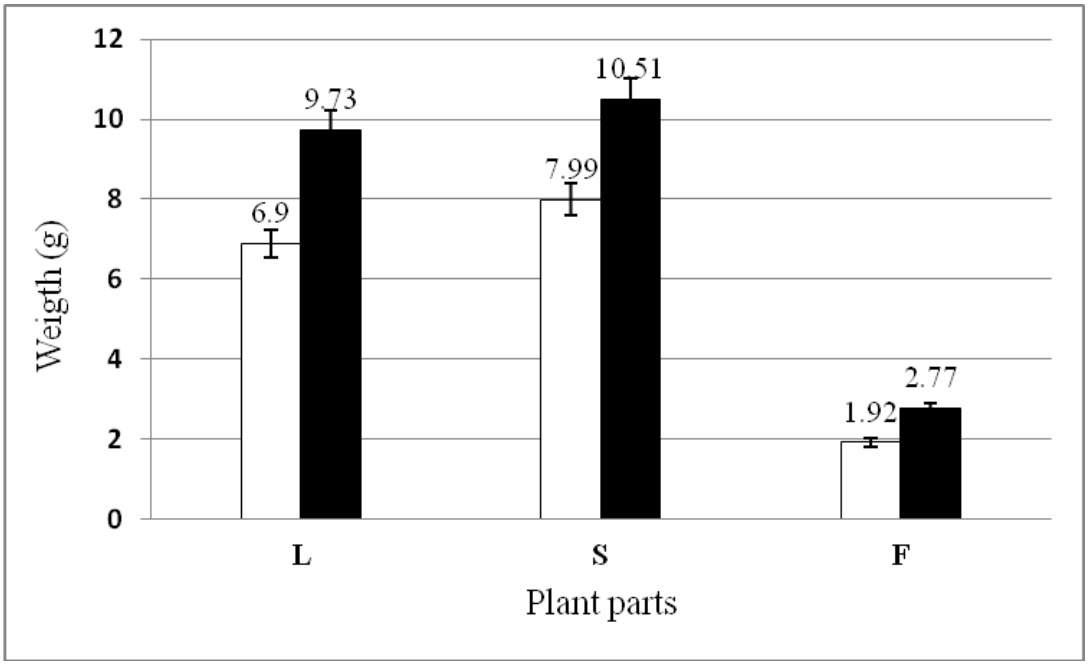

Fig. 3.7 Mean biomass of various plant parts of *Scirpus grossus* grown in ■ fertilized and □ unfertilized peat soil at MARDI Research Station site, Jalan Kebun, Selangor, Malaysia. (L: leaves, S: stems, F: flowers), taken at week 24.

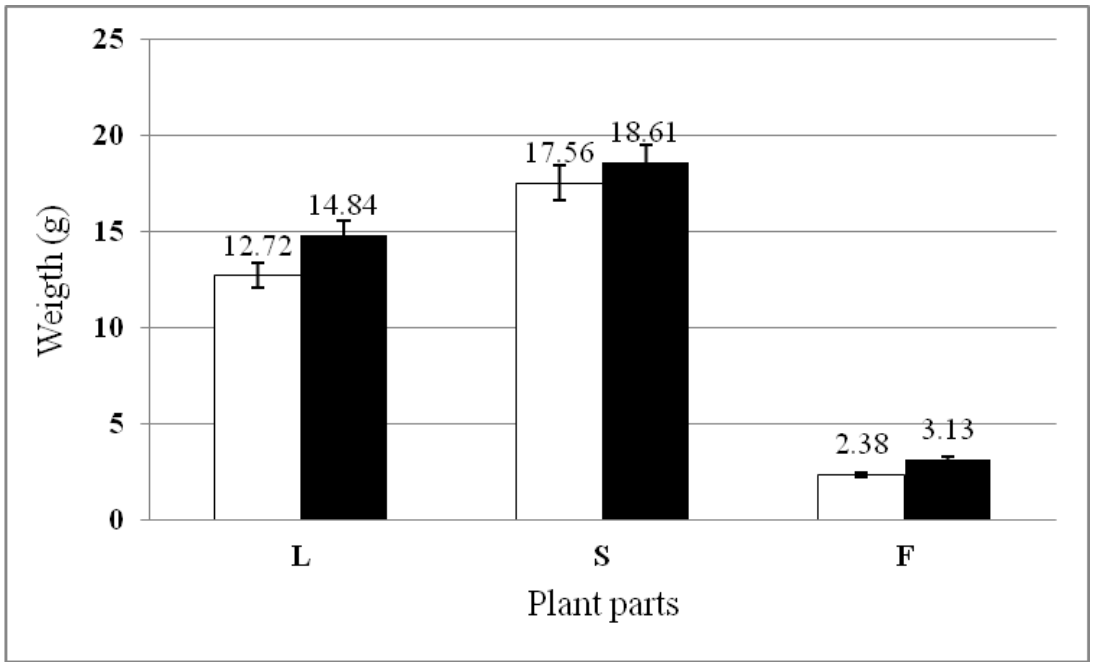

Fig. 3.8 Mean biomass of various plant parts of *Scirpus grossus* grown in ■ fertilized and □ unfertilized paddy soil at MARDI Research Station site, Tanjung Karang, Selangor, Malaysia. (L: leaves, S:stems, F:flowers), taken at week 24.

3.3.1.4 Chlorophyll fluorescence in leaves of *Scirpus grossus* grown on fertilized and unfertilized peat and paddy soils

Chlorophyll fluorescence results, which is indicative of the tissue's photosynthetic capacity, are shown in Fig. 3.9 The Fv/Fm ratios were very similar between the two set of plants. The Fv/Fm values recorded between the fertilized and non-fertilizer grown plants ranged between 0.76 to 0.77. This difference was not significant at $p < 0.05$ (LSD tests). The Anova are summarised in appendix 13 (pp. lxiv).

With regard to chlorophyll fluorescence, the Fv/Fm ratios were very similar between the two sets of plants (Fig. 3.10). The Fv/Fm values recorded between the fertilized and non-fertilizer grown plants ranged between 0.799 to 0.793 (Fig. 3.10). The difference was not significant at $p < 0.05$ (LSD tests). Although the values were higher than the values recorded in *S. grossus* grown in peat soil the difference was not significant between growth on the two soils (Fig. 3.6). The Anova are summarised in appendix 14 (pp. lxv).

The chlorophyll content and chlorophyll fluorescence parameters. (Fv/Fm) can be considered to be "stress indicators" (Maxwell and Johnson 2000), and any significant changes can be taken to indicate a negative/positive impact on the plant of the different fertilizer treatments in this study. However as was observed with chlorophyll content and plant biomass studies above, fertilizer treatment did not significantly alter the chlorophyll fluorescence values. Thus results with on all the three parameters agree that *S. grossus* growth and photosynthetic capability was not affected by the lack of fertilizer in both the soil types.

According to the literature typical Fv/Fm parameter values for dark-adapted non-stressed healthy leaves in C3 plants are in the region of 0,80 to 0,83 (Bown *et al.* 2009; Murchie *et al.* 2009). The Fm/Fv values observed in this study were slightly lower than these values ranging between 0.71-0.79. This probably indicates that the peat

and paddy soil on which the *S. grossus* plants were grown had sufficient macro and micro nutrients to support healthy growth during the period of exprimentation. In a study on the effects of fertilizer on the leaf chlorophyll fluorescence parameters, chlorophyll content in *Carya cathayensis*, Song *et al.* 2011 reported a slight increase in both parameters during the early growth period (30-60 days), but a remarkable decrease in mid and later growth periods (75 - 120 days).

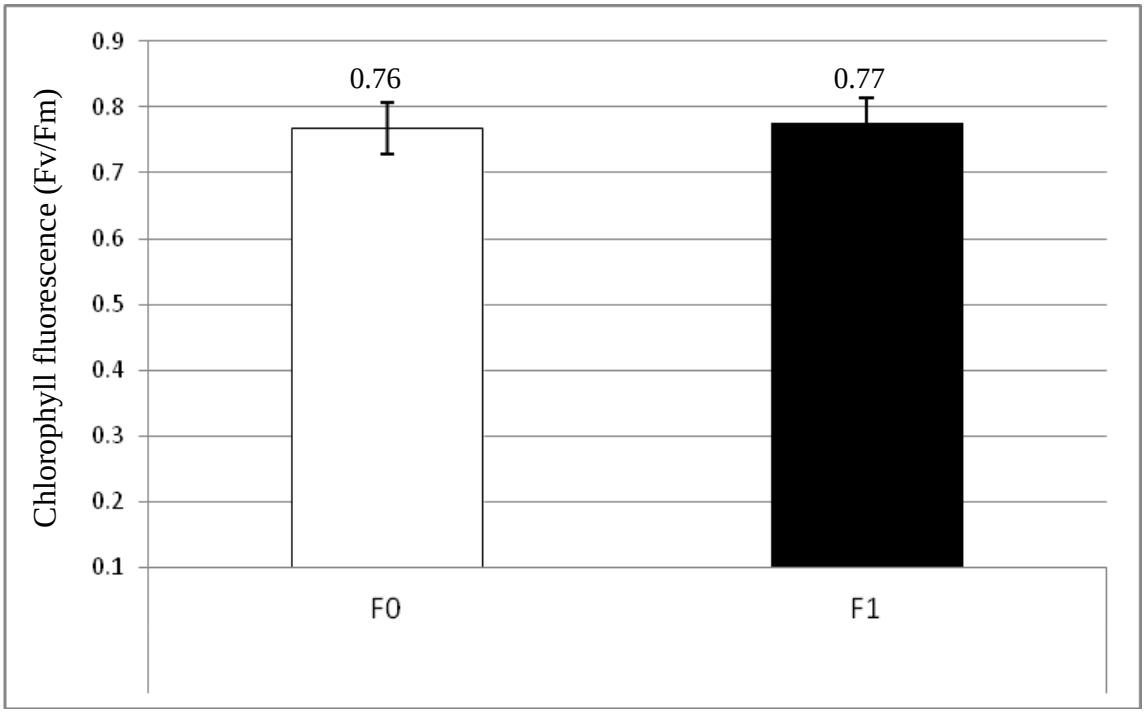

Fig. 3.9 Chlorophyll fluorescence in leaves of *Scirpus grossus* grown in ■ fertilized and □ unfertilized peat soil at MARDI Research Station site, Jalan Kebun, Selangor, Malaysia, taken at week 24.

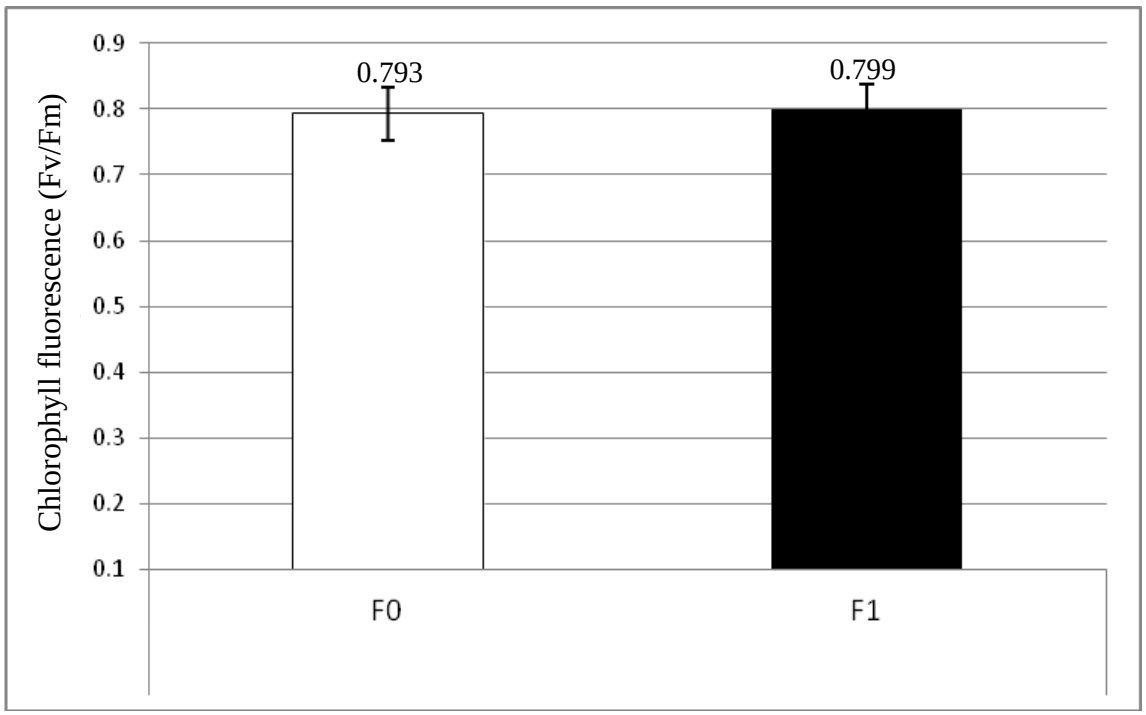

Fig. 3.10 Chlorophyll fluorescence in leaves of *Scirpus grossus* L. grown in ■ fertilized and □ unfertilized paddy soil used at MARDI Research Station site, Tanjung Karang, Selangor, Malaysia, taken at week 24.

3.3.2 Pot Experiments

3.3.2.1 Effect of water depth and different concentration of fertilizer on leaf chlorophyll content in *Scirpus grossus* plants grown in paddy soil.

The results in Table 3.3 show that the chlorophyll content was generally higher in plants grown in fertilized soil. The addition of fertilizers increased the chlorophyll content slightly but the increase was not significant. However there was a clear reduction in the chlorophyll content when the plants were grown under 20 cm of water in the presence of fertilizers. At a water depth of 20 cm, increasing fertilizer amounts led to a large drop in chlorophyll content, after only four weeks, with SPAD readings averaging 18.3 and subsequently leading eventually to death after about 12 weeks with SPAD readings dropping to 2.0 (Fig. 3.11). In the plants that were not treated with fertilizers, the chlorophyll content was not significantly different when grown under different water depths as shown earlier (Table 3.3 and Fig. 3.10). A summary of the results with regard to chlorophyll content are as follows; F0 (0 cm = 36.90, 5 cm = 43.77, 10 cm = 31.73, 20 cm = 38.43) respectively, F2 (0 cm = 48.07, 5 cm = 40.83, 10 cm = 35.60, 20 cm = 13.63), F3X (0 cm = 44.87, 5 cm = 40.00, 10 cm = 48.60, 20 cm = 32.67), F4 (0 cm = 46.57, 5 cm = 34.77, 10 cm = 44.60, 20 cm = 16.03) and at F5 (0 cm = 43.77, 5 cm = 47.67, 10 cm = 45.20, 20 cm = 0.00) respectively. All the results were significant at 0.04% under the influence of two factors together, fertilizer concentration and water depth. The Anova are summarised in appendix 6 (pp. xlix).

The results show chlorophyll content in the leaves of *S. grossus* was subjected to different water depth levels for 16 weeks. As reported above, it was observed that increasing the level of water depth had significant effect on the content of chlorophyll in the leaves of *S. grossus*. However Osborne *et al.* (2002) reported that in *Zea mays* L., there was an inverse relationship between water depth and chlorophyll content, with

chlorophyll content decreasing with increasing water depth. The negative effects of water on photosynthesis has been reported previously by Tripathy *et al*. (1981) and Pandey and Sharma (2002).

This probably indicates that *S. grossus* grows optimally only in a soil in soil inundated with water, such as the paddy soil. Baki (1988) had earlier reported that the growth of *S. grossus* was affected by the depth of inundation with water. Wersal and Madsen (2011) reported significant decline in biomass and plant length as water levels increased, suggesting that submersed leaves alone cannot sustain *M. aquaticum* growth for long periods of time. Earlier, Salvucci and Bowes (1982) reported optimal photosynthetic rates of *M. aquaticum* was observed in the emergent form and *M. aquaticum* cannot remain as a submersed plant for long periods of time as the photosynthetic rate of submersed leaves may not be sufficient to support plant growth.

Previous studies have reported that leaf photosynthetic rates are reduced in plants grown under water probably providing some evidence for an energetic cost involved in heterophyllous plants (Cook and Johnson 1968), However, in this study it was observed that there was a decrease in chlorophyll content in the leaves of *S. grossus* when increasing fertilizer concentrations were added to plants grown under 20 cm water depth. This could be due to several reasons, as has been reported in many studies previously, such as the low rates of photosynthesis observed in *M. aquaticum* as the leaves are flooded for extended periods (Salvucci and Bowes 1982). They suggested that the rate of photosynthesis of the immersed leaves was probably not sufficient to support plant growth. It was also suggested that the growth of the submersed leaves was transient and only utilised for short overwintering periods and times of reduced light and temperature (Sytsma and Anderson 1993a). Another study reported that the presence of algae can lead to reduced hydrocarbon content, and works as a

contraceptive light and thus inhibiting the growth of *Botryococcus braunii* (Deng *et al.* 2012). This was observed (high algae growth) in pots submerged in 20 cm water with high fertilizer concentration.

As mentioned earlier, in this study it was observed that water depth did not affect the chlorophyll content. However when NPK fertilizer was added to plants grown under a depth of 20 cm, a decrease in chlorophyll content was observed, with a greater decrease in increasing fertilizer concentrations and subsequently to the death of the plant. The latter observation could be due to several reasons such as the plant is unable to grow normally under a depth of 20 cm when the fertilizer added becomes toxic as the chemical elements in solution becomes directly readily available to the plant tissues and their high concentration in the tissues will ultimately lead to tissue death.

Table 3.3 Mean chlorophyll content of ramets (m^{-2}) of *Scirpus grossus* plants grown under different NPK concentrations and water depths in paddy soil in Universiti Putra Malaysia, Serdang, Selangor, Malaysia[#*]

	F0 (Control)			
Weeks after Planting	Treatments			
	D1	D2	D3	D4
4	38.09 a	38.24 a	37.87 a	40.97 a
8	39.34 a	42.19 a	35.62 a	43.71 a
12	37.73 a	42.27 a	33.37 a	41.30 a
16	36.90 a	43.77 a	31.73 a	38.43 a
	F2			
Weeks after Planting	Treatments			
	D1	D2	D3	D4
4	40.41 a	44.07 a	42.87 a	56.76 a
8	44.63 a	46.82 a	47.67 a	31.52 a
12	45.07 a	43.47 a	42.30 a	18.20 b
16	48.07 a	40.83 a	35.60 a	13.63 a
	F3			
Weeks after Planting	Treatments			
	D1	D2	D3	D4
4	42.7 a	42.37 a	51.46 a	44.80 a
8	48.91 a	45.07 a	46.27 a	43.34 a
12	46.73 a	42.37 a	48.67 a	35.20 a
16	44.87 a	40.00 a	48.60 a	32.67 a
	F4			
Weeks after Planting	Treatments			
	D1	D2	D3	D4
4	44.90 a	45.46 a	49.36 a	48.20 a
8	48.47 a	49.90 a	49.46 a	39.48 a
12	46.83 a	41.17 a	44.13 a	16.43 b
16	46.57 a	34.77 a	44.60 a	16.03 b
	F5			
Weeks after Planting	Treatments			
	D1	D2	D3	D4
4W	45.03 a	47.72 a	53.19 a	18.28 b
8W	43.97 a	47.20 a	57.67 a	7.90 b
12W	43.87 a	47.83 a	51.13 a	2.00 b
16W	43.77 a	47.67 a	45.20 a	0.00 b

[#]Figures in a column with same lowercase letters for each fertilizer concentration and each water depth are not significantly different at $p<0.01$ (HSD tests).
*F0 (control)(without fertilizer); F2 (50g/500 ml); F3 (75g/750 ml); F4 (100g/1000 ml); F5 (125g/1250 ml)

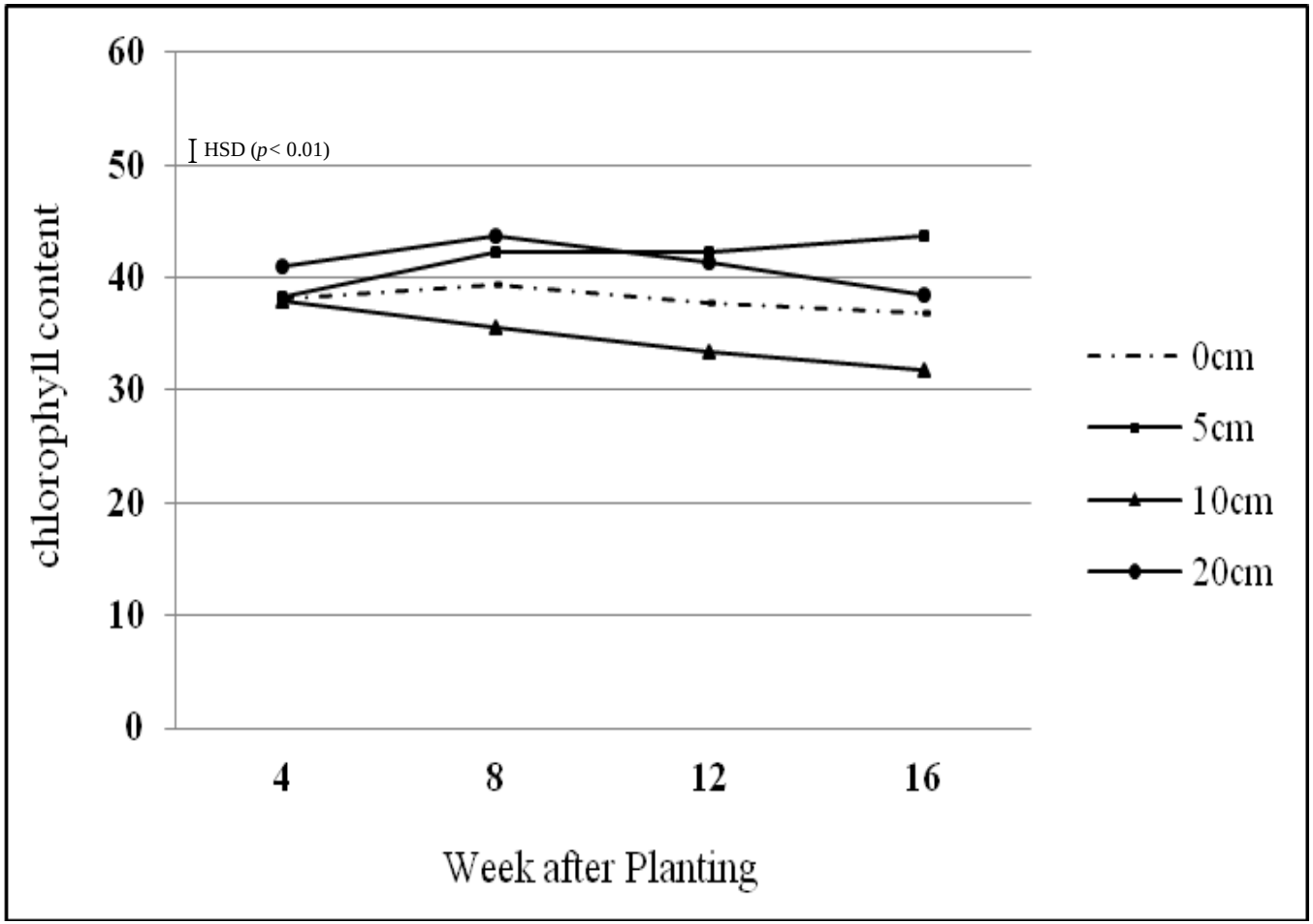

Fig. 3.11a Chlorophyll content in *Scirpus grossus* grown under different water depths (0 cm, 5 cm, 10 cm and 20 cm), without NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia.

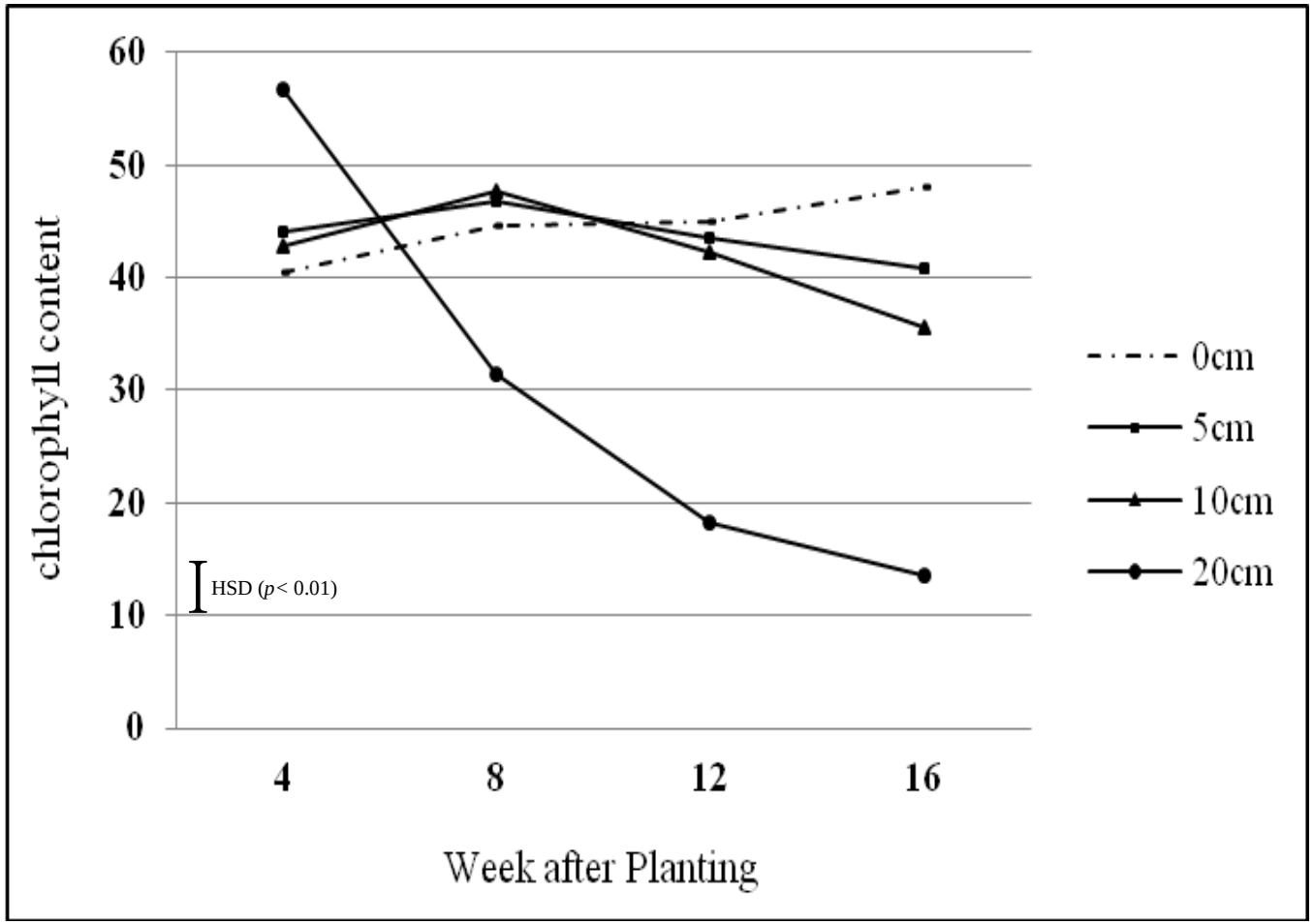

Fig. 3.11b Chlorophyll content in *Scirpus grossus* grown under different water depths (0 cm, 5 cm, 10 cm and 20 cm), with 50 g/ 500 ml NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia.

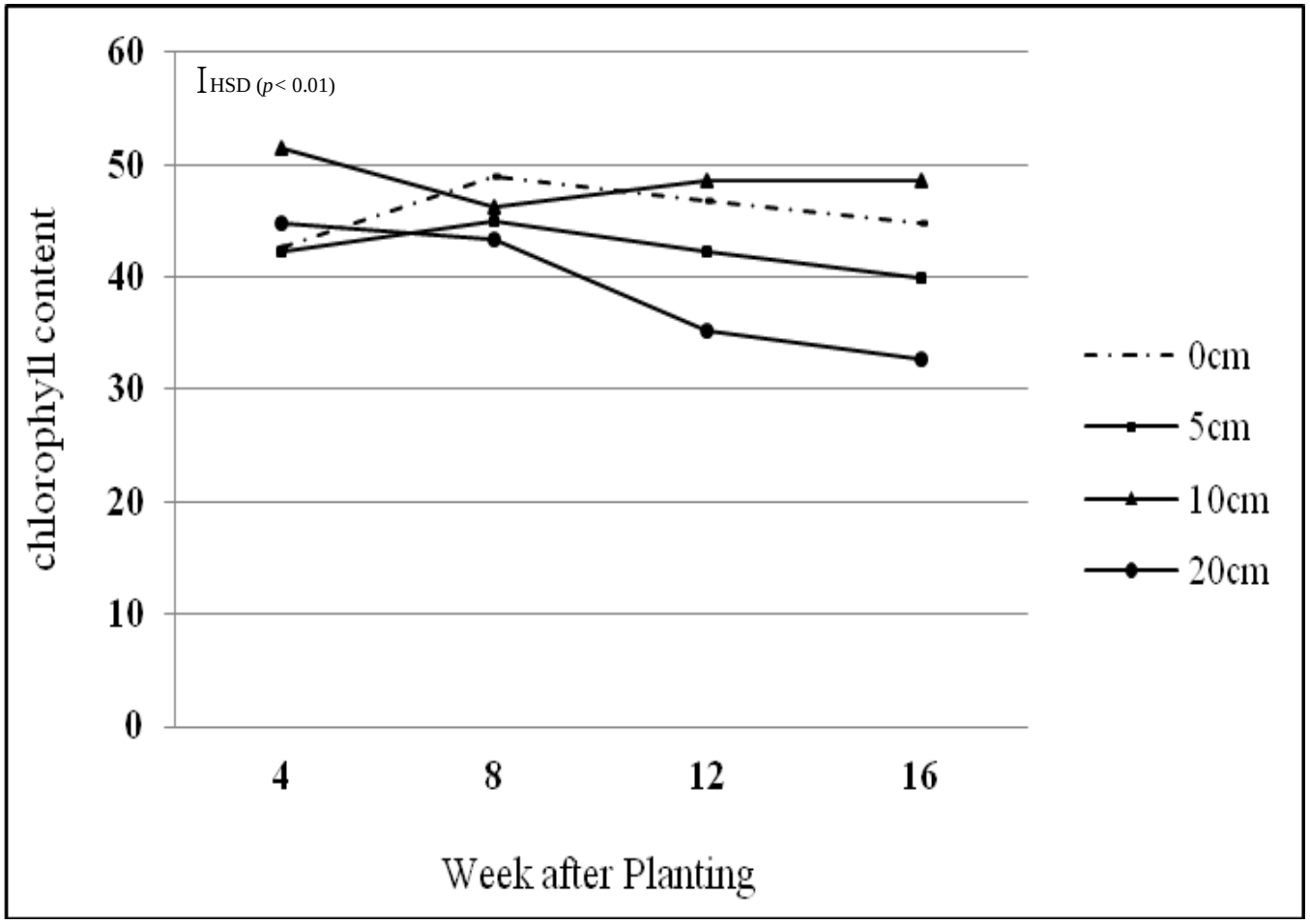

Fig. 3.11c Chlorophyll content in *Scirpus grossus* grown under different water depths (0 cm, 5 cm, 10 cm and 20 cm), with 75 g/ 750 ml NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia.

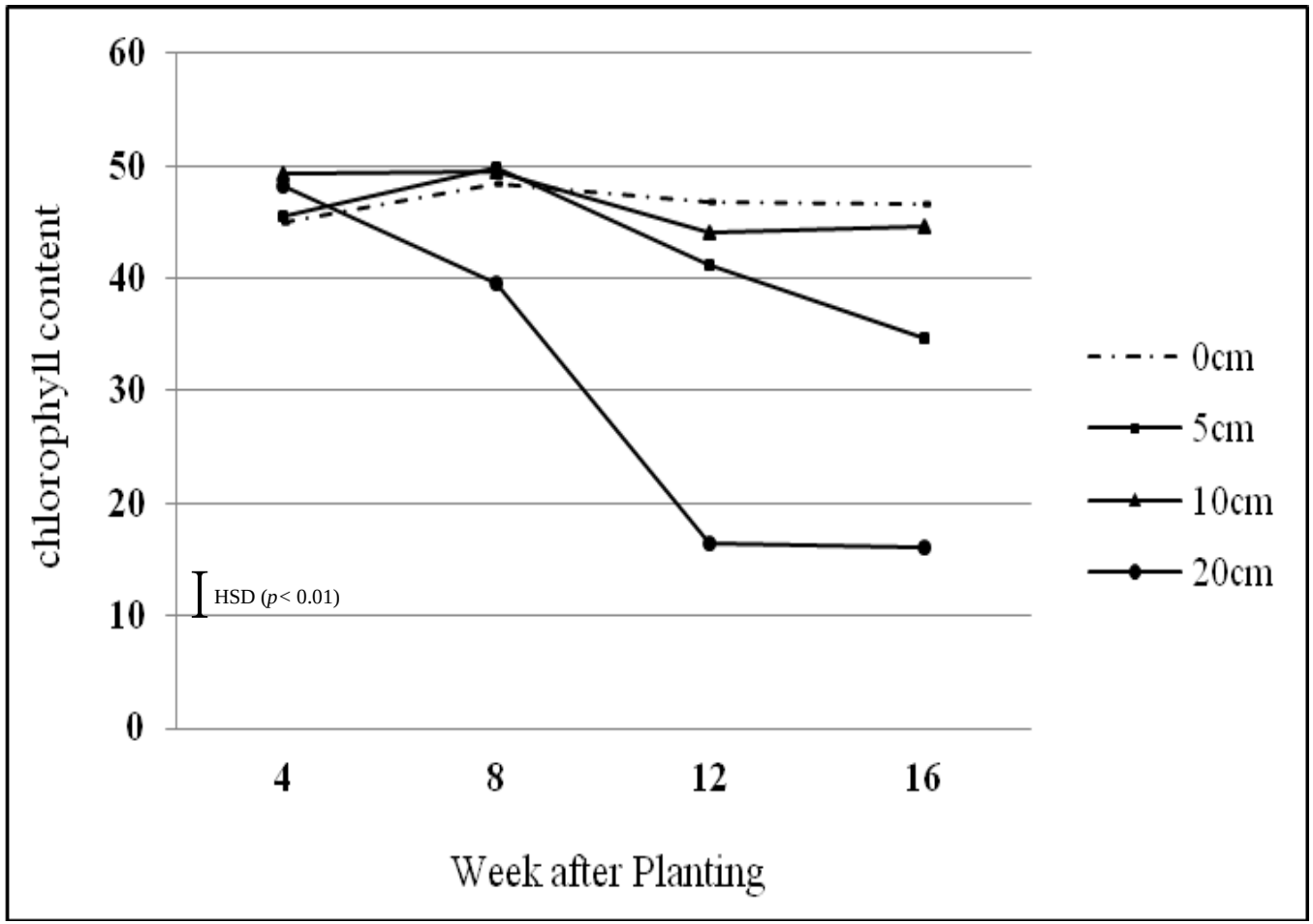

Fig. 3.11d Chlorophyll content in *Scirpus grossus* grown under different water depths (0 cm, 5 cm, 10 cm and 20 cm), with 100 g/ 1000 ml NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia.

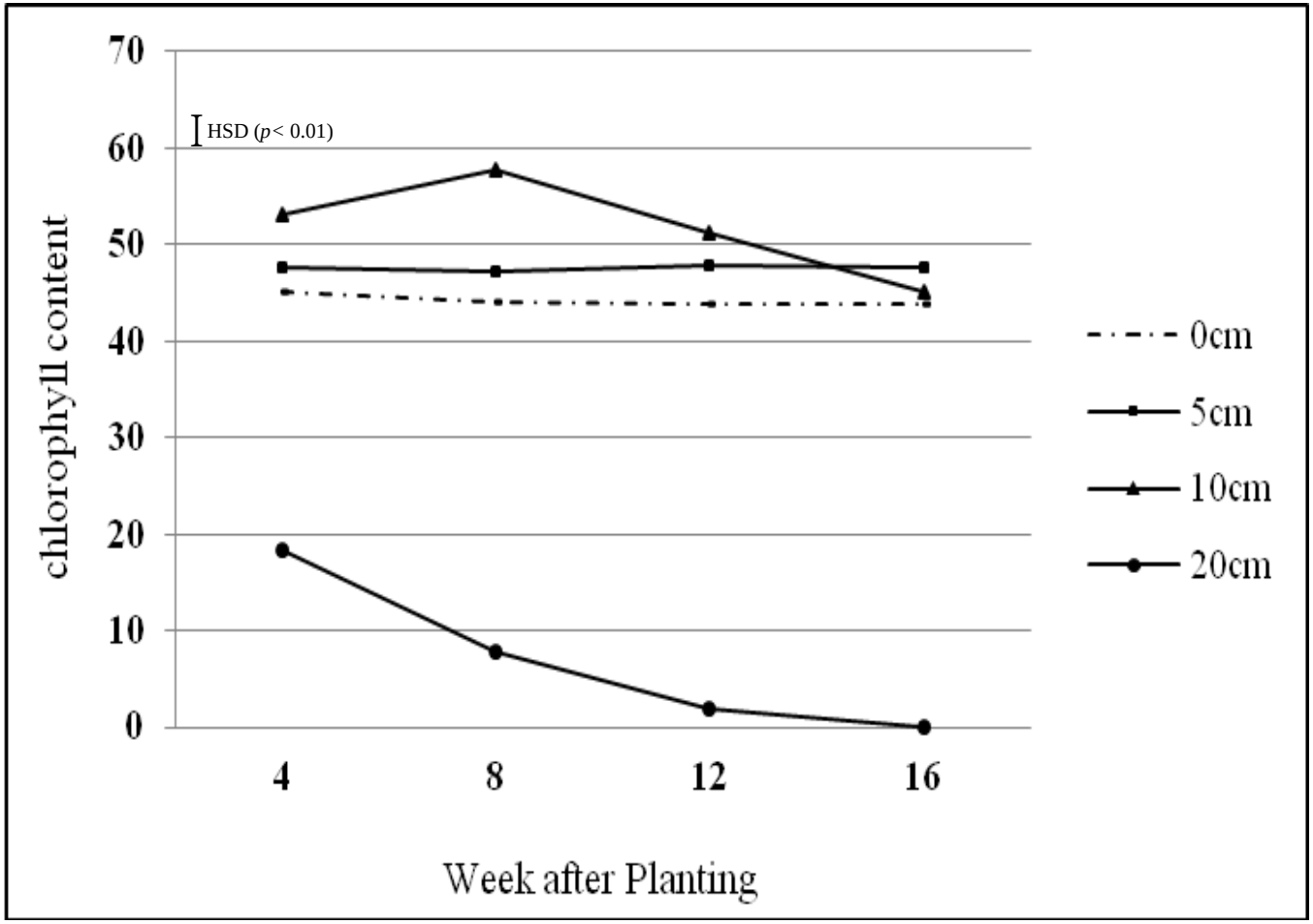

Fig. 3.11e Chlorophyll content in *Scirpus grossus* grown under different water depths (0 cm, 5 cm, 10 cm and 20 cm), with 125 g/ 1250 ml NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia.

3.3.3 Physico-chemical characteristics of fertilized and unfertilized peat and paddy soils

The physico-chemical traits of *S. grossus* plants grown in fertilized and unfertilized in peat and paddy soils are shown in Tables 3.4. Interestingly, the *S. grossus* plants grown on paddy soil had more nutrients compared with *S. grossus* plants grown on peat soil, with regard to several elements particularly, Al, B, Fe and Mn. Logically the plants grown on paddy soil should exhibit better and more robust growth during experimentation. This is shown in the results above, with regard to chlorophyll content, plant biomass and chlorophyll fluorescence whereby a slightly higher, more positive values were seen in *S. grossus* plants grown on paddy soil. A larger difference was observed with regard to plant biomass between *S. grossus* plants grown in peat and paddy soils.

Table 3.4 Physico-chemical characteristics of fertilized and unfertilized peat and paddy soils used at MARDI Research Station, Jalan Kebun and Tanjung Karang, Selangor, Malaysia*

Elements, moisture content, Protein content, pH	Treatments			
	Unfertilized soil (F0)		Fertilized soil (F1)	
	Peat	Paddy	Peat	Paddy
Al	0.0	196.9	0.0	320.5
B	0.0	14.3	0.0	24.0
Ca	2400.0	5600.0	3500.0	4300.0
Cu	4.7	4.8	8.0	4.7
Fe	8.0	200.0	29.0	300.0
K	13600.0	27100.0	18300.0	29600.0
Mg	1900.0	3600.0	3300.0	3000.0
Mn	200.0	1421.6	100.0	1393.0
Na	1200.0	759.1	1400.0	921.0
P	1500.0	1700.0	2300.0	1700.0
S	1700.0	2200.0	2100.0	1600.0
Zn	20.8	27.8	22.6	43.3
Total Nitrogen	9100.0	9000.0	12000.0	9400.0
Protein content	56700.0	4900.0	74800.0	72600.0
Moisture content	7.8%	7.6 %	6.5 %	8.4 %
pH	5.9	5.6	4.3	5.6

*****All values are in ppm, except for moisture content and pH.