A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE

Effect of nitrogen fertilization on carbon accumulation by *Miscanthus* × *giganteus* in both above and below ground

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MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY

DEPARTMENT OF PLANT SCIENCE

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

BY

YEON-HO PARK

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UNDER THE DIRECTION OF PROF. DO-SOON KIM

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ABSTRACT

Effect of nitrogen fertilization on carbon accumulation by *Miscanthus* × *giganteus* in both above and below ground

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Miscanthus is known for its high biomass yield and carbon accumulation, with requirement of relatively low nitrogen fertilizer. Little effort has been made to investigate the effects of nitrogen fertilizer on biomass yield and carbon accumulation. Therefore, this study was conducted to investigate the effects of nitrogen fertilization on biomass yield and carbon accumulation in the above- and below-ground parts of *M.* × *giganteus*. Miscanthus plants were cultivated under different fertilizations, 0, 30, 60, 120, and 240 kg N ha^{-1} $year^{-1}$, for 6 years. Above-ground biomass yield were assessed every year at harvest in early March. Below-ground biomass including rhizomes and roots was harvested and assessed in the 6th year, when above-ground biomass debris was also assessed. Soil was sampled in the 6th year and total carbon content in soil was analyzed. Cumulative above-ground biomass increased with increasing nitrogen fertilizer level with significant differences between non-fertilized treatment and fertilized treatments. Below-ground biomass also increased with increasing nitrogen fertilizer level up to 60 kg N ha^{-1} $year^{-1}$, and thereafter decreased with increasing nitrogen fertilizer level. However, no significant increase in soil total carbon (TC) was observed with nitrogen fertilizer level. In total, carbon accumulated in both above- and below-ground parts of miscanthus for 6 years increased with nitrogen fertilization although soil total carbon was not significantly increased by nitrogen fertilization. Linear regression analysis between nitrogen fertilization and carbon accumulation by miscanthus revealed that miscanthus could accumulate 27.23 t ha^{-1} annually even at no nitrogen fertilization. Carbon accumulation per unit nitrogen fertilization was estimated to be 0.021 t carbon ha^{-1} per kg N ha^{-1}.

Keywords: bioenergy crop, carbon accumulation, *Miscanthus* × *giganteus*, nitrogen fertilization, soil carbon

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1. Introduction

The Kyoto Protocol of the United Nations Framework Convention on Climate Change has been adopted by several worldwide leading countries, implementing the agreement that global warming is “extremely likely” to have occurred by human activity, so reducing atmospheric carbon dioxide is necessary to slow down global warming (Grubb, 1997). As combustion of fossil fuel is regarded as the most problematic human activity that emits most of the carbon dioxide, reduction of fossil fuel consumption by developed countries is being obliged based on the agreement. This obligation proposes another challenge in a sense that reduced amount of fossil fuel usage leads to lack of energy production, which is necessary in maintaining today’s industrialized world. Thus, an alternative energy source which is capable of producing sufficient energy as well as does not release carbon dioxide into atmosphere is needed.

Among number of candidates for the alternative energy source, plant biomass is a potential ideal energy source that can fulfill the both. Plant biomass is produced agriculturally, which makes it possible to be produced at large quantity by utilizing pre-existing agricultural bases, fulfilling enough amount of energy. Plant biomass is produced by photosynthesis, which is in other words that atmospheric carbon is removed and fixed in plant biomass, making it carbon neutral even if plant biomass is completely combusted for energy production. Thus, plant biomass is an ideal energy source that can produce sufficient energy without releasing carbon dioxide into atmosphere. Furthermore, plant biomass is burnable, while other alternative energy sources tend to focus on producing energy only in form of electricity. Burnable fuel is capable of being consumed in pre-existing industrial infrastructure including thermoelectric power plant and internal combustion engine. These traits of plant biomass make it a considerable ideal alternative energy source. However, number of problems still exist in plant biomass. One of problems is that producing plant biomass for energy source may bring food crisis, whether food crop is directly used for energy production or new crop is planted instead of food crop in agricultural land. Other problem is that plant biomass energy may not be truly carbon neutral (Azar, 2006) depending on conditions; for example, if nutritionally rich forestland is destroyed for cultivation of biomass crops, net carbon reduction ability of the land is decreased, making plant biomass energy an another carbon releasing source.

Miscanthus is a biomass crop, which is free from such problems. Miscanthus is a perennial rhizomatous C4 grass which produces large quantity of biomass suitable for biofuel. It requires relatively small resource for its cultivation, can be cultivated in marginal land without intruding pre-existing agricultural land as well as forestlands, able to be harvested for several years with a single propagation, and has reasonable energy input/output balance (Collura, 2006). Furthermore, miscanthus is more than a carbon neutral bioenergy crop in a sense that it can permanently accumulate carbon in below-ground part, including both below-ground biomass and soil (Clifton-Brown, 2004; Foereid. 2004; Hansen, 2004; Zatta, 2014).

When producing miscanthus plants as a bioenergy crop, it is necessary to calculate amount of resources input and output balance. It is commonly known that miscanthus consumes less amount of nitrogen, estimated at 50-70 kg N ha^{-1} $year^{-1}$ for production of 15-18 t DM ha^{-1} $year^{-1}$ (Himken, 1997), which can be considered a low input for large biomass yield. However, the amount of nitrogen consumed for meaningful carbon reduction may differ from that for optimum biomass production, resulting from different carbon fixation ability of miscanthus depending on the level of nitrogen fertilization. For this reason, the effect of nitrogen fertilization on carbon accumulation by miscanthus should be investigated in quantitative way.

This research was conducted to investigate carbon accumulation ability of miscanthus in such way, resulting from altered development and physiology affected by nitrogen fertilization. Result of the study is expected to be utilized not only in scientific fields, but also in deciding bioenergy production policy in context of economical energy production input/output as well as carbon accumulation obliged by the Kyoto Protocol.

2. Literature review

2.1 *Miscanthus* as a promising bioenergy crop

Miscanthus was originally known for its ornamental purpose or as an invasive weed species. Nowadays, as Kyoto Protocol of the United Nations Framework Convention on climate change was agreed by most of leading countries, cut down on fossil fuel consumption was obliged on major leading nations, and led to the research on alternative energy sources. Especially, among various energy sources, bioenergy crop is one of the major energy which is of interest, and among bioenergy crops, miscanthus has number of advantages over others.

Most of all, miscanthus has high biomass yield and energy production. Biomass yield of miscanthus is known to range 10-40 t ha^{-1} $year^{-1}$ in European regions (Jones & Walsh, 2001). Researches on miscanthus biomass yield resulted in 14.8-33.5 t ha^{-1} $year^{-1}$ in Germany (Kahle, 2001), 13.8-16.6 t ha^{-1} $year^{-1}$ in Italy (Salvatore, 2007). Its yield is comparatively higher than that of other bioenergy crops. Along with its high biomass yield, amount of energy that miscanthus produces is also reasonable. Result of research on *M.* × *giganteus* straw and pellet combustion showed that energy yield of miscanthus is 152-326 MJ ha^{-1} $year^{-1}$, and energy balance is 7.7-15.4, concluding it as a promising candidate as an alternative fuel (Collura, 2006). Mixed combustion experiment of pulverized miscanthus biomass with coal in power plant showed that combustion of plant biomass successfully produced enough amount of energy without causing negative effect on the facility (Rudiger, 1996). This result showed that miscanthus successfully produced energy in form of burnable fuel, which differentiates miscanthus from other alternative energy sources that produces energy mainly in electricity. Producing burnable fuel is an important advantage in a sense that burnable fuel is easily consumable in pre-existing infrastructure, including power plants and combustion engines. Furthermore, miscanthus also has high potential to replace large quantity of fossil fuel consumption when cultivated in nationwide scale. According to model 'MISCANMOD', if miscanthus is grown on 10% of suitable land area in EU, 234 TWh could be generated. The amount equals 9% of gross electricity generated in EU in 2000. At the same time, 76 Mt of carbon was reduced, which equals 9% of total EU carbon emission reduction required for Kyoto Protocol baseline levels (Clifton-Brown, 2004).

Miscanthus requires less resources as well as less cost for its cultivation. Miscanthus needs to be propagated by rhizome, which is expensive than seed propagation when compared for a single propagation. However, for its perennial trait, no more expenses for propagation is needed for at least 10 years after first year of establishment (Chung, 2012), resulting in cut down on cost of cultivation.

Miscanthus also has low demand for resources for optimized cultivation. *M. × giganteus*' water use efficiency is 9.5 g kg^{-1} (Beale, 1999). Nitrogen use efficiency of miscanthus was 0.35, which is higher compared to that of triticale and red canary grass, 0.14 and 0.11 t $(kg\ N)^{-1}$ respectively (Lewandowski, 2006). In the same research, energy use efficiency was 54, 26, 13 GJ bioenergy per GJ energy input in miscanthus, triticale, and red canary grass, respectively.

Miscanthus can adapt to various climates. Taking into account that there has been much concern that production of bioenergy crop may bring about ecological and food price issue (Ciaian, 2011; Johansson, 2007), combined with miscanthus' ability to grow in marginal land, this trait may make it possible for miscanthus to utilize more marginal land for cultivation without intruding agricultural land or forestland. Miscanthus species can grow in from lower latitude of Mediterranean region to high latitude of Scandinavian region (Chung, 2012). Not only the miscanthus can grow in higher latitude, but also it does not lose its productivity in low temperature condition. Though it is well known that C4 carbon fixation pathway is suited for photochemical activity in low carbon concentration and high temperature, miscanthus can maintain high photosynthesis rate even in cool climate (Long, 1999).

2.2 Carbon accumulation of miscanthus

Since Kyoto Protocol focuses on cut down on carbon dioxide emission, not only biomass yield and energy potent but carbon accumulation ability of bioenergy crop is also one other important factor in deciding which crop to produce. All crops remove and fix carbon dioxide from the atmosphere, permanently storing it in its biomass, and reducing atmospheric carbon as a result. However, carbon fixation rate varies among crops, and in some cases, crop reduces carbon fixation ability of the land. For instance, converting uncultivated land into corn cultivation resulted in 3-8 t ha^{-1} $year^{-1}$ of carbon loss in soil (Kristina, 2008). Cultivating food crops for purpose of bioenergy production in rainforests, savannas, and grasslands resulted in releasing 17 times more carbon than the carbon reduced by produced bioenergy (Fargione, 2008). Though optimized management in cropland may reduce carbon loss, the amount is small compared to that of carbon loss. Long-term studies regarding various crops, including cereals, legumes and maize, resulted in 0.57 t ha^{-1} $year^{-1}$ of additional carbon sequestration with change to no-till farming, and 0.20 by enhancing crop rotation (West, 2012). Management of maize cropland and switchgrass resulted in 1 t ha^{-1} $year^{-1}$ of increased carbon sequestration (Follett, 2012). Even cultivating perennial plants for bioenergy in former cropland resulted in little amount of increased carbon fixation. Cultivation of various perennial grass resulted in 0.44-1 t ha^{-1} $year^{-1}$ of additional carbon sequestration in average (Don, 2012; Kristina, 2009; Post, 1999). Cultivation of switchgrass in pre-existing farmland resulted in 2.5 t ha^{-1} of carbon accumulation in soil over five years (Liebig, 2008), and insignificant change in soil carbon in change from maize cropland (Garten, 1999). For these reasons, there still exists questions whether cultivating bioenergy crop has remarkable effect in reducing carbon.

To solve such questions, a bioenergy crop which is able to accumulate significant amount of carbon into soil as well as can be cultivated in lands that has low carbon sequestration effect, such as marginal land, is needed. Among number of bioenergy crops, miscanthus fulfills both conditions. Number of studies support the fact that miscanthus can accumulate large quantity of carbon in both below-ground biomass and soil (Clifton-Brown, 2004; Foereid, 2004; Hansen, 2004; Zatta, 2014), making it carbon reducing rather than carbon neutral. Soil has capability of storing mass quantity of carbon, known to be 3.3 times than that of atmospheric carbon and 4.5 times than that of biotic carbon (Lal, 2004). Research took place in Southern Ireland for 16 years of miscanthus cultivation resulted in 20.7 t ha^{-1} $year^{-1}$ of below-ground dry biomass in 0-40cm profiles (Clifton-Brown, 2007). At the same time, total carbon storage in below-ground biomass accounted for 8.8 t C ha^{-1} in 0-30cm profiles. Another research took place in Germany resulted in 1.63 t C ha^{-1} $year^{-1}$ (Kahle, 2001). Since carbon fixation of miscanthus in its rhizomatous and root takes up most of the total carbon fixed by miscanthus, this trait can make miscanthus a major carbon reducing bioenergy crop. Furthermore, miscanthus has higher carbon accumulation ability compared to other crops. It is expected that miscanthus' carbon accumulation ability can play an important role in reducing net atmospheric carbon.

3. Materials & Methods

3.1 Plant materials

M. × *giganteus* rhizomes were planted at 1m x 1m in 10 lysimeters located at the Experimental Farm Station of Seoul National University, Suwon, Korea in March 2011. Nitrogen fertilizer was applied in urea form (CH_4N_2O) at 0, 30, 60, 120, 240 kg N ha^{-1} year^{-1} in every March. Each level of nitrogen fertilizer was applied to two individual lysimeters, of which each lysimeter (4 m x 4 m) was then subdivided into 2 subplots (2 m x 4 m), giving 4 replications for each level of nitrogen fertilization. The 10 lysimters applied with different nitrogen fertilizations were arranged in a completely randomized design.

Weed management was made by spraying S-metolachlor (Dual gold, Syngenta) and dicamba (Banvel, Sungbo Chemical) in the 1st and the 2nd year of this study. Since the 3rd year, no additional weed management was made due to full canopy cover by miscanthus regardless of nitrogen fertilization level.

3.2 Above-ground analysis

3.2.1 Analysis of shoot biomass

Canopy height of miscanthus was assessed in November before harvest. Above-ground shoots were harvested by cutting at the soil surface level from the area of 50 cm x 50 cm in February after overwintering. The harvest from the sampling area was made at three different places in each replication. Dry biomass yield and its components such as number of stems, stem length, stem diameter, number of nodes, and panicle length were then recorded.

3.2.2 Analysis of debris biomass

Biomass debris was harvested from the area of 50 cm x 50 cm in the 6th year of the study. Biomass debris included fallen leaves, stem debris, unharvested stump, and other shoot parts deposited on the ground level, which was not cleared for six years of the stduy. Harvest was made by hand raking until the soil surface was exposed. Harvested biomass debris was dried in a dry oven at 80℃ for two days, and weighed.

3.2.3 Estimation of above-ground carbon accumulation

Carbon quantity of above-ground biomass was estimated by yield of above-ground biomass and carbon content in miscanthus' biomass based on Kahle's study (2001). Kahle concluded that carbon content was estimated at 48.4% in harvested shoot biomass in average. Carbon content of biomass debris was estimated to be 48.3% in the same study, which is defined as "decomposing above-ground biomass residues of the previous year".

3.3 Below-ground analysis

3.3.1 Soil sampling

Soil samples were collected following Zatta's method (2014), in which samples were divided by distance from center of the plant. Sampling was done in March after the 6^{th} year of the study. Soil sampling was made at four points in each replication, and five soil profiles were collected for each point of collection. The four sampling points include 0 cm, 12.5 cm, 25 cm, and 17.7 cm apart from the center of miscanthus plant, representing the center of the plant, quarter the distance between two plants, central point of two plants, and central point of four plants, respectively. The five soil profiles include 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, 80-100 cm depth from soil surface. Steel pipe of 10 cm diameter was used 0-20 cm soil profile, while soil auger (5 cm of inner diameter) was used for the other deeper soil profiles. For collection of 0-20cm profiles, steel pipe was embedded with hammer to reach 20cm depth from soil surface. To precisely embed pipe, upper side of the pipe was marked at 20cm length from the bottom of the pipe. Pipe was twisted for complete separation of soil, and pulled out. Auger was marked at 40, 60, 80, and 100cm from the bottom part to precisely collect profiles. Soil sample was pulled out with auger, and upper half of the soil was removed to avoid mixing of soil from other profiles. Hand separable underground biomass including rhizomes and roots in each sampled soil was separated by hand, washed and oven-dried before recording its dry weight. Soil samples were transferred to plastic trays, mixed, and air-dried for a week. Air-dried samples were then sifted with 2 mm mesh. Additional underground biomass was separated during sifting, and separated biomass was washed, oven-dried, and weighed. Soil was stored at -20 ℃ before analysis.

3.3.2 Analysis of soil carbon content

Prepared soil was thoroughly mixed, and 10g of each sample was pulled out. Soil was finely ground with mortar, and were analyzed with CHNS analyzer (vario MICRO cube, Elementar, Germany) to assess total carbon (TC) and total nitrogen (TN) in percentage.

3.3.3 Estimation of below-ground carbon accumulation

Carbon quantity of below-ground part was estimated by carbon quantity in below-ground biomass and carbon content in soil. Carbon quantity of below-ground biomass was estimated by below-ground biomass yield and carbon content in miscanthus' biomass based on Kahle's study (2001). Kahle concluded that carbon content was estimated at 44.8% in below biomass in average, calculated by carbon content of root and rhizome, and ratio of the two in total below-ground biomass. Carbon quantity in soil carbon was calculated by multiplying total carbon content of soil to bulk density of soil.

4. Results

4.1 Effect of nitrogen fertilization on above-ground biomass

Average shoot biomass yield of miscanthus is as shown in the table below (Table 1). In 4th year, severe drought in summer resulted in reduced yield. Highest shoot biomass was 41, 1159, 2279, 1425, 1975, 2271 g m^{-1} in year 1-6, respectively. In all years, biomass yield showed overall increasing tendency with increasing nitrogen fertilization, except for 1st and 5th year when yield at 240 kg N ha^{-1} $year^{-1}$ was lower than that of nitrogen fertilization of 120 kg N ha^{-1} $year^{-1}$. Lowest shoot biomass was 11, 357, 916, 738, 1200, 2100 g m^{-1} in year 1-6, respectively. In all years, lowest shoot biomass yield was shown in 0 kg N ha^{-1} $year^{-1}$. Result of ANOVA test on above-ground biomass yield in each year showed meaningful difference between treatments, resulting in F-value lower than 0.01 in all years.

Table 1. Harvested shoot biomass of miscanthus in 1 m × 1 m subplot under different nitrogen fertilizations.

Nitrogen fertilization (kg N ha^{-1} $year^{-1}$)	Harvested shoot biomass in 1m x 1m subplot (g)					
	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
0	11	357	916	738	1,200	2,100
30	24	535	1,517	1,042	1,925	2,158
60	41	672	1,638	1,287	1,919	2,133
120	41	1,055	2,025	1,158	1,975	2,413
240	35	1,159	2,279	1,425	1,644	2,771
$LSD_{0.05}$	15.54	242.6	490.5	302.2	297.8	406.4

Yield component was also assessed along with total biomass yield in 6th year (Table 2). Plant height increased to reach its maximum at 30 kg N ha^{-1} $year^{-1}$, and decreased with more fertilization. Panicle length decreased with more nitrogen fertilization, resulting in net stem length of (panicle length excluded from plant height) 331, 368, 366, 360, 343 cm in 0, 30, 60, 120, 240 kg N ha^{-1} $year^{-1}$, respectively. Number of tillers was significantly large in 240 kg N ha^{-1} $year^{-1}$, but couldn't find significant difference in rest of nitrogen fertilization levels. We couldn't find significant difference in leaf age. Stem diameter increased with nitrogen fertilization to reach its maximum at 120 kg N ha^{-1} $year^{-1}$, and decreased at 240 kg N ha^{-1} $year^{-1}$.

Table 2. Yield components of harvested shoot biomass assessed in the 6th year after planting miscanthus grown under different nitrogen fertilizations.

Nitrogen fertilization (kg N ha^{-1} $year^{-1}$)	Total dry weight (g)	Plant height (cm)	Number of tillers	Leaf age	Stem diameter (mm)	Panicle length (cm)
0	2100.0	402.0	46.4	14.6	9.0	71.5
30	2158.3	430.1	43.1	14.9	9.7	62.1
60	2133.3	424.0	45.8	14.5	9.9	57.8
120	2412.5	414.3	43.5	16.1	10.3	54.6
240	2770.8	394.5	55.3	15.6	9.8	51.2
$LSD_{0.05}$	406.4	14.18	7.35	0.74	0.56	7.02

Six years' cumulative Miscanthus above-ground biomass also increased with nitrogen fertilization. Cumulative miscanthus above-ground biomass was 5185±369, 7108±445, 7558±137, 8496±246, 9127±252 g m^{-1} in 0, 30, 60, 120, 240 kg N ha^{-1} $year^{-1}$ respectively. Significant increase in biomass was observed in 30 kg N ha^{-1} $year^{-1}$, and there was no significant increase was observed in other nitrogen fertilization levels.

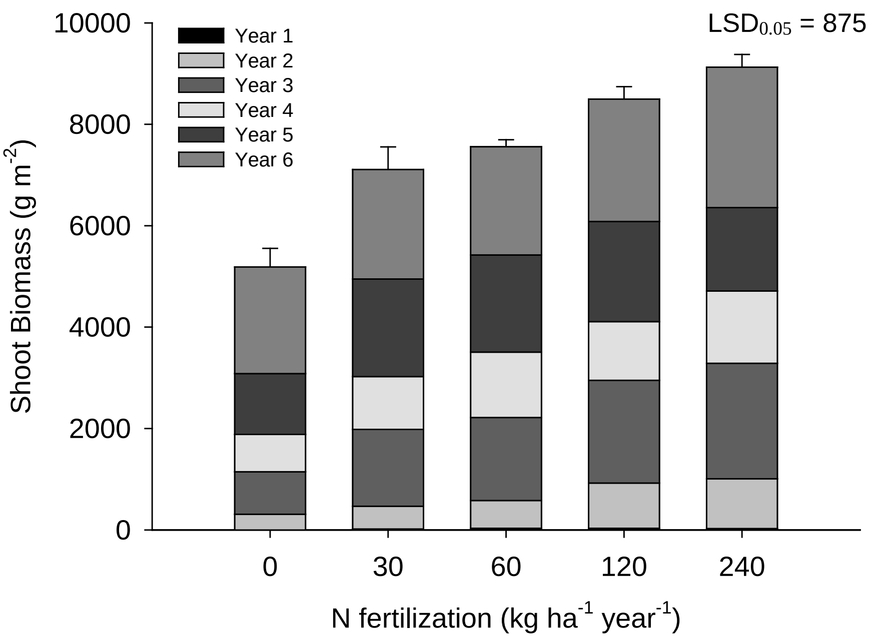

Figure 1. Cumulative shoot biomass of miscanthus grown under different nitrogen fertilizations.

Particularly, result of linear regression analysis on nitrogen fertilization and biomass production of 6th year (Fig. 2) shows that additional nitrogen fertilization had positive correlation to shoot biomass production. The coefficient of biomass production for annual nitrogen fertilization was 0.029 t kg^{-1}.

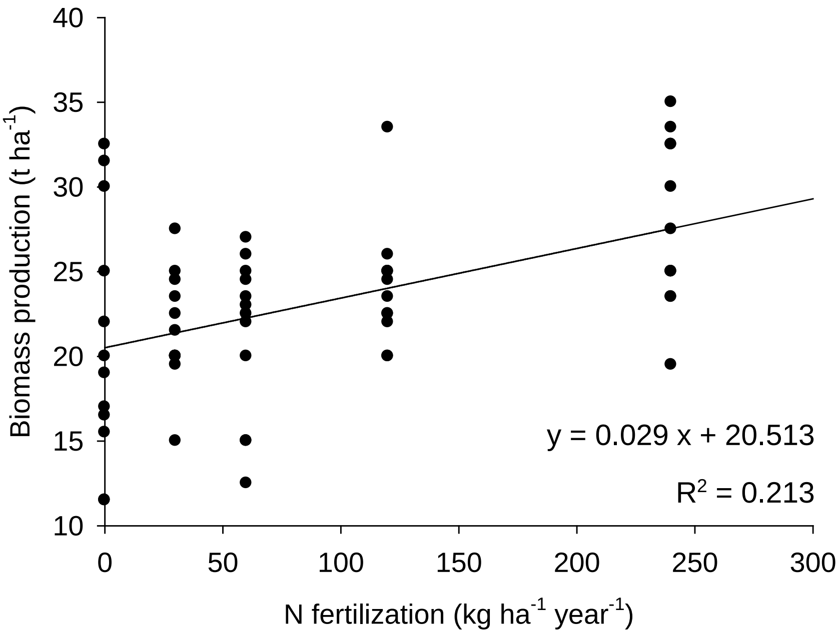

Figure 2. Linear relationship between nitrogen fertilization and biomass production in the 6th year.

4.2 Effect of nitrogen fertilization on below-ground biomass

Most of the below-ground biomass was observed to be distributed at depth of 0-20cm, taking up 99.5% of total underground biomass. Though biomass existed in most 20-40cm profiles, quantity of biomass was small (<0.01g per sample). Small amount (<0.01g) of biomass also existed in 4 samples in 40-60cm profiles, and 16 samples in 60-80cm profiles out of total 80 samples per each profile. No biomass was found deeper than 80cm from soil surface. Below-ground biomass in 0-20cm profile was 16.29±2.51, 24.80±3.17, 25.95±4.93, 17.10±3.84, 21.26±3.58 g in 0, 30, 60, 120, 240 kg N ha^{-1} $year^{-1}$, respectively. Below-ground biomass showed increasing tendency in low nitrogen fertilization, showed highest value in 60 kg N ha^{-1} $year^{-1}$, and decreased as more nitrogen fertilizer was applied. Calculated by surface area of collected samples (circle, 10cm in diameter), below-ground biomass quantity was converted to 20.7, 31.6, 33.0, 21.8, 27.1 t ha^{-1} in 0, 30, 60, 120, 240 kg N ha^{-1} $year^{-1}$, respectively. Below-ground biomass was highest at 60 kg N ha^{-1} $year^{-1}$ (Figure 3). Further increase in nitrogen fertilization reduced below-ground biomass.

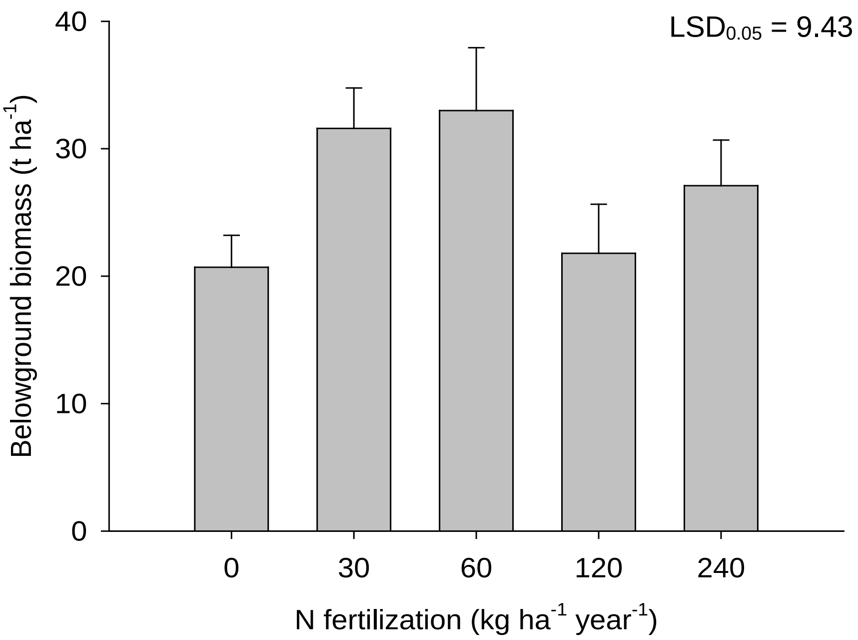

Figure 3. Below-ground biomass of miscanthus grown under different nitrogen fertilizations.

4.3 Effect of nitrogen fertilization on soil total carbon content

Below-ground biomass was excluded when calculating soil TC content, and carbon content only in soil was calculated with elemental analyzer. Soil TC content ranged from 0.57% to 0.64% but was not significantly affected by nitrogen fertilization (Figure 4). No significant difference of soil TC content by distance from plants was observed either.

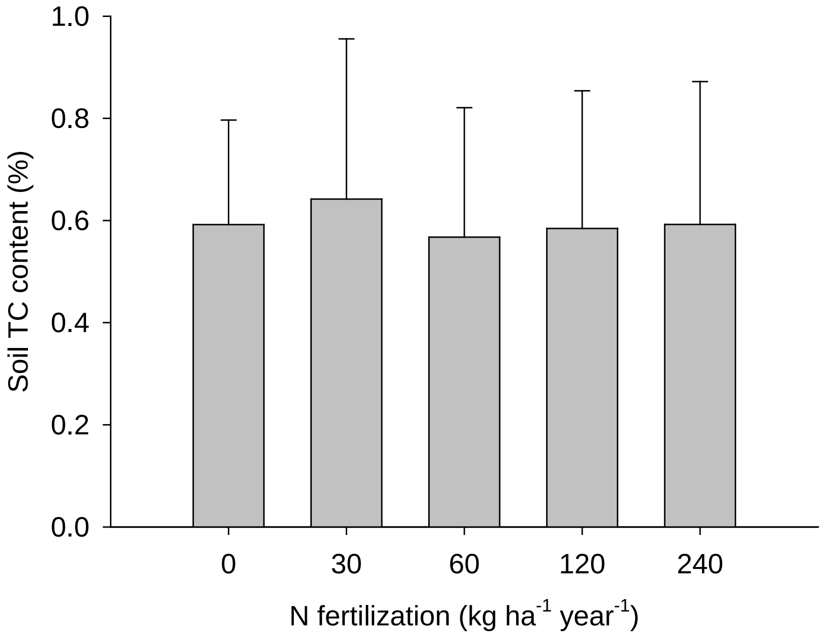

Figure 4. Soil TC content influenced by miscanthus grown under different nitrogen fertilizations.

However, vertical distribution of total soil carbon showed significant difference by soil depth, showing the highest soil TC content of 1.1% at 0-20 cm soil profile and significant reduction to around 0.5% at soil profiles deeper than 20 cm (Figure 5). Interestingly, total carbon content of soil at 0-20cm profile showed more than double in all nitrogen fertilization levels, ranging from 221% to 268% of average TC content of other profiles. This indicates that carbon in shallow soil profile derived from outer source, particularly from decaying rhizomes and biomass debris.

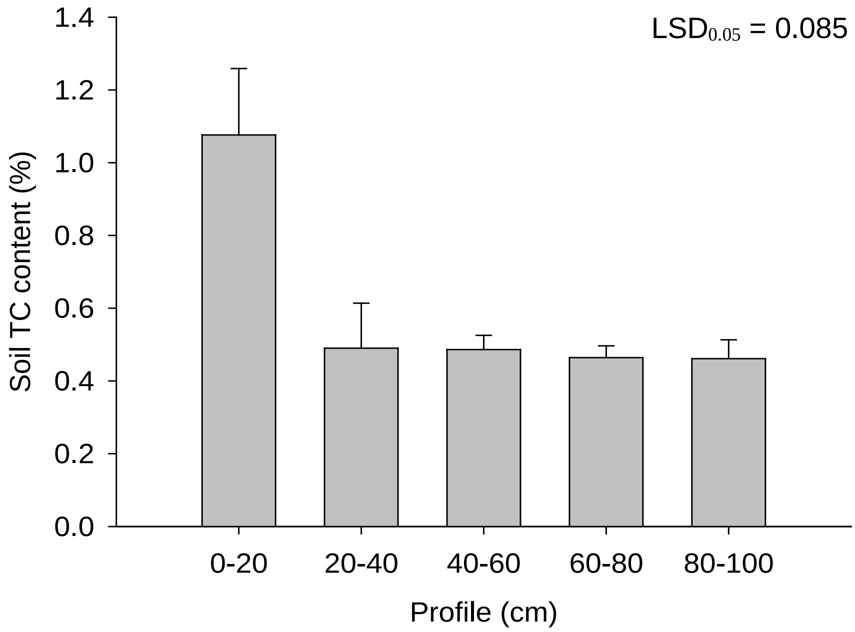

Figure 5. Soil TC content at different profiles.

Since vertical distribution of soil TC content showed significant difference at 0-20 cm soil profiles compared to deeper profiles, TC content in 0-20cm profiles was expected to show significant difference by nitrogen fertilization. The results showed that TC content in soil was highest at nitrogen fertilization of 30 kg ha^{-1} $year^{-1}$ of nitrogen fertilization, resulting in 1.19% of TC content. However, ANOVA result on did not show significant difference by nitrogen fertilization, resulting in F-value>0.51.

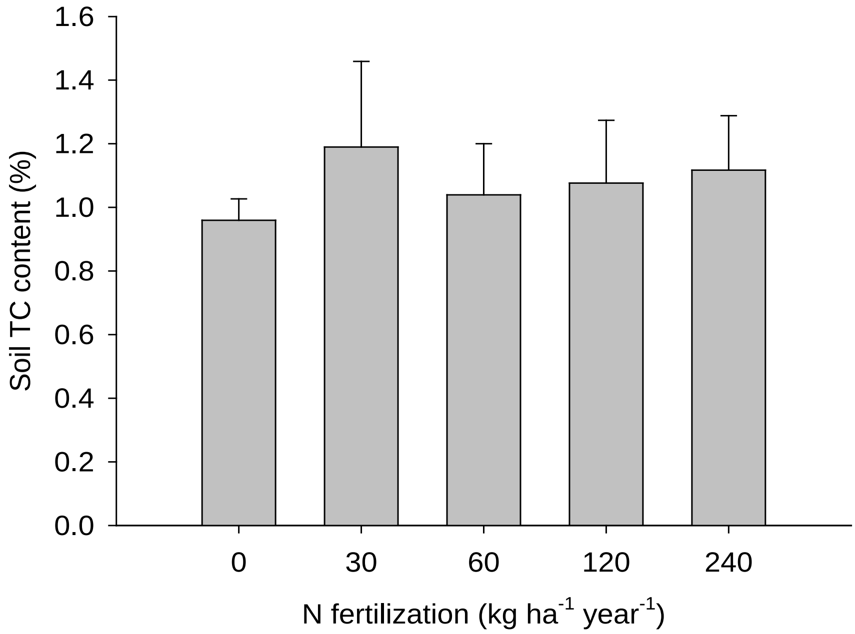

Figure 6. Soil TC content at 0-20cm profiles.

Total below-ground soil quantity in 0-20cm profiles was calculated with carbon quantity in soil at 0-20cm profiles and carbon quantity in below-ground biomass at 0-20cm profiles. Carbon quantity in soil was calculated by multiplying carbon content to bulk density of silty loam soil. Soil bulk density was estimated by classification referring to "unified soil classification system" (Howard, 1986) and bulk density calculated by USDA National Cooperative Soil Survey, National Cooperative Soil Characterization Database (Available online, accessed October/1/2017). Carbon quantity in below-ground biomass was calculated by converting harvested biomass yield to t ha-1, and multiplying it to carbon content of miscanthus biomass suggested in Kahle's experiment (2001), which converted 44.8% of below-ground biomass to carbon quantity. 36.3, 47.5, 42.9, 40.1, 43.4 t ha^{-1} of carbon was accumulated in below-ground part in 0, 30, 60, 120, 240 kg N ha^{-1} $year^{-1}$, respectively. Statistical results, however, resulted in insignificant effect (F-value>0.27) of nitrogen fertilization on total below-ground carbon quantity.

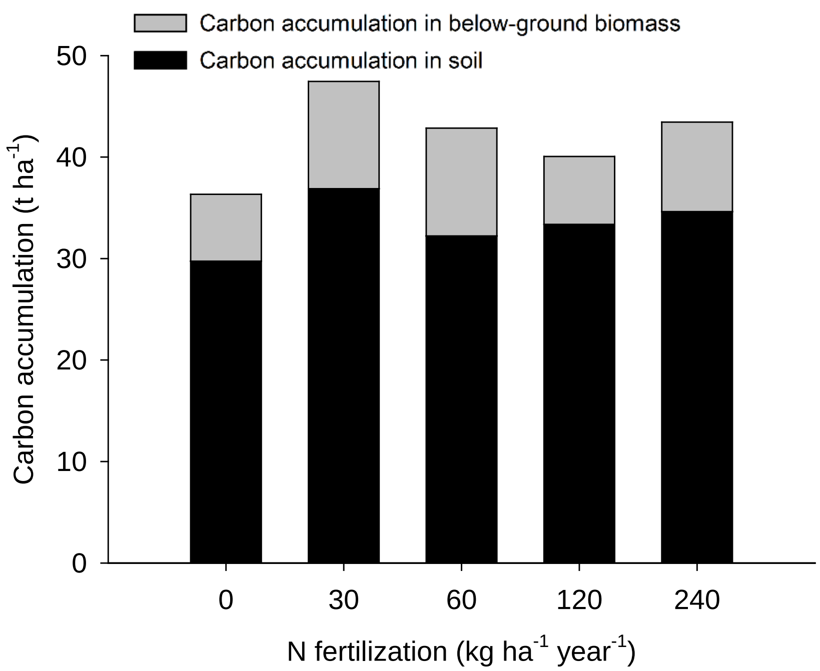

Figure 7. Total below-ground carbon accumulation at 0-20cm profiles, including carbon in soil and carbon in below-ground biomass.

4.4 Effect of nitrogen fertilization on total carbon accumulation by miscanthus

Total carbon sequestration by miscanthus was calculated based on above-ground biomass of 6th year, biomass debris, and below-ground biomass at 0-20cm profiles. Soil TC content was excluded due to its absence of significant results and difficulties in converting TC content into actual carbon quantity. Carbon quantity per area was calculated using carbon content of miscanthus biomass in Kahle's experiment (2001), which converted 48.4% of harvested above-ground biomass, 44.8% of below-ground biomass, and 48.3% of debris biomass to carbon. Total carbon accumulation increased as nitrogen fertilization increased, reaching its maximum at 240 kg N ha^{-1} year^{-1}. Total carbon quantity accumulated in miscanthus biomass was 24.05±2.49, 30.46±2.09, 30.9±1.94, 27.95±1.52, 32.25±2.29 t ha^{-1} in 0, 30, 60, 120, 240 kg N ha^{-1} year^{-1}, respectively. The quantity equals 80.09, 101.43, 102.90, 93.07, 107.39 t ha^{-1} of carbon dioxide fixation.

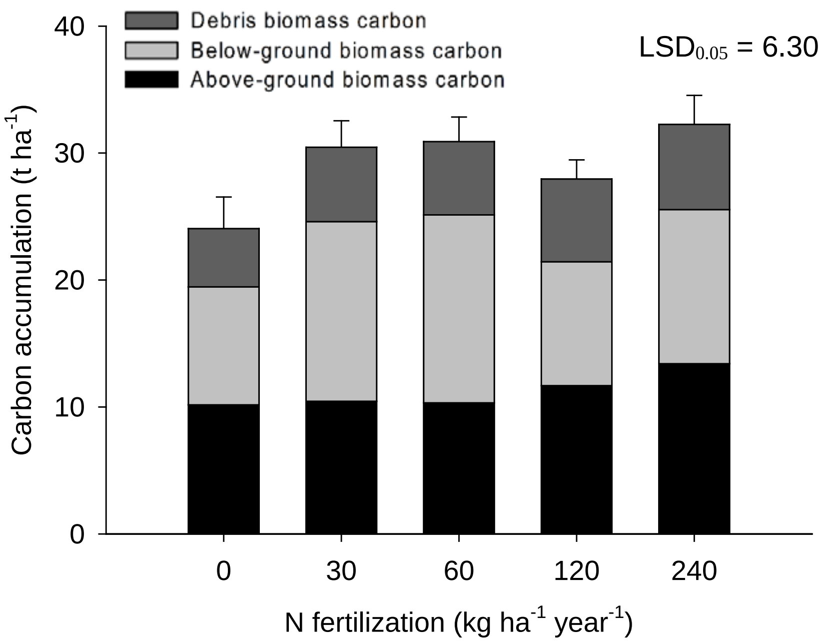

Figure 8. Total carbon accumulation by miscanthus.

Among total carbon quantity in biomass, 9.29, 14.15, 14.80, 9.76, 12.13 t ha^{-1} of carbon was accumulated in below-ground biomass, which takes up 66.1%, 46.5%, 47.9%, 34.9%, 37.6%, respectively. Ratio of carbon accumulation in below-ground biomass was at its maximum at 60 kg N ha^{-1} $year^{-1}$, and the ratio was significantly high in 30, 60 kg N ha^{-1} $year^{-1}$ compared to other treatments.

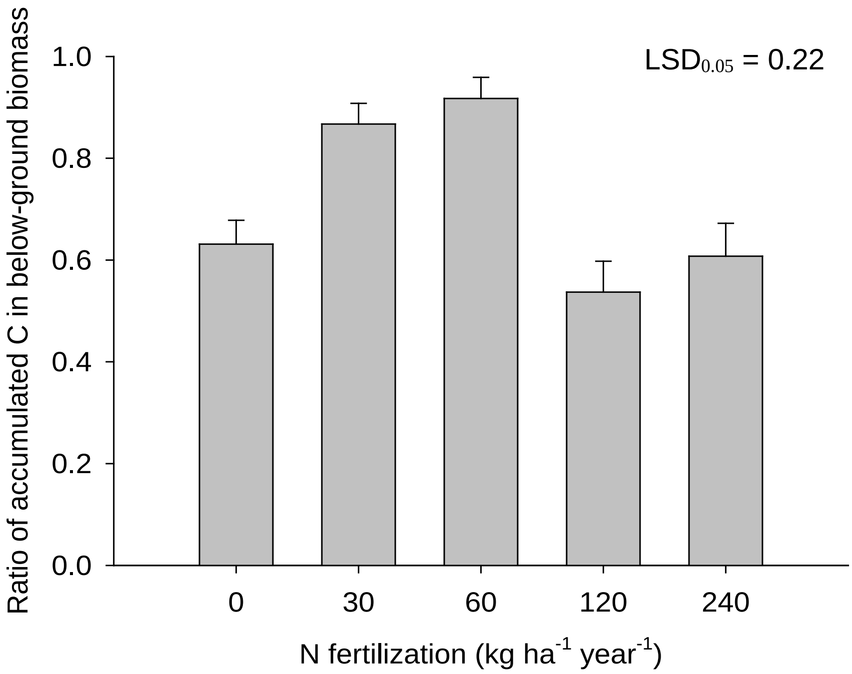

Figure 9. Ratio of carbon accumulated in below-ground biomass among total carbon accumulated in whole plant biomass.

Result of linear regression analysis of annual nitrogen fertilization and total carbon accumulation in miscanthus' biomass is shown as below (Figure 10). However, no significant coefficient between nitrogen fertilization and accumulated carbon in below-ground biomass was observed.

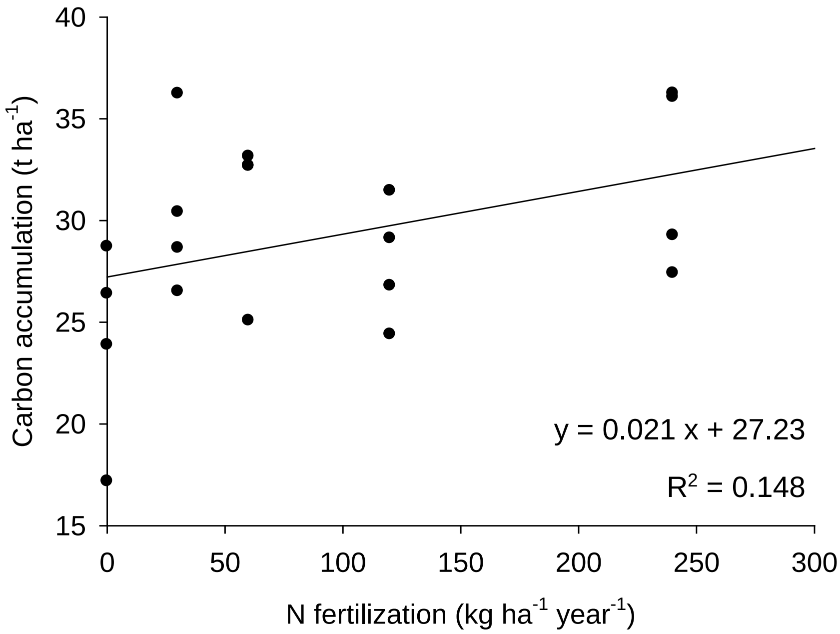

Figure 10. Linear relationship between nitrogen fertilization and total carbon accumulated in above- and below-ground parts of miscanthus for 6 years.

5. Discussion

According to the results, nitrogen fertilization had positive effect on overall biomass production of miscanthus in all treated plots. For above-ground parts of *M.* x *giganteus*, harvested biomass ranged from 21-27.7 t ha^{-1} in 6^{th} year, which is within range of miscanthus yield in European regions of 10-40 t ha^{-1} $year^{-1}$ (Jones & Walsh, 2001; Kahle, 2001; Salvatore, 2007). More nitrogen fertilization led to more above-ground biomass production in all fertilization levels, resulting in more energy production. However, though there was significant difference between biomass production in non-fertilized and fertilized plots, no significant difference was observed among fertilized plots. For below-ground parts of *M.* x *giganteus*, we could find out that below-ground biomass increased with nitrogen fertilization to reach its peak at 60 kg N ha^{-1} $year^{-1}$, and decreased as more fertilizer was added. Below-ground biomass was in range of 16.29-25.95 t ha^{-1}, which is a proximity to range of 17.4-30 t ha^{-1} in Japan (Yazaki, 2004) and 15-30 t ha^{-1} in Germany (Azar, 2006; Kahle, 2001). The most of below-ground biomass (99.5%) was observed in 0-20 cm soil profiles, which corresponds to Hansen's research that rhizome and root was barely observed deeper than 20 cm (Hansen, 2004).

On the other hand, we couldn't find clear relation between nitrogen fertilization and soil TC content in this study. Plant biomass' dedication to permanent carbon fixation in soil derives from decaying underground biomass part. As 25% of below-ground biomass dies annually and contributes to carbon content in soil (Shoji, 1990), soil carbon content was expected to show highest number when below-ground biomass part of miscanthus is at its maximum development. However, no significant difference was observed between treatments due to its high variance. Though TC content in 0-20cm profiles was significantly higher than other profiles and was expected to show significant difference, no significant difference was observed either. Number researches had similar result that change in soil carbon content by miscanthus was not significant. Research conducted in Denmark for 9-16 years with *M.* × *giganteus* resulted in insignificant change in the overall soil organic carbon (Hansen, 2004). The reason for such result was proposed by Zatta (2014). According to Zatta, cultivation of *M.* × *giganteus* resulted in its dedication to soil carbon, resulting in 12% of soil organic carbon deriving from miscanthus. In the same research, however, total soil organic carbon stock did not significantly change. It suggesting that the rhizosphere of miscanthus absorbed easily accessible carbon in soil, resulting in carbon loss rather than accumulation in early development, and more carbon was absorbed and lost when rhizosphere was more developed. As a result, miscanthus derived carbon replaced rather than accumulated, carbon in soil. Because the lifespan of miscanthus is estimated to be over 10 years, soil carbon deriving from decaying below-ground biomass of miscanthus could not be evaluated in relatively short period of time. Even simulation of 20 years of miscanthus cultivation via "RothC" model resulted in insignificant change in SOC quantity (Zatta, 2014). Researches on soil carbon content in miscanthus plantation for longer time, even for number of decades, is needed for analysis on long-term effect of Miscanthus on soil carbon content.

We could also find out that miscanthus had high nitrogen use efficiency in producing utilizable biomass. Results of linear regression analysis showed that biomass production increased by additional nitrogen fertilization was small. Also, ratio of actual stem, excluding panicle length from plant length, increased with increasing fertilization in 6th year but the rate of increase was not significant. Since stem is the main part of plant that can be utilized as bioenergy, this result shows that ratio of highly utilizable part of miscanthus is high enough at low fertilization level taking cost of fertilization into account. The integrated results indicate that miscanthus' nitrogen use efficiency is at its optimum in low nitrogen fertilization.

Taking political and environmental visions into account in deciding miscanthus as a bioenergy crop, both energy balance and carbon reducing effect should be assessed. As number of researches has shown that miscanthus' energy balance is reasonable (Rudiger, 1996; Collura, 2006) and can replace fossil fuel consumption effectively (Clifton-Brown, 2004), energy balance of miscanthus is able to be considered as enough. And so, carbon reducing effect should be considered in more detail. Technically telling, plant biomass energy is carbon neutral, because biomass originates from fixed atmospheric carbon that plant assimilated via photosynthesis pathway. However, plant biomass energy can either be carbon negative or positive, affected by environment and surroundings of bioenergy crop cultivation (Azar, 2006). Ultimate carbon reduction requires permanent fixation of atmospheric carbon. Therefore, only carbon fixed in below-ground biomass and soil can be regarded as miscanthus' contribution to carbon removal since above-ground biomass will be combusted to release fixed carbon into atmosphere. Our study did not show significant carbon accumulation in soil in the form of soil carbon as 6 years of our field study was not long enough for below-ground biomass converted into soil carbon, further studies should be conducted for much longer period to investigate the long-term effect of nitrogen fertilization on miscanthus' carbon sequestration into soil.

6. References

1. **A don, B Osborne, A Hastings, U Skiba, et al**. 2012. Land-use change to bioenergy production in Europe: implications for the greenhouse gas balance and soil carbon. *GCB Bioenergy* 4 (4): 372-391

2. **A Zatta, J C Clifton-Brown, P Robson, A Hastings, A Monti**. 2014. Land use change from C3 grassland to C4 *Miscanthus*: effects on soil carbon content and estimated mitigation benefit after six years. *GCB Bioenergy* 6: 360-370.

3. **B Foereid, A Neergaard, H Høgh-Jensena**. 2004. Turnover of organic matter in a *Miscanthus* field: effect of time in *Miscanthus* cultivation and inorganic nitrogen supply. *Soil Bioenergy & Biochemistry* 36: 1075-1085.

4. **C Azar, K Lindgren, E Larson, K Möllersten**. 2006. Carbon capture and storage from fossil fuels and biomass - costs and potential role in stabilizing the atmosphere. *Climatic Change* 74: 47-79.

5. **C T Garten, S D Wullschleger**. 1999. Soil carbon inventories under a bioenergy crop (switchgrass): Measurement limitations. *Journal of Environmental Quality* 28(4): 1359.

6. **D J A Johansson, C Azar**. 2007. A scenario based analysis of land competition between food and bioenergy production in the US. *Climatic Change* 82: 267-291

7. **E M Hansen, B T Christensen, L S Jensen, K Kristensen**. 2004. Carbon sequestration in soil beneath long-term *Miscanthus* plantations as determined by 13C abundance. *Biomass and Bioenergy* 26: 97-105.

8. **J H Chung, D S Kim.** 2012. *Miscanthus* as a potential bioenergy crop in East Asia. *Journal of Crop Science and Biotechnology* 15 (2): 65-77.

9. **Howard**, K **Amster**. 1986. Soil classification handbook: unified soil classification system. Denver, Colo.: Geotechnical Branch, Division of Research and Laboratory Services, Engineering and Research Center, Bureau of Reclamation.

10. **H Rüdigger, A Kicherer, U Greul, H Spliethoff, K R G Hein**. 1996. Investigations in combined combustion of biomass and coal in power plant technology. *Energy & Fuels* 10: 789-796.

11. **H Yamamoto, J Fujino, K Yamaji**. 2001. Evaluation of bioenergy potential with a multi-regional global-land-use-and-energy model. *Biomass and Bioenergy* 21: 185-203.

12. **I Lewandowski, J C Clifton-Brown, J M O Scurlockc, W Huismand.** 2000. *Miscanthus*: European experience with a novel energy crop, *Biomass and Bioenergy* 19: 209-227.

13. **I Lewandowski, U Schmidt**. 2005. Nitrogen, energy and land use efficiencies of *Miscanthus*, reed canary grass and triticale as determined by the boundary line approach, *Agriculture, Ecosystems and Environment* 112: 335-346.

14. **J Brejda. 1997**. Soil changes following 18 years of protection from grazing in Arizona Chaparrel. *The Southwestern Naturalist* 42(4): 478-487.

15. **J C Clifton-Brown, J Breuer, M B Jones**. 2007. Carbon mitigation by the energy crop, *Miscanthus*. *Global Change Biology* 13: 2296-2307.

16. **J C Clifton-Brown, P F Stampfl, M B Jones**. 2004. *Miscanthus* biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emission. *Global Change Biology* 10: 509-518.

17. **J Fargione, J Hill, D Tilman, S Polasky, P Hawthorne**. 2008. Land clearing and the biofuel carbon debt. *Science* 319 (5867): 1235-1238

18. **J Valentine, J Clifton-Brown, A Hastings, P Robson, G Allison, P Smith**. 2011. Food vs. fuel: the use of land for lignocellulosic 'next generation' energy crops that minimize competition with primary food production. *GCB Bioenergy* 4 (1): 1-19

19. **K J Anderson-Teixeira, S C Davis, M D Masters, E H Delucia**. 2009. *GCB Bioenergy* 1 (1): 75-96

20. **L Ercoli, M Mariotti, A Masoni, E Bonari**. 1999. Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of *Miscanthus*, *Field Crop Research* 63: 3-11

21. **M Grubb, C Vrolijk, D Brack**. 1997. The Kyoto protocol: a guide and assessment [Book]. *Royal Institute of International Affairs Energy and Environmental Programme*, London.

22. **M Himken, J Lammel, Neukirchen, U Czypionka-Krause, H-W Olfs**. 1997. Cultivation of *Miscanthus* under West European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. *Plant and Soil* 189: 117-126.

23. **M A Liebig, M R Schmer, K P Vogel, R B Mitchell. 2008**. Soil carbon storage by switchgrass grown for bioenergy. *Bioenergy Research* 1 (3-4):

215-222.

24. **M V Kok, E Özgür.** 2013. Thermal analysis and kinetics of biomass samples. *Fuel Processing Technology* 106: 739-743.

25. National Cooperative Soil Survey. National Cooperative Soil Characterization Database. Available online. Accessed [October/1/2017].

26. **P Kahle, S Beuch, B Boelcke, P Leinweber, HR Schulten**. 2001. Cropping of *Miscanthus* in central Europe: biomass production and influence on nutrients and soil organic matter. *European Journal of Agronomy* 15: 171-184.

27. **P Ciaian, d' A Kancs**. 2011. Interdependencies in the energy-bioenergy-food price systems: A cointegration analysis. *Resource and Energy Economics* 33: 326-348

28. **P Smith**. 2004. How long before a change in soil organic carbon can be detected? *Global Change Biology* 10: 1878–1883.

29. **R F Follett, K P Vogel, G E Varvel, R B Mitchell, J Kimble**. Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. 2012. *Bioenergy Research* 5(4): 866-875

30. **R Lal**. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 203: 1623-1627.

31. **S A Khan, R L Mulvaney, T R Ellsworth, C W Boast**. 2007. The myth of nitrogen fertilization for soil carbon sequestration. *Journal of Environmental Quality* 36(6): 1821-1832

32. **S Collura, B Azambre, G Finqueneisel, T Zimny, J V Weber**. 2006.

Miscanthus × *giganteus* straw and pellets as sustainable fuels. *Environmental Chemistry Letters* 4 (2): 75-78.

33. **S L Cosentino, C Patan`e, E Sanzone, V Copani, S Foti**. 2007. Effects of soil water content and nitrogen supply on the productivity of *Miscanthus* × *giganteus* Greef et Deu. in a Mediterranean environment. *Industrial Crops and Products* 25: 75-88.

34. **S Shoji, T Kurebayashi, I Yamada**. 1990. Growth and chemical composition of Japanese pampas grass (*Miscanthus sinensis*) with special reference to the formation of dark-colored andisols in the northeastern Japan. *Soil Science and Plant Nutrition* 36 (1): 105-120.

35. **T L Richard**. 2010. Challenges in scaling up biofuels infrastructure. *Science* 329(5993): 793-796.

36. **T O West, W M Post**. 2002. Soil organic carbon sequestration rates by tillage and crop rotation: A global data analysis. *Soil Sciency Society of America 66*: 1930-1946.

37. **W M Post, K C Kwon**. 2000. Soil carbon sequestration and land-use change: Processes and potential. *Global Change Biology* 6: 317-327.

38. **X C Baxter, L I Darvell, J M Jones, T Barraclough, N E Yates, I Shield**. 2014. *Miscanthus* combustion properties and variations with *Miscanthus* agronomy. *Fuel* 117: 851-869.

ABSTRACT IN KOREAN

질소 시비량이 기간테우스 억새의 지상부 및 지하부 탄소 축적에 미치는 영향

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억새는 가장 유망한 바이오 에너지 작물 중 하나로, 교토 의정서에 의거한 화석연료 감축 정책 하에서 억새의 바이오 에너지 생산성과 탄소 축적 능력은 바이오 에너지 생산 정책 결정에 있어 가장 고려해야 할 사항들이다. 하지만 질소 시비량이 이 두 요소에 대해 미치는 영향에 대한 연구는 잘 이루어져 있지 않다. 따라서 질소 시비량이 질소 시비량이 억새의 바이오매스 생산량과 탄소 축적 능력에 미치는 영향을 연구하기 위해 본 실험이 진행되었다. 본 실험에서는 기간테우스 억새(*Miscanthus* × *giganteus*)를 6년간 생육하였으며, 생육 기간 동안 연간 0, 30, 60, 120, 240 kg N ha^{-1}의 질소 시비가 이루어졌다. 매년 지상부 바이오매스를 수확하여 수량 및 수량구성요소를 조사하였으며, 6년차에는 근경부와 뿌리 부분을 포함한 지하부 바이오매스를 수확하여 수량을 조사하였다. 지상부의 잔여 바이오매스(biomass debris)도 6년차에 수확하여 건조중을 조사하였다. 토양 역시 6년차에 수집하여 총 탄소량을 조사하였다.

질소 시비량, 지상부 바이오매스, 지하부 바이오매스, 그리고 토양 중 탄소 함량은 통계적인 분석을 통해 연관관계를 조사하였다. 실험 결과 6년간 지상부 바이오매스의 총량은 질소 시비량 증가에 따라 증가하였으나, 유의미한 차이는 무시비 처리구와 시비 처리구 사이에서만 관찰되었고, 시비구 사이에서는 유의미한 차이를 관찰할 수 없었다. 6년차 바이오매스의 수량 구성요소 조사 결과 질소 시비량이 증가함에 따라 화기 길이가 감소하였으며, 억새의 줄기 부분의 활용도가 높음을 고려하면 이는 바이오매스 전체 활용도가 증가하는 결과이다. 지하부 바이오매스는 60 kg N ha^{-1} $year^{-1}$ 까지는 질소 시비량에 따라서 증가하다가 더 높은 질소 시비량에서는 바이오매스가 점점 감소하였다. 토양 중 탄소 함량은 질소 시비량에 따른 유의미한 차이를 관찰할 수 없었다. 이 결과는 억새의 지하부가 노화 및 분해되기 위한 충분한 시간 동안 실험이 이루어지지 않았으며, 억새가 생육 과정에서 토양 중 존재하던 탄소를 이용하였기에 토양 중 총 탄소량이 유지 혹은 감소하였기 때문에 나타난 것으로 추측된다. 한편 0-20cm 깊이의 토양의 탄소 함량은 다른 깊이의 토양에 비해 탄소 함량이 최소 2배 이상인 것으로 나타났고, 이는 지상부의 영향인 것으로 추정된다. 그러나 0-20cm 토양의 탄소 함량을 단독으로 조사하였을 때에도 질소 시비량에 따른 유의미한 탄소 함량의 변화는 관찰할 수 없었다. 억새의 전체 바이오매스에 저장된 탄소의 총량은 질소 시비량에 따라 증가하는 추세를 보였다. 지하부 바이오매스의 탄소 축적량이 전체에서 차지하는 비율은 질소 시비 증가에 따라 감소하였다. 회귀 선형분석

결과로는 질소 시비량 증가에 따른 지하부 바이오매스의 탄소 축적량의 유의미한 증가는 관찰되지 않았다. 억새의 10년이 넘는 수명을 고려한다면, 실험의 보완을 위해 이 실험과 같은 방법으로 장기적인 실험 수행이 필요할 것으로 생각되며, 이를 통해 억새의 지하부 바이오매스 분해를 통해 유입된 토양 중 탄소 함량을 측정하고, 장기적인 질소 시비량 차이가 억새의 바이오매스 생산성 및 탄소 고정능력에 미치는 영향을 보다 정확히 평가할 수 있을 것으로 보인다.

핵심어: 바이오에너지 작물, 탄소 축적, 기간테우스 억새, 질소 시비, 토양 탄소

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