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Kishor Rimal

Abstract

Increased production of timber lead fertilization of plantation forests thus has become an important woodland management measure. Therefore, the search for an early indication of a more sensitive indicator of soil fertility under different fertilizer treatments change is particularly important. The study of soil microbial biomass will help us to understand the relationship between fertilization and soil fertility. Soil microbial biomass is an important component in carbon cycle of terrestrial ecosystems. It is very sensitive to the changes in soil conditions, and may alter in a short period of time reflecting the soil microorganisms as the prime indicators of soil fertility. The study of different fertilization and soil microbial biomass relationship can provide a theoretical basis for the establishment of rational fertilization system, improve soil quality and achieve sustainable development of forest productivity.

This study was carried out in the experimental plots located at Dongtai Forest Park in Yancheng city of Jiangsu Province, eastern China. In order to understand the effect of different fertilization treatments in the poplar plantations on the soil respiration, soil microbial biomass carbon and nitrogen; an experiment was conducted with six different fertilization treatments i.e. CK (control), T1 (NPK fertilizer), T2 (organic fertilizer), T3 (biochar), T4 (NPK fertilizer plus biochar) and T5 (organic fertilizer plus biochar). Measurements were done during the period of August 2015 to April 2016. The plots were measured for three times for seasonal variation of soil respiration. At same time, the soil were also sampled and analyzed in lab to determine the seasonal variation in soil microbial biomass carbon and nitrogen to fertilization in Poplar Plantations.

Clearly, a direct univariate regression failed to demonstrate a good correlation between CO_2 efflux and the temperature. Similarly, this study found poor correlation between soil moisture and soil respiration during all seasons. The effect of fertilization on soil respiration was significant between control and treatments (r^2=0.347) and within treatments (r^2=0.874). Soil respiration from T4 and T5 vary significantly with the control plots CK ($P<0.05$). There was seen decrease in mean soil respiration for treatments compared to CK for T1, T2 and T3 during the period.

During 2015-09, the mean soil respiration among treatments plots ranged from 2.02 ± 0.06 µmol/m^2/s lowest for T1 to 2.94 ± 0.01µmol/m^2/s highest for T5. Likely, on the month 2015-12, the average soil respiration ranged from 1.16 ± 0.01µmol/m^2/s for CK to 1.47 ± 0.02 µmol/m^2/s for T4. Similarly, on 2016-04, the soil respiration was lowest for CK with 2.28 ± 0.017 µmol/m^2/s and was highest for T5 with 2.66 ± 0.04µmol/m^2/s. There was no any significant variation in soil respiration between treatments ($p>0.05$). The coefficient of variation was highest for T5, 41.7±2.2%, while lowest for T3, 30.6±2.2%. For all treatments the coefficient of variation was found highly fluctuating, but the variation was not found statistically significant ($p>0.05$).

The mean SMBC ranged from 0.93±0.1 to 2.6±0.6 g/kg during the period. The seasonal effect on SMBC variation was found significant ($p<0.01$). Each measurement showed the Control Treatment (CK) has minimum SMBC content than treatments. The overall coefficient of variation within treatment groups was found 11.9%, 26.2% and 25.8% respectively for each set of measurement. . The coefficient of variation was highest for T1, 55±13%, while lowest for T4, 39±3%. For all treatments except T1, the

coefficient of variation was found decreasing as compared to CK, but the variation was not found statistically significant($p>0.05$).

The mean soil microbial biomass nitrogen content also was found higher in treatments than control plots. The effect of fertilization on mean SMBN content was not found significant between control and treatments; and also not significant within treatments ($P>0.05$).

The mean SMBN ranged from 1.63±0.343 to 36.65±7.433 g/kg during the period and the seasonal variation was found significant. ($f=32.927$, $df=2,34$, $p=0.001$). Also, the coefficient of variation was highest for T1, 101±9.7%, while lowest for T5, 81±9.7%. For all treatments the coefficient of variation was found highly fluctuating, but the variation was not found statistically significant ($p<0.05$).

Key words: Soil Respiration, Soil microbial biomass, Fertilization, Poplar plantations

摘要

提高人工林木材的产量，已成为一种重要的林地管理措施，因此，寻找一个更敏感的指标来指示不同施肥处理下土壤肥力的变化尤为重要。对土壤微生物量的研究有助于我们了解施肥与土壤肥力的关系。土壤微生物量是陆地生态系统碳循环的重要组成部分。它对土壤条件的变化非常敏感，并且作为土壤肥力的主要指标的土壤微生物可能会在很短的一段时间内产生变化。土壤微生物组成、结构和性质的变化，可以更直观地反映土壤肥力状况，因此，研究不同施肥和土壤微生物量的关系，可为建立合理的施肥制度、提高土壤质量、实现林业生产力的可持续发展提供理论依据。

本研究在中国东部江苏省盐城市东台森林公园的试验田进行了试验研究。为了了解不同施肥处理对杨树人工林的土壤呼吸、土壤微生物生物量碳和氮的影响，设计了六个不同的施肥处理的实验，即CK（对照），进行T1（氮磷钾肥），T2（有机肥）、T3（生物炭），T4（化肥+生物炭）和T5（有机肥+生物炭）。测量是在2015年8月至2016年4月期间完成。为测定土壤呼吸的季节性变化需在该试验地测量三次。同时对杨树人工林土壤微生物量碳、氮在施肥中的季节变化进行了采样和分析。

显然，一个直接的单因素回归分析不能证明CO_2和温度有较好的相关性。同样，这项研究发现，土壤水分和土壤呼吸在所有季节之间的相关性较差。施肥对土壤呼吸的影响在对照和处理间（R^2=0.347）和各处理之间（R^2=0.874）是显著的。T4和T5的土壤呼吸与对照呈显著相关（P=0.01）。在此期间，T1、T2和T3处理与对照相比，平均土壤呼吸速率下降。

在2015年09月，平均土壤呼吸之间处理介于最低2.02 ± 0.06 μmol/m^2/s（T1）至最高2.94 ± 0.01μmol/m^2/s（T5）。到2015年12月，月平均土壤呼吸介于最低1.16±0.01μmol/m^2/s（CK）至最高1.47±0.02 μmol/m^2/s（T4）。在2016年04月，土壤呼吸最低为2.28±0.017μmol/m^2/s（CK），最高为2.66±0.04μmol/m2/s（T5）。各处理间土壤呼吸没有任何显著的变化（p>0.05）。变异系数是最高的为T5（41.7±2.2%），而最低为T3（30.6±2.2%）。结果表明，所有处理变异系数波动很大，但没有找到有统计学意义的显著性（p > 0.05）。

测量期间，SMBC的平均值从0.93±0.1g/kg到2.6±0.6g/kg之间不等。在SMBC变化的季节性的影响，发现显著（P<0.01）测量值表明对照组的SMBC含量远低于其他处理的SMBC含量。各处理组的总体变异系数分别为11.9%，26.2%和25.8%，分别是每组的测量值。T1处理的总体变异系数最高，为55±13%，T4处理的最低，为39±3%。除了T1处理，其他处理的总体变异系数相比对照组来说是呈上升趋势，但这种变化

的没有差异性（ p>0.05）。与对照处理值相比，其他处理的SMBN平均值比对照处理值高。对照和处理之间，施肥对SMBN含量的影响不显著，各处理间也没有显著性。

试验期间，SMBN含量的平均值从1.63±0.343g/kg 到6.65±7.433g/kg不等，差异性显著（f=32.927, df=2,34,(p=0.001），且T1的总体变异系数最大，是101±9.7%，T5的总体变异系数为81±9.7%，是最小的。所有处理的总体变异系数波动较大，但是这种变化不存在显著差异（ p<0.05）。

关键词：土壤呼吸；土壤微生物生物量；施肥；杨树人工林

Abbreviation and description in this study

Variable	Description	Units
N	Nitrogen	/
P	Phosphorus	/
K	Potassium	
CO_2	Carbon dioxide	/
TC	Total carbon	g*kg-1
TN	Total nitrogen	g*kg-1
TC/TN	Total carbon: Total nitrogen	/
SMBC	Soil microbial biomass carbon	mg*kg-1
SMBN	Soil microbial biomass nitrogen	mg*kg-1
SMBC/SMBN	Soil microbial biomass carbon: soil microbial biomass nitrogen	
CK	Control group	/
T	Treatment	/
NEP	Net Ecosystem Production	/
GPP	Gross Production Potential	/

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Introduction

Since the beginning of the 1950s, population growth, pollution and energy shortages have become increasingly prominent major world crisis, resulting in a substantial reduction in forest area all over the world (Yang, Zhou et al. 2009). With the rapid infrastructural development and also the economic sector; there was raise in the level of per capita consumption, rising demand for wood worldwide. Reduced natural resources and logging ban policy of natural forest in some countries lead to the plantation forestry as a major source for timber supply. In order to reduce the timber demand and supply gap, many countries initiated aggressively large scale plantations and intensive replanting operations. Replanting is inevitable if forests are managed in the long-term intensive business model (Chen, Fang et al. 2012). Increased production of timber lead fertilization of plantation forests thus become an important woodland management measures. Different kinds of fertilizer use improve soil fertility differently and their effects are also not the same. Soil organic matter is an important indicator of soil fertility, but its effect to characterize changes in soil fertility trends may take longer time or even years (Powlson, Prookes et al. 1987). Therefore, the search for an early indication of a more sensitive indicator of soil fertility under different fertilizer treatments change is particularly important.

Soil microorganisms are an important part of the ecosystem. Mineralization, fixation of atmospheric nitrogen, organic matter decomposition, nutrient transformation and supply are the important leading roles (Jenkinson, Andrew et al. 1990). Soil microbial biomass is very sensitive to changing conditions. Substantial changes can occur in a short time, so the number and variation of soil microbial biomass, can be used as an important basis for analysis of soil conditions (SHEN, LIU et al. 2012). The amount soil microbial biomass is considered to be a more sensitive than that of soil organic matter in soil quality evaluation (Yu, Li et al. 1999, Li 2000, Shengxiu 2000).

CO_2 efflux from the soil surface originating as plant and microbial respiration reflects the large part of belowground activity. This ‘soil respiration’ is the main pathway for carbon moving from the ecosystem to the atmosphere and can strongly influence net carbon uptake from the atmosphere, or net ecosystem production (NEP) – the balance between photosynthesis (GPP) and ecosystem respiration. Studies have shown that on average, 80% of GPP is respired back to the atmosphere (Law, Falge et al. 2002) and that about 70% of ecosystem respiration in temperate

forests is from soil (Goulden, Munger et al. 1996, Law, Ryan et al. 1999, Janssens, Lankreijer et al. 2001). Respiration may be more important than photosynthesis in controlling inter annual variability in NEP (Valentini, Matteucci et al. 2000).

Soil microbial biomass performs both the dynamic soil nutrient cycling processes and also a repository of plant effective soil nutrients (Xu 2002). Retaining soil nutrients and release process that alternately process soil microbial growth and death, and therefore the size of the microbial biomass can indicate the soil microorganisms and soil fertility status (Wang, Zhu et al. 2003). Therefore, the study of soil microbial biomass helps us explore the relationship between different systems of fertilization and soil fertility, and for the establishment of rational fertilization system in accordance with improved soil quality and achieve sustainable use of the land.

1.2 Objectives

The goal of this study is to investigate the seasonal change on soil respiration and microbial carbon and nitrogen in poplar plantations fertilized with various types of fertilizers subjected to five different types of fertilization treatments.

The following are the major objectives of this study.

1.2.1 Specific objective

- To analyze the seasonal variation of soil respiration, soil microbial biomass carbon and nitrogen in fertilized poplar plantations.

1.2.2 General objectives

- To examine the temporal variation in soil respiration
- To examine the relationship of temperature and moisture with respect to soil respiration
- To examine the temporal variations in soil microbial carbon and nitrogen
- To examine the microbial biomass content variation by fertilization type

1.3 Literature Review

1.3.1 Soil Respiration and its relation with fertilization practices.

Soil respiration or the efflux of CO_2 from the soil surface, is a major flux of carbon from managed and unmanaged lands with impacts on soil organic matter content, soil quality and

carbon sequestration. Of the terrestrial fluxes in the global carbon cycle, soil respiration is second only to gross primary production and soil respiration is the largest terrestrial source of atmospheric CO_2 (Elderfield 1998). At small scales, approximately 70% of ecosystem respiration in temperate forests is from soil respiration (Law, Ryan et al. 1999, Giardina, Ryan et al. 2003). Soil respiration from forest ecosystems is assumed to be closely matched to the combined inputs from belowground carbon allocation and aboveground plant litter fall (Raich and Nadelhoffer 1989, Giardina, Binkley et al. 2004). Any shifts in forest management practices or natural disturbances disrupting the balance between inputs and soil respiration will impact soil carbon content. Few management activities are focused on factors controlling soil respiration and the effectiveness of management practices on soil quality is usually monitored by long- term changes in soil carbon, but differences are usually small and highly variable (Johnson 1992, Garten 2002).

Intensive forest management practices including fertilization, use of superior genetic material, site preparation, competition control and pest management have greatly increased productivity of forests in the southeastern United States (Stanturf, Kellison et al. 2003); however, it is unclear how forest resource management influences soil respiration and net forest carbon sequestration (Shan, Morris et al. 2001, Davidson and Janssens 2006).

The CO_2 produced at the soil surface results from several respiratory processes, making modeling and interpretation of data complicated. About half the soil respiration is derived from metabolic activity to support and grow roots and associated mycorrhizae (Hanson, Edwards et al. 2000, Högberg, Nordgren et al. 2001). Most of the remainder is associated with heterotrophic respiration from microbial communities using recently produced organic material as an energy substrate (Trumbore 2000, Giardina, Binkley et al. 2004). Only a small fraction about 10% of soil respiration is derived from decomposition of older, more recalcitrant carbon compounds (Gaudinski, Trumbore et al. 2000). The proportion of soil respiration from autotrophic and heterotrophic contributions may vary seasonally and among ecosystems (Hanson, Edwards et al. 2000). Across a range of studies, the heterotrophic contribution varied from 10 to 95% and averaged 54% annually and 40% during the growing season (Hanson, Edwards et al. 2000).

The seasonal variation in temperature, moisture and their multiplying interaction effect has to do a lot with soil respiration. CO_2 efflux is a function of various ecological factors underneath the

soil surface. Soil temperature often does account for a large fraction of seasonal and diel variation in soil CO_2 effluxes, we know from laboratory and field studies that other factors, such as soil water content (Linn and Doran 1984), rates of C inputs to soils (Trumbore, Davidson et al. 1995), and diffusivity (Trumbore, Davidson et al. 1995) also affect CO_2 efflux from soils. Because the soil is a complex medium of an organo-mineral matrix of variable depth, supporting a broad array of plants, animals, and microorganisms; reductionist approaches to modeling individual components of soil processes that are comparable to aboveground canopy physiology models are extremely difficult. So long-term continuous observation and study of forest soil surface CO_2 flux and its influencing factors is an essential part of the whole forest ecological system carbon balance.

1.3.2 Soil Microbial Biomass and their relation to fertilization practices

Soil microbial biomass refers to the total volume of live microorganism less than 5000 μ m. and plays an important role in soil organic matter decomposition. Soil microbial biomass is the most vulnerable to change driven by the material transformation in soil and nutrient cycling. Soil microbial biomass is considered to be the repository of active nutrients, is an important source of nutrients available for plant growth (Ma, Wang et al. 2012, Velmourougane, Venugopalan et al. 2014).

Soil microbial biomass is a small and liable component of soil organic matter. It is thought to exert a key controlling influence on the rate at which N, C and other nutrients cycle through ecosystems (Jenkinson 1988). The interest in estimating soft microbial biomass is related to its function as a pool for subsequent delivery of nutrients, and its rote in structure formation and stabilization of soil and as an ecological marker (Smith and Paul 1990). Soil microbial biomass can be affected by different N management, particularly in the long term (Lovell and Jarvis 1998).

1.3.3 Soil Microbial Biomass Carbon and its relation to fertilization

Soil microbial biomass carbon refers to the total amount of carbon present in all living microorganisms. Microbial biomass carbon is reflected as the size of the microbial biomass accounting about 40% to 45% of the dry matter of microorganisms (Feng, Wang et al. 2006). It is approximately 1% to 5% of soil organic carbon but has a direct or indirect influence in almost all biochemical processes in soil. Soil material in promoting the conversion, energy balance and

biogeochemical cycles play an important role (Chen, Zhen li et al. 1999, Yating, Dong et al. 2010).

Microbial biomass carbon and activity both are concentrated in the upper part of soil organic matter. It is an important source of soil nutrients. Microbial life cycle is through the process of continuous carbon assimilation in the environment; and also outside release of carbon in the atmosphere. As an important characteristics soil quality, the microbial utilization of organic carbon in the soil, maintaining the less amount of energy required for the same microorganisms, reflects the quality of higher utilization and indicates soil environment conducive to the growth of soil microorganisms (Wang, Shen et al. 1996). As the amount of easily decomposed organic matter or organic matter content of the system is high, there is high microbial biomass. These substances provide the energy source for microorganisms (Landgraf and Klose 2002). Increase in the amount of easily decomposable carbon accelerates the growth of microorganisms and improves microbial activity. Conversely, increases the soil carbon complex and also inhibits high microbial respiration (Xiang, Doyle et al. 2008).

Microbial biomass is closely related to the nitrogen content of the soil. The results show that the lack of effective carbon in the soil can limit soil microbial biomass. To evaluate the importance of soil microbial biomass in different ecosystems, in terms of restrictions, soil carbon and nitrogen Wardle (1992) analyzed twenty two different kinds of literature data and figured that microbial biomass carbon is dependent of carbon nitrogen matrix in the soil. There was a significant positive correlation between microbial biomass carbon and nitrogen in the correlation matrix indicating that in most of the ecosystem, soil nitrogen mainly affects fixed carbon microorganisms. Different types of soil microbial biomass and its seasonal changes are mainly concerned with the supply of carbon source (Wang, Shen et al. 1996).

Changes in soil microbial carbon content are greater in topsoil 110 ~ 240 kg per hector and soil organic matter content was positively correlated. It is typically 2% to 5% of soil organic carbon content and varies with different environmental factors and soil ecology. Microbial carbon is greater in arable lands to grasslands to woodlands, consistent with the trends of soil organic matter with soil microbial biomass carbon ranging from 42 ~ 2064 kg per hector accounting for 2% to 4% of soil organic carbon (Zhao 2006).

Microbial biomass carbon content is only a small part of the total soil carbon content. However, the relationship between microbial activity and soil organic carbon is very close. The dynamic change of the decomposition process of organic carbon in soil and microbial biomass carbon change are similar. So the soil microbial activity and soil organic carbon decomposition can be considered to evaluate the strength of the external manifestations (Wang, Zhu et al. 2001). On other hand, the number reflects the microbial biomass size and soil assimilation capacity of mineralized nitrogen is the sign of active soil. Microbial utilization of organic carbon is an important feature to reflect the quality of the soil. The higher utilization rate, the less the required amount of energy to maintain the same microorganism, indicating higher number of soil microorganism, conducive environment for quality growth (Zhao, Cheng et al. 2006). Microbial biomass carbon is closely related to changes in soil organic carbon content, which may be sensitive to changes soil organic carbon content. In addition, changes in soil microbial biomass carbon indicate changes in soil organic carbon content. The ratio of microbial biomass carbon and soil organic carbon is an important indicator to measure accumulation or loss of carbon from an ecosystem (Li 2008).

1.3.4 Microbial Biomass Nitrogen and its relation to fertilization

Microbial biomass nitrogen usually represents 0.5—3.0% of total nitrogen and is a key component of the nitrogen cycle. Microbes are responsible for a significant amount of the work in the system by utilizing organic and inorganic forms of nitrogen for cell growth. The nitrogen stored in the microbial biomass is the most active pool and regulates the amount of biological nitrogen. Microbial community use decayed organic matter (produced from dead plant biomass) as an energy source and in the process break down organic nitrogen to ammonium nitrogen.

Soil microbial biomass nitrogen refers to organisms of size smaller 5000μm (excluding living plant roots) and its chemical composition is mostly protein and polypeptide substances (Qiu, Peng et al. 2006). C / N value is generally ranges from 5 to 6 (Liu, Xiao et al. 2003).

Generally believed that soil microbial biomass nitrogen content is more stable before the crop or in the absence of adequate post-harvest facilities limiting soil organic matter, it depends on how much the level of the soil nitrogen fertility difficulties (Qiu, Peng et al. 2006). Microbial biomass nitrogen content is the basis to reflect the size of the soil nitrogen supply capability. It is the most active soil organic nitrogen component and one of the key aspects of the soil organic - inorganic

nitrogen conversion. Under similar microbial biomass carbon size, microbial biomass nitrogen differences along different soil types and ecology. It is large in arable soil with a content of 40 ~ 385 kg / ha followed by woodlands with 130 ~216 kg / ha and the grasslands with 40 ~ 496kg / ha, the general trend is the arable lands larger than woodlands and the grasslands (Zhou, Chen et al. 2001, Zhao 2006). Results from western and American countries roughly indicate that microbial biomass nitrogen content is generally in the range of 20 ~ 200mg / kg, 3% to 6% of total soil nitrogen (Tang, Jia et al. 2002).

The results of different studies on soil microbial biomass nitrogen accounted for the variation in the proportion of total soil nitrogen. BN/ TN variation is generally 2% to 7%, and is more consistent with the hydrolysis of the nitrogen content in soil. The experimental results from the paddy field of the Dongting Lake area show that the surface soil BN / TN variation was 1.83% - 6.42% (Qin, Ju et al. 2005). Similarly, from long-term eight different fertilization treatments in Rice field from Hunan Province, BN/ TN is 2% - 5%, with an average of 3.6% (Liu, Xiao et al. 2003).

Soil microbial nitrogen and microbial biomass carbon was highly positively correlated. The C/N ratio of soil microbial biomass (about 5-7) is lower than soil organic matter with C/N ratio (about 10 to 12) (Zhao 2006). It also shows that soil microbial nitrogen is important reserve of plant available nitrogen. Inorganic nitrogen when applied to increase C/N ratio in higher straw, follows the process of assimilation and the microbial biomass in combination, can reduce leaching and volatilization of inorganic nitrogen, improving the nitrogen use efficiency (Liu, Xiao et al. 2003).

(Shen, Yu et al. 1994) studied soil microbial biomass nitrogen and soil nitrogen supply relation accordance to combined application of organic and inorganic fertilizers on coastal saline soils. The experiments performed found during growing season in barley showed no soil significant reduction in carbon and nitrogen biomass per unit area before treatments. The fertilization process significantly increased microbial biomass, mainly chemical fertilizer and silkworm manure. The microbial biomass increase is far more for silkworm manure fertilizer treatment, but the increase in soil nitrogen and organic matter content is not obvious. Experiments also found no significant differences between the varieties of fertilizer (ammonium sulfate and urea). The short time application of easily decomposed organic fertilizer in the soil cannot increase soil

nitrogen and soil organic matter content, but it can significantly increase the number and activity of soil microorganisms, thus changing the composition ratio of the original soil organic matter, improving soil nitrogen supply ability.

1.4 Factors affecting Microbial Biomass progress

1.4 .1 anthropogenic factors

- The impact of fertilization on soil microbial biomass

Fertilization as an important means to increase crop yields, improve soil fertility, have a great impact on soil organic matter and microbial biomass. Fertilization influences microbial quantity and diversity as per agricultural practices. Previous studies have shown that soil microbial biomass was significantly higher in fertilized than that without fertilization (Liu, Xiao et al. 2003). Organic fertilizer and straw especially in cereal crops have most significant effect to improve soil microbial biomass (Shan, Luo et al. 2010). Whether be the fertilizer type, straw or manure, soil microbial biomass carbon and nitrogen change in paddy remained synchronized. The soil microbial immobilization depends on soil microbial biomass size itself (Liu, Xiao et al. 2003).

Proper fertilization patterns have an important role to improve soil microbial biomass carbon, nitrogen and phosphorus. The chemical fertilizers with organic nutrient recycling system can significantly improve the soil microbial biomass, soil microbial biomass carbon, nitrogen and phosphorus (Hong xia, Liu et al. 2006). Organic nutrient recycling not only improves soil nutrients, but also strengthen the soil microbial nutrient holding capacity, reduce the loss of fertilizer to improve soil nutrient availability, and promote healthy ecosystems development within soil (Chen, Wang et al. 2005, Ma, Wang et al. 2012, Velmourougane, Venugopalan et al. 2014), their study shows that the long-term application of organic manure or fertilizer can increase soil microbial biomass nitrogen content to the more obvious effects. The application of nitrogen fertilizers (organic or chemical) enhances soil microbial fixed inorganic nitrogen (Han 1996). Research on plantation ecosystem for N disturbance suggests that long-term application of fertilizer can contribute to increased microbial biomass, while long-term application of ammonium sulfate reduces microbial biomass. The phosphorus formerly can promote root growth and development, and latterly with soil acidification causing by physiological stress (Hong xia, Liu et al. 2006).

Recently, research on the use of Biomass charcoal (biochar) is increasing. Biochar is a solid-state carbon-rich material from the organic material under anaerobic conditions of low temperature pyrolysis, black-carbon in the form of existence. Biomass charcoal possesses pore structure, huge surface area, more negatively charged, highly aromatic-based, with strong adsorption characteristics and a high degree of stability (Lehmann, Gaunt et al. 2006, REBECCA 2007, Asai, Samson et al. 2009). Based on these characteristics, the impact of adding biochar on soil properties is becoming a hot topic and a function of increasing attention.

Biochar soil improvement is considered to be an ideal method for a large number of studies have showing that adding biochar can increase the level of soil organic carbon and improve soil fertility (Van Zwieten, Kimber et al. 2010), promote the formation of soil aggregates (Novotny, Hayes et al. 2009), improve adsorption of contaminants and hormones (Kim, Yu et al. 2007, Yu, Ying et al. 2009), provide mineral nutrients for plants (Novak, Busscher et al. 2009) and increase crop yields (Steiner, Teixeira et al. 2007). Some studies have shown that adding biochar in soil can significantly increase soil carbon content. For example, under the same conditions of fertilization, biomass charcoal added substances soils have found to increase the amount of soil organic carbon content (Kuzyakov, Subbotina et al. 2009). Soil microbial biomass is the driving force of soil nutrients release, carbon, nitrogen, phosphorus, sulfur and other transformation and circulation, but the research on biochar on soil microbial biomass influence is still relatively small (Kuang, Jiang et al. 2012, Zhang, Bayou et al. 2012).

Many studies have reported that no-till and minimum tillage method can improve topsoil microbial biomass and soil organic matter compared with the traditional way of farming. The soil samples were taken for a deeper analysis of the case, which show the effect compared between traditional framing, no-till and tillage soils were not significant. Soil microbial biomass carbon and soil organic matter has accumulated in the soil surface. But when sampling to a depth of 23 - 25 cm, this difference disappeared (Wang, Shen et al. 1996). Other research experiments in reduced tillage and no-till also showed soil organic matter and soil microbial biomass increased considerably in surface, but this increase decreases with soil depth (Zhao, Cheng et al. 2006). Different intensity of grazing, the results suggest that heavy pastoral grazing reduces microbial biomass carbon content. It is only the half than light grazing (Zhang, Han et al. 2003). The different land use patterns, floods, droughts and crop rotation, heavy metal pollution, also has a significant impact on soil microbial biomass (Tan, Dai et al. 2006).

1.4.2 Natural environment factors

Microbial life activities consist of a series of biochemical reactions, and these biochemical reactions, in turn, is strongly influenced by temperature, so that the temperature has become an important factor in the number and activity of microorganisms. In general, under the appropriate circumstances, humidity, soil temperature and soil microbial biomass had been reported a positive correlation in between (Li, Ren et al. 2004, Chaofa 2008). In the southern part of the Loess Plateau in semi arid area, total variation of soil microbial biomass nitrogen was highest in summer and lowest in winter. Field test results show that soil microbial biomass and soil temperature have a significant or very significant positive correlation; Alternate freezing and thawing specially in winter days reduce biomass nitrogen and microbial size, but the but these reduction had no significant effect (Li, Ren et al. 2004).

Soil moisture is indispensable to maintain normal metabolic activity of soil microorganisms, microbial biomass is alternating wet and dry as the water changes, that is, when soil moisture increases microbial biomass will rise; On the contrary, the soil tends to drought, microbial volume also decreases (Dongpo, Zhijie et al. 2004, Wang, Han et al. 2008). This is because the humidity is too large, resulting in poor soil aeration, anaerobic hypoxia, inhibited growth and development of soil fungi.

Structure and composition of and soil microbial biomass is basically a natural phenomena. Some scholars have found that between soil microbial biomass nitrogen and soil clay content have a close positive correlation. The unprotected soil microbial biomass mineralization rates were significantly higher than the protected soils. Therefore, some scholars have proposed the concept of carrying capacity of the soil microbial biomass; the amount for a volume of soil which can sustain the microbial properties. Carrying capacity is the ability of the soil to protect the soil microbial stable body mass, the size and soil carbon inputs, sticky grain content, aggregate structure and other factors (Zhou, Chen et al. 2001).

1.5 Prospects of fertilization on poplar plantations

Financial profitability of fertilization in forest plantation has to do mainly with the limitation degree of the nutrients applied. If the nutrients that are limiting growth are applied in plantation sites, then it is highly probable that the operation will be profitable. If applied non-limiting nutrients, then it can even decrease microbial biomass as well as tree growth. Moreover, if any

other factor such as inter or intra specific competition is taking place in plantation site, financial profitability of fertilizer application will be little likely. In such cases, it should be corrected that situation first i.e. thinning (Pineda-Herrera, Ignacio Valdez-Hernandez et al. 2015).

Nutrient deficiencies should be avoided in early stages for poplar, but fertilization and irrigation schedules are very specific to local conditions. Usually a balanced application of nutrients at the start of the growing season is sufficient. Direct foliar applications of nutrients can correct nutrient imbalances that develop during the growing season. An oversupply of nitrogen, however, can alter biomass community, cause the crop to grow too fast, promote formation of sylleptic branches, and delay the onset of dormancy (especiaIly when applied after early August). Excess nitrogen can also increase weed competition. Growers must be able to manipulate crop development by supplying or withholding nitrogen at the right times (Stanturf, Van Oosten et al. 2001).

Materials and Methods

2.1 Study Area

The research trails are located in coastal areas of Dongtai Forest in Yancheng city of Jiangsu Province, eastern China. Its geographical location is 32° 33'N - 32° 57'N, 120° 07'E - 120 ° 53'E. Dongtai forest is located at the Yellow Sea forest park, founded in 1965. It is a subtropical woodland, warm temperate region with maritime monsoon climate and abundant sunshine. Total annual solar radiation in the region is 118 kcal per square centimeter and the annual total of 2255 hours of sunshine. Sunshine was 51%. The annual average temperature is 14.6 ℃ and the frost-free period is 225 days. Average annual rainfall is 1051.0 mm. soils are sandy loam in texture. It is one of several excellent Chinese poplar plantation distribution sites in China. Dongtai, Jiangsu Province Forest is a key coastal protection forest which covers about 4.2 million acres out of which most are artificially planted forest areas. Total growing stock is 14.8 million cubic meters and canopy coverage is 78.1%. Forest vegetation are mainly planted poplar (*Populus deltoides*), dawn redwood (Metasequoia glyptostrodoide) scattered ginkgo (Ginkgo biloba), black locust (*Robinia pseudoacacia L.*), Chinese fir (Cunninghamia *lanceolata*), Paulownia (*Paulownia fortunei*) and willow fir (*Cryptomeria fortunci Hooibrenk*).

A 7-yr-old stand of pure poplar plantations *(Populus deltoides)* comprising alkaline soil with undergrowth vegetation mainly comprised of *Humulus scandens* and *Pteris biaurita* was selected as the study site. The afforestation density of poplar was 3m×5m. In the study area, for the year 2013, the forest canopy coverage was 72%, and the mean tree height was 18.1m with a mean diameter at breast height (DBH) of 16.2 cm (Wang, Tan et al. 2015)

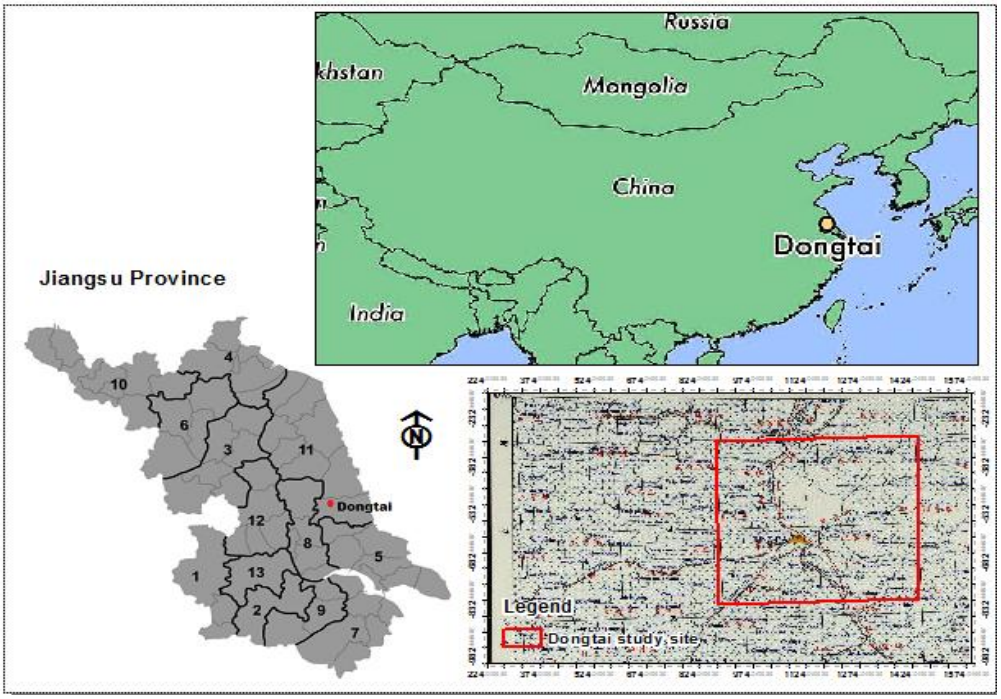

Figure 1 Study area and study site

2.1.1 Plots and experimental settings

An area of approximately 300m × 80m was selected for the experiment. 18 experimental plots of 20m × 12m were established on the basis of identical site conditions at regular intervals. The plots are planted within *populus* clones I-35 poplar (*Populus deltoides* CL '35'), now seven years old at a spacing of 5m × 3 m. Plots were fertilized and management measures undertaken are basically the same. The plots were separated in six groups (treatments) with three replicates each. One group established as CK (Control) and other five were fertilized as T1 (NPK fertilizer); T2 (organic fertilizer); T3 (biological charcoal-biochar); T4 (NPK fertilizer +biological charcoal) and T5 (organic fertilizer + biological charcoal). Completely randomized design was used in two rows of north-south direction. In between rows an isolation buffer zone of 10m was provided and the difference of 6m from plot to plot in rows was maintained. Fertilizers were applied after the sixth month annually. NPK compound fertilizer with nitrogen 15% was applied 25 kg per plot.

Organic fertilizer made of cottonseed and canola waste mass ratio 1: 1 was applied at the rate of 50 kg per plots. Biological charcoal fertilizer also known as Bio-char was applied at the rate of 50kg per plot plots per year per 50 kg. Fertilization method was uniform plowing.

2.2 Data collection and analysis

2.2.1 Field measurements

Soil CO_2 efflux and soil temperatures were measured on each plot at random location. Soil CO_2 efflux was measured using an L16400-09 soil chamber connected to an LI-6400 portable photosynthesis system for data collection. Soil temperatures at 10 cm depth were monitored at each point using thermocouple sensors connected to a LI-6400. The measurement of soil CO_2 efflux started in 2015-09. Soil CO_2 efflux was sampled thrice representing the seasons. Experiments were carried out during 2015-09 to 2016-04 for the period of 7 months. The data were collected three times; twice at start and ending and once at 2015-12.

Soil samples, 0-10 cm, using a soil sampler, were collected from each plot randomly for moisture content and lab analysis. Fresh soil samples were separated into two parts after removing roots and stones and sieving through a 2mm sieve. One part was stored in refrigerator at 4°C and used for analysis of microbial biomass carbon and nitrogen while other part was used to estimate moisture content in the soil. Soil moisture was determined by oven drying and weighting method.

2.2.2 Laboratory Analysis

Microbial biomass carbon and nitrogen were determined by fumigation extraction method. The following were the steps followed for determination of microbial biomass carbon and nitrogen.

1. Fresh sieved soil samples were weighted in 2 parts of 10gm and put into two 50ml small beakers. One set of the beakers were placed in a vacuum desiccators with a 50ml beaker of ethanol-free chloroform and other set is stored at 25℃ room temperature as control.
2. Vacuum desiccators was sealed with Vaseline and let the chloroform boil for about 1min. Then the vacuum pump was use to draw all air out. After the exhaust valve was closed. Then the vacuum desiccators was kept at dark place for 24 hours at room temperature for fumigation

3. After 24 hours the desiccators was removed and the beaker with remaining chloroform was taken out and repeated flushing was done until the smell of chloroform vanished. After, the soils were transferred to centrifuge tube, to be used in the next experiment
4. Then, 1: 4 ratio of 10 gm soil and 40 ml extract - 0.5mol/L of K_2SO_4 solution was maintained in each centrifuge tube and put on a shaking machine to make it fully mixed. Then the tubes were transferred to the centrifuge machine and centrifuged at 3000r / min for 5 min, and the supernatant was filtered.
5. After filtration the extracts were taken into a Shimadzu TOC-VCPN analyzer for determination of total organic carbon, microbial biomass carbon and nitrogen. The formula is:

$$B_{C(N)} = E_{C(N)} / K_{C(N)}$$

Where: $B_{C(N)}$ is soil microbial biomass carbon (nitrogen) content

$E_{C(N)}$ is the difference between carbon difference between carbon (nitrogen) content of the fumigated and non fumigated soil samples

$K_{C(N)}$ is the conversion factor, carbon value of 0.45 or the nitrogen value of 0.54 (Li 2000, Feng, Wang et al. 2006).

2.2.3 Data Analysis

Preliminary experimental data were summarized With Excel 2007. The subsequent analysis and processing was done with statistical software SPSS 20.0. One-way ANOVA for analysis of variance while, using Tukey's post- hoc test and pair wise T-test were used for multiple comparisons (significance level at 0.05). Statistically analyzed data were finished with Sigmaplot 13.0 a graph processing.

Results

3.1 Environmental Characteristics of the soil

3.1.1 Seasonal variation in Soil Temperature due to Fertilization

The soil temperature varies with season of the year. This variation stakes a vital role in shaping the microbial communities in the soil. Dongtai experimental plot also showed seasonal variation in soil temperature. During 2015-09, the mean soil temperature among treatments plots showed only a slight variation ranging from 22.7 ± 0.25°C lowest for T3 to 23.30 ± 0.12°C highest for T4. Likely, on the month 2015-12, the average soil temperature was relatively low and the variation of soil temperature among treatments was also narrow ranging from 7.23 ± 0.07°C for T4 to 7.63 ± 0.12°C for T2. Similarly, on 2016-04, the soil temperature was lowest for CK with 17.68 ± 0.15°C and was highest for T1 with 18.09 ± 0.14°C. There was no any significant variation in soil temperature between treatments for all three sets of observations ($p>0.05$).

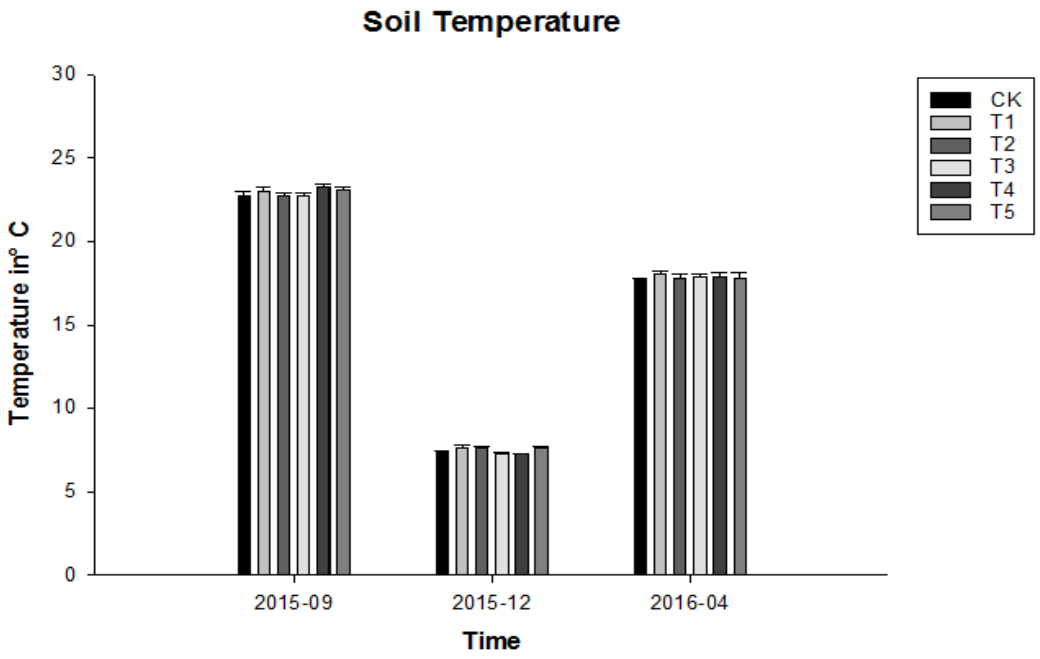

Figure 2 Soil temperature (0-10) cm measured during different seasons

3.1.2 Seasonal variation in Soil Moisture Content due to fertilization

Soil moisture availability shapes the microbial communities. Soil texture, structure and addition of organic matter define the moisture retention within the soil regime. The soil moisture content varied with season of the year. During 2015-09, the mean soil moisture content among treatments plots ranged from 24% ± 0.006 lowest for CK to 28% ± 0.002 highest for T3. Likely, on the month 2015-12, the average soil moisture content ranged from 23.2% ± 0.010 for T1 to 20.5% ± 0.017 for T2. Similarly, on 2016-04, the soil Moisture Content was lowest for T2 with 35% ± 0.003 and was highest for T5 with 37.4% ± 0.009. There was no any significant variation in soil moisture content between treatments for all three sets of observations ($p>0.05$).

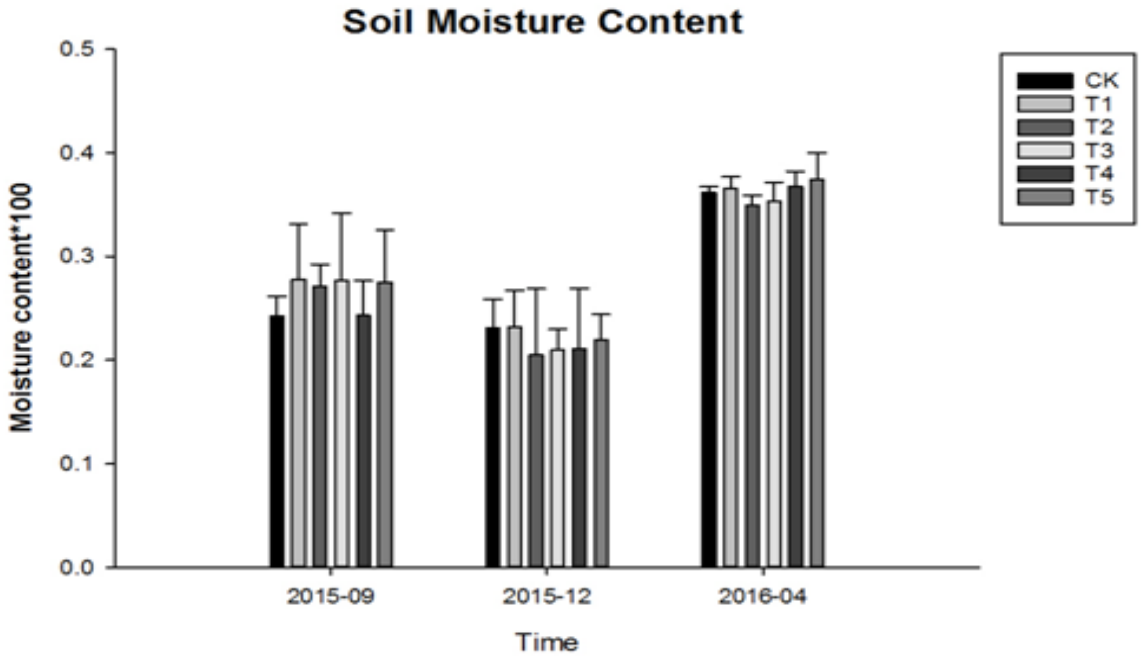

Figure 3 Volumetric soil moisture content (0-10) cm measured in % during different seasons

3.2 Effect of fertilization measures on seasonal dynamics of soil Respiration.

The results showed the seasonal difference in soil respiration. During 2015-09, the mean soil respiration among treatments plots ranged from 2.02 ± 0.06 µmol/m^2/s lowest for T1 to 2.94 ± 0.01µmol/m^2/s highest for T5. Likely, on the month 2015-12, the average soil respiration ranged from 1.16 ± 0.01µmol/m^2/s for CK to 1.47 ± 0.02 µmol/m^2/s for T4. Similarly, on 2016-04, the soil respiration was lowest for CK with 2.28 ± 0.017 µmol/m^2/s and was highest for T5 with 2.66

± 0.04µmol/m^2/s. There was no any significant variation in soil respiration between treatments for all three sets of measurement (p>0.05).

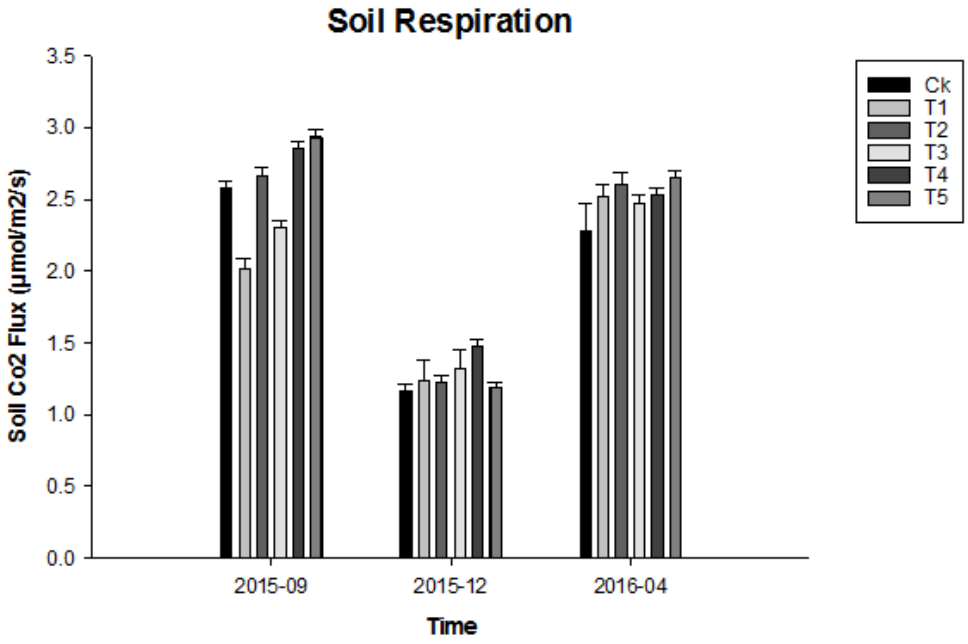

Figure 4 Soil respiration (0-10) cm recorded during different seasons

In an average, the effect of fertilization on mean soil respiration was significant between control and treatments (r^2=0.347) and within treatments (r^2=0.874). Soil respiration from T4 and T5 vary significantly with the control plots CK (P<0.05). There was seen decrease in mean soil respiration for treatments compared to CK for T1, T2 and T3 during the period.

Table 1 Annova tests of soil respiration between- control and treatments effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Control Vs Treatments	.128	1	.128	8.485	.010
Error	.241	16	.015		
Total	80.732	18			
Corrected Total	.369	17			

Table 2 Annova tests of soil respiration between treatments

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatments	.323	5	.065	16.654	.000
Error	.047	12	.004		
Total	80.732	18			
Corrected Total	.369	17			

One way ANNOVA with repeated measurements was performed to test the effect of mean seasonal variation in soil CO_2 efflux. The result showed that the time effect on the mean soil respiration is significant. ($f=207.994$, $df=2,34$, $p<0.01$).

Table 3 Annova tests of soil respiration for seasonal effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
season	19.311	2	9.655	207.944	.000	.924
Error(season)	1.579	34	.046			

A pairwise t-test showed that mean soil respiration for 2015-09 and 2015-12 varied significantly ($t=15.764$, $df=17$, $p=0.001$) and 2015-12 and 2016-04 also varied significantly ($t=-23.801$, $df=17$, $p=0.001$) but the variation between 2015-09 and 2016-04 was statistically insignificant ($p>0.05$).

Variation of coefficient of Soil respiration and its seasonal dynamics of variability under different fertilizer treatments were analyzed for poplar plantations, now at age of eight years old. The coefficient of variation was used to measure the degree of variation in data for each set of observations. For 2015-09 measurements, the overall coefficient of variation within treatment groups was 13±6% with highest 5.2±2% for T1 and lowest 0.4±0.2% for T5. Likely, for 2015-12 measurement, the overall coefficient of variation was 10.4±2.5%. Within treatments it was highest for T1, 1.9±2.8% lowest for T4, 0.5±0.1%. Similarly, for 2016-04, the overall coefficient of variation within treatments was 7±3.4%, it was highest for CK, 12.7±5.6% and lowest for T4, 2±1%.

The following table shows the coefficient of variation of soil CO_2 efflux of 0-10 cm top soil for five different fertilizer treatments and the control separately for the entire research period.
The coefficient of variation was highest for T5, 41.7±2.2%, while lowest for T3, 30.6±2.2%. For all treatments the coefficient of variation was found highly fluctuating, but the variation was not found statistically significant ($p>0.05$).

Table 4 The seasonal variation of coefficient of soil CO_2 efflux under different Fertilization treatments (%)

Treatments	Mean	Std. Error
T1	0.334	0.022
T2	0.376	0.022
T3	0.306	0.022
T4	0.317	0.022
T5	0.417	0.022
CK	0.38	0.022

3.3 Analysis of relation between Soil respiration, Soil temperature and Soil moisture content

Linear regression and curve fitting on soil CO_2 efflux (R) against soil temperature (T), and moisture (M), was performed. The R–T relationship was fitted using three functions including linear, Q_{10} (or exponential), and power functions. The regression results are listed in the Table 1.

Table 5 Univariate regression of CO_2 efflux against soil temperature at 0-10 cm depth

Univariate regression of CO_2 efflux against soil temperature at 0-10 cm depth						
	2015-09		2015-12		2016-04	
Function form	Fitted equation	r^2	Fitted equation	r^2	Fitted equation	r^2
Linear	$R = 0.238T-2.896$	.074	$R =3.163 -0.225T$	.215	$R = 0.020T+2.155$	.003
Exponential	$R = 0.341e^{0.87T}$	.060	$R = 5.390e^{-0.195T}$	.214	$R = 2.044e^{0.011T}$	.005
Power	$R = 0.9624T^{2.005}$	.060	$R =23.453T^{-1.457T}$	.212	$R = 1.461T^{0.187T}$	.004

Similar procedures were performed to derive the relationship between CO_2 efflux and soil moisture. The r^2 for these models are similar to those of temperature functions.

Table 6 Univariate regression of CO_2 efflux against soil moisture at 0-10 cm depth

Function form	2015-09		2015-12		2016-04	
	Fitted equation	r2	Fitted equation	r2	Fitted equation	r2
Linear	R=3.506-3.572M	.079	R =1.739-2.162M	.102	R =1.372+3.143M	.045
Exponential	$R = 3.807e^{-1.531M}$	.087	$R = 1.821e^{-0.1685M}$	.105	$R = 1.582e^{1.267M}$	.037
Power	$R = 0.9624M^{-0.395}$	.083	$R\ 0.734M^{-0.354}$	.104	$R = 3.931M^{0.444}$	.040

Clearly, a direct univariate regression failed to demonstrate a good correlation between CO_2 efflux and the temperature. The differences in r^2 values among all the functions are minimal. The highest valve of r^2 was obtained for the measurement form 2015-12 (r^2=0.21). Similarly, it failed to demonstrate a good correlation between CO_2 efflux and the moisture. The differences in r^2 values among all the functions are minimal. The highest valve of r^2 was obtained for the measurement form 2016-04 (r^2=0.08) showing very poor correlation. The widely accepted relation for temperature and soil respiration is explained by exponential functions but here result suggests that any of these functions, with its simplicity and convenience for curve-fitting can be a good choice to represent the R–T and R-M relationship.

The measurements done for different months were averaged for the period for each treatment. The average soil CO_2 effux against temperature shows that the data naturally fall into two groups separated by the linear function line.

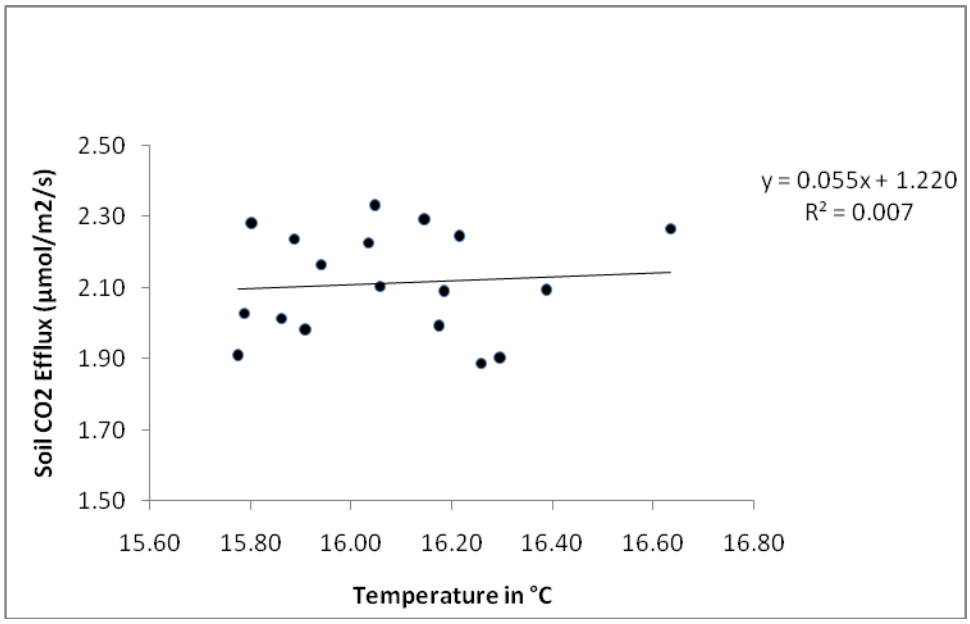

Figure 5 Average plot CO_2 efflux versus soil temperature

The first group covers all the data samples with moisture ranging from 26 to 28% and the second with moisture above 28%. When linear regression for R–T relationship was once again performed on each group, the correlation coefficients were improved considerably for both equations. The r^2 for Group one is 0.48 and for Group two it was 0.06, contrasting to the r^2 value of 0.007 before grouping. Regression after grouping of the data results in two equations:

$$R= 2.581-1.663M \quad (1)$$

$$R =0.618M-7.739 \quad (1a)$$

$$R =3.060- 3.286M \quad (1b)$$

The moisture may affect the slopes of the linear functions. To test this assumption, a test was performed to test a significance differences between the two coefficients of regression, ANCOVA was carried out to check the homogeneity of the slopes of the linear Equations (1a) and (1b). The null hypothesis is that the two slopes of equations (1a) and (1b) are the same and this hypothesis was accepted with a t-test (p=0.14).

The insignificant difference in the slopes of the functions warrants a combined equation to represent the R–T relationship. The parameters of the combined equation are derived using a statistical procedure of multiple regression analysis. The combined equation is:

$$R=1.520 + 0.35T + 0.19M \quad (1c)$$

f(T) = T 0.35 represents the isolated temperature dependence of soil respiration rate. When it is removed from the CO_2 data (subtracting the CO_2 efflux data by T 0.35) the residuals are once again fitted with a linear function. There was no change in r^2 value 0.16. This means for the entire experimental plots irrespective of the treatments applied, only 16% of the variation in the residuals after temperature effect is removed can be explained by moisture. The two variables moisture and temperature solely cannot provide significant variations. So, formulation of a combined linear function with main and interactive effect of temperature and moisture can therefore, represent the much better combined effect of soil moisture and temperature (r^2=0.23).

$$R = 2.003 +0.57T -3.186M+0.003TM \quad (1d)$$

Where, R is CO_2 efflux, f(T) and f(M) are the temperature and moisture effect respectively, and f(O) is effect of other factors

The applicability of this relation is restricted to larger areas as it is not able to explain a large range of variability which may due to various ecological factors interacting below ground.

3.4 Effect of fertilization measures on seasonal dynamics of soil microbial biomass carbon and nitrogen

3.4.1 Soil Microbial Biomass Carbon

The mean Soil Microbial biomass carbon was calculated for each season. The following bar shows the distribution of SMBC for seasons across treatments.

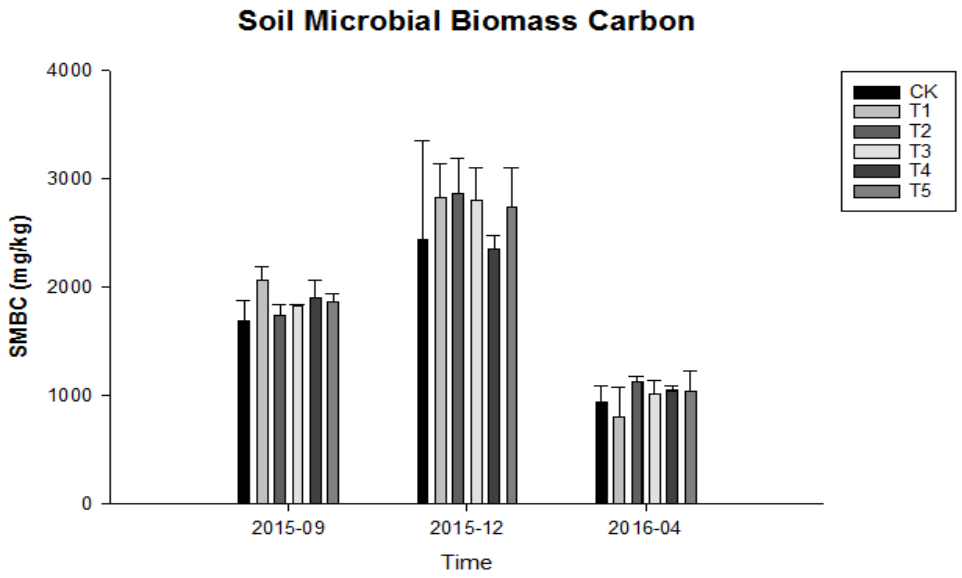

Figure 6 The seasonal variation of the SMBC under different fertilization treatments

The bar shows that Soil SMBC content in 2015-12 was in maximum then gradually decreased for 2015-09 and in 2016-04 was in minimum. It showed increase for 3 months from August to December, but in April it showed a small decrease. The mean SMBC ranged from 0.93±0.1 to 2.6±0.6 g/kg during the period. Each measurement showed the Control Treatment (CK) has minimum SMBC content than treatments. Analysis of variance showed that SMBC content for different treatment do not vary significantly for all three sets of observation (P >0.05). One way ANNOVA with repeated measurements was performed to test the effect of seasonal variation in SMBC content. The result showed that the test of homogeneity of variance was voided. So to access time effect on the mean SMBC size, a Greenhouse-Geisser correction was applied and the variation then was found significant. (f=54.454, df=1.18, 20.06, p<0.01).

Table 7 Annova tests of SMBC for seasonal effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
seasons	Greenhouse-Geisser	25336682.363	1.180	21463512.731	54.454	.000	.762
Error(seasons)	Greenhouse-Geisser	7909822.821	20.068	394156.677			

A pairwise t-test showed that mean SMBC for 2015-09 and 2015-12 varies significantly (t=-4.402, df=17, p=0.001), 2015-12 and 2016-04 also varies significantly (t=-12.893, df=17, p=0.001) and also the variation between 2015-09 and 2016-04 is statistically significant (t=-12.893, df=17, p=0.05). The following table shows the mean SMBC calculated for seasons and their distribution across treatments.

Table 8 SMBC measured distribution across different treatment types

Soil Microbial Biomass Carbon (Cmic) (mg/kg)				
Treatments	**Months**	**Mean**	**Std. Error**	**Std. Deviation**
T1	2015-09	2063.61	127.25	220.41
	2015-12	2824.58	317.21	549.42
	2016-04	806.71	265.84	460.44
T2	2015-09	1741.93	104.03	180.19
	2015-12	2870.15	315.47	546.41
	2016-04	1123.03	53.63	92.89
T3	2015-09	1821.90	15.58	26.99
	2015-12	2804.57	296.71	513.91
	2016-04	1011.47	130.31	225.70
T4	2015-09	1900.82	164.18	284.38
	2015-12	2354.84	117.88	204.18
	2016-04	1045.56	44.86	77.69
T5	2015-09	1864.15	71.79	124.34
	2015-12	2733.90	362.08	627.15
	2016-04	1038.20	194.15	336.28
CK	2015-09	1689.07	187.58	324.90
	2015-12	2438.55	913.69	1582.56
	2016-04	935.01	159.17	275.69

In this study, microbial biomass carbon content and its seasonal dynamics of variability under different fertilizer treatments were analyzed for poplar plantations, now at age of seven years old. The coefficient of variation was used to measure the amount of degree of variation in a data in the each statistical observation. The overall coefficient of variation within treatment groups was found 11.9%, 26.2% and 25.8% respectively for each set of measurement.

The coefficient of variation can be used to indicate the degree of seasonal variation of soil microbial biomass that fluctuates with the season's severity. The following table shows the coefficient of variation of SMBC of 0-10cm soil for five different fertilizer treatments and the control separately for the research period. The coefficient of variation was highest for T1,

55±13%, while lowest for T4, 39±3%. For all treatments except T1, the coefficient of variation was found decreasing as compared to CK, but the variation was not found statistically significant($p>0.05$).

Table 9 The coefficient of seasonal variation of soil SMBC under different Fertilization treatments (%)

Treatments	Mean	Std. Error
T1	0.55	0.13
T2	0.46	0.04
T3	0.48	0.08
T4	0.39	0.03
T5	0.45	0.10
CK	0.50	0.21

3.4.2 Soil Microbial Biomass Nitrogen

The mean Soil microbial biomass nitrogen was calculated for each season. The following bar shows the distribution of SMBN for seasons across treatments

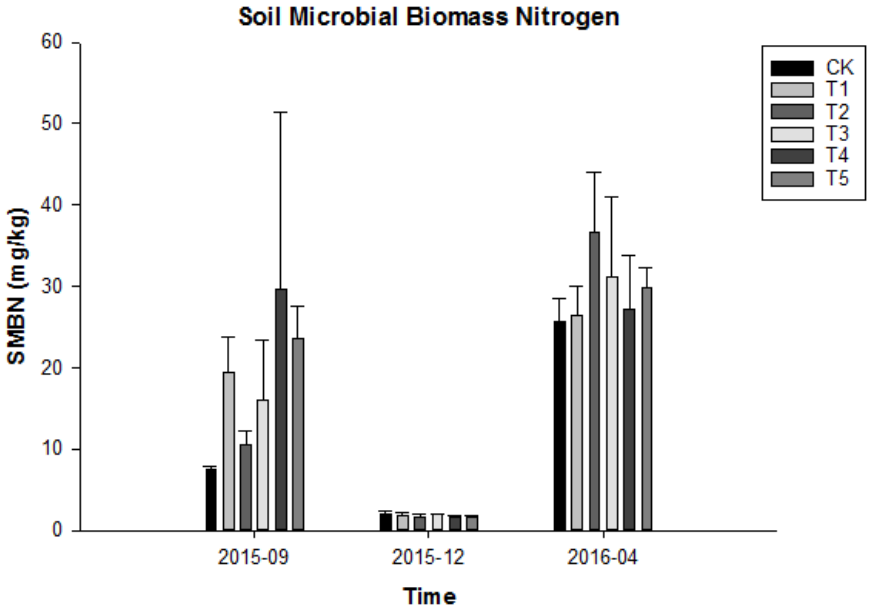

Figure 7 The seasonal variation of the SMBN under different fertilization treatments

The bar shows that SMBN content in 2016-04 was in maximum then gradually decreased for 2015-09 and in 2015-12 was in minimum. There is increase in SMBN content during warmer

days in April and it starts to decrease slowly and reaches minimum in winter. The mean SMBN ranged from 1.63±0.343 in colder days of December to 36.65±7.433 g/kg in warmer days of April.

Analysis of variance showed that SMBN content for different treatment not vary significantly for all three sets of observation (P >0.05). One way ANNOVA with repeated measurements was performed to test the effect of seasonal variation in SMBN content and the variation was found significant. (f=32.927, df=2,34, p<0.01).

Table 10 Annova tests of SMBN for Seasonal effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
seasons	6945.890	2	3472.945	32.927	.000	.660
Error(seasons)	3586.147	34	105.475			

A pairwise t-test showed that mean SMBN for 2015-09 and 2015-12 varies significantly (t=-4.402, df=17, p=0.001), 2015-12 and 2016-04 also varies significantly (t=-12.099, df=17, p<0.001) and also the variation between 2016-04 and 2015-09 is statistically significant (t=2.979, df=17, p=0.008). The following table shows the mean SMBC calculated for seasons and their distribution across treatments.

Table 11 SMBN measured distribution across different treatment types

Soil Microbial Biomass Nitrogen Nmic (g/kg)				
Treatments	**Months**	**Mean**	**Std. Error**	**Std. Deviation**
CK	2015-09	7.44	0.37	0.64
	2015-12	2.11	0.29	0.50
	2016-04	25.62	2.99	5.18
T1	2015-09	19.54	4.26	7.38
	2015-12	1.80	0.44	0.76
	2016-04	26.48	3.61	6.25
T2	2015-09	10.54	1.80	3.12
	2015-12	1.63	0.34	0.59
	2016-04	36.65	7.43	12.87
T3	2015-09	16.03	7.37	12.76
	2015-12	1.99	0.11	0.19
	2016-04	31.24	9.79	16.96
T4	2015-09	29.62	12.71	37.60
	2015-12	1.72	0.22	0.38
	2016-04	27.23	6.61	11.46
T5	2015-09	23.57	3.92	6.80
	2015-12	1.72	0.07	0.13
	2016-04	29.79	2.43	4.22

Microbial biomass carbon content and its seasonal dynamics of variability under different fertilizer treatments were analyzed for poplar plantations, now at age seven years old. The coefficient of variation was used to measure the degree of variation in data for each set of observations.

The overall coefficient of variation within treatment groups was found high as 90.4% and all treatments have higher value than the control, for 2015-09 measurements. Likely, for 2015-12 and 2016-04 measurement, the coefficient of variation was 23.9% and 32.8% respectively.

The following table shows the coefficient of variation of SMBC of 0-10cm soil for five different fertilizer treatments and the control separately for the research period. The coefficient of variation was highest for T1, 101±9.7%, while lowest for T5, 81±9.7%. For all treatments the coefficient of variation was found highly fluctuating, but the variation was not found statistically significant ($p<0.05$).

Table 12 The coefficient of seasonal variation of SMBN under different Fertilization treatments (%)

Treatments	Mean	Std. Error
T1	.866	.097
T2	1.107	.097
T3	.905	.097
T4	.964	.097
T5	.812	.097
CK	1.046	.097

Discussion

Fertilization has been shown to increase (Tyree, Seiler et al. 2006), decrease (Maier and Kress 2000) or have no effect on soil respiration (Pangle and Seiler 2002) indicating that fertilization may alter soil respiration through controlling moisture by retention of water at lower moisture stresses (Grahammer, Jawson et al. 1991).

The fertilization effect on soil respiration was significant between control and treatments. There was seen increase in mean soil respiration between control and treatments during the period due to fertilization. Fertilization may increase soil respiration, stimulation of productivity and litter fall inputs. For example, (Tyree, Seiler et al. 2006) determined that differences in soil respiration among fertilization treatments were positively correlated to stand biomass in 33-year-old loblolly pine.

There are evidences also to support reductions in soil respiration with fertilization. N fertilization may lower energetic maintenance requirements of microbes and favor microorganisms that are more efficient in the use of nutrient resources, thereby reducing heterotrophic respiration (Moscatelli, Lagomarsino et al. 2005).

Soil respiration has been found to be both sensitive and insensitive to changes in soil moisture (Maier and Kress 2000, Maier, Albaugh et al. 2004). Lower moisture may reduce soil respiration (Qi, Xu et al. 2002). There have been many discrepancies in representing the effect of moisture on soil CO_2 efflux. Although most results show a positive correlation between soil moisture and CO_2 efflux e.g. (Orchard and Cook 1983, Rout and Gupta 1989, Epron, Farque et al. 1999, Leiros, Trasar-Cepeda et al. 1999), some found the opposite is true, particularly when soil

moisture are high (Davidson, Belk et al. 1998, Gulledge and Schimel 2000). This study also found poor correlation between moisture content and soil respiration during all seasons. But the correlation for control groups was higher than treatments showing some sort of fertilization effect on soil respiration. This study shows decrease in moisture content between control and treatment.

The weak correlations between soil respiration and temperature and between soil respiration and moisture are due to the fact that the soil CO_2 efflux is an overall effect of multiple factors including moisture and temperature. The correlation between soil CO_2 efflux and any single factor may be affected by other factors. The weak correlation may also be caused by the characteristic combination of temperature and moisture. High temperature associated with low moisture in summer and low temperature associated with high moisture in winter. When one of the two factors is too limiting, it becomes the control factor and the other factor has little effect. Therefore, direct regression of the soil CO_2 efflux against a single factor is inadequate to show the contribution of individual factors. Thus, isolation of the effect of each of the two factors is required (Xu and Qi 2001).

The effect of temperature on soil respiration rate has been treated commonly. Higher temperature may reduce soil respiration (Qi, Xu et al. 2002). Our result that moisture does not affect the temperature dependence of soil CO_2 efflux suggests that, in our system under study, moisture and temperature independently, but simultaneously, affect soil CO_2 efflux rate, although soil moisture and temperature often correlated and that the two factors simultaneously affect soil CO_2 efflux. The results derived in this study are based on the temporal change of soil CO_2 efflux, temperature and moisture, and 'temporal' represents the seasons here. The $r^2 = 0.23$ means that the temporal variation of soil CO_2 efflux is explained by the simultaneous variations of temperature and moisture. The regression result by no means implies the general contribution of the variables to soil CO_2 efflux.

The study shows various fertilizer measures have no significant contribution to improve average microbial biomass carbon content than control. This is mainly due to types of fertilization and quantity. N addition may result in the decreasing of SMBC (Sarathchandra, Ghani et al. 2001, Treseder 2008). One time application of nitrogen fertilizer cannot spring up the microbial community (Magill and Aber 1998). Fertilization showed poor effect on soil microbial biomass

carbon, indicating that chemical fertilizer on soil microbial activity does not have a promoting effect may be due various interactive effects of soil environment. This finding contradicts with findings from (Chen, Fang et al. 2012), the highest SMBC content in summer for poplar plantations in northern Jiangsu coastal area showing a positive relation between SMBC and temperature. But for this study, the finding is opposite.

Although microbial biomass nitrogen is rare in content, it is an important part of the microbial biomass. Soil microbial biomass management measures are extremely sensitive to changes in soil fertility with the environmental quality showing a rapid indication on importance of SMBN. The results show that different fertilizer treatments could increase soil microbial biomass nitrogen differently across seasons, but its average effect is not found significant between controls and fertilized plots.

Seasonal dynamics of soil microbial biomass nitrogen and soil microbial biomass carbon trend is similar; except that the minimum and maximum values for 2015-09. When SMBC was high SMBN was low. This finding resembles with the findings from (Chen and He 2002) and (Li, Ren et al. 2004). Their Microbial Biomass Research also shows higher SMBN in summer and low in winter. There was seen a negative relation between SMBC and SMBN. When SMBC was high, SMBN was low.

Soil microbial biomass C:N ratio may reflect the structural information of microbial communities (Lovell, Jarvis et al. 1995, Guo, Liu et al. 2013), C: N ratio lower its unit weight of soil organic matter, contains higher levels of microbial biomass carbon (Huang , 2008).This study shows that a average C: N ratio and average SMBC are highly correlated (r^2=73.5) while average C: N ratio and average SMBN have poor correlation. But the variation in C: N ratio is not significant within treatments or between seasonal measurements. Application of organic fertilizer, organic manure and biomass carbon mixed fertilizer increases the amplitude of lower soil microbial biomass carbon only in a smaller fraction (Sabahi, Veisi et al. 2010) but some others find this inconsistent. (Nicolardot, Recous et al. 2001) believe that soil microbial biomass carbon ratio and the input of organic carbon nitrogen ratio has an excellent positive correlation. However, some studies show that the application of organic carbon on soil microbial biomass carbon nitrogen ratio has little effect, as (Kushwaha, Tripathi et al. 2000) reported that straw reduces the microbial biomass carbon and nitrogen ratio.

Researchers (Kallenbach and Grandy 2011) performed a meta- analysis and figured out that addition of soil organic carbon (not including biochar) in soil can affect the soil microbial biomass but that affect do not alters the soil microbial carbon nitrogen ratio significantly. Changes in soil microbial biomass carbon ratio, generally considered to be caused by the different composition of soil microbial ecology. Some researchers (Steinbeiss, Gleixner et al. 2009, Grossman, O'Neill et al. 2010, Liang, Lehmann et al. 2010) considered the application of biochar has a significant impact on soil microbial community structure. During this study of eight month, the application of biochar did not significant vary the soil microbial carbon nitrogen ratio, may the change in soil microbial community needs analysis of soil further depth.

Conclusion

Reforestation and aforestation of retreated coastal land is gaining high popularity all over China, especially with *populous deltoids*, a fast growing species to meet its forest product demands. Fertilizing the crops is a common practice. Fertilization with interaction of seasonal components like temperature and moisture content can alter the belowground ecology of microorganisms. This study focused on soil respiration and microbial biomass carbon and nitrogen to get decisive information on rational fertilization practice. Accurately defining the soil respiration with seasonal variation and defining the relation is always challenging. Even under the best controlled conditions, Factors that influence the soil $C0_2$ flux has not been defined properly. Fertilization effect alters the seasonal trends of soil respiration with respect to temperature complicating prediction and developing a highly fitted model.

The seasonal variation in temperature, moisture and their multiplying interaction is only able to define only a lower percentage of variability of with soil respiration. CO_2 efflux is a function of various ecological factors underneath the soil surface. Seasonal variation in soil microbial biomass carbon due to fertilization can be significant but many researches have also shown poor effect. The results from across the world have suggested that different fertilizer treatments could increase soil microbial biomass nitrogen differently across seasons and also there was seen a negative relation between SMBC and SMBN. Further, a long term analysis will be exploring the relationship between different systems of fertilization and soil fertility, and for the establishment of rational fertilization system in accordance with improved soil quality and achieve sustainable land use.

Annexes

Annex 1

Soil Temperature in °C				
Treatments	**Month**	**Mean**	**Std. Error**	**Std. Deviation**
CK	2015-09	22.73	0.28	0.49
	2015-12	7.33	0.10	0.17
	2016-04	17.68	0.15	0.26
T1	2015-09	23.03	0.22	0.38
	2015-12	7.61	0.16	0.27
	2016-04	18.09	0.14	0.25
T2	2015-09	22.77	0.18	0.31
	2015-12	7.63	0.12	0.21
	2016-04	17.82	0.28	0.48
T3	2015-09	22.70	0.25	0.44
	2015-12	7.26	0.07	0.13
	2016-04	17.86	0.18	0.31
T4	2015-09	23.30	0.12	0.20
	2015-12	7.23	0.07	0.12
	2016-04	17.88	0.23	0.40
T5	2015-09	23.13	0.15	0.25
	2015-12	7.58	0.13	0.23
	2016-04	17.75	0.59	1.02

Annex 2

Soil Moisture Content%				
treatments	months	Mean	Std. Error	Std. Deviation
CK	2015-09	24.0%	.006	.010
	2015-12	23.1%	.008	.013
	2016-04	36.2%	.002	.003
T1	2015-09	28.0%	.015	.026
	2015-12	23.2%	.010	.017
	2016-04	36.6%	.004	.007
T2	2015-09	27.0%	.006	.010
	2015-12	20.5%	.017	.029
	2016-04	35.0%	.003	.005
T3	2015-09	28.0%	.020	.035
	2015-12	21.0%	.005	.009
	2016-04	35.3%	.006	.011
T4	2015-09	24.0%	.010	.017
	2015-12	21.1%	.016	.027
	2016-04	36.8%	.005	.009
T5	2015-09	27.7%	.015	.025
	2015-12	21.9%	.007	.012
	2016-04	37.4%	.009	.016

Annex-3

Soil CO_2 Efflux ($\mu mol/m^2/s$)

Treatments	Months	Mean	Std. Error	Std. Deviation
CK	2015-09	2.58	0.04	0.07
	2015-12	1.16	0.01	0.01
	2016-04	2.28	0.17	0.29
T1	2015-09	2.02	0.06	0.11
	2015-12	1.24	0.09	0.15
	2016-04	2.51	0.08	0.13
T2	2015-09	2.67	0.05	0.09
	2015-12	1.23	0.00	0.01
	2016-04	2.60	0.08	0.14
T3	2015-09	2.30	0.04	0.08
	2015-12	1.32	0.08	0.15
	2016-04	2.48	0.05	0.09
T4	2015-09	2.86	0.02	0.04
	2015-12	1.47	0.02	0.04
	2016-04	2.53	0.03	0.05
T5	2015-09	2.94	0.01	0.01
	2015-12	1.18	0.03	0.05
	2016-04	2.66	0.04	0.07

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