STUDIES ON THE CHANGES IN SOIL ORGANIC MATTER AND RICE YIELD WITH MANURE APPLICATION AND CLIMATE CHANGE BASED ON THE OVER 30 YEARS LONG-TERM EXPERIMENT AND STATISTICAL DATA IN YAMAGATA, JAPAN

NGUYEN SY TOAN

Division: Biocontrol and Bioenvironmental Sciences

Specialty: Bioproduction Science

The United Graduate School of Agricultural Sciences

Iwate University

(YAMAGATA UNIVERSITY)

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By

NGUYEN SY TOAN

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土壌有機物と稲収量の年次変動に及ぼす有機物施用と気候変動の影響：山形県における長期連用試験と長期統計データからの解析

Nguyen Sy Toan

岩手大学大学院 連合農学研究科

生物生産科学専攻

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English Abstract

Global climate change has been observed over several decades, which can have a great effect on agricultural production and food security. There are many studies on the effect of climate change on rice yield all over the world. However the responses of rice growth and soil organic matter (SOM) decomposition to climate change during long-term periods (over 30 years) have not been reported in single rice paddy ecosystem, especially in Asian countries. On the other hand, observations from over 30 years long-term in-situ experiments can provide full understanding of the characteristics and functional changes occurring in soils across time with climate change. In addition, it is also helpful to predict soil productivity and carbon stocks under future climate change scenarios. Natural stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) are widely used to study the dynamic of SOM in various plant-soil ecosystems, but itis rarely applied in long-term experiment in submerged rice paddies. Therefore, three studies were carried out in this thesis to fully understand how climate change and long-term manure application affect SOM and rice yield based on the over 30 years long-term single rice experiment and statistical data in Yamagata Prefecture, Japan.

In the first part, statistical data was used to test the changes in climate, rice yield as well as their correlations in four areas in Yamagata Prefecture (Murayama, Mogami, Okitama and Shonai) from 1982-2017. The results showed that temperatures have been increased significantly in all of four areas ($P<0.05$), strongly in summer season during rice growing stages. Long-term observation of its impacts on rice yield and yield components were recorded from 1982-2017 in four main rice production areas. Results showed air temperatures in crop season had strong positive correlation with rice yield in the four areas. There was also strong

positive effect of temperatures on rice yield in its growth stages. Among 4 yield components, 1000-grain weight and ripening percentage had high positive correlations with rice yield, while the panicle/m^2 and spikelet/panicle did not show a significant correlation. The long period for TP (transplanting day)-HD (heading day) resulted in decreasing yield, while HD-MT (maturing day) duration had no correlation. Our results indicated that rice growth stages could be considered as a strong tool to evaluate the effect of climate change on rice yield.

In the second part, response of rice yield to climatic parameters was also addressed, in combination with 32 years archive soil samples were used to investigate the changes in SOM components and its mineralization potential in a single rice paddy during 32 years long-term application of inorganic fertilizers and organic matter from 1983-2014, which was located at the Yamagata Integrated Agricultural Research Center, Yamagata, with five treatments as [1) PK, 2) NPK, 3) NPK + rice straw (RS), 4) NPK + rice straw compost (CM1), and 5) NPK + overdosed rice straw compost (CM3)]. The results for rice yield shows that yield was enhanced by rising temperatures in early summer (Jun-July), and by the application of mineral fertilizer and organic matter. The results indicated that application of rice straw and compost significantly increased soil organic carbon, total nitrogen (SOC and TN) ($P<0.05$).Meanwhile, without RS and CM did not resulted in significant change in SOC and TN contents. The increase of SOM in RS, CM1 and CM3 also was observed in relatively compared with NPK control. It was interesting that $\delta^{13}C$ of all treatments decreased annually, as the effects of RS, CM and the residue from rice plant. The high negative correlation of $\delta^{13}C$ values and SOC was found in this experiment, proving that $\delta^{13}C$ could be a SOM tracer. The change in $\delta^{15}N$ was not as clear as $\delta^{13}C$, but RS application led to decrease $\delta^{15}N$, while CM application tended to increase this value, compared to the rest of 5 treatments. Surprisingly, available P increased significantly in all treatments (P<0.001). In addition, an anaerobic incubation experiment was conducted in laboratory to identify the effects of long-term application of organic matter and mineral fertilizers application on the changes in soil C decomposition and N mineralization in a rice paddy. Decomposed C（CO_2 and CH_4 productions）and mineralized N（NH_4^+ -N production）potentials were measured after 4 weeks anaerobic incubation of soil samples at 30℃ in submerged condition. The results showed that the mean ratio decomposed C to mineralized N (Dec-C/Min-N) lower as the application of organic matters after anaerobic incubation. The mean ratios of Dec-C/Min-N were varied from 5.6 to 6.2. The research gave us a better understanding of C decomposition and N mineralization after long-term organic matter and mineral fertilizers application.

In the third part, the labile organic matter through extractable pools by hot water and water extracted methods were carried out to understand how the quality and quantity of labile organic matter were affect by long-term organic matter application. Soil samples were collected after a 31-years long-term experiment as shown above. The amounts of hot water extracted organic carbon and nitrogen (HWEOC and HWEON) at 80℃ and 16 hours, water extracted organic carbon and nitrogen (WEOC and WEON) at room temperature, and their $\delta^{13}C$ and $\delta^{15}N$ were measured for both surface (0-15 cm) and subsurface (15-25 cm) layers. The ratios of soil to water were 1:1.5 and 1:10 for both hot water and water extraction procedures. The results showed that the amounts of extracted organic carbon and nitrogen (EOC and EON) from hot water and water extraction had a high correlation with thosein bulk soil, which increased with the organic matter application, compared to NPK treatment. The $\delta^{13}C$ values in HWEOC and WEOC ranged from -28.3 to -26.4‰, similar to the original rice straw and rice straw compost. There was no correlation between $\delta^{13}C$ values and amounts of

HWEOC (or WEOC). Meanwhile, the $\delta^{13}C$ values in bulk soils ranged from -25.7 to -23.2‰, and decreased after long-term application of organic matters for both RS and CM treatments, compared to the NPK treatment. These results demonstrated that HWEOC and WEOC were originated from rice plants photosynthesis and the organic matter application, but not from the original bulk soil. The significant positive correlations between amounts of hot water or water extracted organic C (or N) and available N ($P<0.001$) implied that not only HWEOC, but also WEOC, HWEON and WEON could be used as integrated indexes for soil quality in this long-term experiment.

In conclusion, this study showed that the warming phenomenon had been observed in four areas in Yamagata Prefecture, mostly happened in summer rather than winter. As the results, rice yields were affected strongly with the temperature in crop season (June-September), as well as in two growth stage of rice plant (Transplanting-Heading-Maturing) ($P<0.05$). The application of rice straw and compost increased SOM and soil fertility. Interestingly, stable $\delta^{13}C$ could be useful and sensitive carbon tracer, and affected by its C pools. More attentions should be paid on the labile pools of organic matter in long time duration.

日本語要旨

地球温暖化は数十年間にわたって観察されており、農業生産にも影響を与えている。気候変動が稲収量に及ぼす影響についてはいくつかの研究があるが、30 年以上の長期間にわたって、稲の各生育段階への影響は限られている。一方、30 年以上の長期連用試験から得た結果は、土壌生産性と土壌および環境相互作用や、気候変動に伴って土壌特性と機能性の変化などの予測に役に立っている。様々な植物 •土壌生態系における土壌有機物の動態変動を研究するために炭素と窒素の安定同位体自然存在比($\delta^{13}C$ と $\delta^{15}N$)が広く利用されているが、湛水条件での水田長期連用実験には安定同位体アプローチはまだ応用されていない。そこで、本研究では、山形県における 30 年以上の長期間にわたる長期連用試験と官署統計データに基づいて、気候変動と有機物施用が土壌有機物と稲収量にどのように影響するかを明らかにするために 3 つの研究を行った。

研究 1 では、1982～2017 年の 36 年間に、山形県の 4 地域（村山、最上、置賜、庄内）における気候、稲収量およびそれらの相関関係の変化を統計的に分析した。その結果、稲作期の夏期には 4 つの地域でも気温が著しく上昇していることが示された($P<0.05$)。また、1982 年から 2017 年にかけて、4 地域における稲収量および収量構成四要素への影響を分析した。その結果、稲栽培期間中の気温が 4 つの地域でも稲収量と強い正の相関を示した。一方、休耕期間の気温が稲収量に悪影響を及ぼす傾向があることも示した。4 つの稲収量構成要素のうち、千粒重と登熟歩合は稲収量と正の相関を示したが、面積あたり穂数と穂あたり籾数は有意な相関を示さなかった。以上の結果より、稲生育の各段階における気候変動が稲収量に与える影響は異なることを明らかにした。

研究 2 では、1983 年から 2014 年までの 32 年間の無機と有機物肥料を長期施用した水田における土壌炭素 •窒素およびそれらの無機化能の変化を調べるために、32 年間のアーカイブ土壌試料を用いて分析を行った。山形県農業総合研究センター構内にある長期連用試験の 5 つの処理区、即ち（1）PK、2）NPK、3）NPK +稲わら(RS)、4）NPK +稲わら堆肥(CM1)、5）NPK +過剰稲わら堆肥(CM3)を研究対象とした。その結果、稲わらや堆肥の施用は土壌有機態炭素(SOC)、全窒素(TN)が有意に増加した(P<0.05)。一方、長期稲栽培の影響で、すべての処理の $\delta^{13}C$ の値が、年々低下してきた。 また $\delta^{13}C$ 値と SOC の高い負の相関があるから、$\delta^{13}C$ が土壌有機物の変化指標として可能であることを明らかにした。 $\delta^{15}N$ の変化は $\delta^{13}C$ のような明確的な変動がなかったが、5 つの処理の中に、RS 施用区の $\delta^{15}N$ は他処理区より低下したことを見出した。さらに可給態リンはすべての処理区において長期連用期間に伴って有意に増加した(P<0.001)。水田土壌炭素分解および窒素無機化の変化に対する有機物の長期施用の影響を調べるために、室内での嫌気培養実験も行った。湛水条件下 30℃で 4 週間の嫌気培養後、易分解性炭素(Dec-C、CO_2 と CH_4 生成量)および無機化窒素（Min-N、$NH4^+$-N 生成量）を測定した結果、5 つの処理区の中に、Dec-C/Min-N 値の順は、PK> NPK> CM1> RS> CM3 であり、有機物施用処理区がやや低かった。平均的に Dec-C/Min-N は約 5.6～6.2 であった。

研究 3 では、易分解性有機物の質と量がどのように長期有機物連用に影響されるかを明らかにするために、熱水抽出法を用いて抽出可能な不安定な有機物の測定を行った。研究 2 で述べた長期連用試験の水田圃場において、31 年目の秋に採取した土壌試料を

供試した。80℃16時間熱水で抽出した有機態炭素と窒素(HWEOCとHWEON)、室温で抽出した水抽出有機態炭素と窒素(WEOCとWEON)およびそれらの$\delta^{13}C$と$\delta^{15}N$の値を、表層(0-15cm)と下層(15-25cm)に分けて測定した。また抽出するとき、土壌と水の割合は1：1.5と1:10で、両方とも行った。その結果、熱水抽出有機態炭素と窒素の量は、NPK処理と比較して有機物施用で増加し、土壌のSOCとTNと高い相関を示した。HWEOCとWEOCの$\delta^{13}C$値は、元の稲わらと稲わら堆肥と同様に-28.3～-26.4‰であった。$\delta^{13}C$値とHWEOC（またはWEOC)の量との間に相関はなかった。一方、土壌炭素の$\delta^{13}C$値は、-25.7～-23.2‰の範囲であり、NPK処理と比較してRS処理およびCM処理の両方で有機物の長期適用後に低下した。これらの結果は、HWEOCおよびWEOCの炭素源は、元土壌からではなく、イネの光合成および有機物施用に由来することを明らかにした。本研究では熱水抽出および水抽出土壌有機態炭素と窒素は、可給態Nとの間に有意な正の相関($P<0.001$)があるので、HWEOCだけでなく、WEOC、HWEONとWEONも土壌性質の変化を判断する指標として可能であることを明らかにした。

全体を纏めると、山形県の4つの地域における温暖化は、最近の30年間の間に確実に進んでいる。また夏季の温暖化の進行は冬季より激しい。稲収量およびイネの2つの生育段階（移植から出穂と出穂から収穫）の期間は、その年の6月から9月までの温度に強く影響される($P<0.05$)。また、稲わらと稲わら堆肥の施用は土壌有機物を増加させると同時に土壌の窒素肥沃度を促進させる。さらに、易分解性有機物と水溶性有機物などの土壌炭素源において、炭素安定同位体の自然存在比の$\delta^{13}C$は、有用かつ敏感性

がある炭素トレーサーであることを本研究で明らかにした。なお、長期間にわたる水田土壌中の易分解性有機物の動態変動についてはさらなる研究が必要である。

Abbreviation

Abbreviation	Full name
LTE	Long term experiment
CH_4	Methane
CO_2	Carbon oxide
Dec-C	Decomposition Carbon
Min-N	Nitrogen mineralization
Net Dec-C	Difference between anaerobic and aerobic decomposition carbon
Net Min-N	Difference between anaerobic and aerobic nitrogen mineralization
DOC	Dissolve organic carbon
HWEOC	Hot water extracted organic carbon
WEOC	Water extracted organic carbon
HWEON	Hot water extracted organic nitrogen
WEON	Water extracted organic nitrogen
EOC	Extracted organic carbon
EON	Extracted organic nitrogen
Av-P	Available phosphorous
EOM	Extracted organic matter
SOM	Soil organic matter
NH_4^+-N	Ammonium nitrogen
NO_3^--N	Nitrate nitrogen
SOC	Soil organic carbon
TN	Total nitrogen
$\delta^{13}C$	The abundance of ^{13}C in per mill relative to VPDB
$\delta^{15}N$	The abundance of ^{15}N in per mill relative to atmosphere
WFPS	Water-filled pore space
RS	Rice straw
CM	Rice straw compost

TP	Transplanting day
HD	Heading day
MT	Maturing day
Mean air T	Mean air temperature
Max air T	Maximum air temperature
Min air T	Minimum air temperature
Mean soil T	Mean soil temperature
Min soil T	Minimum air temperature
Mean H	Mean humidity
Min H	Minimum humidity
CS	Crop season (from transplanting-maturing)
OS	Offseason (from previous October to current April)
Fig.	Figure

Chapter I General Introduction

1.1. Paddy rice yield

Globally, rice (*Oryza sativa* L.) is one of the most important crops of the world, providing over 18.8% of the world's caloric consumption (Table 1.1). Asia accounts for 90% of the world rice production (FAO, 2016). The rice consumption in Japan recent decades, but still maintain as daily food. The rice harvested area, production and yield increased gradually in the last 5 decades.

Table 1.1 World caloric consumption of main foods (FAO, 2016)

Item	kcal/capita/ day	Percentage (%)
Rice (Milled Equivalent)	541	18.8
Wheat and products	527	18.3
Sugar (Raw Equivalent)	200	6.9
Maize and products	147	5.1
Milk - Excluding Butter	138	4.8
Pigmeat	124	4.3
Total	2884	100

From 1961-2016, harvested area, production, rice yield increased 39, 244 and 148%, respectively. These increases are due to the applications of new rice variety, fertilizer and cultivation technique. However, in Japan, harvested area and production have decreased since yield remains stable in the last 35 years (1982-2016).

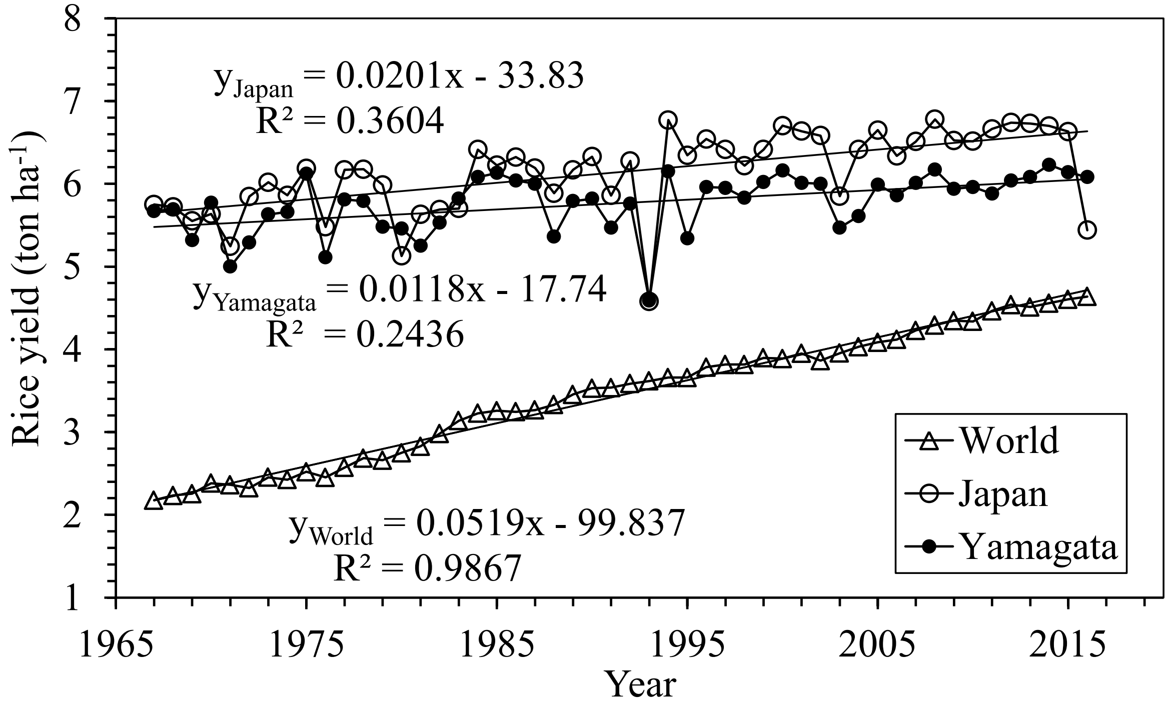


Fig. 1.1 Rice yield of Yamagata, Japan and World from 1967-2016 (FAO, 2016)

In Japan, rice is the most common full filled in paddy field, which occupy 85% of the land cultivation area. However, due to the shortage of area, rice production of Japan ranks 13th in the world rice production nation (FAO, 2017), with about 8 million of rice production. In recent decades, rice harvested area in Japan tends to decrease from 3.31 to 1.48% from 1961-2016 (FAO, 2017). It is noticeable that Japan paddy rice yield has been vary less changed.

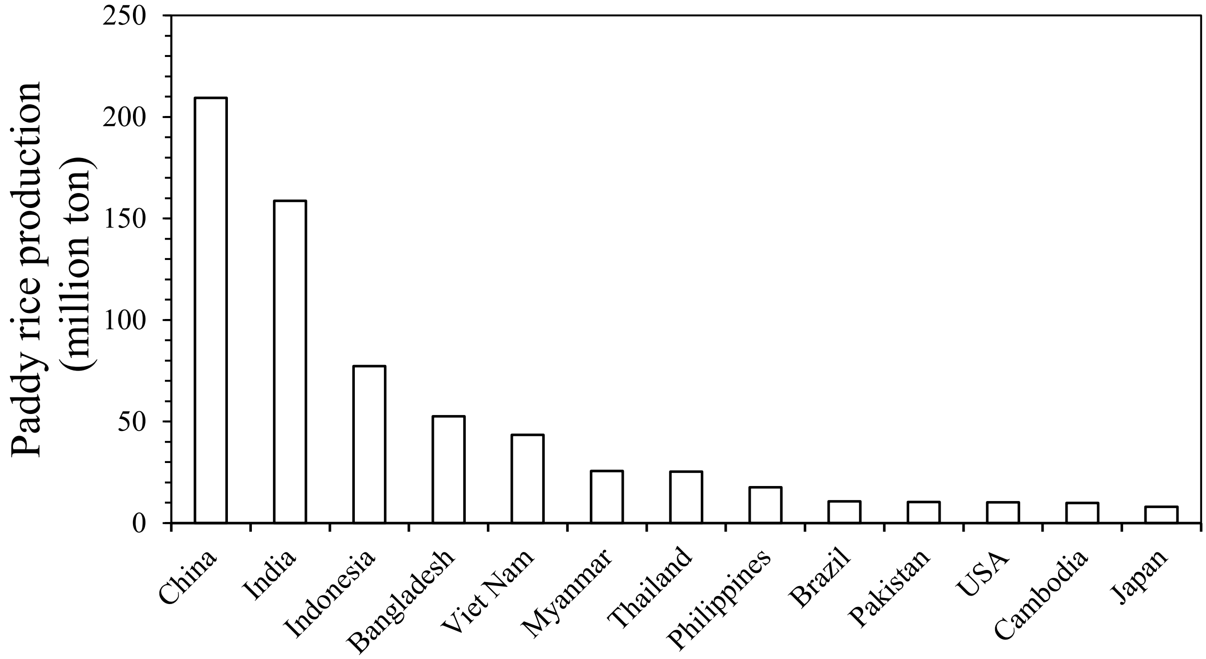


Fig. 1.2 Top rice paddy production countries (FAO, 2016)

1.2. Global climate change

Climate has been changing recently through its parameters. As comparing to the baseline of 1951-1980, the annually air temperature changes have been recorded as remarkable increase (Fig. 1.2). In the last recent three decades, temperatures were always recorded as higher than baseline. This strongly emphasizes that temperature has been increasing in global scale. It is reported that the globally averaged temperature of the earth surface has been increased by 0.3-0.6℃ from the late 19 century to 1990 decade (Folland and Karl, 2001). It is also proved that global surface temperature is rising in prior of 2 decades (Hansen et al., 2010a). Several researches on project of climate change due to warming confirm this situation (Iizumi et al., 2010b; Kurihara et al., 2005a).

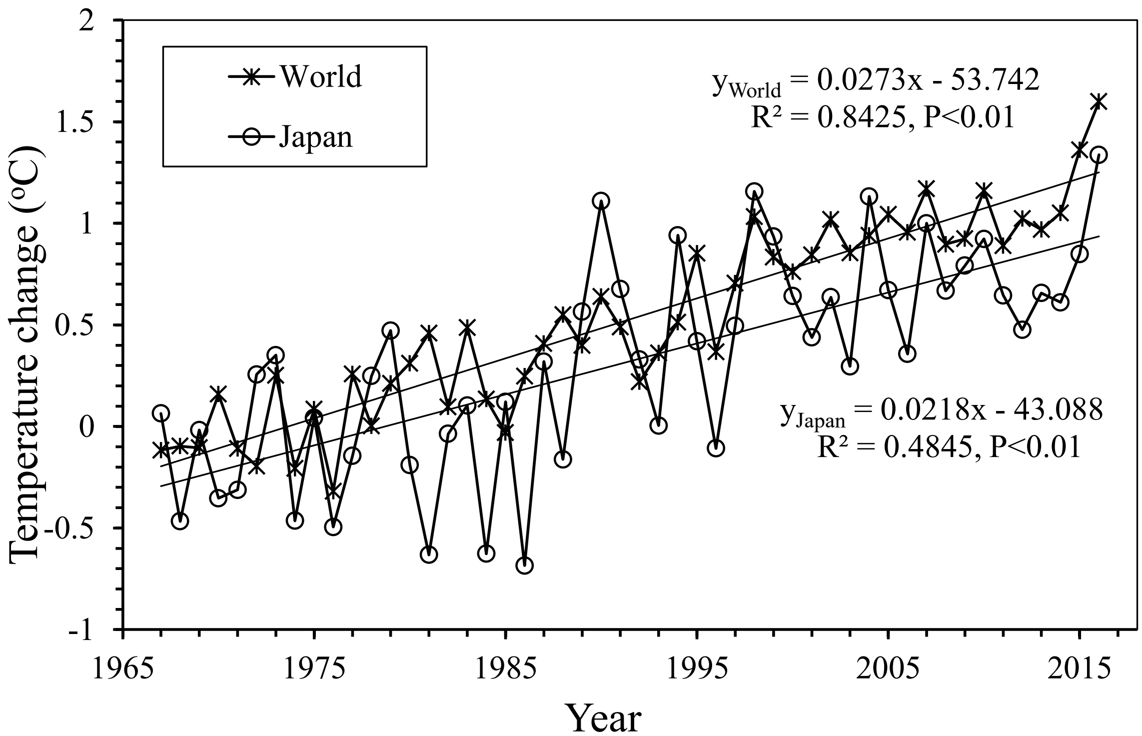


Fig. 1.2 Geological annually temperature change from 1967-2017. Period 1951-1980 was used as baseline (FAO, 2018)

1.3. Role of long-term experiment in agriculture

Long-term experiment (LTE) is widely carried out in many countries. A numerous LTEs in paddy rice paddy are carried out in Japan. Although there are many LTEs, the reports on the changes of soil organic matter (SOM) during long-term period are limited with full results. There are number of researches on LTEs showed the changes in SOM after long period (Cheng et al., 2016a; Cheng et al., 2016b; Nishida et al., 2007; Senbayram et al., 2008), however, most of the researches did not show the archive result. Therefore, our 32 years continuous soil samples from LTE could be ideal materials to investigate the responses of SOM content and rice yield to climate change. We are also the first one who conducted incubation of LTE soil in paddy rice field, for long period of time to evaluate SOC and N mineralization potentials affected by LTE.

The results obtained from incubation with several continuous years could be significantly meaningful to access the SOM dynamics.

1.4. Soil fertility response to long-term rice straw and compost application

Rice straw (RS) is mostly returned to the field as a manure after harvesting in Japan. Rice straw is generally cut into small species and spread on the rice field. Rice straw return is favorable to keep more carbon stock since RS contains 40% carbon by the biomass. However, RS normally takes few years to be decomposed, especially due to cold winter in Japan. As a result, the effect from RS to the field, not direct in the same year. The other recommendation is to firstly make RS become compost (CM) elsewhere, and then apply into the field. However, this may need more work. Therefore, the application of RS and CM play an important role in rice growth, and SOM content. The effects of RS on rice yield and soil properties were reported in many studies (Cheng et al., 2016a; Iqbal, 2016; Mahmoud et al., 2009; Nakajima et al., 2016). However, in this study we have the long annual soil samples, which could deplete the effect of environmental variability, to obtain the more precise observation.

1.5. Changes in soil $\delta^{13}C$ and $\delta^{15}N$ response to mineral fertilizers and manures application

The $\delta^{13}C$ value in soil is important parameter to powerfully trace C derived from different sources. According to the difference in pathways of photosynthesis, plants can be generally divided into two types, C_3 and C_4. The $\delta^{13}C$ values in C_3 plants such as rice and cotton approximately range between -35 ~ -26‰. C_4 plants such as corn and sorghum have $\delta^{13}C$ values of about -19 ~ -12‰. Application of fertilizer or materials which are derived from C_3 and C_4 plants also can alter soil $\delta^{13}C$ value. The question remain here, is how fast the soil $\delta^{13}C$ value change? Many LTEs are conducted to study the changes in $\delta^{13}C$ values. For example, Senbayam (2008) reported 34 years long time applied organic matter decreased $\delta^{13}C$ values from -24.3 to -27.3‰, which close to C_3 plant values. I t has been reported that soil $\delta^{13}C$ values were greatly changed by animal manure, mineral fertilizer application (Bol et al., 2005). Although the change speeds of $\delta^{13}C$ values are quite slow in soil, soil $\delta^{13}C$ values could be greatly affected by environmental factors.

The response of soil $\delta^{15}N$ is more complex compared to $\delta^{13}C$. The soil $\delta^{15}N$ of CM was reported to be higher than soil (Choi et al., 2017). RS material left over harvesting will be enriched by $^{14}NH_4$ from NPK fertilizer, lead to decrease their $\delta^{15}N$. As a result, when we mix soil with RS, the soil $\delta^{15}N$ would be decreased. In the LTE in this study, except PK treatment, NPK fertilizer was applied in other treatments.

1.6. Objectives and outlines of this study

Climate changes have been temporally and spatially observed over several decades. There are still poorly understood in its frequency, magnitude and trend. Studies on rice yield and SOM affected by climate and LTE in paddy ecosystem can give us diversity of perspective results. The effect of climate change on rice paddy should be looked at different sides of aspects, from the easy observable rice growth, to the changes in soil properties, which can't realize easily by clear phenomenon. Based on this concept, our study attempted to split into three main parts.

The first part was aimed to determine the changes in air temperatures and their impacts on rice growth. Here we mainly focus on rice yield and its four components (panicle/m^2, spikelet/panicle, 1000-grain weight, ripening percentage). The effects analyzed based on growing stages is supposed to be more effective than using monthly climate changes. We plan to test the impact of temperatures on transplanting, heading, maturing date. Then we have chance to compare the correlations of temperature changes in monthly and growing stages (Chapter II).

In the second part, we targeted to determine the changes in yield and SOM under different fertilizer applications in long period. The rice yield was recorded form 1982-2014, together with the climatic factors on the same period. The archive soil samples were annually collected from LTE from 1983-2014 with eight different fertilizers applications. Since soil not only plays important role in supplying nutrient to rice plants, but also can be considered as a huge carbon storage pool. Understanding the dynamics of SOM stock in long term can give us some agricultural strategies to keep carbon balance between soil and the atmosphere (Chapter III)

Finally, in the last part, our purpose was to investigate the changes in labile pools of SOM by two different extraction methods. Since the amount of SOM can’t change quickly due to climate change such as global warming. In contrast, the labile C pools, might be more sensitive to environmental variables. Extraction methods with hot water and water were selected to separate different labile C pools. We want to test the correlations of extracted OM with the bulk soil, and with two incubation methods (Chapter IV).

Chapter II Response of rice paddy cultivation to rising temperatures in Yamagata prefecture, northeastern Japan

2.1. Introduction

Rice (*Oryza sativa*) is one of the most important crops of the world, providing over 18.8% of the world's caloric consumption (FAO, 2017). Rice yield is among of the most considerable issues of the global food security. Growing rice is affected strongly by the changes of climate. The four components as panicle number, spikelet number, 1000-grain weight and ripening percentage are the main factors that decide the rice yield. Researches on the effects of climate on rice growth and rice yield in Japan have been widely conducted in recent decades. It is predicted that next 20 year mean yield in northeastern Japan will be decreased due to the impact of climate (Iizumi et al., 2010b).

The global warming phenomenon has been addressed in variable locations recently (Fitzgerald and Resurreccion, 2009a; Hansen et al., 2010b; Kim et al., 2013; Lobell and Field, 2007a). Mean, maximum, and minimum air temperatures are of the most common weather parameters affect the clime change. In Japan, rising temperatures also observed and reported the similar trend (Iizumi et al., 2010a; Tsukaguchi and Iida, 2015; Zakaria et al., 2015). The annual mean air temperature of Japan is reported to significant increased 1.4 °C (P<0.001) in the last 40 years (1977-2016), relatively to (1982-2010) period as standard, according to the Japan meteorological station.

Rice growth is strongly responded to surface air temperatures. The optimum for rice growth is around 30°C. Over threshold temperature during heading stage may lead to spikelet sterility (Ishimaru et al., 2016), or effect on flowering period (Matsui et al., 2015a). Researches on rice yield in Japan, mostly focus on summer season, as the rice cultivar mostly grow in this season showed the negative effects from temperature stress (Iizumi et al., 2010a; Ishimaru et al., 2011a; Morita et al., 2016; Zakaria et al., 2015). Although there is high correlation between climatic factors and rice yield in summer season, the main factor should be the climate change during growing stages, which started from transplanting - heading - maturing time. The previous study on Apple in Japan show the strong effect of rising air temperature during budding and flowering time in 38 years (Shen and Kobayashi, 2017). However, the lack of growing stage data may restrict such kinds of researches. Up to date, there is no report on long-term effects of climate changes in long-term in different growth stages of paddy rice.

In this study, our objectives are to: (1) determine the response of rice growth (yield, components, phenological events) to temperature changes and (2) determine the effects of geographical conditions to rice growth from 1982-2017 in northeast Japan.

2.2. Material and methods

2.2.1. Researched sites records of growth stages and temperatures

Yamagata is divided into 4 main regions: Murayama, Mogami, Okitama, and Shonai. Detail temperatures, sunshine, rainfall and observation location for 4 sites showed in Fig. 2.1 and Table 2.1. Rice has been cultivated in several centuries in this area. The main variety for cultivation is Haenuki, which accounts for about 57-67% of the total rice cultivar area in four regions in 2017. We start the collected data time in 1982-2017.

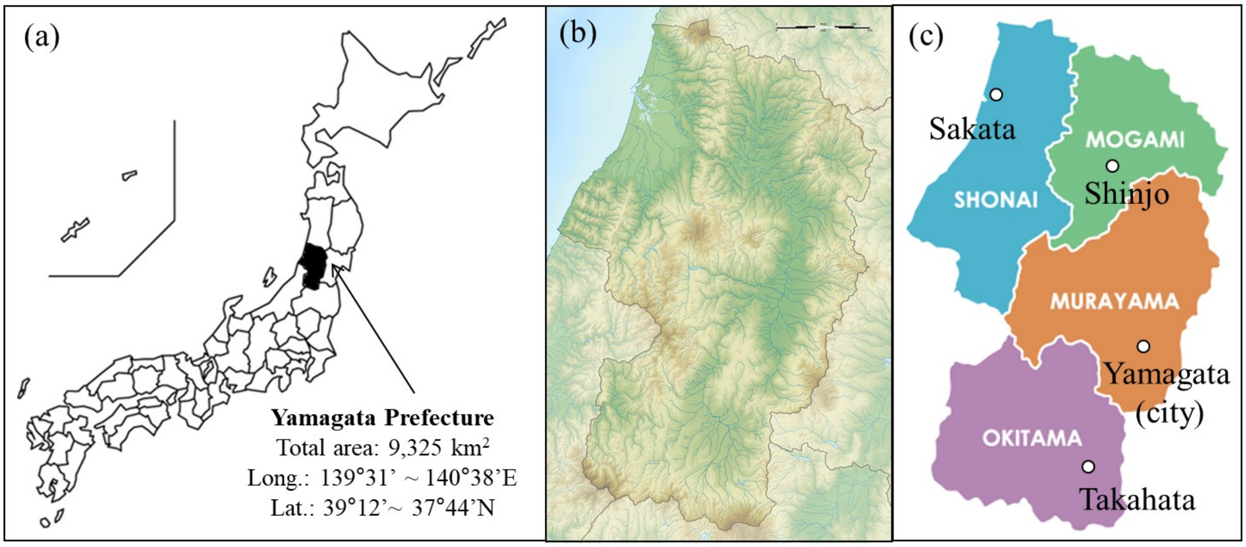


Fig. 2.1. The maps of Yamagata Prefecture in Japan (a) and its geographical (b) and administrative districts (c). The meteorological stations located on Yamagata city, Shinjo city, Takahata town and Sakata city are representative of four regions of Murayama, Mogami, Okitama and Shonai in map (c). Data from https://www.pref.yamagata.jp/ou/kikakushinko/020052/tokeijoho.html

As divided by 4 regions, and there is a considerable difference on meterological and geographical properties among them. Mogami and Sakata are at low elevation and more northernsince Okitama and Murayama are more southern and at higher elevation.

Table 2.1. General geographical and climate properties of 4 investigated sites

Site	Temperature observation			Average of annual temperatures, sunshine and rainfall during period of 1981-2010				
	Long (East)	Lat (North)	Elev (m)	Mean air Temp (°C)	Max air Temp (°C)	Min air Temp (°C)	Sunshine (hrs)	Rainfall (mm)
Muyamama (Yamagata city)	140°20.7'	38°15.3'	153	11.7	16.7	7.5	1613	1163
Mogami (Shinjo city)	140°18.7'	38°45.4'	105	10.7	15.3	6.7	1323	1856
Okitama (Takahata town)	140°12.4'	38°0.2'	220	10.8	16.1	6.3	1542	1235
Shonai (Sakata city)	139°50.6'	38°54.5'	3	12.7	16.5	9.1	1552	1892

2.2.2. Analysis of the trends in phenological parameters

We analyze the trends of rice yield and four main components: panicle number (panicle/m^2), spikelet number (spikelet/panicle), 1000-grain weight (g/1000 grain), and ripening percentage (% of ripening grain). The growth duration of rice were divided into transplanting-heading, heading-maturing and transplanting-maturing (TP-HD, HD-MT and TP-MT). More detail on three phenological event days showed in Table 2.3.

The averages of mean air temperatures, maximum air temperature, and minimum air temperature during two growth stages (TP-HD) day and heading- maturing day (HD-MT) and the total of two stages transplanting-maturing day (TP-MT). We collected the temperatures data based on the average of transplanting, heading and maturing day in four sites from 1982-2017.

2.2.3. Analysis of the relationship between the rice yield, yield components and air temperatures

The observed temperatures time series collected every day from transplanting to heading, and maturing day. The average of temperatures was used to test the correlation with the changes of rice yield and its components. The linear equation:

$$Y = ax + b,$$

where Y is yield and yield components, a is increasing slope (trend), x is temperatures and a is constant index. The coefficient correlation (R) between yield (yield components) and temperatures trend were also obtained with statistical three levels: $P<0.05$, 0.01 and 0.001 probability.

2.2.4. Analysis of the relationship between the temperature trend effects on the growth duration

The duration of growth stages are correlated with the record of temperatures during the same time. And the June-July, August-September are chosen as highest correlated to TP-HD and HD-MT stage. The slope (trend), correlation (R) and *P* values also tested for statically significance by fitting the linear equation mentioned above.

2.3. Results

2.3.1. Temporal trends in rice yield and yield components

Rice yield and yield, panicle number, spikelet number, 1000-graind weight, and ripening percentage data is showed in Table 2.2. Mogami showed the lowest yield (5.6 ton ha^{-1}), while the rest of sites showed same rice yield (5.9 ton ha^{-1}) on average of 36 years. Among 4 locations, two sites showed the significant increase ($P<0.05$), with the trend of 0.018 and 0.013 ton $year^{-1}$ in Murayama and Shonai, while Mogami and Okitama did not increase rice yield in the same period.

For the trends of four yield components, panicle number and spikelet number declined while 1000-grain weight and ripening percentage increased after 36 years. The average of 36 years of the panicle number, spikelet number, 1000-grain weight and ripening percentage ranged from 447-524 panicle m^{-2}, 63-68 spikelet $panicle^{-1}$, 21.1-21.4 g and 84.0-86.8%, respectively. Panicle number declined significantly in Shonai location only, with the trend of 3.23 panicle $year^{-1}$ ($P<0.001$), while spikelet number decreased significantly in Mogami and Okitama with trend of 0.18 and 0.17 spikelet $year^{-1}$.

Table 2.2 Temporal changes in rice yield and its components in 4 regions from 1982-2014

Site	Rice yield			Panicle number			Spikelet number			1000-grain weight			Ripening percentage		
	Mean	Trend	R	Mean	Trend	R	Mean	Trend	R	Mean	Trend	R	Mean	Trend	R
	ton per ha	ton per ha per year		panicle per m^2	panicle per year		spikelet per panicle	spikelet per panicle per year		gam	gam per year		%	% per year	
Murayama	5.9 (0.4)	0.02	0.52**	495 (25)	-0.22	-0.09	68 (3)	-0.09	-0.29	21.1 (0.6)	0.03	0.61***	84.9 (4.8)	0.308	0.67***
Mogami	5.6 (0.5)	0.01	0.26	447 (20)	0.32	0.17	70 (4)	-0.18	-0.53***	21.4 (0.7)	0.05	0.65***	84.0 (6.5)	0.217	0.35*
Okitama	5.9 (0.4)	0.01	0.38*	467 (24)	-0.74	-0.33	71 (4)	-0.16	-0.48**	21.3 (0.7)	0.04	0.66***	85.4 (5.3)	0.351	0.70***
Shonai	5.9 (0.3)	0	0.06	524 (45)	-3.23	-0.75***	63 (3)	0.07	0.23	21.2 (0.8)	0.05	0.73***	86.8 (4.2)	0.262	0.66***

The figures in parentheses show standard deviation of the mean. The *, **, and *** represent at P<0.05, 0.01, and 0.001 probability level, respectively

2.3.2. Trends transplanting, heading, maturing date and growth duration

The trends in transplanting, heading, maturing date and growth duration showed in Table 2.3 and Figure 2.3. Transplanting date becomes lately, and significant changes were observed in Okitama and Shonai (P<0.05) with the trend of 0.08-0.16 day year^{-1}. In contrast, the heading date, become earlier, and showed significant change in Murayama (0.13 day year^{-1}). For the maturing date, no significant changes were observed in all locations.

Table 2.3 Temporal changes in Transplanting, Heading and Harvesting date (top) and growth stage duration (bottom)

Site	Transplanting date			Heading date			Maturing date		
	Mean (date)	Trend (day yr^{-1})	R	Mean (date)	Trend (day yr^{-1})	R	Mean (date)	Trend (day yr^{-1})	R
Murayama	May 20	0.04	0.29	August 8	-0.13	-0.38*	October 12	-0.21	-0.19
Mogami	May 20	0.06	0.21	August 7	-0.09	-0.24	October 15	-0.20	-0.19
Okitama	May 19	0.16	0.69***	August 7	-0.08	-0.23	October 14	-0.15	-0.14
Shonai	May 12	0.08	0.42*	August 7	-0.10	-0.28	October 18	0.21	0.19

Site	Transplanting-Heading duration			Heading-Maturing duration			Transplanting-Maturing duration		
	Mean (days)	Trend (day yr^{-1})	R	Mean (days)	Trend (day yr^{-1})	R	Mean (days)	Trend (day yr^{-1})	R
Murayama	79	-1.2	-0.46**	55	1.8	0.53***	134	-0.1	-0.03
Mogami	79	-0.9	-0.36*	55	1.1	0.36*	134	-0.1	-0.05
Okitama	80	-1.4	-0.58***	56	1.4	0.49**	136	-0.3	-0.14
Shonai	87	-1.2	-0.46**	54	0.2	0.07	140	-0.6	-0.30

The *, **, and *** represent at P< 0.05, 0.01, and 0.001 probability level, respectively.

For the growth duration, TP-HD duration declined with the increase of HD-MT duration (P<0.05), except TP-MT duration in Shonai. The average of transplanting date in Murarama, Mogami, and Okitama are in 79-80 days, which about 1 week shorter than in Shonai (87 days).

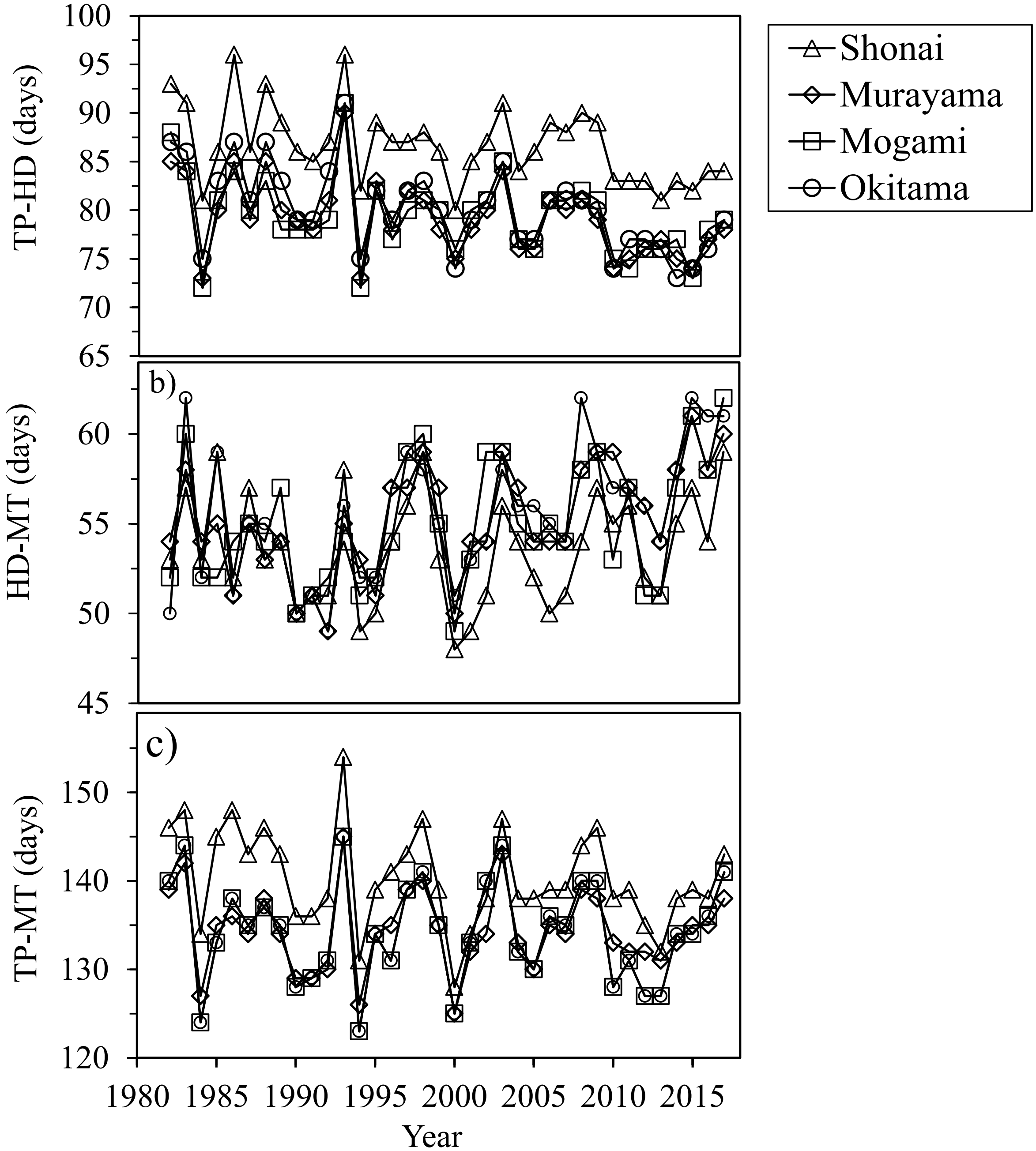


Fig. 2.2. Temporal changes of Transplanting-Heading (a), Heading-Harvesting (b) and Heading-Harvesting (c) duration of four regions in Yamgata from 1982-2017

However, HD-MT duration in all locations is similarly and ranged in 54-56 days. As a result, the total growth duration (TP-MT) did not show significant change in all sites.

2.3.3. Temporal changes of temperatures

The average of temperatures during two growth stages were analyzed transplanting, heading and maturing day, and trends of data showed in Table 2.4.

Table 2.4 Mean and temporal trend of temperatures in different growth stages

Growth stages	Mean air temperature			Maximum air temperature			Minimum air temperature		
	Mean (℃)	Trend (℃ yr^{-1})	R	Mean (℃)	Trend (℃ yr^{-1})	R	Mean (℃)	Trend (℃ yr^{-1})	R
Transplanting-Heading									
Murayama	21.4 (0.9)	0.039	0.48**	26.9 (1.0)	0.049	0.51**	16.9 (0.8)	0.04	0.51**
Mogami	20.5(0.9)	0.052	0.64***	25.8(1.1)	0.064	0.61***	16.3(0.8)	0.051	0.65***
Okitama	20.6 (0.9)	0.067	0.77***	26.0 (1.3)	0.095	0.78***	15.9 (0.8)	0.059	0.75***
Shonai	20.8 (0.7)	0.049	0.70***	24.8 (0.8)	0.051	0.66***	17.2 (0.8)	0.052	0.73***
Heading-Maturing									
Murayama	21.9 (1.3)	0.022	0.17	27.3 (1.6)	0.023	0.16	17.9 (1.3)	0.019	0.15
Mogami	21.5(1.5)	0.021	0.1430	26.7(1.7)	0.029	0.179	17.9(1.2)	0.017	0.147
Okitama	20.8 (1.5)	0.015	0.100	26.3 (1.8)	0.042	0.25	17.4 (1.2)	0.018	0.16
Shonai	23.2 (1.3)	0.03	0.24	27.5 (1.4)	0.03	0.22	19.7 (1.2)	0.033	0.28
Transplanting-Maturing									
Murayama	21.7 (0.9)	0.032	0.36*	27.1 (1.1)	0.039	0.36*	17.3 (0.9)	0.032	0.38*
Mogami	20.9(1)	0.039	0.429**	26.1(1.2)	0.049	0.436**	16.9(0.8)	0.038	0.52**
Okitama	20.7 (1)	0.044	0.46**	26.1 (1.3)	0.071	0.57***	16.5 (0.8)	0.043	0.57***
Shonai	21.7 (0.8)	0.043	0.54***	25.8 (0.9)	0.045	0.51**	18.2 (0.8)	0.046	0.61***

The figures in parentheses show standard deviation. The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. R indicates for Coefficient Correlation.

The average of mean air temperature ranged from 20.5-21.4 ℃ in TP-HD duration, which is lower than in heading-maturing stage (20.8-23.2℃). In all locations, increasing trends showed in TP-HD stage but no change in HD-MT stage. For the whole stage, increasing trend were observed ($P<0.05$).

2.3.4. Relationship between yield (yield components) and temperatures

The relationship between rice yield and temperatures showed in Table 2.5. In TP-HD stage and TP-MT stages, except for Shonai, rice yield and temperatures had closed relationship. Except Shonai, among two stages, sensitivity temperature (Sens) ranged from 0.11-0.31 ton $°C^{-1}$. In shonai, the Sens ranged from 0.02-0.11 ton $°C^{-1}$, which is lowest compared to the other sites. The correlation of all sites largely ranged from 0.06-0.72, with the highest in Murayama and lowest in Shonai.

Response of yield components to temperatures also represented in this Table 2.5. Panicle number showed the negative correlation with temperatures, but with less pronounces relationship. Interestingly, Shonai region showed the strongest correlation with temperature changes, especially in TP-HD stage, which Sens ranged from -25.51 to -44.65 with R (-0.46 to -0.75), while the other sites showed in 0.26 to -14.42 with R (-0.01 to -0.50). However, in TP-HD stage, only Shonai shows strong significant relationship with temperatures. Spikelet number showed very low correlation with temperatures, which almost no significance in all sites, at all growth stages. In contrast, the 1000-grain weight and ripening percentage had high positive correlation with temperatures, and also similar trend with rice yield. In both components, R showed higher values in TP-HD stage (ranged in 0.40 to 0.76) than HD-MT stage (ranged in 0.12 to 0.54). Similar trend in Sens also reported in 1000-grain weight and ripening percentage, which showed the Sens ranged in (0.37 to 0.57 and 0.12 to 0.22 g $°C^{-1}$) and (2.28 to 4.09 and 1.26 to 2.34% $°C^{-1}$), in TP-HD and HD-MT stage, respectively. The overall for two growth stage also confirmed high correlation between 1000-grain weight, ripening percentage and temperatures (R ranged in 0.42 to 0.69, $P<0.05$ in both components).

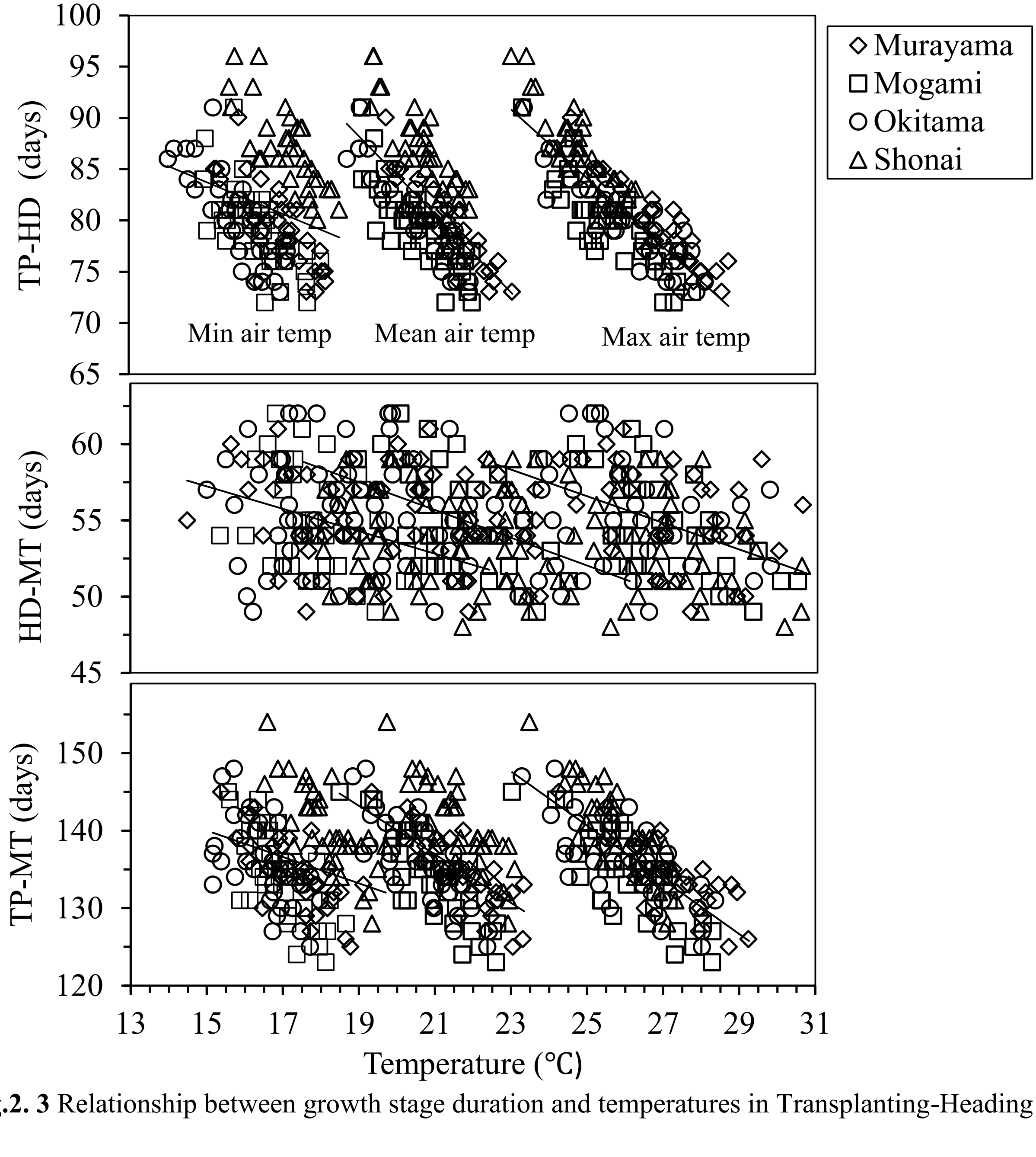


Fig.2. 3 Relationship between growth stage duration and temperatures in Transplanting-Heading (a), Heading-Maturing (b) and Transplanting-Maturing stage (c)

Table 2. 5 Responses of yield and yield components to temperatures changes

Yield and components	Transplanting-Heading stage						Heading-Maturing stage						Transplanting-Maturing stage					
	Mean air temp		Max air temp		Min air temp		Mean air temp		Max air temp		Min air temp		Mean air temp		Max air temp		Min air temp	
	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R	Sens (°C^{-1})	R
Yield (ton ha^{-1})																		
Murayama	0.28	0.65***	0.27	0.72***	0.23	0.50**	0.15	0.52**	0.13	0.54***	0.14	0.48**	0.26	0.65***	0.23	0.69***	0.24	0.56***
Mogami	0.30	0.49**	0.31	0.63***	0.15	0.24	0.21	0.59***	0.17	0.43**	0.22	0.47**	0.35	0.63***	0.32	0.64***	0.33	0.47**
Okitama	0.24	0.62***	0.19	0.68***	0.18	0.42*	0.11	0.47**	0.11	0.56***	0.11	0.36*	0.22	0.63***	0.19	0.70***	0.22	0.49**
Shonai	0.08	0.23	0.09	0.29	0.02	0.06	0.07	0.36*	0.07	0.38*	0.07	0.34*	0.10	0.35*	0.11	0.40*	0.08	0.24
Panicle number (panicle m^{-2})																		
Murayama	-6.38	-0.22	-3.82	-0.16	-9.7	-0.32	-6.65	-0.36*	-5.39	-0.34*	-6.14	-0.33*	-8.85	-0.34*	-6.33	-0.29	-10.49	-0.38*
Mogami	-1.74	-0.07	0.26	0.01	-4.45	-0.18	-3.28	-0.25	-2.23	-0.15	-2.53	-0.14	-4.34	-0.21	-1.35	-0.07	-5.71	-0.21
Okitama	-8.93	-0.35*	-4.89	-0.260	-14.42	–0.50**	-3.24	-0.21	-3.59	-0.27	-2.38	-0.12	-7.59	-0.32	-5.56	-0.31	-11.27	-0.38*
Shonai	-35.85	-0.58***	-25.51	-0.46**	-44.65	-0.75***	-6.47	-0.19	-5.13	-0.16	-8.08	-0.22	-23.21	-0.43**	-17.61	-0.36*	-32.38	-0.58***
Spikelet number (spikelet panicle^{-1})																		
Murayama	0.08	0.02	-0.29	-0.09	0.18	0.05	0.39	0.17	0.40	0.20	0.33	0.14	0.38	0.11	0.20	0.07	0.39	0.11
Mogami	-0.02	0.00	-0.02	-0.01	-0.15	-0.03	0.78	0.33	0.30	0.11	0.74	0.23	0.80	0.21	0.19	0.06	0.65	0.13
Okitama	-0.93	-0.24	-0.89	-0.31	-0.62	-0.14	0.37	0.16	0.17	0.09	0.27	0.09	-0.05	-0.02	-0.33	-0.12	-0.19	-0.04
Shonai	1.01	0.23	0.56	0.14	1.54	0.36*	0.19	0.08	0.14	0.06	0.24	0.09	0.64	0.17	0.38	0.11	1.05	0.26
1000-grain weight (g)																		
Murayama	0.39	0.60***	0.37	0.68***	0.36	0.53***	0.15	0.37*	0.12	0.34*	0.14	0.34*	0.32	0.54***	0.28	0.56***	0.31	0.5**
Mogami	0.46	0.53***	0.41	0.61***	0.37	0.40*	0.18	0.37*	0.22	0.38*	0.20	0.31	0.40	0.52**	0.41	0.59***	0.49	0.49**
Okitama	0.57	0.74***	0.43	0.76***	0.53	0.62***	0.12	0.26	0.16	0.40*	0.04	0.06	0.38	0.54***	0.35	0.65***	0.38	0.42*
Shonai	0.56	0.53***	0.47	0.51**	0.52	0.51**	0.12	0.21	0.12	0.22	0.13	0.21	0.40	0.44**	0.36	0.43**	0.43	0.45**
Ripening percentage (%)																		
Murayama	3.51	0.63***	3.33	0.70***	3.40	0.58***	1.95	0.54***	1.64	0.53***	1.84	0.51**	3.34	0.65***	2.92	0.68***	3.38	0.62***
Mogami	3.59	0.48**	3.42	0.58***	2.28	0.29	2.29	0.54***	2.05	0.42*	2.34	0.41*	3.98	0.59***	3.62	0.59***	3.97	0.46**
Okitama	4.09	0.71***	3.07	0.74***	4.02	0.63***	1.26	0.37*	1.44	0.49**	1.49	0.33*	3.20	0.61***	2.79	0.69***	4.03	0.60***
Shonai	3.78	0.65***	3.21	0.62***	3.64	0.65***	1.44	0.45**	1.28	0.44**	1.66	0.49**	3.18	0.64***	2.83	0.62***	3.59	0.68***

The *, **, and *** represent at P<0.05, 0.01, and 0.001 probability level, respectively. Sens and R indicated for Sensitivity and Coefficient Correlation

2.3.5. Relationship between growth stage duration and temperature changes

Table 2.6 and Fig. 2.2 show the response of growth duration due to temperature change. In TP-HD stage, there are very high correlation coefficients, ranged from -0.74 to -0.92 and sensitivity ranged from 3.0 to 4.8 day °C^{-1}. The R correlation in mean and maximum air temperatures is similar to each other, and higher than in minimum air temperature.

Table 2. 6 Response growth stage duration due to temperatures changes

Growth stage	Mean air temp		Maximum air temp		Minimum air temp	
	Sensitivity (day °C^{-1})	R	Sensitivity (day °C^{-1})	R	Sensitivity (day °C^{-1})	R
Transplanting-Heading stage						
Murayama	-4.1	-0.90***	-3.4	-0.89***	-3.5	-0.74***
Mogami	-4.0	-0.84***	-3.2	-0.84***	-3.4	-0.68***
Okitama	-4.3	-0.91***	-3.0	-0.89***	-4.3	-0.81***
Shonai	-4.8	-0.87***	-4.2	-0.85***	-4.1	-0.76***
Heading-Maturing stage						
Murayama	-0.6	-0.26	-1.2	-0.56***	-1.1	-0.42*
Mogami	-1.2	-0.52**	-0.6	-0.28	-0.6	-0.21
Okitama	-0.9	-0.38*	-0.9	–0.50**	-0.8	-0.31
Shonai	-1.1	-0.48**	1.6	0.22	2.4	0.28
Transplanting-Maturing stage						
Murayama	-3.8	-0.78***	-3.2	-0.78***	-3.6	-0.71***
Mogami	-4.9	-0.82***	-3.8	-0.79***	-5.4	-0.71***
Okitama	-4.2	-0.77***	-3.0	-0.73***	-3.6	-0.53***
Shonai	-5.4	-0.83***	-4.9	-0.82***	-5.2	-0.76***

The *, **, and *** represent at $P < 0.05$, 0.01, and 0.001 probability level, respectively.

In HD-MT stage, similar negative correlation also confirmed, but with less correlated coefficients. As a result, in the whole duration from transplanting-maturing, timing declined with the rising temperature with Sens ranging in -3.0 to -5.4 day $°C^{-1}$ and correlation ranging in -0.53 to -0.83 ($P<0.001$).

2.4. Discussion

Generally, paddy rice yield in Japan in recent decades is much stable compared to the world rice yield. The temporal correlation (R) of Japan and world average rice paddy yield in the period 1982 - 2017 was 0.398 and 0.994 (FAO, 2017). Our 4 researched sites, changes in yield are also similar to Japan average (Table 2.2). The average yield increased about 10% rice yield , while the world rice yield increased 113%, (about 11 times higher) in the last 50 years from 1967-2016 (FAO, 2018). This may be explained by that the demand of rice production in Japan is declined recent decade; as a result, there is no need to increase rice yield. This situation suggests that rice yield changed in recent time in Japan should be mainly affected from changing climate, not by practice technique or fertilizer application trend, which is a main reason for the strong increasing rice yield in other countries. In four observed regions, the main rice cultivar is Haenuki, which accounts for average of 62% of total paddy rice cultivation area in 2017.

It is clearly that temperature in growth season (from transplanting-maturing) in all sites rose significantly. But it is noticeable that, rising temperature only significantly observed in TP-HD stage, but not in HD-MT stage. We also observed that trend of temperature of June-July and August-September in the same period (which most correlated relatively to TP-HD and TP-HD). That is no surprise to see the same trend of temperature in June-July and August-September (Table 2.7).

Table 2.7 Mean and temporal changes of monthly temperatures in summer in 4 regions in Yamagata in 1982-2017 period

	Mean air temp			Maximum air temp			Minimum air temp		
	Mean (°C)	Trend (°Cyear^{-1})	R	Mean (°C)	Trend (°Cyear^{-1})	R	Mean (°C)	Trend (°Cyear^{-1})	R
June-July									
Murayama	21.8 (1.0)	0.06	0.57***	27.2 (1.3)	0.07	0.56***	17.5 (1.0)	0.06	0.64***
Mogami	20.9 (0.9)	0.06	0.58***	25.9 (1.3)	0.07	0.54***	16.8 (0.8)	0.05	0.62***
Okitama	21.0 (1.0)	0.06	0.61***	26.3 (1.3)	0.10	0.69***	16.7 (0.8)	0.05	0.56**
Shonai	21.7 (0.9)	0.05	0.56***	25.6 (1.0)	0.06	0.53***	18.3 (0.9)	0.06	0.61***
August-September									
Murayama	22.6 (1.0)	0.03	0.26	27.9 (1.3)	0.04	0.24	18.6 (1.0)	0.03	0.27
Mogami	21.9 (1.0)	0.03	0.24	27.1 (1.3)	0.04	0.28	17.9 (1.0)	0.03	0.24
Okitama	21.9 (1.0)	0.04	0.31	27.3 (1.5)	0.08	0.44*	17.5 (1.0)	0.04	0.26
Shonai	23.4 (1.0)	0.04	0.30	27.6 (1.2)	0.04	0.30	19.7 (1.0)	0.04	0.30
June-September									
Murayama	22.2 (0.9)	0.05	0.49**	27.5 (1.1)	0.05	0.45**	18 (0.8)	0.05	0.55***
Mogami	21.4 (0.8)	0.04	0.48**	26.5 (1.1)	0.06	0.48**	17.4 (0.7)	0.04	0.52**
Okitama	21.4 (0.8)	0.05	0.55***	26.9 (1.2)	0.10	0.64***	17.2 (0.6)	0.03	0.27
Shonai	22.5 (0.8)	0.05	0.49**	26.6 (0.9)	0.05	0.46**	19.0 (0.8)	0.05	0.54***

The figures in parentheses show standard deviation of the mean. The *, **, and *** represent at $P < 0.05$, 0.01, and 0.001 probability level, respectively.

The warming summer also reported by (Dorrepaal et al., 2003), which lead to increasing winter snow cover, could affect the crop cultivation in Netherlands. However, the other study in Japan showed that surface air temperature in summer is predicted to increase 1℃ while winter will increase 2℃ in the period of 100 years from 1981-2100 (Kurihara et al., 2005b). The record of annual temperature in Yamagata prefecture from 1890-2017 showed the increasing trend of 1.25, 0.8 and 1.89℃ in 100 years for mean, maximum and minimum air temperature. In our four locations in 1982-2017, the trend in mean, maximum and minimum temperatures were not large different among them. Although there are number of researches to report the rising temperatures in summer, there is no report to compared together trend of temperature in monthly record and rice growth stages in more than 3 decades. To our knowledge, we are the first to report the temperature in TP-HD stage in Japan paddy rice increased, but not in HD-MT stage, and this result is tightly high correlated to June-July and August-September periods.

There is no surprising that temperatures changes in summer season had strong correlation with the rice yield in four researched regions. Numerous researches also showed that warming summer affect strongly on rice yield via growing stages (Ishimaru et al., 2011a; Ishimaru et al., 2016; Kim et al., 2013; Matsui et al., 2015b). However, all reports focused on the negative effect on rice yield, and their components. In our research, a positive correlation with the increase of temperature was observed. It is explained that in cold regions, where growing temperature is lower than optimum temperature condition, increasing temperature can enhance rice plant growth via photosynthesis. The four regions in this research has an average temperature in transplanting-maturing period around 20.7~21.7℃ (**Table** 2. 4), considered as lower than optimum temperature for rice plant growth. This is also confirmed in the other researches (Ghadirnezhad and Fallah, 2014; Yoshida, 1973). Temperature responses of photosynthesis was optimum in the range of 30-35℃ (Nagai and Makino, 2009), which is much higher in our research locations. In our research cultivar, main variety is Haenuki which is belonging to *Japonica* cultivar. (Fukui et al., 2015) reported the growth rate of 10 common Japanese rice varieties, showed the optimum of growth rate of Haenuki is also around 30-35℃. This result is also confirmed by (Hatfield and Prueger, 2015), which mentioned that temperature would decline rice yield when maximum temperature reach over 33℃. In our researched sites, the temperature during growth stage is about 10 degrees below optimum temperature as mentioned above. A research by (Weng and Chen, 1987) on *Japonica* and *Indica* varieties showed that elevating temperature from 20 to 30℃ in summer could increase photosynthesis rate of 22-42%, while respiration rate increases 116-128%. The previous report by (Yoshida, 1973) mentioned that early growth stages rate increase with the increase of temperature from 22-31℃. Among the 4 rice yield components, the spikelet number per panicle had negative correlation with increasing temperatures. This could be explained by that low temperature will prolong flowering stage, therefor produce more spikelet per panicle. The inversely correlation between spikelet number per panicle and temperature also showed in previous study (Yoshida, 1973). As conclusion, therefore, it is expected to increase rice biomass response to rising temperatures in our researched sites.

The response of rice growth to temperature also leads to change in growth duration. As mentioned above, rising temperature in our enhance rice growth via increasing photosynthesis and respiration rate, since the temperature in 4 sites is much lower than the optimum condition. Therefore, the rising temperature would shorten the growth duration. Our result shows that strong correlation in TP-HD stage, which confirms the hypothesis. In HD-MT stage, the correlation with temperature is much less significant. This could be explained that after vegetative stage, effect of temperature become weaker. The duration of HD-MT is reported to be vary less in common rice cultivars in Japan by (Fukui et al., 2015), which is similar to our result (Table 2.6).

We also notice that temperature at transplanting and maturing date in four sites is vary widely compared to heading date (**Fig.** 2.4). This proves that heading stage is more sensitive than maturing stage. It is also interesting that maximum temperature in heading date of four sites are vary less, and no different among them, but more vary in mean and minimum air temperature. This result confirms that rice plants response to maximum air temperature is more sensitive than mean and minimum air temperature. The increase of minimum temperature is reported to be linked to global climate changes, while maximum temperature are effected by local condition (Hatfield and Prueger, 2015). The rising minimum air temperature tend to reduce rice yield, while rising maximum temperature lead to enhance rice yield in tropical and subtropical Asia (Welch et al., 2010). However, in our research, we find that rice yield also response positively with minimum air temperature. But, interestingly, the correlation between plant growth stages, rice yield with minimum air temperature is clearly lower than with mean and maximum temperatures (Table 2.6 and Fig.2. 5).

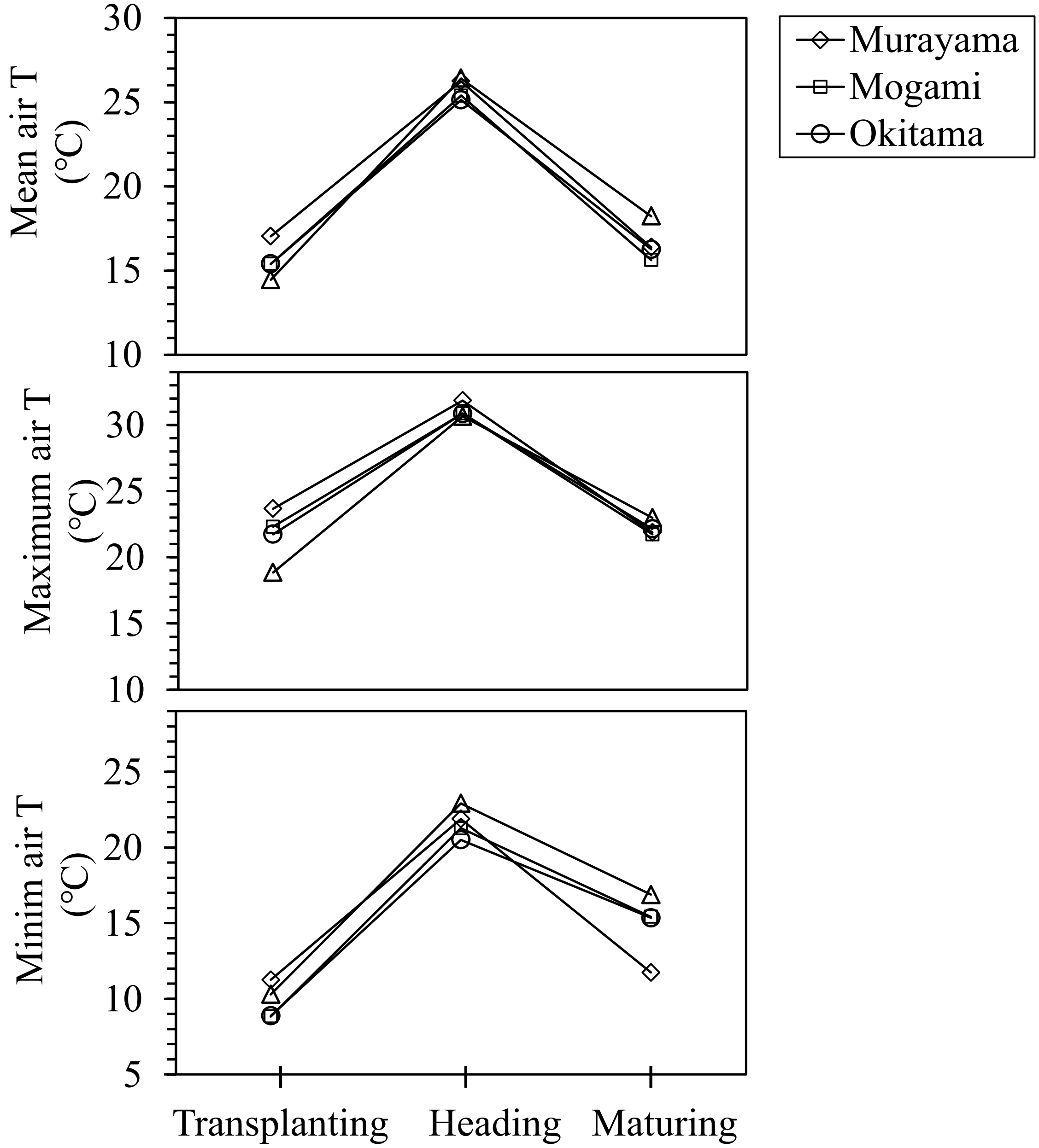


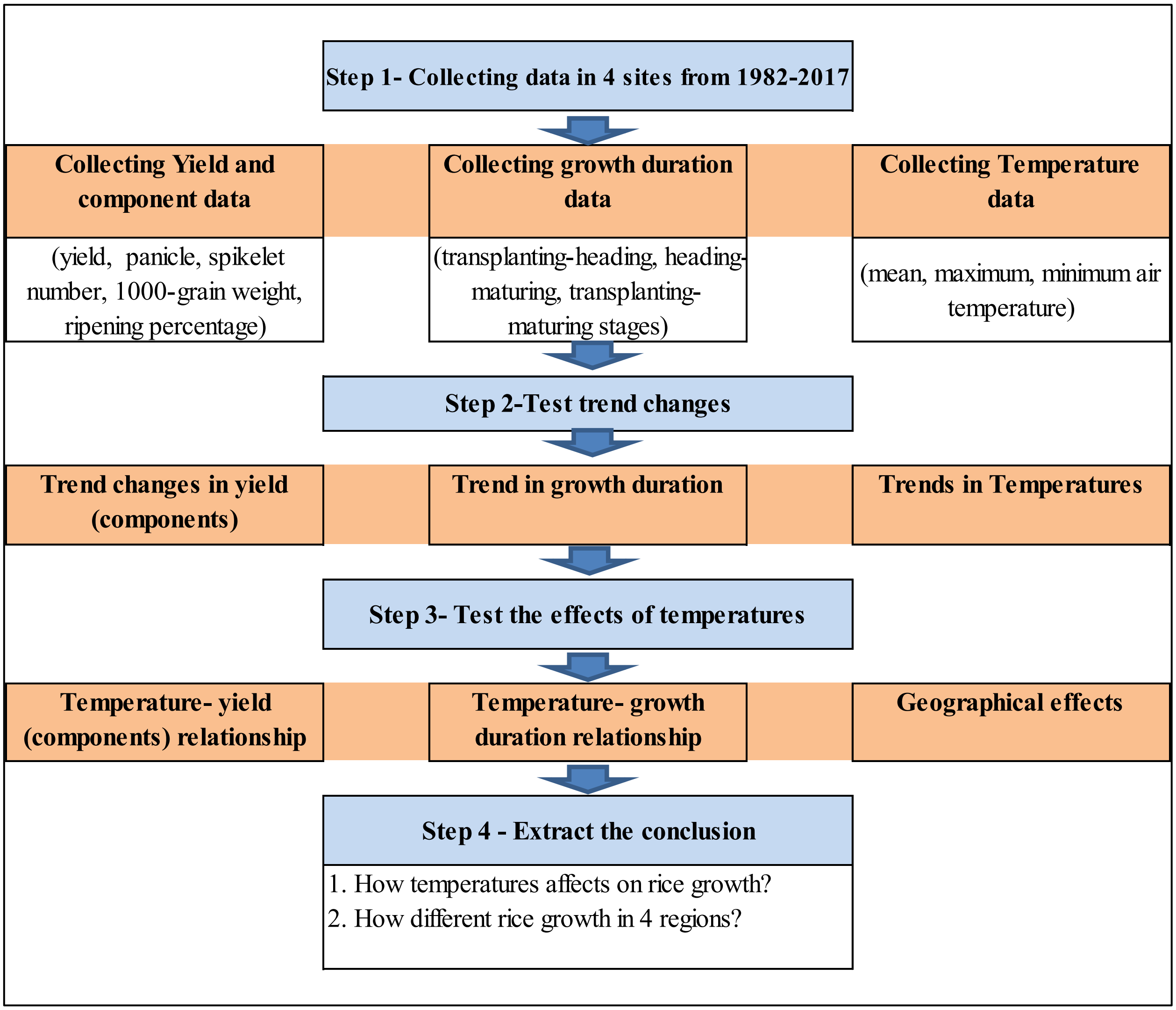
Fig. 2. 4. Temperature at transplanting, heading and harvesting date of four location.

Although four study sites belong to Yamagata prefecture, a distinguishable geographic property is noticeable. (Fukui et al., 2015; Wada, 1952) reported that rice cultivars from the lower latitudes (North) were more sensitive to temperature than the higher latitude places (South). In present study, Murayama and Okitama has the lower Latitudes (38°15.3' N and 38°0.2' N) which is considerable lower than Mogami and Shonai location (38°45.4' N and 38°54.5' N). Low altitude also lead to less sensitive to temperature, because at this elevation level, plant growth temperature is higher, which closer to optimum temperature (Jing and Jichao, 2012). The Shonai site shows the lesser changing trend (about 6 to 9 times slower than other sites, Table 2.2). Notice that Shonai is located at the lowest elevation and highest latitude in among sites (2. 1), make rice plant become less sensitive to the change of environment. This may explain the higher correlation of rice yield, yield components in Muyamara and Okitama, and the lowest sensitive to temperature showed in Shonai (Table 2.5, Table 2.6). The reason of this trend contributes to that the place where close to latitude origin is also tend to receive more photoperiod (Fukui et al., 2015).

2.5. Conclusion

Temperatures have been increasing significantly in four researched sites, highly correlation in early growing stage. Rising temperatures led to enhance rice yield, 1000-grain weight and ripening percentage, but not in number of panicle and spikelet. Response of rice growth to temperature depends on growing stage timing, as well as geographical location. As a result, phenological stages clearly declined with the higher temperature in vegetative stage, but less pronounce in productive stage. The response of rice growth to minimum temperature is less sensitive to mean and mean temperature. Our results highlight that rising temperature in cold regions contribute to positive effects on paddy rice cultivation.

Chapter 2- General process



Chapter III Changes in soil organic matter with long-term applications of mineral fertilizers and organic manures

3.1. Introduction

Rice is one of the most important cereal crops. In Asia, it is the most consumption food. The world rice paddy cultivated area occupies 159.8 million ha, which produce 741 million ton for production (FAO, 2017). It is estimated rice production and harvested areas in Asia contributes 90 and 88% of the global total. Climate changes in Yamagata, northeastern Japan were observed and reported in Chapter 2. We also addressed the responses of rice yield to rising temperatures in 4 regions of Yamagata (Murayama, Mogami, Okitama, Shonai). However, since there is several rice varieties were practiced, caused the large errors to final conclusion. The changes of varieties in large real rice practiced regions are normally inevitable. Therefore, in this study we challenge to test the response of rice yield from specific variety at a long-term experiment.

Study on rice mainly related to the rice paddy soil condition, which account for a huge factor of the ecosystem. Carbon and Nitrogen are two key elements of soil organic matters in soil which affect strongly on the soil fertility. Researches on soil organic carbon and nitrogen, therefore, widely conducted in rice paddy field (Cheng et al., 2016a; Iqbal, 2016; Koyama and Hayashi, 2017; Nakajima et al., 2016).

Long-term experiments (LTE) play a key role in term of observing slowly changing trends, may not observed in short term (Soler et al., 2014). At the beginning, LTE focused on yield stable, then across the time, further purposes had been conducted (Körschens, 2006 ; Rasmussen et al., 1998; Senbayram et al., 2008). In Asia, many LTEs in rice paddy field have been implemented (Azuma et al., 2015; Cheng et al., 2016a). Carbon (C) which contribute about 58% of SOM, and nitrogen (N) which are source for nutrient of plants, are two of the most important organic elements (Balesdent J et al., 1988; Senbayram et al., 2008). There are numerous of researches on LTEs have been carried on (Körschens, 2006 ; Richter et al., 2007). Beside the quantity of C and N, natural stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) is widely used to study the dynamic of SOM. The change in $\delta^{13}C$ and $\delta^{15}N$ in soils are closely related to the organic matter source and its discriminations during the cycling processes (Nakajima et al., 2016; Werth and Kuzyakov, 2010; Yoneyama et al., 2001). Researches on $\delta^{13}C$ and $\delta^{15}N$ on agricultural soils had been done with limited result (Nishida et al., 2007; Nishida and Sato, 2015; Senbayram et al., 2008; Yoneyama et al., 2001). However, there is no report on their quality with regards to their stable isotope values continuously in annually over decades on rice paddy.

Incubation is an easy and convenient method to understand the soil organic matter dynamic. The carbon decomposition (Dec-C) as well as nitrogen mineralization (Min-N) confirmed a high correlation with the amount of SOM, and also with rice yield (Cheng et al., 2016a; Cheng et al., 2007; Nakajima et al., 2016). Although there are many experiments on incubation, but there is no research showing the continuous changes of rice yield relatively with changes in SOM with over 30 year period. Therefore, the objectives of this study were to determine the changes in the rice yield and SOM (for both C and N), their $\delta^{13}C$ and $\delta^{15}N$ values and the relationships among all C and N parameters for the soil samples from the long-term (31 years) application of two types of organic matter (rice straw (RS) and its compost (CM)) combined with NPK fertilizers in a single rice paddy for a cold temperate region in Japan. Our hypothesis was that application of RS and CM may accelerate the quantity and quality of SOM, leading to enhancement of rice yield. At the same time, the changes in $\delta^{13}C$ and $\delta^{15}N$ values in soils under long-term fertilization practices are associated with originally inputted materials.

3.2. Material and Methods

3.2.1. Site description and field management

The long-term experimental field is located at the Yamagata Integrated Agricultural Research Center, Yamagata, Japan (38 ^{o}15'N, 140^{o}15'E), a typical humid temperate climate zone. The mean annual air temperature was 11.7 °C and mean annual precipitation was 1163 mm over a period of 30 years (1981–2010), according to data recorded at Yamagata Meteorological Station. LTE was started in May 1982 with the first rice growing season. The soil is classified as an Inceptisol by US Soil Taxonomy (Cheng et al., 2007). The initial soil had a pH (H_2O) of 5.56, SOC of 8.9 g kg^{-1}, and TN of 1.1 g kg^{-1} at the depth of 0–12 cm. More information about this LTE can be found in Yamagata Agricultural Research Station (1983).

Soil samples were collected from five plots of 100 m^2 under different fertilizations: (1) PK, (2) NPK, (3) NPK + rice straw (RS), (4) NPK + rice straw compost (CM1), and (5) NPK + overdosed rice straw compost (CM3). Three fertilizers, ammonium sulfate, monocalcium phosphate and potassium chloride applied annually corresponded to the rate 80 kg N: 68 kg P_2O_5: 83 kg K_2O ha^{-1}.

Table 3.1. Mineral fertilizer and organic matters application in the long-term experiment at every rice growth season.

Treatment	N	P_2O_5	K_2O	Rice straw	Rice straw Compost
	kg ha^{-1}			Ton ha^{-1}	
PK	0	68	83	0	0
NPK	80	68	83	0	0
RS	80	68	83	6	0
CM1	80	68	83	0	10
CM3	80	68	83	0	30

Rice straw and rice straw compost in RS and CM1 treatment were applied at annual rates of 6 and 10 Mg ha^{-1}, equivalent to the average yield of rice straw and compost returning to the field after the harvest. An annual rate of 30 Mg ha^{-1} of rice straw compost was applied in CM3 treatment, three times larger than regular application rate (CM1). Detailed properties of RS and CM are shown in Table 3.2. RS was left on the soil surface after harvest and during the winter season. CM was produced outdoors for about 2-3 years until the volume stabilized. RS compost was incorporated in the CM1 and CM3 treatments, while the NPK fertilizers were broadcasted before soil puddling and rice transplanting. Rice straw and rice straw compost were incorporated at the plowed layer (0-15cm). Average C and N contents and natural stable C isotope of rice straw and compost are presented in Table 3.2. In 1994, due to effects of erosion, the part of top soil was lost. To recover the soil organic matter content, a guest soil had been added in all experiment.

Table 3.2. Water, organic C and total N contents, and $\delta^{13}C$ values of the organic matters used in the long-term experiment at every rice growth season.

	Rice straw (RS)	Straw compost (CM)	NPK fertilizer	Guest soil
Water content (%)	10.0	76.3†	-	-
Total C (g kg^{-1}dry weight)	387	216†	-	32.9*
Total N (g kg^{-1}dry weight)	6.0	16.7†	19.0*	2.4*
C/N	64.5	12.9	-	13.83
$\delta^{13}C$ (‰)	-28.30	-27.41	-	-23.87
$\delta^{15}N$ (‰)	1.63	5.85	0.28	3.55

†Water, organic C and total N contents are average values from rice straw compost samples analyzed in 1996, 2002, and 2013. The values without † showed the data was only analyzed in 2013. Guest soil collected in 2017 nearby orniginal place to take before. NPK fertilizer is Haenuki special for Nitrogen (15:17:15 ratio).
*Total C and N calibrated from isotope measurement.

3.2.2 Climatic parameter collection

`Climatic data were collected from the research site from 1982-2014. Mean air temperature (Mean air T), maximum air temperature (Max air T), minimum air temperature (Min air T), mean soil temperature (Mean soil T), minimum soil temperature (Min soil T), sunshine, radiation, mean humidity (Mean H), and Min humidity (Min H) have been recorded to test the trend and effects on rice yield. Three period June-July, August-September, and June-September (abbreviated as Jun-Jul, Aug-Sep, and Jun-Sep) are considered as closed to the Transplanting-Heading, Heading-Maturing, and Transplanting-Maturing stages (Abbreviated as TP-HD, HD-MT, and TP-MT stages). As we found in four regions in Yamagata, temperatures in June-July, August-September could representative for phenological events, which addressed in Chapter 2. The average of climatic data in 1982-2014 in LTE site was described in Fig. 3.1.

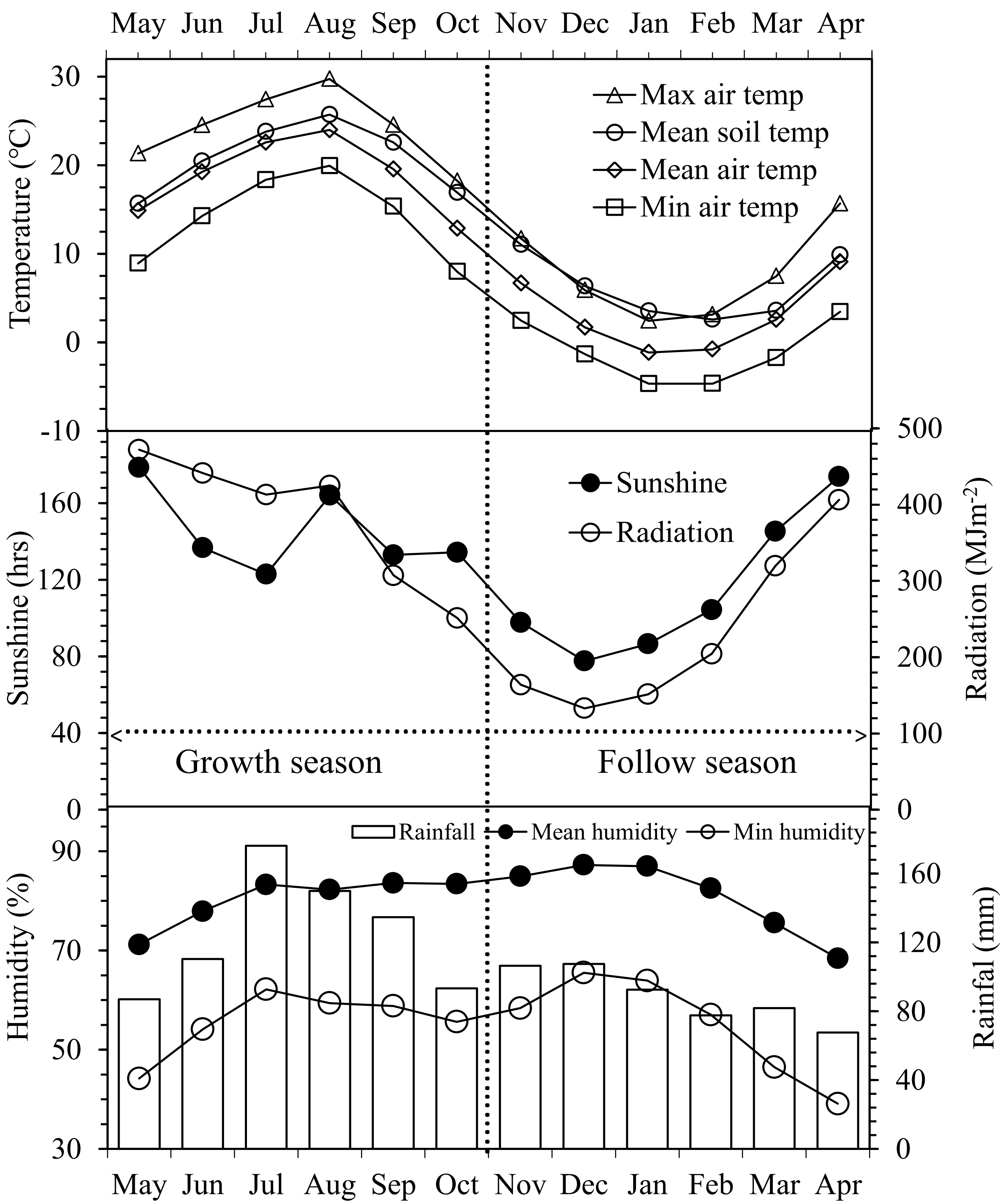


Fig. 3.1 The average of temperatures (air and soil temperatures) (top), sunshine and radiation (middle) and rainfall and humidity (bottom) from 1982-2014 at LTE location.

3.2.3. Soil sampling and analysis

Soil samples were collected annually after harvesting, for 33 years of continuous rice cultivation. Plough layer (15 cm) was decided for sampling from 1983-2014. For every year sampling, soil samples were air-dried and sieved (⌀<2 mm) and finally stored at room temperature. A total of 165 samples from 5 treatments were collected for analysis. For the determination of stable isotope C and N, soil samples were finely ground by Hi-Speed Vibration Sample Mill (Model TI-200, CMT, Iwaki, Japan).

Air-dried soils were used to measure SOC and TN content by dry combustion according to "Soil Normal Analysis Methods" (JSSSPN 1986) using a CN-900 Analyzer (Sumika Chemical Analysis Service, Japan). The $\delta^{13}C$ values of ground soil samples were measured by using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Flash 2000, Delta V Plus; Thermo Scientific, Germany). Mineral N [ammonium-nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N)] in the air dried soils was extracted by shaking with 10% potassium chloride (KCl) for 30 min. The extracted solutions were filtered and stored in a deep freezer (–18°C) until analysis. Both NH_4^+-N and NO_3^--N contents were measured by a colorimetric method (the nitroprusside method and hydrazine reduction method (JSSSPN 1986).). One part of air dry soil was extracted with 0.002 N H_2SO_4 solution to measure available phosphorus (Av-P) (JSSSPN 1986).

3.2.4. Aerobic incubation experiment

Each soil sample (5 g on an oven-dried) was weighed into a 68-mL serum bottle and adjusted to 60% water-filled pore space (WFPS). Each bottle was capped with a butyl rubber stopper with aluminum seal and the air inside was replaced with pure air (O_2+ N_2) gas. All soil samples in the sealed bottles were incubated at room temperature (25ºC). After 2 weeks, soil samples were taken out of the incubator, to measure the CO_2 and CH_4 productions. The CO_2 and CH_4 concentrations in the headspace of each bottle were determined by gas chromatography using GC-8A and GC-2014 (Shimadzu, Kyoto, Japan) with TCD and FID detectors, respectively (Cheng et al. 2007). The CO_2 and CH_4 concentrations in the headspace were used to calculate CO_2 and CH_4 productions after correcting for gaseous dissolution in the incubation water by Henry's Law coefficient (Cheng et al., 2006). The C decomposition (Dec-C) was estimated from the sum of CO_2 and CH_4 productions. After the gases were measured, the incubated soil was immediately extracted with 30 ml 10% KCl solution by shaking for 30 mins on a reciprocal shaker. The amounts of NH_4^+-N in the soil extracts were measured as described above. The net N mineralization was calculated as the amount of NH_4^+-N in soil after anaerobic incubation minus the amount of NH_4^+-N in air-dried soil.

3.2.5. Anaerobic incubation experiment

After 2 weeks of aerobic incubation, each soil sample (5 g net air dry soil) was amended with 10 mL distilled water. Each bottle was capped aluminum seal and the air inside was replaced with pure N_2 gas. All soil samples in the sealed bottles were incubated at 30ºC. After 4 weeks of anaerobic incubation, soil samples were taken out to measure the CO_2 and CH_4 productions. The CO_2 and CH_4 concentrations in the headspace of each bottle were determined by gas chromatography using GC-8A and GC-2014 (Shimadzu, Kyoto, Japan) with TCD and FID detectors, respectively(Cheng et al., 2007). After the gases were measured, soil was extracted with 20 ml 15% KCl solution by shaking for 30 mins on a reciprocal shaker. The amounts of NH_4^+-N in the soil extracts were measured as described above. The N mineralization was calculated as the amount of NH_4^+-N in soil after anaerobic incubation minus the amount of NH4+-N in air-dried soil.

3.2.6. Modified RothC model application

To estimate the change of SOC, we use RothC model to compare with the measured data. We applied the modified RothC referenced from (Shirato et al., 2004).

3.2.7. Statistical analysis

Since there were no plot replications for each treatment, statistical analysis was not carried out in this paper. All data from the three pseudo-replicates were only used for the calculation of the average value and standard deviation for each treatment. Correlation coefficients were used to assess the significance of the inter-relationships among the main parameters.

3.3. Results

3.3.1. Changes in rice yield

Table 3.3 shows mean and temporal changes in rice yield from LTE. Average yield during 1982-2014 of all treatments ranged from 3.1-6.5 ton ha^{-1}, with the lowest yield showed in PK (3.1 ton ha^{-1}), and highest rice yield in CM3 treatment (6.5 ton ha^{-1}). Except PK, all treatments showed the significant increase in rice yield (P<0.01). The increasing trend has the same trend with rice yield, which showed highest in CM3 and lowest in PK treatment.

Table 3.3 Changes in yield and yield difference relatively to NPK after 33 years applications of inorganic fertilizer and organic matter

Treatment	Mean and temporal changes in rice yield (ton) in 1982-2014 period			Differences to NPK treatment (%) in 1982-2014 period		
	Mean (ton)	Trend (ton yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
PK	3.1 (0.5)	0.016	0.29	-46.8 (8.7)	0.035	0.04
NPK	5.9 (0.5)	0.027	0.52**	-	-	-
RS	6.1 (0.6)	0.042	0.67***	4.1 (6.4)	0.238	0.36*
CM1	6.2 (0.7)	0.053	0.73***	5.7 (8.4)	0.427	0.49**
CM3	6.5 (0.7)	0.055	0.74***	11.6 (8.9)	0.424	0.46**

The *, **, and *** represent at P<0.05, 0.01, and 0.001 probability level, respectively

As NPK treatment is considered as the control treatment, we compared the other treatments to control (Table 3.3). Except PK treatment showed the decrease in rice yield (-46.8%), the RS, CM1 and CM3 showed increase by 4.1, 5.7, 11.6% compared to NPK treatment, respectively (P<0.05).

3.3.2. Changes in climatic parameters in LTE site from 1982-2014

Changes in climatic parameters showed in Table 3.4. Mean air T, Min air T and Mean soil T showed the increase trends in summer time. It is also observed that Jun-Jul temperatures increased significantly (except Max air T), but less significant in Aug-Sep period. For the whole crop season (CS), only Mean air T and Mean soil T increased with the trend of 0.4 and 0.03℃ $year^{-1}$. Sunshine time and rainfall have the same negative correlation coefficients, especially in crop season. Radiation shows less significant change. Meanwhile, Mean H increased at the same time but Min H shows vary less change compared to mean humidity.

Table 3.4 Change in climatic parameters in LTE site from 1982-2014

	Mean air temp		Max air temp		Min air temp		Mean soil temp		Sunshine		Radiation		Rainfall		Mean humidity		Min humidity	
	Trend (°C yr^{-1})	R	Trend (°C yr^{-1})	R	Trend (°C yr^{-1})	R	Trend (°C yr^{-1})	R	Trend (hrs yr^{-1})	R	Trend (MJ m^{-2} yr^{-1})	R	Trend (dm yr^{-1})	R	Trend (% yr^{-1})	R	Trend (% yr^{-1})	R
Jan	0.01	0.09	0.01	0.06	0.04	0.21	0.03	0.35	-1.70	-0.60***	1.79	0.5**	-0.19	-0.11	0.14	0.42*	-0.07	-0.10
Feb	0.02	0.12	0.04	0.25	0.04	0.19	0.02	0.23	-1.71	-0.54**	0.31	0.10	-0.91	-0.33	0.13	0.42*	-0.08	-0.13
Mar	0.02	0.15	0.03	0.20	0.02	0.16	0.01	0.08	-2.10	-0.72***	1.21	0.30	-3.04	-0.85***	0.13	0.26	0.14	0.20
Apr	-0.01	-0.08	0.01	0.08	0.02	0.13	-0.01	-0.06	-1.35	-0.37*	-0.30	-0.08	-2.84	-0.57***	0.09	0.17	0.09	0.11
May	0.02	0.23	-0.02	-0.18	0.01	0.13	-0.01	-0.11	-2.43	-0.58***	-1.66	-0.30	-3.48	-0.71***	0.15	0.39*	-0.05	-0.10
Jun	0.06	0.55***	0.00	0.00	0.05	0.41*	0.02	0.17	-1.37	-0.34	-0.29	-0.05	-1.61	-0.35*	-0.04	-0.17	-0.36	-0.64**
Jul	0.05	0.36*	0.02	0.13	0.05	0.35*	0.05	0.40*	-2.60	-0.57***	2.22	0.27	-3.29	-0.47**	0.11	0.42*	0.09	0.15
Aug	0.00	-0.03	-0.08	-0.42*	-0.03	-0.30	0.00	0.01	-3.04	-0.55***	0.67	0.07	-4.49	-0.65***	0.13	0.41*	-0.05	-0.07
Sep	0.05	0.41*	0.02	0.14	0.03	0.26	0.07	0.57***	-0.19	-0.08	-2.14	-0.24	-0.69	-0.20	-0.07	-0.28	-0.18	-0.32
Oct	0.06	0.54**	0.03	0.30	0.07	0.48**	0.09	0.69***	-1.15	-0.53**	1.64	0.28	-2.12	-0.65***	0.12	0.48*	0.03	0.06
Nov	0.03	0.25	0.03	0.18	0.02	0.12	0.08	0.67***	-0.78	-0.48**	0.76	0.17	-1.46	-0.61***	0.15	0.54**	0.23	0.47*
Dec	-0.02	-0.13	-0.04	-0.25	-0.02	-0.15	0.04	0.38*	-1.77	-0.74***	3.06	0.62***	-1.79	-0.79***	0.31	0.69***	0.25	0.37
Year	0.02	0.43*	0.00	0.06	0.02	0.33	0.03	0.6***	-20.20	-0.76***	7.42	0.26	-26.19	-0.87***	0.12	0.59**	0.00	0.01
Jun-Jul	0.06	0.54**	0.01	0.10	0.05	0.45**	0.03	0.35*	-3.97	-0.55***	1.92	0.18	-4.90	-0.54**	0.03	0.18	-0.13	-0.30
Aug-Sept	0.03	0.25	-0.03	-0.27	0.00	0.01	0.04	0.38*	-3.23	-0.49**	-1.47	-0.12	-5.18	-0.65***	0.01	0.05	-0.11	-0.26
Jun-Sep	0.04	0.47**	-0.01	-0.09	0.02	0.33	0.03	0.39*	-7.20	-0.57***	0.45	0.03	-10.08	-0.67***	0.02	0.14	-0.12	-0.35
Oct-Apr	0.02	0.26	0.02	0.22	0.03	0.29	0.05	0.60***	-10.51	-0.76***	7.53	0.55**	-12.38	-0.89***	0.21	0.70***	0.08	0.22

The *, **, and *** represent at P<0.05, 0.01, and 0.001 probability level, respectively

3.3.3. Response of rice yield to climatic changes at LTE site from 1982-2014

The relationship between rice yield and temperatures shows in Table 3.5.

Table 3.5. Responses of yield (ton ha^{-1}) to temperatures changes at LTE site in 1982-2014

	Year		Jun-Jul		Aug-Sept		June-Sept		Oct-Apr	
	Sens (ton °C^{-1})	R	Sens (ton °C^{-1})	R	Sens (ton °C^{-1})	R	Sens (ton °C^{-1})	R	Sens (ton °C^{-1})	R
Mean air temperature (°C)										
PK	0.19	0.19	0.26	0.46**	-0.07	-0.12	0.13	0.20	0.12	0.16
NPK	0.24	0.27	0.32	0.64***	0.14	0.28	0.32	0.54**	0.04	0.06
RS	0.37	0.34	0.31	0.52**	0.15	0.24	0.32	0.44**	0.16	0.20
CM1	0.48	0.38*	0.43	0.6***	0.14	0.19	0.39	0.46**	0.19	0.20
CM3	0.27	0.21	0.41	0.56***	0.07	0.10	0.33	0.38*	0.08	0.08
Maximum air temperature (°C)										
PK	-0.18	-0.23	-0.11	-0.24	-0.15	-0.31	-0.17	-0.31	0.02	0.03
NPK	-0.04	-0.05	0.02	0.05	0.00	0.00	0.01	0.02	0.03	0.05
RS	-0.04	-0.05	-0.02	-0.03	-0.08	-0.16	-0.06	-0.11	0.08	0.12
CM1	0.02	0.02	0.00	0.01	-0.22	-0.35*	-0.14	-0.19	0.17	0.21
CM3	-0.23	-0.22	-0.04	0.01	-0.28	-0.45**	-0.21	-0.31	0.03	0.04
Min air temperature (°C)										
PK	-0.10	-0.13	-0.06	-0.11	-0.11	-0.18	-0.14	-0.19	0.01	0.02
NPK	0.08	0.12	0.15	0.31	-0.03	-0.05	0.13	0.19	0.05	0.11
RS	0.20	0.23	0.22	0.38*	0.02	0.04	0.24	0.29	0.10	0.18
CM1	0.25	0.24	0.26	0.37*	-0.16	-0.19	0.15	0.15	0.17	0.24
CM3	0.03	0.03	0.22	0.31	-0.22	-0.27	0.06	0.06	0.05	0.06
Mean soil temp (°C)										
PK	0.18	0.18	0.18	0.30	0.10	0.18	0.16	0.25	0.15	0.19
NPK	0.34	0.39*	0.25	0.44**	0.29	0.56***	0.31	0.54**	0.12	0.18
RS	0.63	0.6***	0.30	0.46**	0.30	0.48**	0.35	0.50**	0.30	0.38*
CM1	0.70	0.53**	0.32	0.41*	0.28	0.38*	0.35	0.42*	0.42	0.43*
CM3	0.48	0.37	0.32	0.40*	0.26	0.34	0.33	0.40*	0.33	0.34

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. Sens and R indicated for Sensitivity and Coefficient Correlation

Mean air T shows significant in June-July period (correlation coefficients ranged in 0.46-0.64), but not significant effect on late period (Aug-Sep). Max air T and Min air T shows less significant in both periods. Meanwhile, Mean soil T shows significant increase in both Jun-Jul and Aug-Sep in three treatments (NPK, RS and CM1). The year temperatures and Offseason temperatures does not affect yield of all treatments, except RS and CM1. Consider for the crop season (Jun-Sep), Mean air T and Mean soil T shows positive effects (except PK).

The effects of sunshine, radiation, rainfall, Mean H and Min H show in Table 3.6. We observed that only sunshine and radiation showed negative effects correlation with rice yield, in year and Offseason, for RS and CM application treatments. In crop season, there is varying less of interaction between these climatic parameters and rice yield.

3.3.4. Changes in SOC, TN, and available P

In this study SOC, TN, Available P (Av-P) was directly measured from initial soil samples. Additionally, SOC was estimated by the RothC model to test the common model with the measurement results. In 1995, due to soil lost from erosion, additional soil was added, therefore, caused the gap change before and after 1994. We hence analyzed based on two main periods: 1983-1994 and 1995-2014. However, to set NPK treatment as control, we compared the differences to NPK in percentage. This method could deplete the changed gap made by additional soil. The results are showed below.

Table 3. 6 Responses of yield (ton ha^{-1}) to sunshine, radiation, rainfall, humidity changes at LTE site in 1982-2014

	Year		Jun-Jul		Aug-Sept		June-Sept		Oct-Apr	
	Sensitivity	Correlation	Sensitivity	Correlation	Sensitivity	Correlation	Sensitivity	Correlation	Sensitivity	Correlation
Sunshine	(ton $100hr^{-1}$)	R	(ton $100hr^{-1}$)	R	(ton $100hr^{-1}$)	R	(ton $100hr^{-1}$)	R	(ton $100hr^{-1}$)	R
PK	-0.05	-0.26	-0.12	-0.15	-0.13	-0.15	-0.08	-0.17	-0.09	-0.21
NPK	-0.05	-0.27	-0.12	-0.18	0.08	0.10	-0.02	-0.05	-0.08	-0.23
RS	-0.12	-0.5**	-0.34	-0.4*	-0.14	-0.15	-0.15	-0.31	-0.20	-0.45*
CM1	-0.13	-0.47**	-0.31	-0.30	-0.15	-0.14	-0.14	-0.25	-0.24	-0.45*
CM3	-0.15	-0.53**	-0.42	-0.41*	-0.27	-0.24	-0.22	-0.36*	-0.25	-0.45*
Radiation	(ton GJ^{-1})	R	(ton GJ^{-1})	R	(ton GJ^{-1})	R	(ton GJ^{-1})	R	(ton GJ^{-1})	R
PK	-0.12	-0.06	-0.55	-0.09	-0.84	-0.12	-0.44	-0.12	-0.19	-0.05
NPK	-0.15	-0.09	-0.20	-0.04	0.38	0.06	0.03	0.01	-1.25	-0.34
RS	-0.92	-0.48*	-1.86	-0.28	-1.80	-0.23	-1.18	-0.29	-2.50	-0.59***
CM1	-1.44	-0.59**	-2.80	-0.35*	-2.98	-0.32	-1.86	-0.38*	-3.39	-0.64***
CM3	-1.39	-0.58**	-2.56	-0.32	-2.96	-0.32	-1.77	-0.36*	-3.04	-0.58**
Rainfall	(ton dm^{-1})	R	(ton dm^{-1})	R	(ton dm^{-1})	R	(ton dm^{-1})	R	(ton dm^{-1})	R
PK	-0.02	-0.09	0.10	0.19	-0.09	-0.21	-0.01	-0.04	-0.10	-0.24
NPK	-0.01	-0.04	0.09	0.19	-0.10	-0.25	-0.02	-0.07	0.04	0.11
RS	0.03	0.14	0.15	0.26	-0.08	-0.16	0.02	0.05	0.09	0.21
CM1	0.01	0.05	0.20	0.29	-0.19	-0.32	-0.02	-0.05	0.13	0.25
CM3	0.04	0.13	0.15	0.21	-0.15	-0.25	-0.02	-0.05	0.18	0.32
Mean himidi	(ton per %)	R	(ton per %)	R	(ton per %)	R	(ton per %)	R	(ton per %)	R
PK	-0.16	-0.39	-0.02	-0.07	-0.07	-0.22	-0.08	-0.21	-0.12	-0.45*
NPK	0.03	0.10	-0.01	-0.03	-0.08	-0.31	-0.08	-0.23	0.03	0.11
RS	0.08	0.21	0.01	0.04	-0.03	-0.12	-0.02	-0.06	0.04	0.17
CM1	0.09	0.38*	0.03	0.08	-0.03	-0.08	0.00	0.00	0.03	0.08
CM3	0.10	0.21	-0.02	-0.05	-0.04	-0.11	-0.06	-0.11	0.06	0.19
Min humidity	(ton per %)	R	(ton per %)	R	(ton per %)	R	(ton per %)	R	(ton per %)	R
PK	-0.15	-0.45*	-0.06	-0.35	-0.05	-0.25	-0.08	-0.37	-0.09	-0.39
NPK	-0.07	-0.26	-0.09	-0.61**	-0.06	-0.39	-0.12	-0.63**	-0.03	-0.14
RS	-0.01	-0.04	-0.07	-0.43*	-0.03	-0.15	-0.08	-0.37	0.00	0.02
CM1	-0.07	-0.18	-0.07	-0.36	-0.08	-0.35*	-0.12	-0.46*	-0.04	-0.16
CM3	-0.02	-0.04	-0.08	-0.37	-0.06	-0.45**	-0.11	-0.40	-0.01	-0.03

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. Sens and R indicated for Sensitivity and Coefficient Correlation

3.3.5. Changes in SOC

a) Changes of SOC from measurement

SOC content is shown in Table 3.7 and Fig. 3.2. It was clear that CM3 and PK treatments show the highest and lowest in both periods.

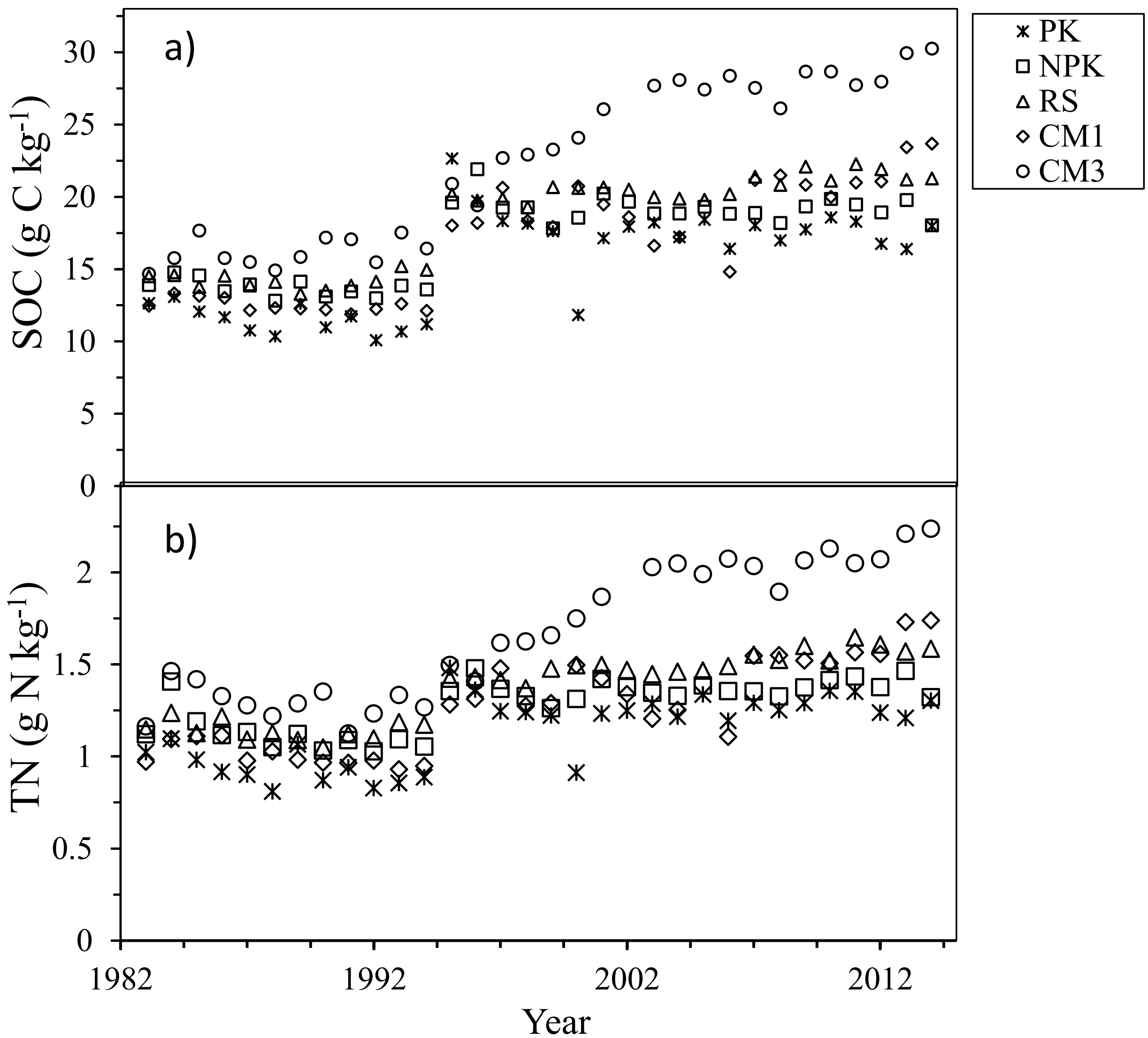


Fig. 3.2 Changes in SOC (a) and TN (b) of bulk soil during 32 years of long-term application of mineral fertilizers and organic matters

Since the soil was added by guest soil from 1995, we observed a gap between 1994 and 1995. In the period of 1983-1994, average of SOC were ranged from 11.5 to 16.1 g C kg^{-1}, meanwhile, increasing trend ranged in (-0.17~0.11 g C yr^{-1}). However, only PK and CM1 show the significant change ($P<0.05$). In 1995-2014 periods, SOC ranged in 17.7 to 26.2 g C kg^{-1}. RS, CM1 and CM3 increased significantly with the trend ranged in 0.10~0.46 g C yr^{-1}), while PK and NPK do not show significant change.

Changes in differences relatively to NPK treatment in the whole period (1983-2014) show in Table 3.7 and Fig. 3.3. The different mean and trend show highest in CM3 and lowest in PK treatments, ranged from -11.0~29.6 % and 0.29~1.55 % yr^{-1}, respectively. Except PK, remain treatments showed significant positive trend with $P<0.001$, compared to NPK treatment.

b) Changes of SOC obtained by RothC model

The results of SOC estimated by RothC model is shown in Table 3.7 and Fig. 3.8. In 1982-1994 period, average of SOC ranged from 12.0~17.1 g C kg^{-1}, with increasing trend ranged in (-0.102~0.132 g C yr^{-1}). Differently from measurement, all treatments show high correlation coefficient ($P<0.001$). PK and NPK show negative trend, while the rest of treatments show positive trend. For1995-2014 period, average of SOC ranged from 18.0~22.5 g C kg^{-1}. Although PK treatment shows the lowest in SOC (18.0 g C kg^{-1}) which similar to measurement, however, the highest SOC was RS treatment, not CM3 treatment. All changes are significant at $P<0.001$.

Table 3.7 Changes of SOC content and difference relatively to NPK treatments in LTE experiment from 1983-2014. The figures in parentheses show standard error of the mean.

Treatment	Mean and temporal changes of SOC (g C kg^{-1}) in 1983-1994 period			Mean and temporal changes of SOC (g C kg^{-1}) in 1995-2014 period			Differences to NPK treatment (%) from 1983-2014 period		
	Mean (g kg^{-1})	Trend (g yr^{-1})	R	Mean (g C kg^{-1})	Trend (g yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
By Measurement									
PK	11.5 (1.0)	-0.17	-0.63*	17.7 (1.9)	-0.08	-0.25	-11.0 (9.0)	0.29	0.30
NPK	13.7 (0.6)	-0.08	-0.50	19.2 (0.9)	-0.04	-0.29	—	—	—
RS	14.2 (0.6)	0.03	0.18	20.7 (0.8)	0.10	0.74***	6.2 (6.8)	0.41	0.56***
CM1	12.5 (0.5)	-0.08	-0.62*	19.6 (2.3)	0.21	0.57*	-2.0 (12.0)	0.83	0.65***
CM3	16.1 (1.0)	0.11	0.40	26.2 (3.1)	0.46	0.90***	29.6 (17.9)	1.55	0.83***
By RothC Model									
PK	12.0 (0.4)	-0.102	-0.995***	18.0 (0.9)	-0.146	-0.989***	-8.1 (4.9)	0.35	0.668***
NPK	13.9 (0.2)	-0.066	-0.995***	18.9 (0.6)	-0.106	-0.989***	—	—	—
RS	17.0 (1.2)	0.322	0.995***	22.5 (1.1)	0.176	0.989***	20.7 (9.8)	0.42	0.399*
CM1	13.3 (0.1)	0.034	0.996***	18.8 (0.1)	-0.013	-0.986***	-2.0 (3.5)	0.35	0.940***
CM3	17.1 (0.5)	0.132	0.995***	22.1 (0.5)	0.079	0.990***	19.4 (6.6)	0.11	0.157

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. R indicates for Coefficient Correlation

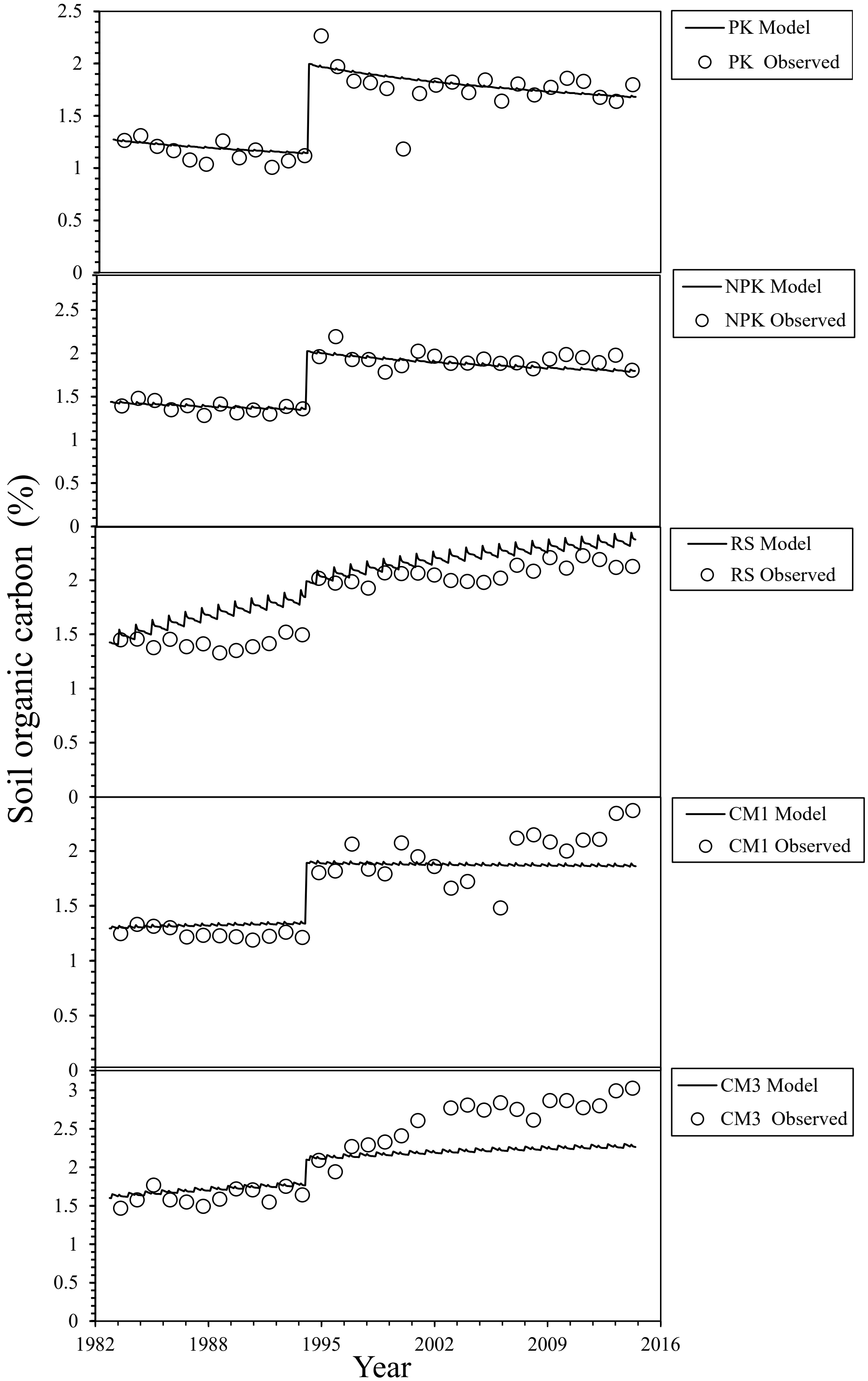


Fig. 3.8 Change of SOC by measurement and by RothC model

Table 3.7 shows the changes in differences relatively to NPK treatment. RS treatment shows the highest (20.7%), while lowest showed in PK (-8.1 %). The increasing trend ranged in 0.11~0.42 g C yr^{-1}, which is lower than real measurement. Excluding CM3, all treatments shows significant increase at $P<0.05$ (RS treatment) and $P<0.001$ (PK and CM1 treatments).

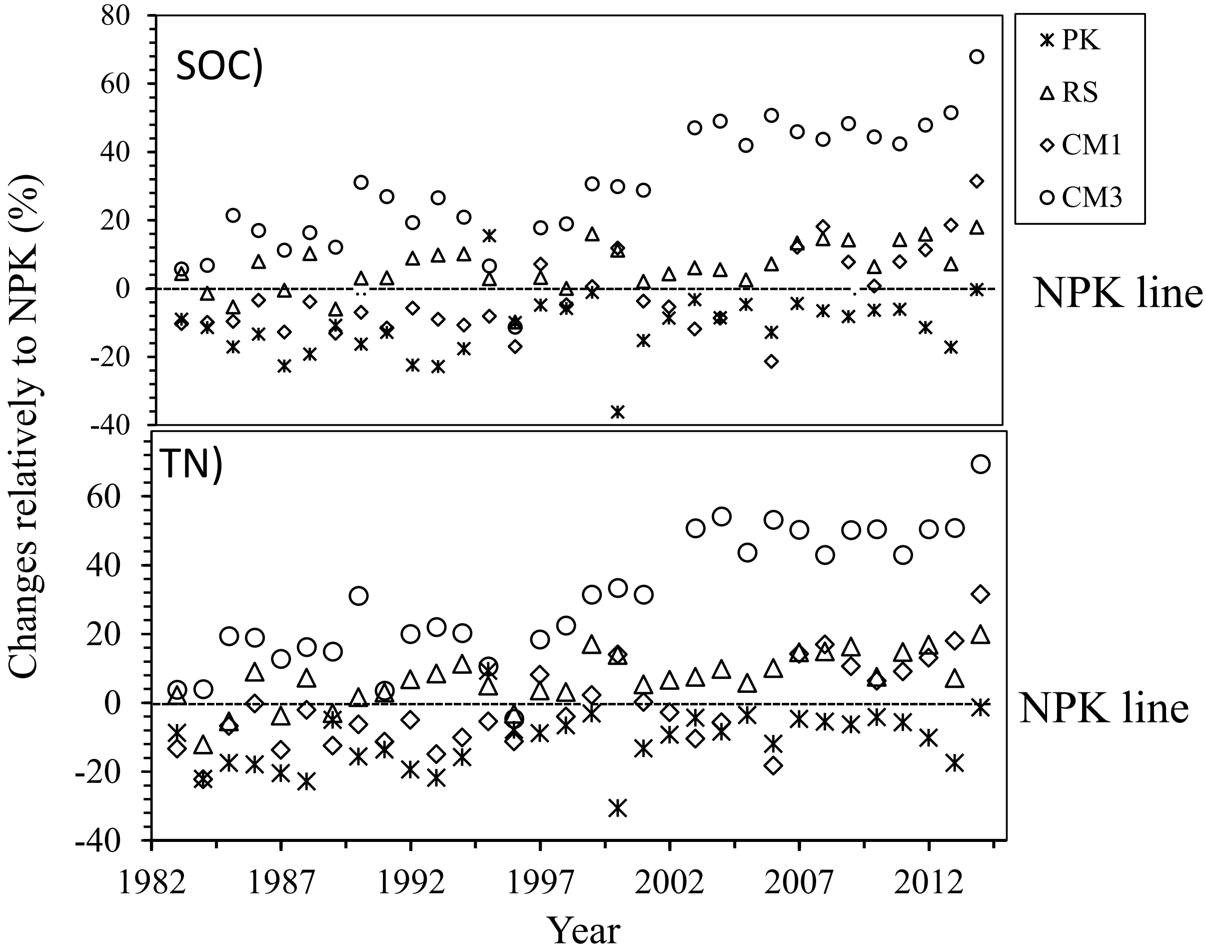


Fig. 3.3 Changes difference SOC (TN) relatively to NPK control of bulk during 32 years of long-term application of mineral fertilizers and organic matters

3.3.6. Changes in TN

TN contents are shown in Table 3.8 and Fig. 3.2. Similarly to pattern in SOC, CM3 treatments show the highest TN in both periods. In the period of 1983-1994, average of TN were ranged from 0.93 to 1.29 g N kg^{-1}, meanwhile, increasing trend are all negative and ranged in (-0.018~0.004 g N yr^{-1}). Significant changes observed in PK, NPK and CM1, but not in RS and CM3 treatments. In the follow period 1995-2014, mean TN ranged from 1.26~1.91 g N kg^{-1},and all trends are positive, ranged from 0.000~0.037 g N yr^{-1}). Application of RS and CM shows significant changes (P<0.01), but not in PK and NPK treatments.

Changes in differences to NPK treatments are shown in Table 3.8 and Fig. 3.3. The different mean and trend show highest in CM3 and lowest in PK treatments, ranged from -11.1~30.2 % and 0.37~1.72 % yr^{-1}, respectively. All treatments show significant positive trend, with PK at P<0.05 and remain treatments at P<0.001.

Table 3.8 Changes of TN content and differences relatively to NPK treatments in LTE experiment from 1983-2014. The figures in parentheses show standard error of the mean.

Treatment	Mean and temporal changes of TN (g N kg^{-1}) in 1983-1994 period			Mean and temporal changes of TN (g N kg^{-1}) in 1995-2014 period			Differences to NPK treatment (%) from 1983-2014 period		
	Mean (g kg^{-1})	Trend (g kg^{-1} yr^{-1})	R	Mean (g kg^{-1})	Trend (g kg^{-1} yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
PK	0.93 (0.09)	-0.016	-0.607*	1.26 (0.11)	0.000	0.016	-11.1 (8.1)	0.37	0.431*
NPK	1.12 (0.10)	-0.018	-0.711**	1.37 (0.05)	0.002	0.186	—	—	—
RS	1.14 (0.06)	-0.004	-0.235	1.50 (0.07)	0.011	0.861***	6.9 (7.3)	0.56	0.712***
CM1	1.01 (0.06)	-0.012	-0.873***	1.43 (0.17)	0.019	0.661**	-1.0 (12.5)	0.95	0.72***
CM3	1.29 (0.10)	-0.007	-0.571	1.91 (0.24)	0.037	0.919***	30.2 (19.0)	1.72	0.860***

The *, **, and *** represent at P<0.05, 0.01, and 0.001 probability level, respectively. R indicated for Coefficient Correlation

3.3.7. Changes in available P

The change in Av-P is presented in Table 3.9 and Fig. 3.5. Interestingly, we did not observe the gap between to period 1983-1994 and 1995-2014 as shown in SOC and TN. All the treatments show the significant increase (P<0.001). The mean of Av-P of all treatments from 1983-2014 ranged from 23.9 to 35.9 (mg P kg^{-1}), shows highest in CM3 and lowest in NPK treatments. The RS showed the highest trend, compared to the rest of treatments (1.02 mg year^{-1}), while lowest trend shows in PK treatment (0.69 mg year^{-1}). The correlation coefficients of the trend in all treatments are very high, which ranged from 0.84 to 0.95.

Table 3.9 Changes of available P and differences relatively to NPK treatments from 1983-2014. The figures in parentheses show standard deviation of the estimates

Treatment	Mean and temporal changes in available P (mg P kg^{-1})			Differences to NPK treatment (%) from 1983-2014 period		
	Mean (mg P kg^{-1})	Trend (mg year^{-1})	R	Mean (%)	Trend (% year^{-1})	R
PK	26.6 (7.6)	0.69	0.86***	13.8 (17.0)	-0.72	-0.4*
NPK	23.9 (7.3)	0.75	0.95***	—	—	—
RS	26.6 (10.4)	1.02	0.92***	9.4 (19.1)	0.96	0.47**
CM1	28.6 (9.4)	0.83	0.84***	22.5 (20.5)	-0.59	-0.270
CM3	35.9 (9.5)	0.85	0.84***	55.6 (26.9)	−1.50	-0.53**

Changes in different Av-P to NPK treatment in percentage show the decreased trend in PK, CM3 (P<0.05), but increased trend in RS treatment. However, no significant trend observed in CM1 treatment. On the averages of 32 years, all treatments shows higher compared to NPK control, but not significant in CM1 treatment. The highest value shows in CM1 (55.6% increase). However, the trend of PK, CM1, and CM3 treatments show the decreased trend, while RS treatment shows increased trends. The correlation coefficients ranged from -0.53 to 0.47, which is much lower than in changes in Av-P mentioned above.

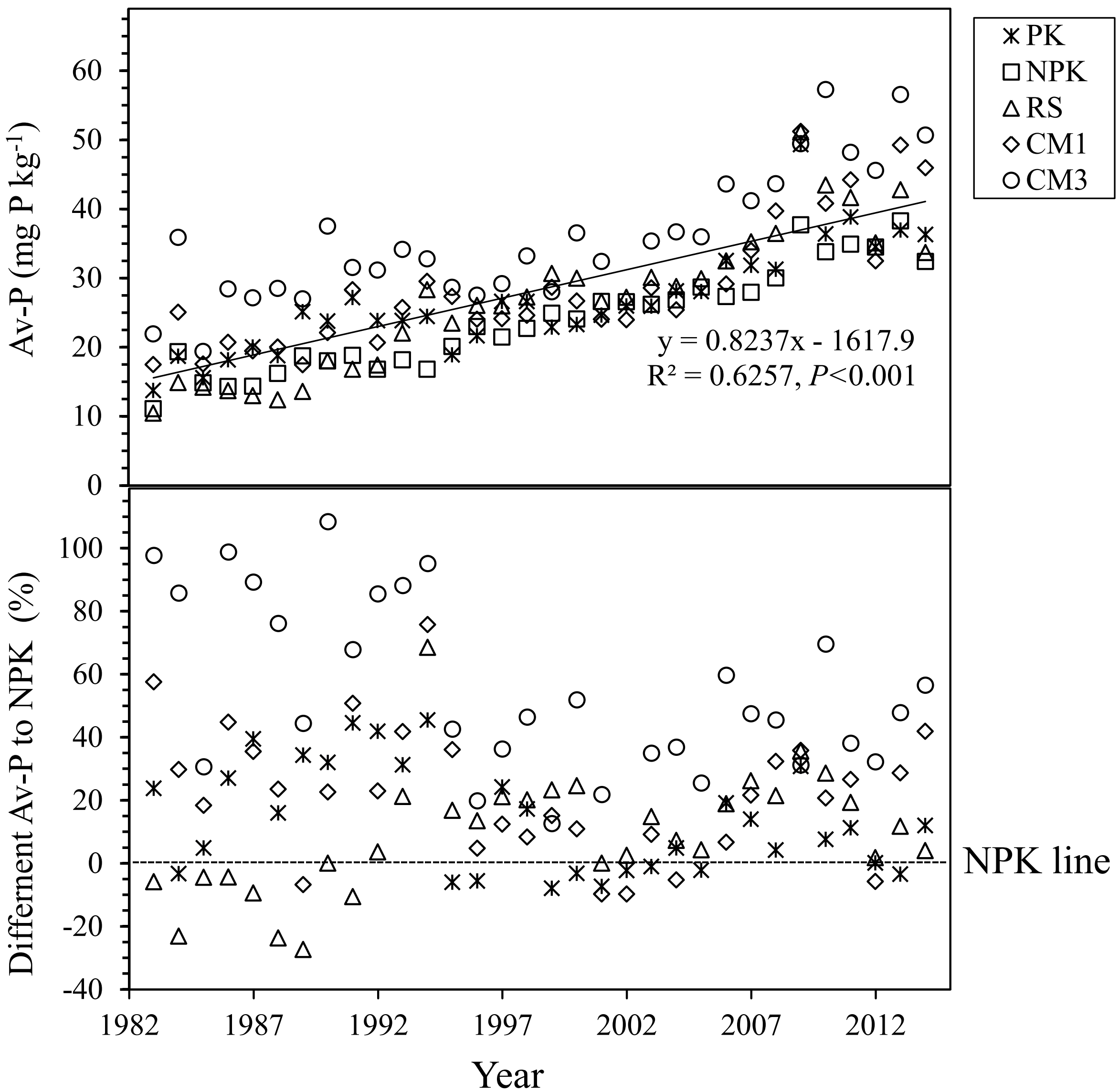


Fig. 3.5. Changes in the amounts of available P from bulk soil after 31 years long-term application of mineral fertilizer and organic matters.

3.3.8. Changes in soil $\delta^{13}C$ and $\delta^{15}N$

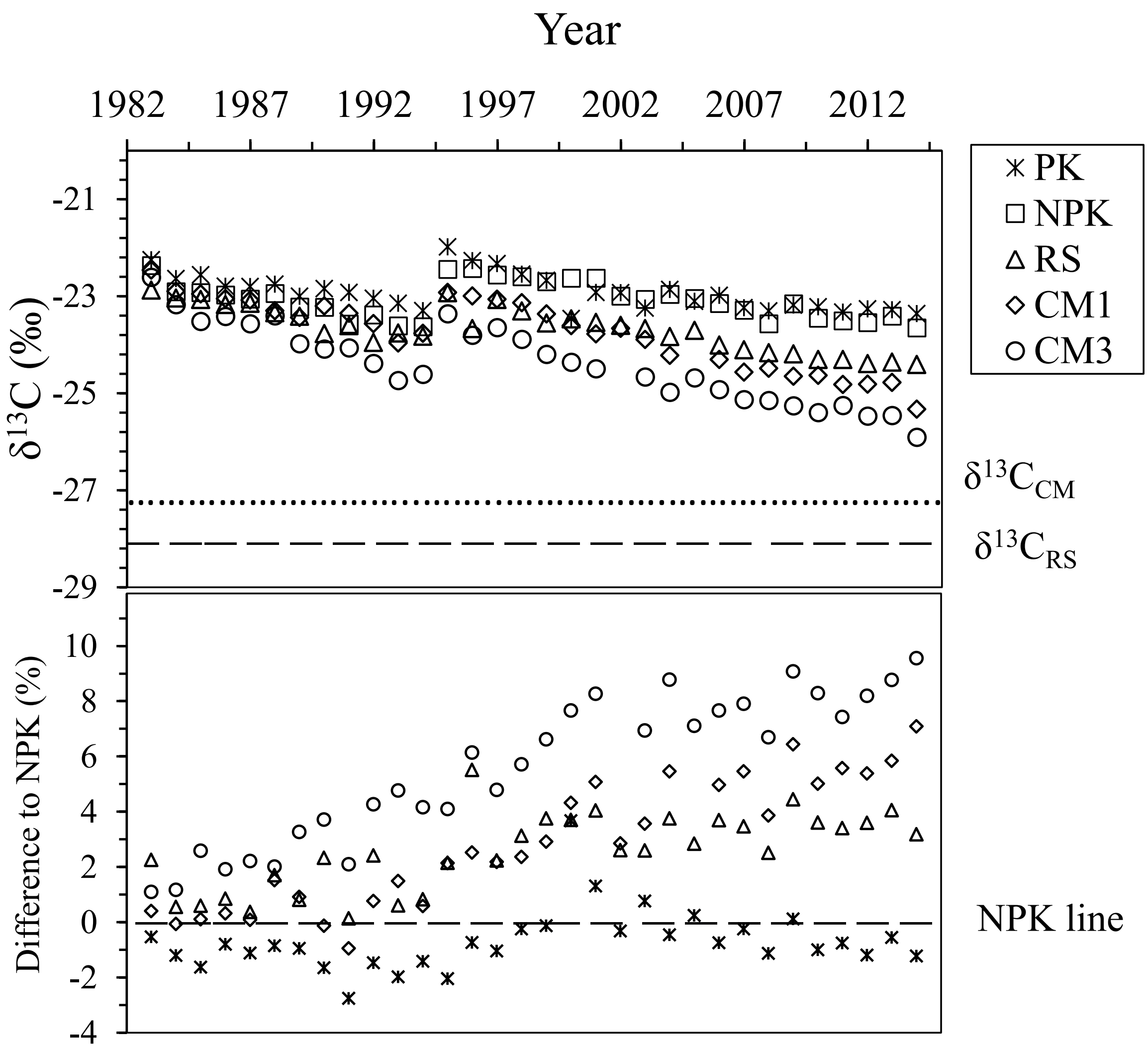
Changes in soil $\delta^{13}C$

The results for $\delta^{13}C$ are shown in Table 3.10 and Fig. 3.6. All values for $\delta^{13}C$ decreased significantly ($P<0.001$) in both periods (before and after adding guest soil). In period 1983-1994, $\delta^{13}C$ decreased with after 12 years ($P<0.001$), and the average $\delta^{13}C$ values ranged from -22.8 to -23.8‰. A change of trend has the similar pattern, which decreased -0.166 to -0.072‰ yr^{-1}. PK treatment shows the highest of mean $\delta^{13}C$ (-22.8‰), while CM3 shows the highest value $\delta^{13}C$ (-23.8‰). Similarly, in the subsequent period 1995-2014, $\delta^{13}C$ values decreased ($P<0.001$) significantly and average values and changed trend of 20 years ranged from -23.0 to -24.7‰, and -0.109 to -0.044‰ yr^{-1}, respectively. The highest and lowest of mean $\delta^{13}C$ in this period is PK and CM3 treatments, which is similar to 1983-1994 period. For the trend of $\delta^{13}C$, PK shows highest trend, but lowest trend is CM1, not CM3 treatment as previous period. Compared to NPK control treatment, we found that except PK, all treatments showed significant decrease $\delta^{13}C$, from 2.5 to 5.6% at $P<0.001$ probability.

Table 3.10 Changes in $\delta^{13}C$ and difference of $\delta^{13}C$ compared relatively to NPK (1983-2014). The figures in parentheses show standard deviation of the estimates.

Treatment	Mean and temporal changes of $\delta^{13}C$ (‰) in 1983-1994 period			Mean and temporal changes of $\delta^{13}C$ (‰) in 1995-2014 period			Differences to NPK treatment (%) from 1983-2014 period		
	Mean (‰)	Trend (‰ yr^{-1})	R	Mean (‰)	Trend (‰ yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
PK	-22.8 (0.3)	-0.072	-0.93***	–23.0 (0.4)	-0.044	–0.80***	-0.7 (1.1)	0.03	0.270
NPK	-23.2 (0.4)	-0.095	-0.93***	–23.0 (0.4)	-0.049	-0.96***	—	—	—
RS	-23.4 (0.4)	-0.095	-0.95***	-23.8 (0.4)	-0.062	-0.95***	2.5 (1.4)	0.10	0.71***
CM1	-23.3 (0.4)	-0.106	-0.94***	-24.1 (0.7)	-0.106	-0.98***	2.8 (2.3)	0.23	0.93***
CM3	-23.8 (0.6)	-0.166	-0.96***	-24.7 (0.7)	-0.099	-0.97***	5.6 (2.6)	0.26	0.94***

The *, **, and *** represent at P<0.05, 0.01, and 0.001 probability level, respectively. R indicates for Coefficient Correlation


Year
1982
1987
1992
1997
2002
2007
2012
-21
-23
-25
-27
-29
δ13C (‰)
δ13CCM
δ13CRS
PK
NPK
RS
CM1
CM3
10
8
6
4
2
0
-2
-4
Difference to NPK (%)
NPK line

Changes in soil δ^15^N

As shown in **Fig. 3.7**, changes of isotope $\delta^{15}N$ did not follow linear correlation, analysis was therefore not implemented.

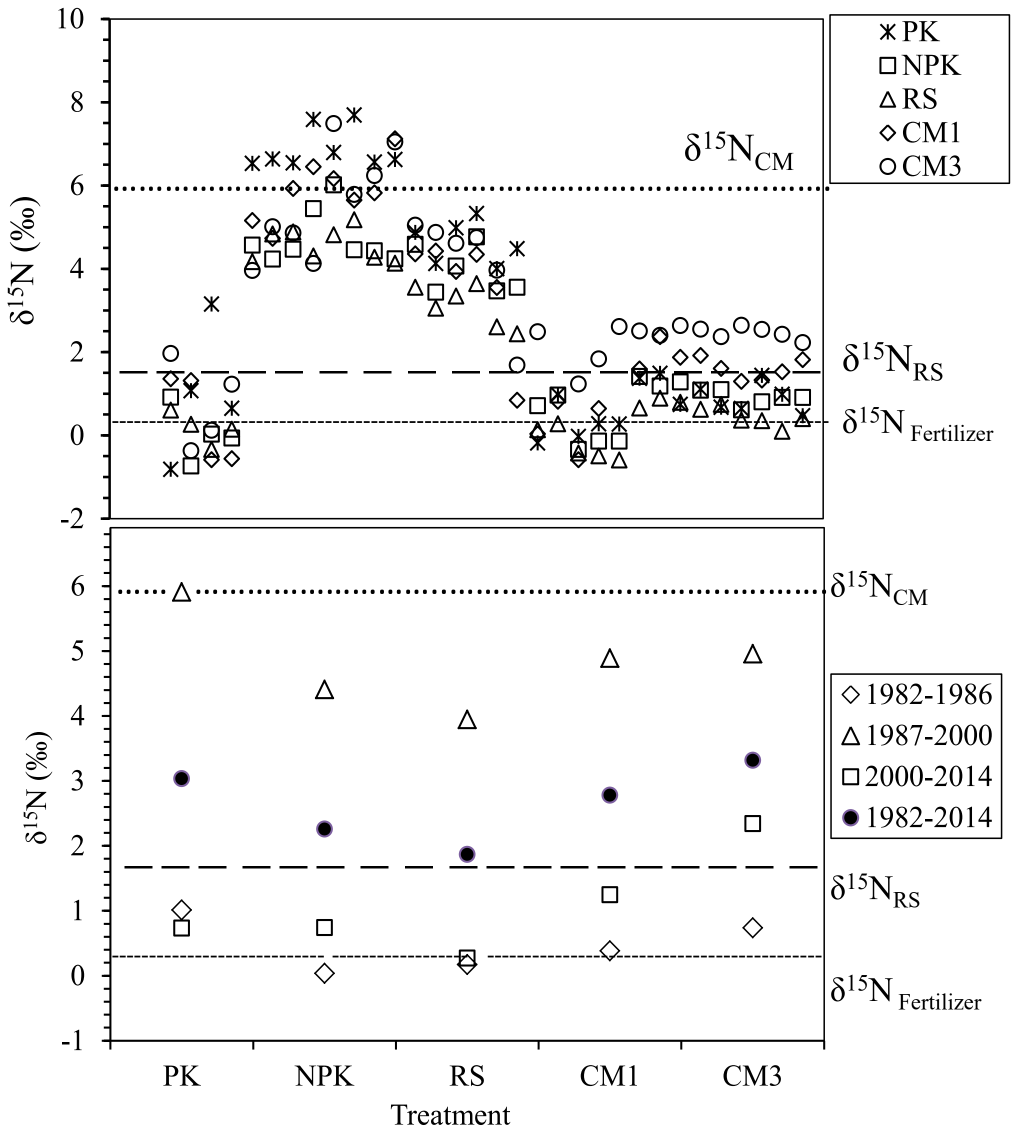


Fig. 3.7 Changes in difference $\delta^{15}N$ values relatively to three shift changes of builk soil during 32 years of long-term application of mineral fertilizers and organic matters. Three dash lines represent for Compost, Rice straw and NPK fertilizer applied in treatments.

The data distributes into three main areas, based on 1982-1986, 1987-2000 and 2001-2014 periods. The mean of $\delta^{15}N$ in three periods ranged in 0.04~1.01, 3.94~5.91 and 0.27~2.34, respectively. Overall of 1983-2014 period, $\delta^{15}N$ ranged from 1.87~3.32. In all periods, RS shows lowest value, while CM3 treatment indicates the highest values on the averages.

3.3.9. Changes in Dec-C and Min-N

Carbon decomposition (De-C) was calculated from aerobic and anaerobic incubation. It was calculated as the total carbon emission from (CO_2, CH_4) from aerobic incubation and anaerobic incubation (Table 3.11 and Fig. 3.9). The average of De-C of all treatments from aerobic and anaerobic incubation ranged from 285~435 and 588~1044 mg C kg^{-1}, respectively. The Net Dec-C between anaerobic and aerobic incubation shows Dec-C ranged from 303~666 g C kg^{-1}, and shows significant decrease at $P<0.001$. The lowest Dec-C in both two kind of incubation and two periods were PK, while the highest showed in CM3 treatment. The anaerobic incubation confirmed significant changes in Dec-C ($P<0.05$).

Table 3.11 Mean and temporal and changed trend of decomposition carbon. The figures in parentheses show standard error of the mean. Since data from 1983-1994 was out un-normal from anaerobic incubation, we do not obtain trend and R analysis

Treatment	Mean and temporal changes in Dec-C (mg C kg^{-1}) in 1983-1994 period			Mean and temporal changes in Dec-C (mg C kg^{-1}) in 1995-2014 period			Differences to NPK treatment (%) in 1983-2014 period		
	Mean (mg kg^{-1})	Trend (mg yr^{-1})	R	Mean (mg kg^{-1})	Trend (mg yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
Aerobic incubation									
PK	285 (45)	-9.9	-0.80**	286 (49)	-5.8	-0.70***	-11.7 (10.4)	0.02	0.02
NPK	332 (40)	-6.0	-0.54	319 (42)	-5.7	-0.81***	—	—	—
RS	367 (27)	-2.6	-0.34	415 (48)	-2.8	-0.35	23.6 (15.4)	1.25	0.76***
CM1	276 (28)	-4.3	-0.56	348 (30)	1.0	0.21	0.4 (19.1)	1.78	0.88***
CM3	378 (32)	4.8	0.53	435 (44)	2.2	0.31	29.5 (23.4)	1.94	0.79***
Anaerobic incubation									
PK	836 (263)	–	–	588 (92)	-11.6	-0.74***	-8.5 (13)	-0.55	-0.40*
NPK	861 (206)	–	–	662 (75)	-10.8	-0.86***	—	—	—
RS	804 (274)	–	–	801 (69)	-8.2	-0.70***	11.7 (22.3)	1.55	0.65***
CM1	782 (294)	–	–	680 (65)	-6.2	-0.58**	-1.6 (17.2)	0.70	0.39*
CM3	1044 (233)	–	–	829 (76)	-6.4	-0.51*	24.6 (13.7)	0.40	0.28
Net Dec-C (Difference between anaerobic and aerobic incubation)									
PK	551 (231)	–	–	303 (48)	-5.8	-0.72***	-6.0 (20.6)	-1.01	-0.46**
NPK	529 (199)	–	–	343 (38)	-5.2	-0.80***	–	–	–
RS	437 (258)	–	–	386 (39)	-5.4	-0.83***	2.4 (30.3)	1.56	0.48**
CM1	506 (277)	–	–	332 (58)	-7.3	-0.77***	-3.7 (25.9)	-0.20	-0.07
CM3	666 (237)	–	–	394 (68)	-8.6	-0.77***	19.9 (22.2)	-0.93	-0.40*

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. R indicates for Coefficient Correlation

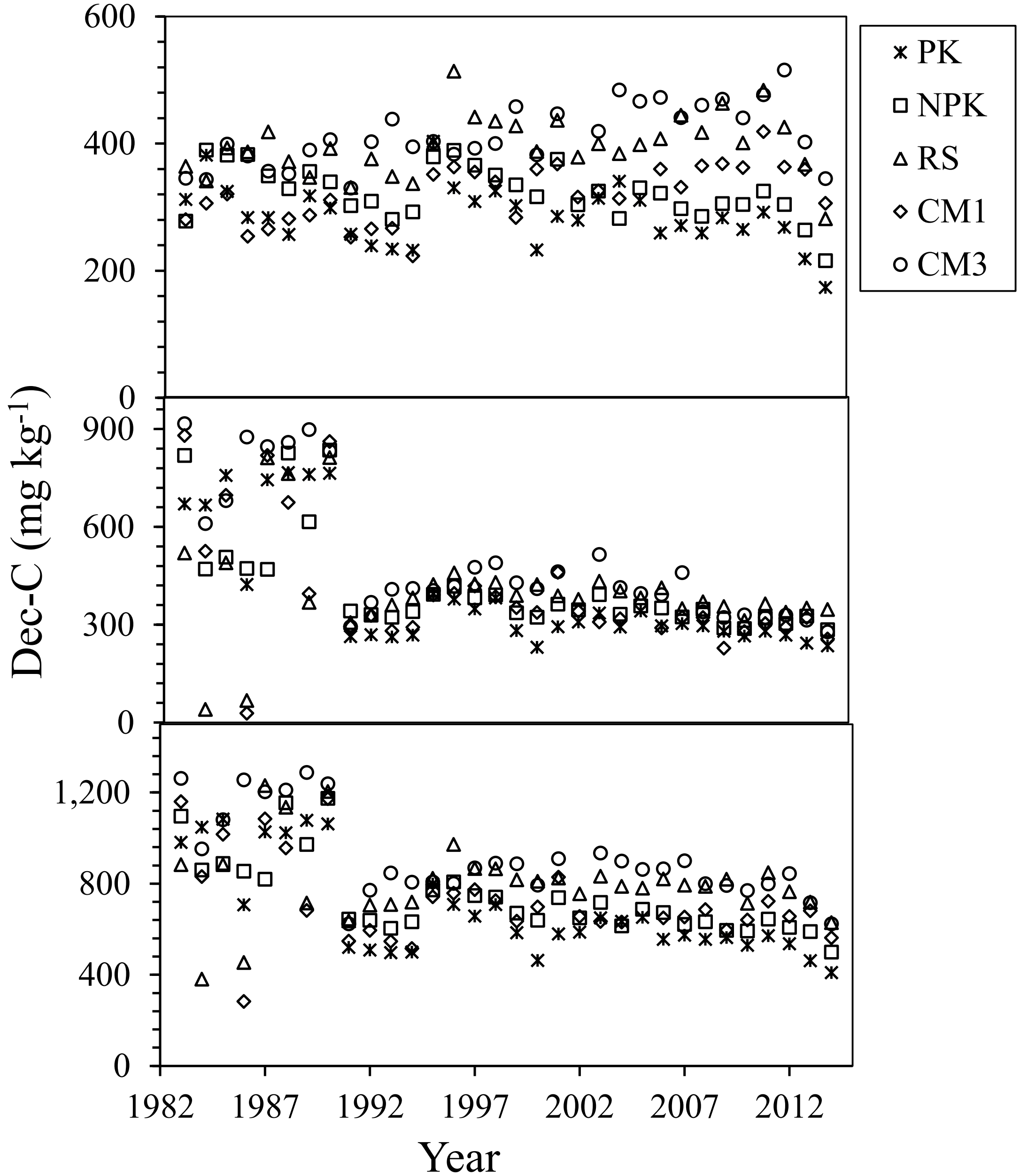


Fig. 3.9 Carbon decomposition after 2 weeks aerobic incubation (top), 4 weeks anaerobic incubation, and total decomposition (bottom)

For both 2 periods, the average of Min-N ranged from 40~86, 97~156 and 36~88 mg N kg^{-1}, for aerobic, anaerobic incubation and net Min-N, respectively (Table 12 and Fig. 3.10). In both aerobic and anaerobic incubation, PK shows the lowest while CM3 shows the lowest of mean Min-N.

Table 3.12 Mean and temporal changed trend of mineralization nitrogen. The figures in parentheses show standard error of the mean.

Treatment	Mean and temporal changes in Min-N (mg N kg^{-1}) in 1983-1994 period			Mean and temporal changes in Min-N (mg N kg^{-1}) in 1995-2014 period			Differences to NPK treatment (%) in 1983-2014 period		
	Mean (mg kg^{-1})	Trend (mg yr^{-1})	R	Mean (mg kg^{-1})	Trend (mg yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
Aerobic incubation Min-N									
PK	66 (28)	-6.3	-0.81**	40 (11)	-1.1	-0.6**	7.5 (31.8)	-1.59	-0.46**
NPK	52 (16)	-3.6	-0.79**	40 (8)	-0.9	-0.63**	—	—	—
RS	58 (19)	-2.9	-0.540	51 (11)	-1.1	-0.54*	23.0 (21.7)	0.72	0.30
CM1	71 (28)	-5.6	-0.69*	53 (10)	-0.4	-0.27	35.4 (17.5)	0.56	0.29
CM3	86 (24)	-3.3	-0.470	68 (12)	1.0	0.5*	74.7 (55.3)	2.63	0.44*
Anaerobic incubation Min-N									
PK	97 (20)	-0.8	-0.14	98 (23)	-2.5	-0.63**	-8.6 (16.3)	-0.34	-0.19
NPK	104 (17)	0.9	0.18	110 (23)	-2.7	-0.69***	–	–	–
RS	126 (17)	1.9	0.38	131 (22)	-1.9	-0.51*	22.2 (18)	0.27	0.14
CM1	115 (12)	-1.4	-0.38	123 (19)	-1.0	-0.31	14.6 (21.3)	0.52	0.22
CM3	138 (24)	0.5	0.08	156 (23)	-1.1	-0.28	41.6 (31.1)	0.89	0.27
Net Min-N (Difference between anaerobic and aerobic incubation Min-N)									
PK	36 (20)	4.5	0.76**	59 (16)	-1.4	-0.51*	-21.9 (23.8)	0.98	0.39*
NPK	51 (25)	4.4	0.63*	70 (18)	-1.8	-0.6**	–	–	–
RS	68 (21)	4.8	0.80**	80 (16)	-0.9	-0.310	24.5 (40.4)	-0.15	-0.03
CM1	42 (23)	4.5	0.70*	70 (15)	-0.5	-0.220	-2.2 (38.4)	1.30	0.32
CM3	52 (21)	3.8	0.62*	88 (27)	–2.0	-0.450	19.9 (59.0)	0.72	0.12

The *, **, and *** represent at *P*<0.05, 0.01, and 0.001 probability level, respectively. R indicated for Coefficient Correlation

3.3.10. Relationships among SOC, TN, De-C and Min-N

The relationship among stock of SOC, TN, Dec-C and Min-N is shown in Table 3.13. There is strong negative correlation between SOC and TN in both periods (R ranged in -0.79 to -0.36‰). Dec-C and Min-N show positive correlation with SOM. The correlation of Dec-C and Min-N with SOM in aerobic incubation confirms stronger relationship compared with anaerobic incubation, ranged in 0.07 to 0.32 and 0.66 to 0.77, respectively.

The mean and temporal change of Dec-C/SOC and Min-N/TN ratio shows in Table 3.14. Mean Dec-C/SOC in 1983-1994 and 1995-2014 period ranged from 0.653 to 0.725 and 0.322-0.389 g kg^{-1}, respectively. No analysis was done in 1982-1994, but for following period high negative trend was observed in Dec-C/SOC at $P<0.001$. Similar result observed for Min-N/TN ratio trend, which mean Min-N/TN ratio ranged in 0.078 to 0.088 g kg^{-1}, and R ranged in -0.80 to -0.68 ($P<0.001$).

Changes in C/N ratio in bulk soil and from incubation showed in Table 3.15, Fig. 3.4 and Fig.3.12. The mean of C/N of bulk soil ranged from 12.29 to 12.57 and from 3.75 to 14.04 in 1983-2014 and 1995-2014 periods, respectively. The increased trend happened in first period, but decreased after adding guest soil. For the aerobic incubation, Dec-C/Min-N is much lower, ranged from 4.33 to 8.43 in both periods. CM1 and CM3 show the lower ratios of Dec-C/Min-N compared to other treatments. For aerobic incubation, this ratio ranged in 6.85 to 8.86 and 5.40 to 6.22 for 1983-1994 and 1995-2014 period, respectively.

Table 3.13 Correlation among SOC, TN, $\delta^{13}C$, $\delta^{15}N$, Dec-C and Min-N from 1982-2014

	SOC	TN	$\delta^{15}N$	$\delta^{13}C$	Min-N (Aerobic)	Min-N (Anaerobic)	Net Min-N	Dec-C (Aerobic)	Dec-C (Anaerobic)
1982-1994 period									
SOC	1.00								
TN	0.92***	1.00							
$\delta^{15}N$	-0.28*	-0.47***	1.00						
$\delta^{13}C$	-0.55***	-0.36**	-0.46***	1.00					
Min-N (Aerobic)	0.31*	0.58***	-0.60***	0.20	1.00				
Min-N (Anaerobic)	0.54***	0.71***	-0.37**	-0.27*	0.71***	1.00			
Net Min-N	0.19	0.01	0.43***	-0.58***	-0.59***	0.15	1.00		
Dec-C (Aerobic)	0.77***	0.76***	-0.33*	-0.32*	0.29*	0.40**	0.05	1.00	
Dec-C (Anaerobic)	0.07	0.10	-0.06	0.28*	0.11	-0.09	-0.26*	0.26*	1.00
1995-2014 period									
SOC	1.00								
TN	0.99***	1.00							
$\delta^{15}N$	0.11	0.04	1.00						
$\delta^{13}C$	-0.72***	-0.79***	0.20*	1.00					
Min-N (Aerobic)	0.64***	0.63***	0.28**	-0.47***	1.00				
Min-N (Anaerobic)	0.58***	0.55***	0.35***	-0.31**	0.72***	1.00			
Net Min-N	0.35***	0.33***	0.29**	-0.11	0.31**	0.88***	1.00		
Dec-C (Aerobic)	0.68***	0.66***	0.20*	-0.47***	0.73***	0.79***	0.59***	1.00	
Dec-C (Anaerobic)	0.32**	0.26**	0.32**	0.07	0.40***	0.65***	0.62***	0.59***	1.00

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively.

Table 3.14 Mean and temporal and changed trend of Dec-C/SOC and Min-N /TN ratio after anaerobic incubation. The figures in parentheses show standard error of the mean. Since data from 1983-1994 was out un-normal from anaerobic incubation, we do not obtain trend and R analysis for Dec-C.

Treatment	Mean and temporal changes in 1983-1994 period			Mean and temporal changes in 1995-2014 period			Differences to NPK treatment (%) in 1983-2014 period		
	Mean ($g\ kg^{-1}$)	Trend ($g\ yr^{-1}$)	R	Mean ($g\ kg^{-1}$)	Trend ($g\ yr^{-1}$)	R	Mean (%)	Trend ($\%\ yr^{-1}$)	R
Dec-C/SOC ratio ($g\ kg^{-1}$)									
PK	0.725 (0.218)	—	—	0.333 (0.038)	-0.0050	-0.77***	3.6 (17.3)	-0.98	-0.53**
NPK	0.630 (0.161)	—	—	0.344 (0.033)	-0.0049	-0.86***	—	—	—
RS	0.571 (0.206)	—	—	0.389 (0.043)	-0.0060	-0.82***	5.1 (20.4)	1.06	0.49**
CM1	0.628 (0.237)	—	—	0.351 (0.053)	-0.0066	-0.75***	1.2 (18.7)	-0.10	-0.05
CM3	0.653 (0.165)	—	—	0.322 (0.054)	-0.0083	-0.94***	-2.6 (13.4)	-0.83	-0.59***
Min-N/TN ratio ($g\ kg^{-1}$)									
PK	0.106 (0.019)	0.0005	0.090	0.078 (0.017)	-0.0020	-0.68**	2.9 (18.7)	-0.79	-0.38*
NPK	0.095 (0.015)	0.0016	0.370	0.080 (0.017)	-0.0020	-0.70***	—	—	—
RS	0.112 (0.015)	0.0017	0.380	0.088 (0.016)	-0.0019	-0.71***	13.9 (17.6)	-0.31	-0.16
CM1	0.116 (0.010)	-0.0001	-0.040	0.087 (0.015)	-0.0017	-0.70***	15.4 (19.5)	-0.56	-0.26
CM3	0.108 (0.014)	0.0006	0.150	0.083 (0.016)	-0.0022	-0.80***	8.4 (21.5)	-0.68	-0.29

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. R indicates for Coefficient Correlation

Table 3.15 Mean and temporal changes of C to N ratio of bulk soil and incubation. The figures in parentheses show standard error of the mean.

Treatment	Mean and temporal changes in C/N ratio in 1983-1994 period			Mean and temporal changes in C/N ratio in 1995-2014 period			Differences to NPK treatment (%) in 1983-2014 period		
	Mean	Trend (yr^{-1})	R	Mean	Trend (yr^{-1})	R	Mean (%)	Trend (% yr^{-1})	R
Bulk soil (SOC/TN)									
PK	12.35 (0.32)	0.023	0.25	14.00 (0.53)	-0.062	-0.70***	-0.4 (3.0)	-0.04	-0.11
NPK	12.29 (0.61)	0.108	0.63*	14.04 (0.33)	-0.048	-0.86***	—	—	—
RS	12.48 (0.37)	0.061	0.59*	13.76 (0.21)	-0.028	-0.77***	-1.0 (2.1)	-0.09	-0.37*
CM1	12.43 (0.50)	0.073	0.52	13.74 (0.26)	-0.031	-0.73***	-1.4 (2.5)	-0.06	-0.21
CM3	12.57 (1.02)	0.154	0.55	13.75 (0.20)	-0.027	-0.79***	-0.4 (4.8)	-0.13	-0.25
Aerobic incubation (Dec-C/Min-N)									
PK	5.20 (1.67)	0.349	0.63*	7.59 (1.65)	0.103	0.37	-14.6 (26.5)	1.45	0.50**
NPK	6.71 (1.57)	0.382	0.84**	8.14 (1.13)	0.039	0.21	—	—	—
RS	6.89 (1.90)	0.287	0.53	8.43 (1.50)	0.139	0.55*	4.3 (19.9)	0.20	0.09
CM1	4.33 (1.31)	0.265	0.70*	6.71 (1.10)	0.083	0.46*	-24.7 (12.8)	0.95	0.68***
CM3	4.72 (1.22)	0.221	0.63*	6.6 (1.29)	-0.065	-0.30	-21.0 (19.5)	0.21	0.10
Anaerobic incubation (Dec-C/Min-N)									
PK	8.86 (4.60)	—	—	6.22 (1.39)	0.052	0.22	-1.9 (27.1)	0.57	0.19
NPK	8.75 (3.63)	—	—	6.17 (0.83)	0.049	0.35	—	—	—
RS	6.98 (2.89)	—	—	6.21 (0.78)	0.036	0.27	-5.0 (19.6)	0.83	0.39*
CM1	6.85 (2.77)	—	—	5.61 (0.67)	-0.012	-0.11	-11.7 (19.1)	0.20	0.10
CM3	7.84 (2.37)	—	—	5.40 (0.66)	0.004	0.03	-8.3 (18.1)	-0.50	-0.26

The *, **, and *** represent at $P<0.05$, 0.01, and 0.001 probability level, respectively. R indicates for Coefficient Correlation

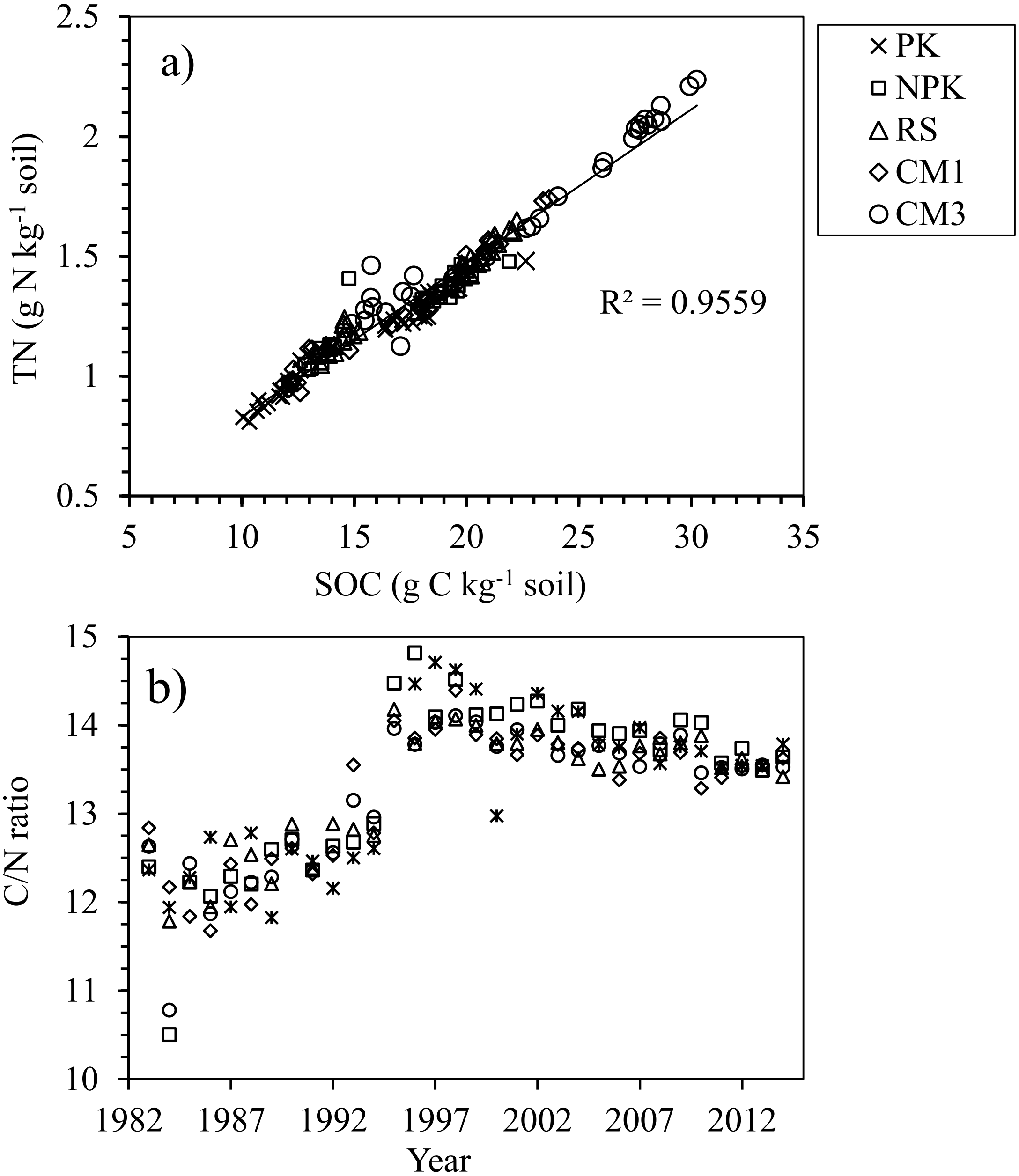


Fig. 3.4 The C and N correlation (a) and their ratios (b) of bulk soil after 32 years of long-term application of mineral fertilizers and organic matters

3.4. Discussion

3.4.1. Changes in rice yield and climatic in LTE

Rice yield of 5 treatments ranged from 3.1 to 6.5 ton ha^{-1}, however, except PK, the other treatment has similar yield to Murayama area in the same period (5.92 ton ha^{-1}, as shown in Chapter 2). It is notice that LTE located in Murayama area, therefore, the rice yield is as our hypothesis. Data from FAO (2017) also paddy rice yield of Japan from 1982-2016 is around 6.31 ton ha^{-1}, which is among the LTE yield range. Therefore, our LTE field could be representative for Japan. Although field was reconstructed in 1994 by adding guest soil, there was no shift of rice yield after, confirmed that rice yield mostly maintained by mineral fertilizer and organic matter application. Compared to NPK control treatment, the application of RS increased about 4.1%, which is not considerable as clear difference. The treatment CM1 increased 5.7%, while CM3 treatment increased 11.6% higher than NPK treatment. The effect of rice straw compost was reported to enhance paddy rice yield and soil quality (Mahmoud et al., 2009; Watanabe et al., 2009). Rice straw amendment also contributed to increase nitrogen availability in rice paddy (Iqbal, 2016). The previous study on paddy rice with application of 6 Mg, which is lower than in CM1 treatment showed that rice straw compost could replace a part of fertilizer (Watanabe et al., 2009). However, application of compost in CM3 treatment was three times higher than normal recommended application.

The LTE location is getting hot in summer season, mainly in June-October. This is also the time for rice growth in this site. This result confirmed with our results in Chapter 2, which clarify the climate changes in 4 regions in Yamagata, including Murayama. The effect of Mean air T on rice yield in June-July, closely to transplanting-heading stage, similar to our result reported in Chapter 2. Interestingly, Mean soil T shows the same effect as Mean air T, which positively increase rice yield with rising temperature in whole crop season. However, it is interesting that PK treatment did not response to temperature change. It is explained that rice yield in this treatment is very low, so the effect from temperature was depleted by the effect of nutrient deficiency. The positive effects of temperatures on rice yield were as our expectation. As we discussed in Chapter 2, Yamagata was cold region, which temperature in crop season is under optimum temperature for rice plant photosynthesis (around 30℃). The rising of Min air T as reported to reduce rice yield in tropical and subtropical Asia, while rising Max air T promoted it (Welch et al., 2010). However, we don't see such a trend in LTE site. It is noticed that all regions in this study was in hot temperate climate, which temperatures are much higher than in our research. Therefore, warming phenomenon effect on both positive and negative way, depend on the location. The effect of other climatic parameters in LTE seemed to be depleted (Table 3.6). The less pronounce effects of them could be explained by that LTE has been well managed by sufficient irrigation system, as a result, reduce effect from rainfall as well as humidity.

3.4.2. Changes in SOC, TN, Av-P

In our experiment, we applied long-term inorganic and organic matter by applying mineral fertilizers and rice straw/rice straw compost. The previous researches were reported that applying inorganic and organic matters could contribute to regulate the storage of C and N in the soil (Körschens, 2006 ; Senbayram et al., 2008; Thord Karlsson et al., 2003). Our results showed that application of mineral fertilizer-only could not maintain the SOC and TN content. However, combination with RS and CM led to increase significantly SOC and TN (Table 3.7, Table 3.8). It was also clear that driven trend for C and N were similar to each other. NPK fertilizer has been widely used in agricultural production, so setting NPK treatment as control was reasonable to compare with other treatments. Our previous study conducted in the same soil also reported that after 30 years, SOC content increased relatively with application of rice straw and compost (Cheng et al., 2016b). Other research also showed that 3 years applying rice husk charcoal to an Andosol paddy field could increase the stock of soil C (Koyama and Hayashi, 2017). Therefore, our result was as expected. We observed that compost application had higher changing trend of SOC and TN than RS application. RS compost was also reported to decrease soil salinity, together with enhancing SOM content (Mahmoud et al., 2009). The increasing slopes in our research were in the order as CM3>CM1>RS>NPK>PK. PK and NPK treatments showed the negative slopes (-0.081 and -0.043), while the other treatments showed significantly positive slopes. This result once again, confirmed that only inorganic fertilizer is not enough to maintain the soil C stock. The average of SOC in CM1 was lower that RS in 1995-2014 period (19.6 vs 20.7 mg C kg^{-1}), however, increasing trend of CM1 was higher (0.215 and 0.105). Though there was a gap when guest soil added in 1995, however, we used calculated the difference of SOC and TN relatively to NPK treatment could delete the gap. The same pattern also showed the increase trend of RS, CM1 and CM3 to NPK control treatment. It was also surprised us the trend of Av-P did not showed big difference after adding new soil in 1995, which happened in SOC and TN. This may be interpreted that guest soil had the similar Av-P. Mahamoud et al (2009) also reported that RS compost enhanced Av-P of soil. However, other studies showed that rice straw application reduced Av-P paddy soil (Iqbal, 2016; van Asten et al., 2005). Av-P with 25% RS added treatment was reported to be optimum ratio (Iqbal, 2016).

RothC model is the common method to predict changes in SOC stock in long period. The model principle was based on the input and output of C pools. In our study, we applied the modified RothC model (Shirato et al., 2004) since one crop season and one winter snow cover season are the typical agriculture style in current Japan. The results from model and from the observed measurements were well fitted before adding new soil. It was unpredicted that after addition of guest soil, the CM1 and CM3 treatment didn't fit well the model, while the other treatments confirmed good fitted results. One of the possibilities is that the real decomposition rate of RS compost may be faster than described by model. Previous researches applied RothC showed that RothC model could be a good tool to predict soil C changes in both low land and upland in Japan (Shirato and Taniyama, 2003; Shirato et al., 2011). However, the large different in real observation also reported when applied RothC (Li et al., 2016; Peltre et al., 2012), with the uncounted factors not considered (pH and EC), giving difficulty in well-fitting the model. Our experiment showed the decrease in pH, while increased EC in compost application treatment, stronger than others (Cheng et al., 2016b). Altogether, this makes the application of RothC model need further modified.

3.4.3. Changes in stable isotopes of $\delta^{13}C$ and $\delta^{15}N$

Since we applied RS and its compost, and their $\delta^{13}C$ and $\delta^{15}N$ were lower than of the soil. The soil samples in 1983, which was closed to original soils (LTE started in 1982) had the $\delta^{13}C$ values ranging from -23.9 to -22.3‰. It is well known that $\delta^{13}C$ in C_3 and C_4 plants had are approximately -26 and -13‰, respectively. This indicated that the original soil was mixed up between C_3 and C_4 plant; with the dominance contribute from C_3 plant (estimated about 70%). Since the $\delta^{13}C$ of RS and CM were -28.3 and -27.4‰, lower than the original soil. As a result, we expected the $\delta^{13}C$ in RS, CM1, and CM3 treatments would decrease strongly. The decreasing values of $\delta^{13}C$ in PK and NPK treatments, mostly affected by the plant residues such as the stubbles and roots after harvest. The decrease of $\delta^{13}C$ due to manure application in long-term was also found by Senbayram et al (2008), which reported that the $\delta^{13}C$ values decreased from -24.3 to -27.3‰ after 34 years of RS application. Other researches also mentioned the organic matter changed the $\delta^{13}C$ of soil after long period of fertilizer application (Bol et al., 2005; Yoneyama et al., 2001; Zhao et al., 2002).

The changes in $\delta^{15}N$ value in our samples were unpredictable. However, the $\delta^{15}N$ in RS treatment is generally lower than the other treatments, while CM3 tends to have the highest $\delta^{15}N$ value. The average of $\delta^{15}N$ of all treatments ranged from 1.9 to 3.3‰, which was in the range of $\delta^{15}N$ of RS and CM. The previous study also showed the $\delta^{15}N$ in soil samples from unfertilized, animal manure and mineral fertilizer are from 4.4 to 5.8‰, which also in values of RS and CM range (Bol et al., 2005). Other work also reported that rice straw compost application in paddy soil in Tokohu region (Japan) kept the $\delta^{15}N$ constant, while the treatment without CM decreased the $\delta^{15}N$ (Nishida et al., 2007; Nishida and Sato, 2015). The $\delta^{15}N$ of CM in our study was 5.85‰, which is higher than in RS (1.69‰) (Table 3.2). Furthermore, the NPK fertilizer also contributes to affect value of soil isotope. The $\delta^{15}N$ of NPK fertilizer was 0.28‰.

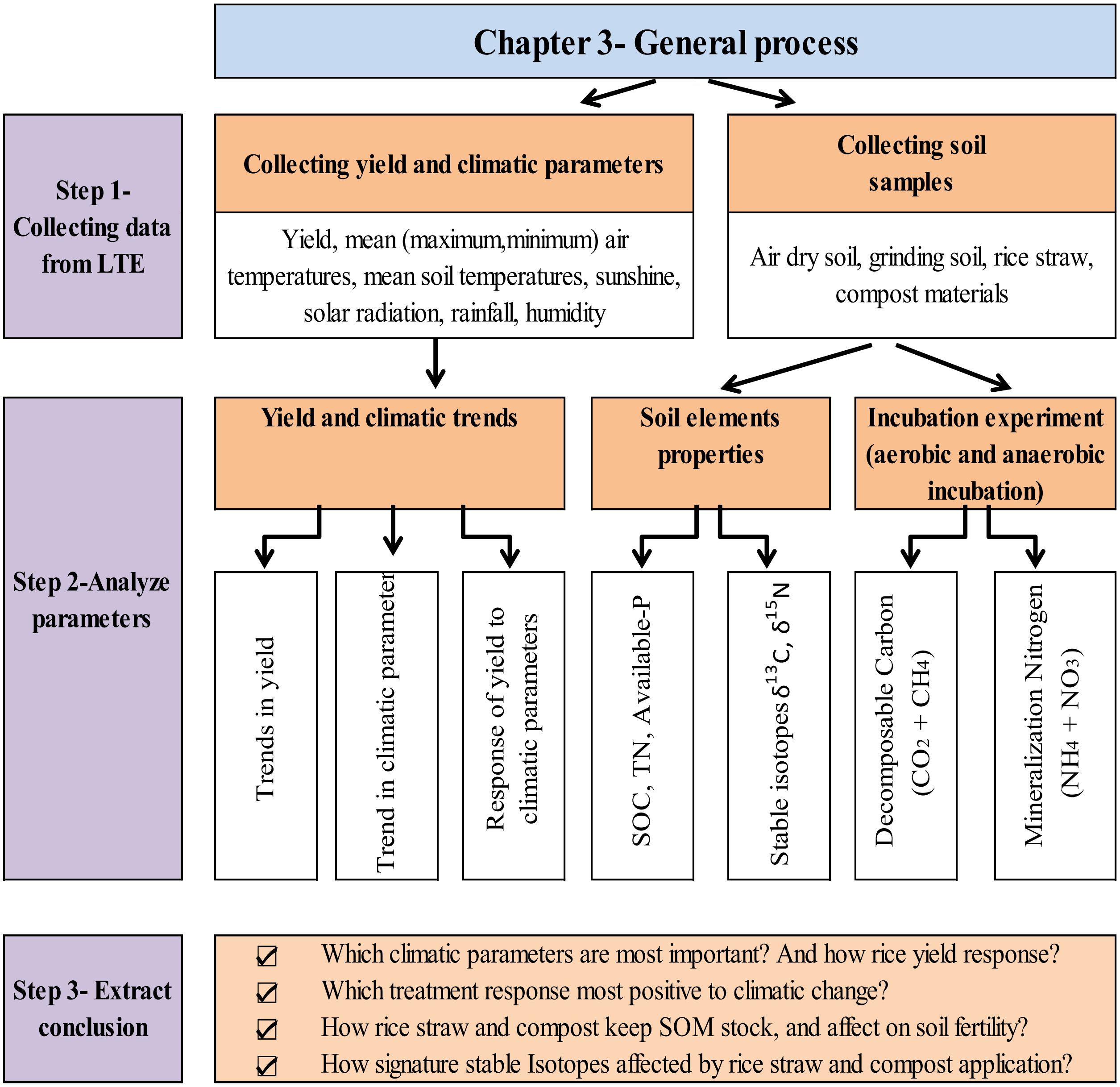
3.4.4. Changes in Min-N, De-C of air bulk soil from incubation

Unlike SOC and TN stock, we observed that min-N and De-C did not show continuous increasing. In our experiment, the NO_3^--N was not detected, so Min-N was all contributed from

NH_4^+-N. The absence of NO_3^--N could be explained by the sampling time. The nitrification process was suppressed due to long duration of wet condition. As a result, NO_3^--N was not detected in in this study. Compared to NPK, only CM3 was significant increase Min-N, while RS treatment also showed the low *P* value (*P*=0.07). The mean ratios of De-C to Min-N was around 6.23 to 7.20, which was much lower than in those bulk soil C/N ratio (about 13.31 on average), indicating that the easily decomposed organic matter mainly contributed from soil microbial biomass and sol protein (Azuma et al., 2015; Rillig et al., 2007). The previous research on the same soil also confirm this (Cheng et al., 2016a). The low De-C of PK treatment could be due to the lack of N fertilizer, which not applied in this treatment.

3.5. Conclusion

Rising temperatures in June-July has been addressed in LTE site over period of 1982-2014. Rice yield of all treatments (except PK) had positive correlation with temperature $P<0.05$. With long-term applied mineral fertilizer and organic matter, changes in SOC, TN, Av-P, C decomposition and N mineralization were clearly observed reference to NPK and also over 32 years. The highest SOC and TN contents were shown in CM3, follow by CM1, RS, NPK and PK. RothC model was well fitted to mineral fertilizer and RS treatments, but didn't fit well for the rice straw compost application treatment. The Av-P increased in all treatments except CM1, and application of RS and CM increased Av-P relative to NPK treatment. The increased trend was highest in RS and the lowest in PK. The $\delta^{13}C$ values decreased in all treatments, and get close to those values of RS and CM. Meanwhile, $\delta^{15}N$ were more unstable, however, decrease by RS and increased by CM application. The De-C/Min-N ratio after anaerobic and aerobic incubation are about 5.9 and 7.2, which is lower than in bulk soil (C/N~13.32), indicated eased decomposed organic matters compared to bulk soil.


Chapter 3- General process
Step 1- Collecting data from LTE
Collecting yield and climatic parameters
Yield, mean (maximum,minimum) air temperatures, mean soil temperatures, sunshine, solar radiation, rainfall, humidity
Collecting soil samples
Air dry soil, grinding soil, rice straw, compost materials
Step 2-Analyze parameters
Yield and climatic trends
Soil elements properties
Incubation experiment (aerobic and anaerobic incubation)
Trends in yield
Trend in climatic parameter
Response of yield to climatic parameters
SOC, TN, Available-P
Stable isotopes δ13C, δ15N
Decomposable Carbon (CO2 + CH4)
Mineralization Nitrogen (NH4 + NO3)
Step 3- Extract conclusion
☑ Which climatic parameters are most important? And how rice yield response?
☑ Which treatment response most positive to climatic change?
☑ How rice straw and compost keep SOM stock, and affect on soil fertility?
☑ How signature stable Isotopes affected by rice straw and compost application?

Chapter IV Changes in extraction organic matter after 32 years of mineral fertilizer and organic matters application

4.1. Introduction

LTE started more than one hundred years ago, with the first LTE initiated in 1843 at Rothamsted Experimental Station in England. Although the initial purpose of these LTEs was to understand the change in agro-systems productivity, it broadened over time to assess other impacts such as ecosystem perturbations, and global environmental changes on agro-systems sustainability (Körschens, 2006 ; Rasmussen et al., 1998; Senbayram et al., 2008). Compared to the early LTEs carried out in Europe and USA for uplands which were started before the 1900s (Körschens, 2006), the oldest LTE for lowlands rice (*Oryza sativa* L.) was started in 1926 in Anjo, Aichi Prefecture, Japan. Most of the LTEs in rice paddies have been conducted in Asian countries where rice is considered as the main staple food (Azuma et al., 2015; Cheng et al., 2016a; Cheng et al., 2016b).

C and N are two of the most important organic elements in SOM which determine soil sustainability and environmental impacts. Studying the C and N cycles in LTEs soils is crucial to understand the changes in soil quality (Balesdent J et al., 1988; Senbayram et al., 2008). Among the different fractions of SOM, water extracted organic matter (WEOM) represents a small fraction, involved in many soil processes (Chantigny et al., 2014; Guigue et al., 2014). WEOM also contains an important source of nutrients for soil microorganisms (Marschner and Bredow, 2002). WEOM obtained at room temperature can extract a substantial amount of SOM. However, to maximize the amount of WEOM from air-dried soils, hot water extracted organic matter

(HWEOM) obtained at elevated temperature had been recommended by Sparling et al. (1998). Hot water extracted organic C and N (HWEOC and HWEON) from air-dried soils have been used as useful indicators of upland soil N availability in Japan, with an extraction temperature of 80 °C and a duration of 16 hours (Curtin et al., 2006; Ghani et al., 2003). The HWEOC (N) of air-dried paddy soils showed high positive correlation with the available N measured by anaerobic incubation method at 30℃ for 4 weeks (Azuma et al., 2015).

Natural stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) are widely used to investigate the dynamic of SOM and WEOM, as mentioned in chapter 3. Although many LTEs researches on C and N dynamics focused on the quantity (or amount) of HWEOC and WEOC, there is no report on their quality with regards to their stable isotope values in rice paddy.

Therefore, the objectives of this study were to determine the changes in the amounts of HWEOM and WEOM (focus on C and N), their $\delta^{13}C$ and $\delta^{15}N$ values and the relationships among all C and N parameters for the soil samples from the long-term (31 years) application of two types of organic matter (rice straw and rice straw compost) combined with NPK fertilizers in a single rice paddy in a cold temperate region in Japan. Our hypothesis was that application of RS and CM may accelerate the amount of extractable organic matter. At the same time, their $\delta^{13}C$ and $\delta^{15}N$ values would change relatively to their values of input materials.

4.2. Materials and methods

4.2.1. Site location and treatments

Site location and treatments were described in section 3.2.1, chapter 3.

4.2.2. Soil sampling and analysis

Soil samples were collected in November 2012, after 31 years of continuous rice cultivation. Soil samples were divided into surface (0-15 cm) and subsurface (15-25 cm) layers. The depth of the surface layer was chosen to correspond to the plowed layer (0-15cm) where fertilizers and organic matters were incorporated. The sampling depth of the subsurface layer was decided down to 25 cm as rice roots were observed at this depth. Nine soil cores were collected from each of the five plots, and three cores were mixed to make up one composite sample for soil analysis. Three samples were then obtained for each treatment plot. Samples were air-dried and sieved (⌀<2 mm) before analysis. For the determination of stable carbon isotopes, samples were finely ground by Hi-Speed Vibration Sample Mill (Model TI-200, CMT, Iwaki, Japan).

Air-dried soils were used to measure SOC and TN content and $\delta^{13}C$ and $\delta^{15}N$ as described in chapter 3.

4.2.3. Hot water and water extracted C and N measurements

Hot water-extracted organic carbon (HWEOC) and hot water-extracted organic nitrogen (HWEON) were obtained by adapting the method of (Ghani et al., 2003). Two soils to water ratios for extraction were carried out (1:10 and 1:1.5). To obtain 1:10 and 1:1.5 ratio extractions, amounts of 3 and 20 g of air-dried soils were placed in 50 ml centrifuge tubes and shaken by hand for 2 minutes with 30 ml deionized water and then placed in a water bath at 80 ^{o}C for 16 hrs. After cooling, the tubes were centrifuged at 3000 rpm for 30 minutes. The supernatant was filtered through a prewashed 0.45 µm membrane filter (Millex-HV, Merck Millipore Ltd, Cork, Ireland). The supernatant was split into two subsamples for (i) the analysis of dissolved organic carbon (DOC) and total dissolved nitrogen as water extracted nitrogen (WEN) by thermal oxidation (TOC VCPH with TNM-1, Shimadzu, Japan), and (ii) the determination of inorganic N (NH_4^+-N and NO_3^--N) by a colorimetric method (JSSSPN 1986; Cheng *et al.* 2016). Hot water extracted organic nitrogen (HWEON was calculated as the difference between HWEN) and inorganic-N. The remaining solution was freeze-dried (FDU-2200, EYELA, Tokyo Rikakikai Co., Ltd, Japan) to measure the $\delta^{13}C$.

The WEOC and WEON were obtained by shaking 3 g of air-dried soil samples with 30 ml deionized water for 30 minutes (Shaking baths SB-13, AS One Co., Ltd, Japan), then centrifuged and filtered as the hot water extracted samples. The water extractions were done at room temperature. The amounts and $\delta^{13}C$ of WEOC, and the amounts of WEON were determined similarly to those of hot water extractions.

4.2.4. Data calculations and analysis

Data calculations and analysis is described as shown in section 3.5 chapter 3.

4.3. Results

4.3.1. SOC and TN contents, and the $\delta^{13}C$ values

All treatments with organic matter amendment highly increased SOC and TN contents in both surface and subsurface layers compared to NPK. Both SOC and TN contents slightly decreased in PK at 3-5% (Fig. 4.1). $\delta^{13}C$ values in SOC ranged from -25.7 to -23.2‰ for both layers. It decreased after long-term OM application for RS and CM compared to NPK in both surface and subsurface layers. The differences in $\delta^{13}C$ values between surface and subsurface layers were larger in RS and CM than the NPK and PK (Fig. 4.1).

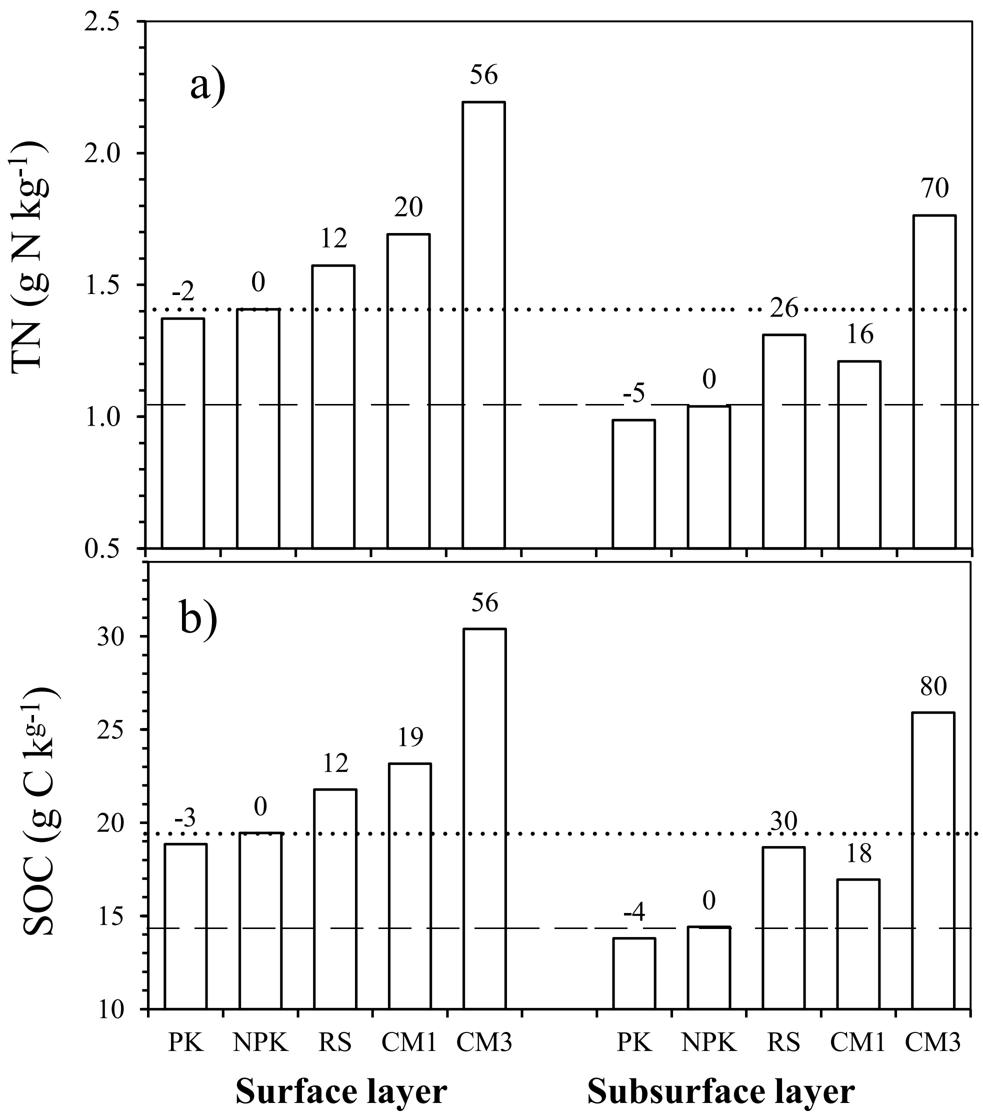


Fig. 4.1. Changes in SOC (a) and TN (b) in surface (0-15 cm) and subsurface (15-25 cm) layers after 31-year application of mineral fertilizers and organic matters. The numbers shown above the bars for each treatment indicate the percentage (%) changes in the SOC or TN compared with NPK treatments (presented as dotted line and solid line).

4.3.2. Hot water and water extracted organic carbon

The changes in the amounts and $\delta^{13}C$ values of HWEOC and WEOC from the soil samples in surface and subsurface layers after 31 years of long-term experiment treated with mineral fertilizers and organic matter are shown in Table 3.1. Long-term application of rice straw and compost increased the amounts of HWEOC and WEOC compared to control NPK in both surface and subsurface layers. In surface layers, the amounts of HWEOC and WEOC ranged from 466 to 702 mg C kg^{-1} and from 210 to 320 mg C kg^{-1}, in 1:10 extraction ratio, and ranged from 287 to 462 mg C kg^{-1} and from 113 to 187 mg C kg^{-1} in 1:1.5 extraction ratio, respectively. In subsurface layers, the amounts of HWEOC and WEOC ranged from 334 to 602 mg C kg^{-1} and from 125 to 263 mg C kg^{-1}, in 1:10 extraction ratio, and ranged from 179 to 360 mg C kg^{-1} and from 81 to 161 mg C kg^{-1},in 1:1.5 extraction ratio, respectively .The percentages of HWEOC and WEOC relative to the SOC for the both layers ranged from 2.31 to 2.84% and from 0.90 to 1.36%, in 1:10 extraction ratio, and from 1.30 to 1.78% and from 0.58 to 0.77%,in 1:1.5 extraction ratio, respectively (Table 3.3). Compared to the larger variability of the HWEOC and WEOC amounts, the $\delta^{13}C$ values of HWEOC and WEOC in both extraction ratios were more similar and ranged from -28.3 to -26.9‰ for surface layer and from -27.6 to -26.4‰ for subsurface layer, respectively (Fig.3.2). These values were obviously lower than the $\delta^{13}C$ values of SOC.

Table 4.1. Changes in the amounts of extracted organic C by two extracted ratios from soil samples in surface (0-15 cm) and subsurface (15-25 cm) layers after 31 years long-term different fertilizer applications.

Treatment	1:1.5 extraction ratio				1:10 extraction ratio			
	HWEOC	WEOC	ΔEOC	HWEOC /WEOC	HWEOC	WEOC	ΔEOC	HWEOC /WEOC
	(mgC*kg^{-1})				(mgC*kg^{-1})			
Surface Layer (0-15cm)								
PK	287	113	174	2.53	466	210	255	2.21
NPK	292	125	168	2.34	496	221	275	2.24
RS	387	168	219	2.30	618	297	321	2.08
CM1	357	154	204	2.33	568	264	304	2.15
CM3	462	187	276	2.48	702	320	382	2.19
Subsurface Layer (15-25cm)								
PK	179	81	98	2.22	334	125	209	2.68
NPK	212	102	110	2.08	379	148	231	2.56
RS	283	131	153	2.17	485	196	290	2.48
CM1	268	123	145	2.18	456	191	265	2.38
CM3	360	161	199	2.23	602	263	339	2.29

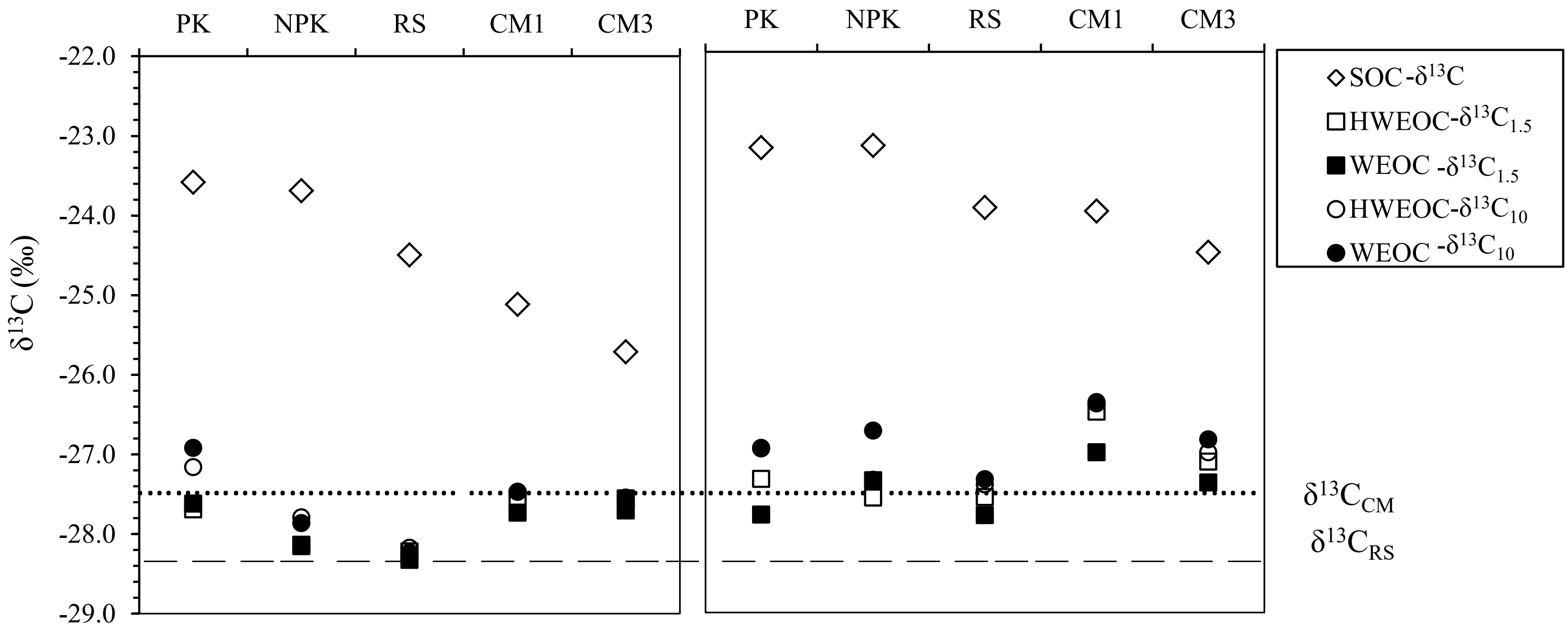


Fig.4.2. Changes in $\delta^{13}C$ values in SOC and EOC in surface (0-15 cm) and subsurface (15-25 cm) layers after 31-year application of mineral fertilizers and organic matters. HWEOC- $\delta^{13}C_{1.5}$ and WEOC- $\delta^{13}C_{1.5}$ indicated to 1:1.5 extracted ratios, HWEOC- $\delta^{13}C_{10}$ and WEOC- $\delta^{13}C_{10}$ indicated to 1:10 extracted ratio. The dot line and solid line presented for rice straw compost and rice straw materials application

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4.3.3. Hot water and water extracted organic nitrogen

Among the total HWEN and WEN, NO_3^--N was undetected in both hot water and water extractions. The amounts of HWEN and WEN changed from both layers after 31 years of long-term experiment treated with mineral fertilizers and organic matters (Table 3.3). The amounts of HWEON and WEON in surface layer ranged from 22.5 to 41.8 mg N kg^{-1} and from 10.4 to 16.6 mg N kg^{-1}, in 1:10 extraction ratio, and ranged from 8.1 to 21.6 mg N kg^{-1} and from 3.6 to 6.6 mg N kg^{-1}, in 1:1.5 extraction ratios, respectively. In the subsurface layer the amounts of HWEON and WEON ranged from 15.3 to 32.5 mg N kg^{-1} and from 6.0 to 13.1 mg N kg^{-1}, in 1.10 extraction ratio, and from 10.7 to 19.8 mg N kg^{-1} and from 4.1 to 7.3 mg N kg^{-1}, in 1:1.5 extraction ratio, respectively (Table 3.3). Long-term application of rice straw and compost increased the amounts of HWEON and WEON compared to control NPK in both surface and subsurface layers, similar to HWEOC and WEOC. The percentages of HWEN and WEN related to the TN for both layers ranged from 1.55 to 2.05% and from 0.61 to 0.82%, for 1:10 extraction ratio, and from 0.51 to 1.30 % and from 0.26 to 0.86%, respectively (Table 3.2).

Table 3.2. The percentage of extracted C (N) and decomposed C (mineralized N) to the SOC (TN) for the soil samples from different long-term mineral fertilizer and organic matter treatments at surface (0-15 cm) and subsurface (15-25 cm) layers

Layers and treatments	1:1.5 extracted ratio		1:10 extracted ratio		Dec-C*	1:1.5 extracted ratio		1:10 extracted ratio		Min-N*
	HWEOC	WEOC	HWEOC	WEOC		HWEN	WEN	HWEN	WEN	
	per SOC (%)					per TN (%)				
Surface layer (0-15 cm)										
PK	1.52	0.60	2.47	1.12	2.61	0.77	0.26	1.64	0.76	8.39
NPK	1.50	0.64	2.55	1.14	2.83	0.73	0.25	1.74	0.80	7.44
RS	1.78	0.77	2.84	1.36	2.98	0.51	0.30	1.80	0.79	8.31
CM1	1.54	0.66	2.45	1.14	2.62	0.93	0.33	1.91	0.81	8.06
CM3	1.52	0.61	2.31	1.05	2.27	0.98	0.30	1.91	0.76	6.92
Average	1.57	0.66	2.52	1.16	2.66	0.79	0.29	1.80	0.78	7.82
Subsurface layer (15-25 cm)										
PK	1.30	0.58	2.42	0.90	2.39	1.08	0.55	1.55	0.61	7.18
NPK	1.47	0.71	2.63	1.03	1.91	1.23	0.57	1.88	0.66	6.21
RS	1.52	0.70	2.60	1.05	2.18	1.11	0.56	1.68	0.65	6.98
CM1	1.58	0.73	2.69	1.13	1.85	1.30	0.86	2.05	0.81	6.80
CM3	1.39	0.62	2.32	1.01	2.02	1.12	0.64	1.84	0.74	6.26
Average	1.45	0.67	2.53	1.02	2.07	1.17	0.64	1.80	0.70	6.69

The NH_4^+-N amounts were lower than 5 mg N kg^{-1} soil among 5 treatments in both layers. NH_4^+-N amounts were higher in hot water extracts than in water extracts. The difference between total extracted N (HWEN or WEN) and inorganic N (NO_3^--N and NH_4^+-N) was the organic N (HWEON or WEON). The percentages of extracted organic NH_4^+-N to total extracted N (EN) ranged from 8.2 to 13.0% for hot water extraction and 6.5 to 14.5% for water extraction, in both extraction ratios, respectively (Table 4.3).

Table 4.3. Changes in the amounts of HWEN and WEN, and hot water and water soluble NH_4-N from the soil samples by two extracted ratios at surface (0-15 cm) and subsurface (15-25 cm) layers after 31 years long-term experiment treated with mineral fertilizers and organic matters.

Layers and treatments	1:1.5 extracted ratio						1:10 extracted ratio					
	Amount of ETN		Amount of NH_4-N		Percentages of NH_4-N to ETN		Amount of ETN		Amount of NH_4-N		Percentages of NH_4-N to ETN	
	by hot water	by water	by hot water	by water	by hot water	by water	by hot water	by water	by hot water	by water	by hot water	by water
	(mg N kg^{-1})				%		(mg N kg^{-1})				%	
Surface layer (0-15 cm)												
PK	12.12	4.25	1.5	0.6	12.3	14.6	25.08	11.47	2.6	1.0	10.3	9.0
NPK	11.76	4.21	1.5	0.6	12.5	14.9	27.12	12.10	2.7	0.8	9.8	6.7
RS	15.34	5.39	2.0	0.7	13.0	13.7	31.97	13.70	3.7	1.3	11.5	9.3
CM1	17.46	6.27	1.7	0.8	9.9	12.0	35.61	14.84	3.3	1.1	9.2	7.5
CM3	23.98	7.50	2.4	0.9	9.9	12.2	46.47	18.06	4.7	1.5	10.1	8.2
Subsurface layer (15-25 cm)												
PK	11.74	4.57	1.1	0.4	9.0	9.6	17.45	6.64	2.1	0.6	12.2	9.6
NPK	14.19	5.21	1.4	0.6	10.2	11.0	22.04	7.41	2.5	0.6	11.5	7.5
RS	16.00	6.03	1.5	0.6	9.1	10.0	24.97	9.55	3.0	1.0	12.1	10.6
CM1	17.12	6.50	1.4	0.6	8.2	8.8	28.01	10.52	3.2	0.7	11.5	6.5
CM3	21.79	8.16	2.0	0.8	9.0	10.4	36.71	14.31	4.2	1.2	11.5	8.4

*The data of Dec-C and Min-N were referred from Cheng *et al*. (2016), which were measured by anaerobic

4.3.4. C to N ratios

C/N ratio of the bulk soil was similar for both surface and subsurface layers, ranging from 13.7 to 13.9 and from 13.9 to 14.7, respectively (Table 4.4). For the water extracted organic matter, the ratio of HWEOC/HWEON for both extraction ratios for both layers ranged between 16.7 and 29.0, while the ratio of WEOC/WEON ranged from 19.3 to 35.6. There was no clear difference of the ratios between hot water and water extraction or between surface and subsurface layers. Ratios of HWEOC/HWEON and WEOC/WEON were slightly higher in RS treatment than others.

Table 4.4 The ratios of SOC/TN, HWEOC/HWEON, WEOC/WEON, ΔWEOC/ΔWEN (difference between hot water and water extractions), Dec-C/Min-N (C decomposition potential/N mineralization potential) from the soil samples at surface (0-15 cm) and subsurface (15-25 cm) layers after 31 years treated with mineral fertilizers and organic matters.

Treatment	SOC/TN	1:1.5 extraction ratio			1:10 extraction ratio			Dep-C/ Min-N*
		HWEOC/ HWEON	WEOC/ WEON	ΔEOC/ ΔEON	HWEOC/ HWEON	WEOC/ WEON	ΔEOC/ ΔEON	
Surface Layer (0-15cm)								
PK	13.7	27.0	30.7	17.3	20.8	20.2	11.9	4.18
NPK	13.8	28.4	35.3	17.4	20.3	19.6	11.6	5.1
RS	13.8	29.0	35.6	29.9	21.8	24.0	11.9	4.6
CM1	13.7	22.7	28.4	13.6	17.5	19.3	9.7	4.6
CM3	13.9	21.6	28.4	13.3	16.9	19.3	9.5	4.3
Subsurface Layer (15-25cm)								
PK	14.0	16.8	19.6	9.6	21.8	20.8	14.2	4.6
NPK	13.9	16.7	22.0	9.0	19.5	21.7	12.2	4.1
RS	14.3	19.5	24.1	10.9	22.1	23.0	13.8	4.2
CM1	14.0	17.1	20.8	9.6	18.4	19.5	11.0	4.1
CM3	14.7	18.1	22.0	10.5	18.5	20.0	10.8	4.4

4.3.5. Relationships between C and N contents and their isotopic enrichments

We combined data of both surface and subsurface layer of two extraction ratios (n=20) for the relationship analysis. There were significant positive correlations among all C and N pools for bulk soil, hot water and water extractions (Table 4.5). The values of $\delta^{15}N$ showed in Fig. 4.3. Conversely, there were significant negative correlations between $\delta^{13}C$-SOC and the C contents of bulk soil, or amounts of HWEOC and WEOC. However, there were no correlation between $\delta^{13}C$ values and the amounts of HWEOC (or WEOC).

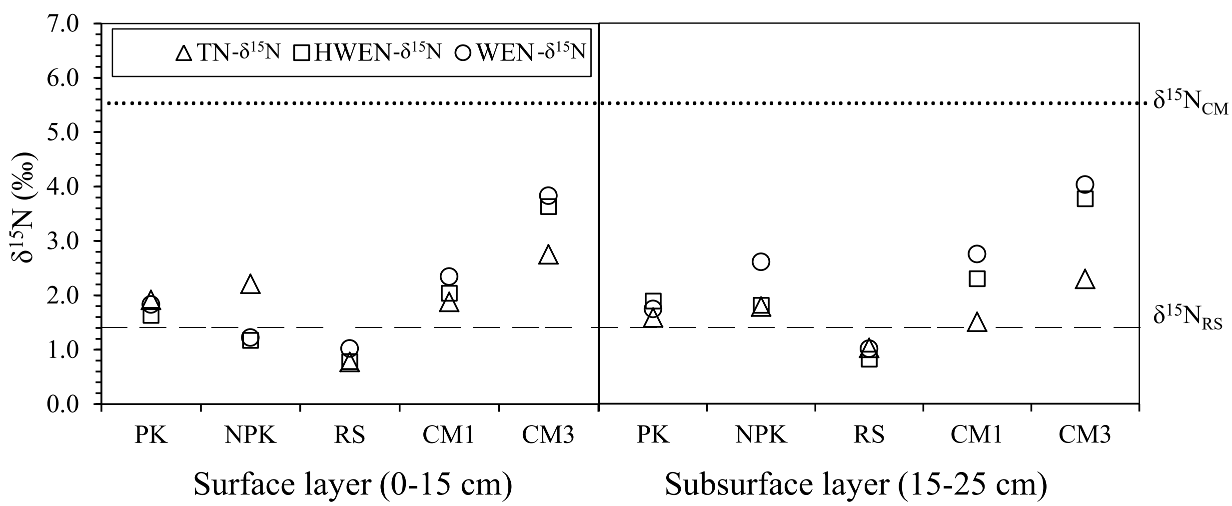


Fig. 4.3. Changes $\delta^{15}N$ values of TN and EN (by 1:1.5 extracted ratio) at surface (0-15 cm) and subsurface (15-25 cm) layers after 31 years application of mineral fertilizers and organic matters. The dot line and solid lines present the $\delta^{15}N$ of RS compost and RS materials application.

Table 4.5. Values of correlations between the amounts and stable isotopes of the parameters related to C and N for soil samples from different long-term mineral fertilizer and organic matter treatments at surface (0-15 cm) and subsurface (15-25 cm) layers. The cells with yellow and red color showed P<0.05 and P<0.01, respectively (combined two ratios, n=20).

	SOC	TN	HWEOC	WEOC	HWEN	WEN	SOC-δ^{13}C	HWEOC-δ^{13}C	WEOC-δ^{13}C	TN-δ^{15}N	HWEN-δ^{15}N	WEN-δ^{15}N
SOC	1.000											
TN	0.995***	1.000										
HWEOC	0.648**	0.652**	1.000									
WEOC	0.645**	0.650**	0.984***	1.000								
HWEN	0.599**	0.594**	0.945***	0.945***	1.000							
WEN	0.520*	0.518*	0.933***	0.952***	0.98***	1.000						
SOC-δ^{13}C	-0.923***	-0.937***	-0.626**	-0.631**	-0.593**	-0.507*	1.000					
HWEOC-δ^{13}C	-0.229	-0.267	-0.097	-0.139	0.101	0.073	0.160	1.000				
WEOC-δ^{13}C	-0.267	-0.299	0.049	-0.021	0.217	0.181	0.257***	0.883***	1.000			
TN-δ^{15}N	0.526*	0.526*	0.216	0.181	0.317	0.254	-0.355	0.107	0.167***	1.000		
HWEN-δ^{15}N	0.600	0.564	0.408	0.399	0.782**	0.765**	-0.504	0.534	0.536***	0.757*	1.000	
WEN-δ^{15}N	0.546	0.512	0.378	0.389	0.788**	0.779**	-0.47	0.562	0.634***	0.713*	0.974***	1.000

4.3.6. Absorbance $SUVA_{254}$

The specific absorbance of $SUVA_{254}$ result was shown in Fig. 4.4. In 1:1.5 ratio, $SUVA_{254}$ ranged from 10.6~17.4 and 13.9~30.0 L mg C cm^{-1} for HWE and WE, respectively. Meanwhile, for 1:10 extraction ratio, SUVA254 ranges from 7.5~13.4 and 10.4~23.3 L mg C cm^{-1} for HWE and WE, respectively. Interestingly, RS shows the lowest result for both extraction ratios, and for both layers. It is also noticeable that WE has higher $SUVA_{254}$ compared to HWE, especially in subsurface layer. The result also figures out the 1:1.5 extraction ratio has higher $SUVA_{254}$ than 1:10 extraction ratio.

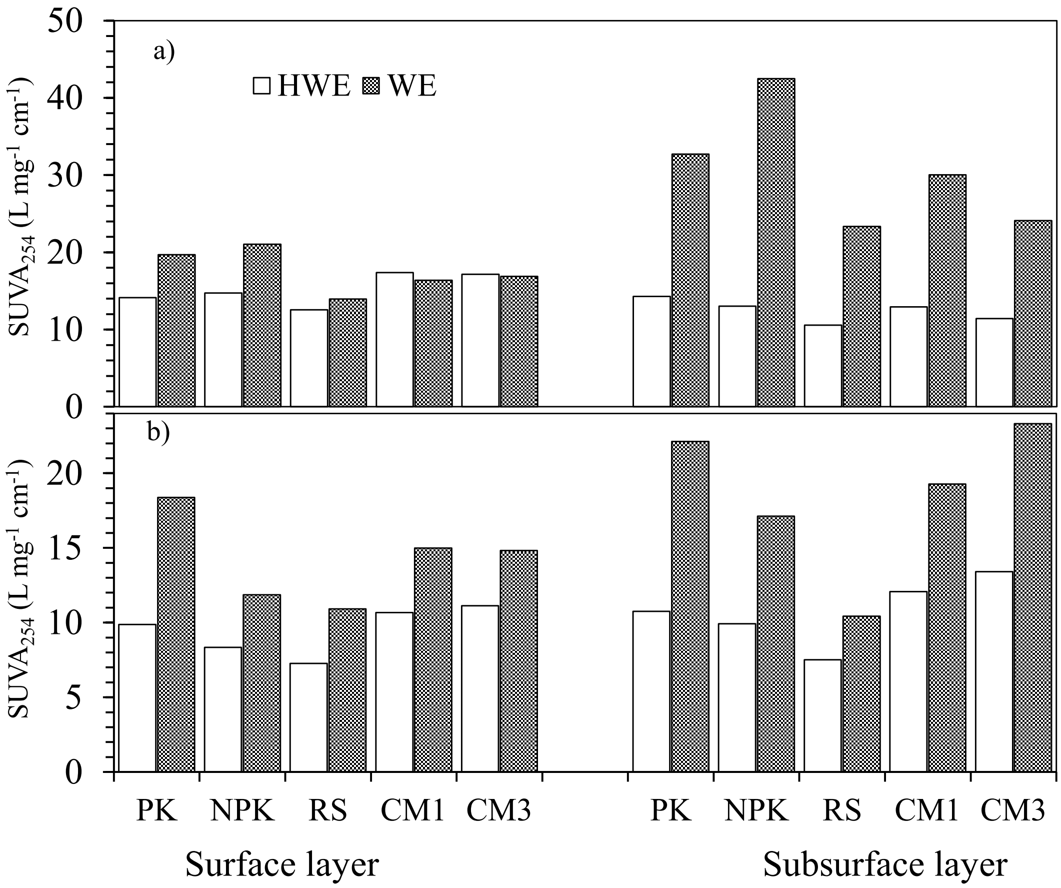


Fig.4.4. Ultraviolet-visible (Specific UV absorbance at 254 nm) by hot water and water extraction at (a) 1:1.5 and (b) 1:10 extracted ratio.

4.3.7. Comparing between 1:1.5 to 1:1.10 extraction ratios

The 1:1.10 extraction ratio can extract more amount in extracted carbon and nitrogen. Similarly, the amount of Ammonium amount extracted from 1:10 also higher than 1:1.5 extraction ration. However, there is no difference in isotope $\delta^{13}C$. The increase in HWEOC and HWEON ranged from 1.52-1.87 and 1.43-2.38 times, respectively. However, the ratios in HWEOC- $\delta^{13}C$ and WEOC-$\delta^{13}C$ were varied from 0.97 to 1.00%, which can be considered as no change.

Table 4.6. The ratios of 1:10 to 1:1.5 extracted ratio C and N for soil samples from different long-term mineral fertilizer and organic matter treatments.

Treatments and layers	HWEC	WEOC	HWEON	WEON	HWE-(NH_4-N)	WE-(NH_4-N)	HWEC-$\delta^{13}C$	WEC-$\delta^{13}C$
Surface layer (0-15 cm)								
PK	1.62	1.86	2.11	2.87	1.74	1.67	0.98	0.97
NPK	1.70	1.77	2.38	3.15	1.81	1.29	0.99	0.99
RS	1.60	1.77	2.12	2.67	1.84	1.71	1.00	1.00
CM1	1.59	1.72	2.06	2.49	1.89	1.48	1.00	0.99
CM3	1.52	1.71	1.94	2.52	1.96	1.62	1.00	0.99
Sub surface layer (15-25 cm)								
PK	1.87	1.55	1.43	1.45	2.02	1.46	0.99	0.97
NPK	1.79	1.45	1.53	1.48	1.75	0.96	0.99	0.98
RS	1.71	1.50	1.51	1.57	2.06	1.68	0.99	0.98
CM1	1.70	1.55	1.58	1.66	2.31	1.20	1.00	0.98
CM3	1.67	1.63	1.64	1.79	2.15	1.42	1.00	0.98

4.4. Discussion

4.4.1. The $\delta^{13}C$ values in bulk soil, HWEOC and WEOC

Generally, the differences in $\delta^{13}C$ values of SOC were originated from plant tissues by C_3 and C_4 plants. The $\delta^{13}C$ of bulk soil depends on the proportions of C_3 plant-derived C and C_4 plant-derived C in the soils. The $\delta^{13}C$ values of C_3 and C_4 plant-derived C are -27‰ and -13‰ on the average, respectively (Yoneyama et al., 2001). Although we did not have the initial soil samples from the start of this LTE in spring of 1982, the soil samples taken from the surface layer (0-15 cm) of NPK treatment plot after the second years of rice growth (in the autumn of 1983) was -22.4‰, corresponding to about 30% of the total SOC derived from C_4 plants based on mass balance method (Yoneyama et al., 2001). In 1994, the treatment plots of this LTE were reset by inputted guest soil to maintain the depth of plow layers which parts of soils were lost due to erosion. The average of $\delta^{13}C$ values of the five treatment soils shifted to -23.4~-22.0‰ in 1995 from -24.6 to -23.3‰ in 1994 (Fig. 4.2). In 2012 after 31 years of rice cultivation, the $\delta^{13}C$ values of bulk soil ranged from -25.7 to -23.2‰ in both layers. The soil $\delta^{13}C$ largely decreased with the application of rice straw and rice straw compost compared with treatments applied with mineral fertilizers only (PK and NPK). It was as per our hypothesis since the $\delta^{13}C$ values of rice (C_3 plant) straw and compost were -28.3 and -27.4‰ , which were much lower than -22.4‰ (values for NPK plots in 1983 were close to the original soil) and the guest soil in 1994 (the $\delta^{13}C$ value of guest soil was lower than-22.0‰. The $\delta^{13}C$ also decreased in PK and NPK treatments after the 31 years of mineral fertilizer application. The decrease in $\delta^{13}C$ in all treatments in 2012 was due to the incorporation of C_3 plant-derived C into the soil after long-term rice cultivation. Similar results were reported by (Senbayram et al., 2008) in the England upland soil and (Zhao et al., 2002) in a rice paddy soil in Japan. In PK and NPK treatments, although the RS was removed from the plots every year after harvest, the rice root exudates released into the soil during the rice growing period, as well as rice stubbles and roots which could not be completely removed from the field, thereby contributing to more input of new rice-derived C into soil. This mechanism may explain the decreasing $\delta^{13}C$ measured in PK and NPK treatments in bulk soil. Similar but less pronounced trend was observed in the subsurface soil layer. This result can be attributed to the organic material applications because the distribution of rice roots was abundant in the surface layer. These results implied that the SOC turnover was faster in the surface layer than in the subsurface layer, despite of the higher SOC content.

The $\delta^{13}C$ in HWEOC and WEOC (less than -26‰ for both surface and subsurface layers, Fig. 4.2) were closer to the $\delta^{13}C$ of the RS (-28.3‰) and CM (-27.4‰) and lower than the $\delta^{13}C$ in SOC (more than -26‰, Fig.4.2). It is also reported that the $\delta^{13}C$ in water extracts was significantly less negative than in bulk SOC by (Guigue et al., 2015). These results indicated that sources of both HWEOC and WEOC were mostly from the rice plant photosynthesis (including rice straw and rice straw compost applications), but not from the original soil. The significant correlation (R = 0.883, P < 0.001) of $\delta^{13}C$ values with HWEOC and WEOC also indicated that C sources were from similar organic matters (Table 4.5).

4.4.2. Amounts of hot water and water extraction matter

Dissolved organic C (DOC) or WEOC is highly mobile in soils and is reported to be an important available source of energy for microorganisms, usually accounting for less than 2% of the SOC (Chantigny et al., 2014; Guigue et al., 2015). Although there are many analytical procedures to separate DOC from soils, both WEOC and HWEOC (80 ^{o}C and 16 hrs) are frequently used (Chantigny et al., 2014; Guigue et al., 2014; Moriizumi and Matsunaga, 2011; Nakanishi et al., 2012). Our results showed that that hot water at 80 ^{o}C is more efficient to extract DOC from soil. Our research also showed that the amounts of decomposed C (Dec-C) were larger than WEOC, but less than HWEOC (Fig.3.3), the Dec-C was calculated from CO_2 and CH_4 productions from the air-dried soil incubated anaerobically at 30ºC for 4 weeks (Cheng et al., 2016a). This result implied the HWEOC could not be decomposed completely during anaerobic incubation. On the other hand, there were significant correlations among SOC, HWEOC and WEOC (P <0.001, Table 4.5), implying that both HWEOC and WEOC can be used as indexes to represent SOC changes in the long-term rice cultivation.

Table 4.7. The best parameters obtained from the anaerobic incubation experiment at 30°C in 8 weeks for measuring the C decomposition potential (Co) and N mineralization potential (No) for the soil samples from different long-term mineral fertilizer and organic matter treatments at surface (0-15 cm) and subsurface (15-25 cm) layers. The curves were modeled by first-order model as [C (or N) = Co (or No) × (1-exp (-k×t)]. Original data referred from Cheng et al. (2016).

Layers and treatments	C decomposition			N mineralization		
	Co (mg C kg^{-1})	k_c † (wk^{-1})	R^{2*}	*No* (mg N kg^{-1})	k_n † (wk^{-1})	R^{2*}
Surface layer (0-15 cm)						
PK	491	0.486	0.994	118	0.306	0.993
NPK	553	0.437	0.981	109	0.417	0.990
RS	660	0.549	0.978	142	0.599	0.991
CM1	612	0.614	0.977	133	0.697	0.973
CM3	705	0.485	0.986	163	0.574	0.972
Subsurface layer (15-25 cm)						
PK	326	0.647	0.991	71	0.297	0.987
NPK	279	0.457	0.983	68	0.346	0.981
RS	402	0.361	0.993	96	0.676	0.978
CM1	322	0.811	0.990	79	0.661	0.988
CM3	511	0.498	0.994	117	0.602	0.979

† k_c *and* k_n are the rate constants for the first-order reaction models.

There are many studies on the extractable organic N and dissolved organic N in soils to predict N mineralization, N leaching, and to evaluate agricultural (nutrient) management practices (reviewed by (Ros et al., 2009). HWEN (or WEN) could be divided into two parts of organic N and inorganic N (NO_3^--N and NH_4^+-N). The NO_3^--N was not detected in all hot water or water extractions in this study, implying that the nitrification activity was ignored in this rice paddy LTE soils. The amounts of hot water or water extracted NH_4^+-N were lower in the extractions, less than 5 mg N kg^{-1} soil, and led to the organic N accounting for the main distribution of the HWEN and WEN in the extractions. The organic N and NH_4^+-N increased by hot water extractions were consistent with previous studies (Azuma et al., 2015; Curtin et al., 2006).

Different to Dec-C, the amounts of mineralized N (Min-N) were larger than those of HWEON and WEON (Table 4.3). Min-N was calculated by NH_4^+-N production from the air-dried soil incubated anaerobically at 30 ºC for 4 weeks (data referred from (Cheng et al., 2016a). Normally, the amounts of Min-N obtained from anaerobic incubation of the air-dried rice paddy soil at 30ºC for 4 weeks is considered as available N for rice cultivation in Japan (JSSSPN, 1986). Our results showed that there were significant positive correlations between available N and amounts of hot water or water extracted organic C (or N) among 5 treatments and 2 layers and two extraction ratios (all $P<0.001$, n=20), which implied that not only HWEOC, but also WEOC, HWEON and WEON could be used as integrated indexes for soil quality in this long-term experiment.

4.4.3. The ratios of SOC/TN, HWEOC/HWEON and WEOC/WEON

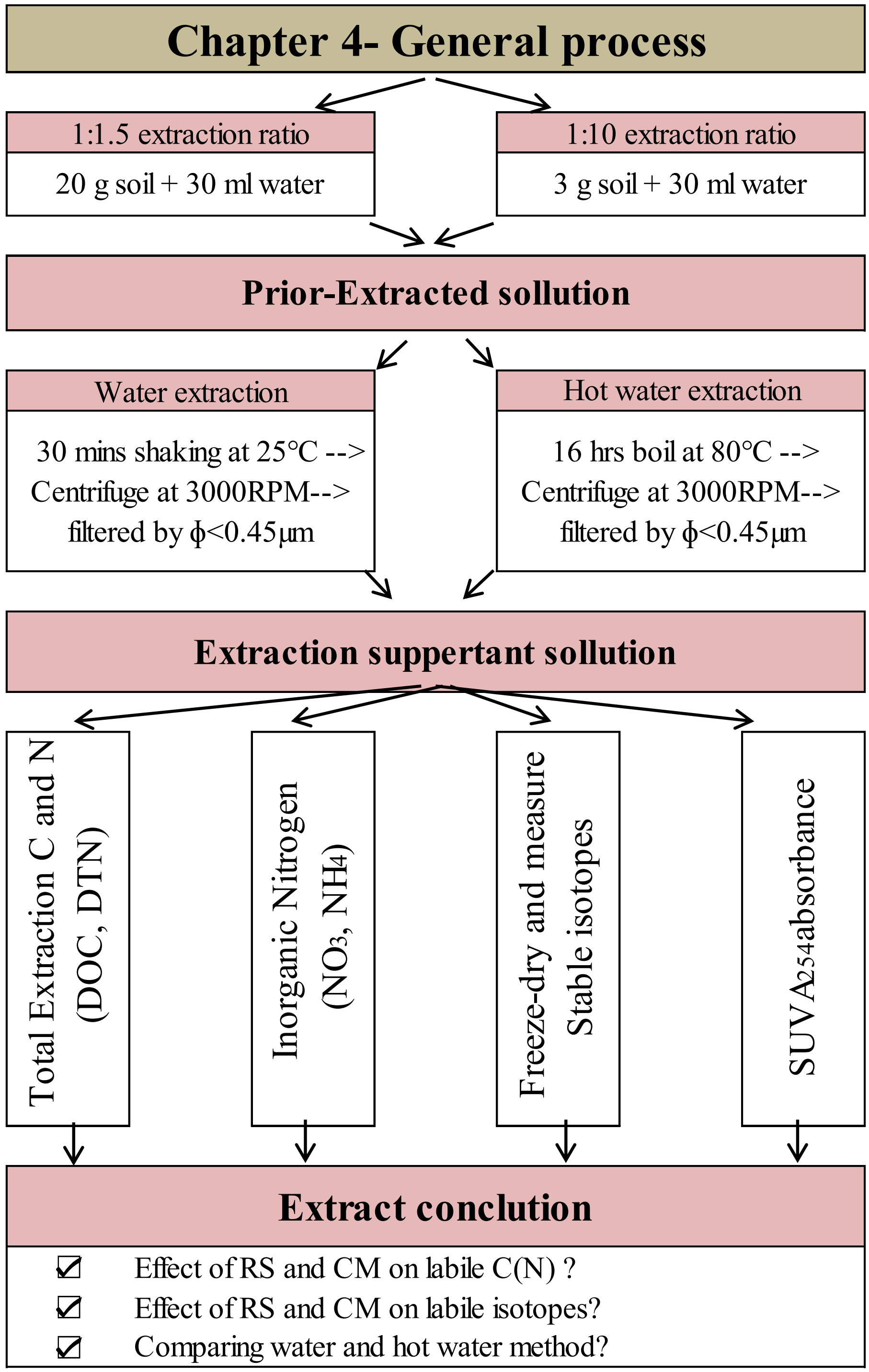
The C/N ratio of organic matter is a key indicator related to C decomposition and N mineralization potentials, as well as instant immobilization in rice paddy. C/N ratio of rice straw decreased from 50-70 to around 10 in a paddy soil after the straw was buried into the soil during five years, as reported in a study based on glass fiber bag method (Shiga *et al*. 1985). There should be larger variations in C/N ratios among the intermediate products from plant tissue and SOC decomposition, which dissolved in the hot water or water extracted solutions. Our results showed that there were large differences among SOC/TN, HWEOC/HWEON, WEOC/WEON, and Dep-C/Min-N (Table 4.3) (Cheng et al., 2016a). Compared with ratios of SOC/TN around 13.7 to 14.7, ratios of WEOC/WEON were the highest (ranging from 19.3 to 35.6), followed by HWEOC/HWEON (ranging from 16.7 to 29.0). The higher C/N ratio of the dissolved organic matter in hot water and water extractions indicated that the organic matter was on the process of soil humification or on both decomposition and humification processes simultaneously. Interestingly, the ratios of HWEOC/HWEON and WEOC/WEON for the RS treatment were higher than those of PK, NPK, and CM treatments. We interpreted this as the result of the large quantities of C in RS (2090 kg C ha^{-1} $year^{-1}$) applied to the RS plot. Compared to the high ratios of HWEOC/HWEON, WEOC/WEON, the C/N ratio of Dec-C to Min-N was very low, ranging from 4.2 to 6.1, indicating that the decomposing organic matter during the anaerobic incubation process was from soil microbial biomass and soil protein (Moriizumi and Matsunaga, 2011; Rillig et al., 2007).

However, HWEOC/HWEON and WEOC/WEON were higher in this study than those reported by previous research (Azuma et al., 2015), which most of HWEOC/HWEN and WEOC/WEN were around 10~15. The reason could be due to the different extraction and calculation methods. Firstly, the EOC/EON should be higher than the EOC/EN, EN was the total of EON and NH_4^+-N. Secondly, (Azuma et al., 2015) extracted the organic N and NH_4^+-N by 1% K_2SO_4 solution (addition of 5 mL 10% K_2SO_4 solution to 50 mL water solution) and filtered by filter paper (No. 5C, Toyo Roshi Co., Tokyo, Japan) in their studies. For measuring δ^{13}C values of HWEOC and WEOC, we filtered the extraction solutions directly by prewashed 0.45 μm membrane filter (Millex-HV, Merck Millipore Ltd, Cork, Ireland). So NH_4^+-N concentration in hot water and water extraction should be less than that extracted with 1% K_2SO_4 solution in our study, even if we put EON and NH_4^+-N together, the WEOC/WEN was still low in this study .

4.5. Conclusion

In this study, we evaluated the effects of long-term application of mineral fertilizers and/or organic matters on soil C and N contents and δ^{13}C from three SOM pools of bulk soil, hot water and water extraction. Amounts of hot water and water extracted C and N were highly correlated with SOC and TN. The δ^{13}C values in bulk soil decreased with RS and its compost application. Conversely, the δ^{13}C values of hot water and water extractions were similar among treatments and close to the δ^{13}C of RS and CM. We suppose that long-term application of rice straw and rice straw compost and temperature did not alter the values of δ^{13}C in mobile pools. Based on these results, we propose that hot water and water extraction could be considered as two useful methods to estimate SOC and TN content in bulk soil, but their δ^{13}C is affected by material application, and do not imply any correlation with those of bulk soil after long duration.

Further researches on C and N contents and their stables isotopes from different pools in long-term rice experiments should be carried out to understand clearly the underlying mechanism.


Chapter 4- General process
1:1.5 extraction ratio
20 g soil + 30 ml water
1:10 extraction ratio
3 g soil + 30 ml water
Prior-Extracted sollution
Water extraction
30 mins shaking at 25°C -->
Centrifuge at 3000RPM-->
filtered by ϕ<0.45μm
Hot water extraction
16 hrs boil at 80°C -->
Centrifuge at 3000RPM-->
filtered by ϕ<0.45μm
Extraction suppertant sollution
Total Extraction C and N
(DOC, DTN)
Inorganic Nitrogen
(NO3, NH4)
Freeze-dry and measure
Stable isotopes
SUVA254absorbance
Extract conclution
Effect of RS and CM on labile C(N) ?
Effect of RS and CM on labile isotopes?
Comparing water and hot water method?

Chapter V General discussion and conclusions

LTE has been widely used in term of many purposes. In our research, a continuous 32-year paddy rice experiment presents the relationship among rice growth with climate changes, and changes in SOM decomposition. The impact of climatic factors on paddy rice had been reported widely elsewhere, but there is still limited on focusing on the real growth stage in long period of years. Therefore, more accurately understanding the effect of climate change and fertilization on rice growth and SOM content according to the plant phenological events (tillering, flowering, heading…) gives us a good chance to see clearly mechanisms of climate effects.

SOM contributes the dominant source of C pool. Study on SOM changes in long-term period (several decades) can help us to understand C stock in global scale. C and N are two key elements in soil which affect the soil fertility. C also can make a great contribution to current global warming through enhance greenhouse gas emissions. Generally, the changes in soil C and N stocks are in large of variety under experimental field condition. Thus, understanding the changes in SOC and TN during long-term continuous period is ideal to predict the climate change in the future.

5.1. Rice yield was advanced by elevated temperature in growth stage period

Several studies confirmed the warming effects on agricultural practices (Fitzgerald and Resurreccion, 2009b; Hansen et al., 2010a; Kim et al., 2013; Lobell and Field, 2007b). Due to cold winter condition, rice cultivation in Japan is mainly practiced in summer season,. Although many studies showed the negative effects of global warming (Ghadirnezhad and Fallah, 2014; Ishimaru et al., 2011b; Ishimaru et al., 2016), increasing temperature was found to enhance the rice yield in our research. We supposed that in our research location, which annual mean temperatures are less than 13℃, and crop season mean temperatures are less than 25℃, which still under optimum temperature for rice growth, especially for rice photosynthesis, which best response present in 30-35℃ (Nagai and Makino, 2009). The rainfall and sunshine didn't make show significant contributions to the rice yield t, mainly due to well manage of Japan irrigation system. We found that increasing temperatures during two growing stages (TP-HD and HD-MT) strongly enhanced rice yield. In four parameters of rice yield, 1000-grain weight and ripening percentage are two main factors determining the rice yield, while the number of spikelet per area and spikelet per panicle didn't affect yield.

5.2. Mineral fertilizer combined organic matter application enhanced SOC and TN contents while decreased $\delta^{13}C$ but less pronounce effects on $\delta^{15}N$ values

Agricultural soil is considered as the main source of C storage in terrestrial ecosystem. Therefore, increase of soil C and N storage could reduce the greenhouse gas emissions in the atmosphere. Our study on LTE with 5 different inorganic fertilizers combined with RS and RS compost pointed out that soil C storage could be significant increased by fertilization. It is also strongly confirmed the rice straw and compost promote carbon stored in soil more than only inorganic fertilizer application (PK and NPK). The incubation experiment also showed the RS and CM applications reduced De-C/Min-N ratio indicated easier decomposable matters. Additionally, $\delta^{13}C$ values of soil decreased in all treatments. More rapid decrease of $\delta^{13}C$ value was found in rice straw and compost application. Our result was reconfirmed by previous works (Bol et al., 2005; Senbayram et al., 2008).

The change of $\delta^{15}N$ value, as we observed, was unstable and unpredictable. However, it is obvious that RS material tend to decrease $\delta^{15}N$ value, while CM increases the value of $\delta^{15}N$. Therefore, the general effect from RS and CM as well as mineral fertilizer, is as our expectation, and confirmed as previous study (Choi et al., 2017). The $\delta^{15}N$ value of NPK fertilizer in this study was 0.28, which is similar to common synthetic nitrogen fertilizers, normally ranged in -2 to 2‰ (Bateman and Kelly, 2007). The effects of fertilizer combined RS and CM was predicted to be the main factors decide the soil $\delta^{15}N$. However, the shift of $\delta^{15}N$ in this experiment left us further unclear mechanism.

5.3. Hot water and water-extracted carbon after long-term applied rice straw and compost affected on amount of EOC, but unclear on their $\delta^{13}C$ values

Carbon is one of the most important organic elements for plant growth and it also greatly determines soil sustainability and environmental impacts. Our previous study in the same LTEs soil showed that SOC in air dry soil increased with organic matter application (Cheng et al. 2017), confirming the same results with other researches (Ding et al. 2014; Menšík et al. 2018). However, there are limited researches on labile carbon pools from LTEs, which contains an important source of nutrients for microorganisms in soil (Marschner and Bredow 2002). For tracing the sources and dynamics of SOC, natural stable carbon isotope ($\delta^{13}C$) is widely used. The change in $\delta^{13}C$ in soils and soil solutions are based on the C source and its discriminations during the cycling processes (Yoneyama et al. 2001; Werth and Kuzyakov 2010). Although many LTEs research on C and N dynamics, there is no report regarding to their $\delta^{13}C$ in rice paddy soil.

Our findings showed that the amounts of EC and EN have high correlation with total bulk soil C and N, as well as C decomposition and N mineralization potentials. This result suggested that labile pools of SOM in long-term period could be considered as an indicator of soil quality. Interestingly, the $\delta^{13}C$ of HWEOC and WEOC didn't alter slowly as happened in bulk soil. As a result, the source is pointed to the organic matter application (rice straw and compost). Therefore, $\delta^{13}C$ in different pools, especially in labile pools remain further studied.

5.4. Conclusions

The responses of, rice yield and SOM decomposition to long-term fertilization application associated with, climate changes are briefly concluded as below:

1) Temperatures in Yamagata increased significantly in the last three decades, and strongly in rice growing period. The warming weather enhanced rice yield, confirmed by summer season as well as two growing stages (TP-HD and HD-MT period).

2) Long-time application of RS and compost enhance rice yield, as well as SOC and TN storage in paddy soil, as well as increasing soil fertility.

3) Hot water and water extracted C and N could present high correlation with the SOC, TN of bulk soil in long-term. Therefore, studies on different SOM pools (labile and stable pools) should be paid more attention to understand the long-term response of C storage to climate change.

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Nguyen Sy Toan

Tsuruoka City, Yamgata Prefecture, Japan

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References

Azuma, H., Uezono, I., Nohara, S., S, T., and N, K. (2015). Rapid method for estimating nitrogen in air-dried paddy soils based on the total organic carbon (TOC) extracted from oven-dried soils shaken in water. *Soil Science and Plant Nutrition*, 10.

Balesdent J, Wagner GH, and A, M. (1988). Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. *Soil Sci. Soc. Am*, 7.

Bateman, A. S., and Kelly, S. D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes Environ Health Stud* **43**, 237-47.

Bol, R., Eriksen, J., Smith, P., Garnett, M. H., Coleman, K., and Christensen, B. T. (2005). The natural abundance of 13C, 15N, 34S and 14C in archived (1923-2000) plant and soil samples from the Askov long-term experiments on animal manure and mineral fertilizer. *Rapid Commun Mass Spectrom* **19**, 3216-26.

Chantigny, M. H., Harrison-Kirk, T., Curtin, D., and Beare, M. (2014). Temperature and duration of extraction affect the biochemical composition of soil water-extractable organic matter. *Soil Biology and Biochemistry* **75**, 161-166.

Cheng, W., Padre, A. T., Sato, C., Shiono, H., Hattori, S., Kajihara, A., Aoyama, M., Tawaraya, K., and Kumagai, K. (2016a). Changes in the soil C and N contents, C decomposition and N mineralization potentials in a rice paddy after long-term application of inorganic fertilizers and organic matter. *Soil Science and Plant Nutrition* **62**, 212-219.

Cheng, W., Padre, A. T., Shiono, H., Sato, C., Nguyen-Sy, T., Tawaraya, K., and Kumagai, K. (2016b). Changes in the pH, EC, available P, SOC and TN stocks in a single rice paddy after long-term application of inorganic fertilizers and organic matters in a cold temperate region of Japan. *Journal of Soils and Sediments* **17**, 1834-1842.

Cheng, W., Yagi, K., Akiyama, H., Nishimura, S., Sudo, S., Fumoto, T., Hasegawa, T., Hartley, A. E., and Megonigal, J. P. (2007). An empirical model of soil chemical properties that regulate methane production in Japanese rice paddy soils. *In* "J Environ Qual", Vol. 36, pp. 1920-5.

Cheng, W., Yagi, K., Sakai, H., and Kobayashi, K. (2006). Effects of Elevated Atmospheric CO2 Concentrations on CH4 and N2O Emission from Rice Soil: An Experiment in Controlled-environment Chambers. *Biogeochemistry* **77**, 351-373.

Choi, W.-J., Kwak, J.-H., Lim, S.-S., Park, H.-J., Chang, S. X., Lee, S.-M., Arshad, M. A., Yun, S.-I., and Kim, H.-Y. (2017). Synthetic fertilizer and livestock manure differently affect δ 15 N in the agricultural landscape: A review. *Agriculture, Ecosystems & Environment* **237**, 1-15.

Curtin, D., Wright, C. E., Beare, M. H., and McCallum, F. M. (2006). Hot Water-Extractable Nitrogen as an Indicator of Soil Nitrogen Availability. *Soil Science Society of America Journal* **70**, 1512.

Dorrepaal, E., Aerts, R., Lissen, J. H. C. C., Callaghanw, T. V., and Logtestijn, R. S. P. V. (2003). Summer warming and increased winter snow cover affect Sphagnum fuscum growth, structure and production in a sub-arctic bog. *Global Change Biology* **10**, 93-105.

FAO (2017). FAOSTAT database. http://faostat.fao.org/. Vol. 2017.

Fitzgerald, M. A., and Resurreccion, A. P. (2009a). Maintaining the yield of edible rice in a warming world. *Functional Plant Biology* **36**, 1037-1045.

Fitzgerald, M. A., and Resurreccion, A. P. (2009b). Maintaining the yield of edible rice in a warming world. *Functional Plant Biology* **36**, 9.

Folland, C. K., and Karl, T. R. (2001). Observed Climate Variability and Change. 84.

Fukui, S., Ishigooka, Y., Kuwagata, T., and Hasegawa, T. (2015). A methodology for estimating phenological parameters of rice cultivars utilizing data from common variety trials. *Journal of Agricultural Meteorology* **71**, 77-89.

Ghadirnezhad, R., and Fallah, A. (2014). Temperature Effect on Yield and Yield Components of Different Rice Cultivars in Flowering Stage. *International Journal of Agronomy* **2014**, 1-4.

Ghani, A., Dexter, M., and Perrott, K. W. (2003). Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology and Biochemistry* **35**, 1231-1243.

Guigue, J., Lévêque, J., Mathieu, O., Schmitt-Kopplin, P., Lucio, M., Arrouays, D., Jolivet, C., Dequiedt, S., Chemidlin Prévost-Bouré, N., and Ranjard, L. (2015). Water-extractable organic matter linked to soil physico-chemistry and microbiology at the regional scale. *Soil Biology and Biochemistry* **84**, 158-167.

Guigue, J., Mathieu, O., Lévêque, J., Mounier, S., Laffont, R., Maron, P. A., Navarro, N., Chateau, C., Amiotte-Suchet, P., and Lucas, Y. (2014). A comparison of extraction procedures for water-extractable organic matter in soils. *European Journal of Soil Science* **65**, 520-530.

Hansen, J., Ruedy, R., Sato, M., and Lo, K. (2010a). Global Surface Temperature Change. *Reviews of Geophysics* **48**.

Hansen, J., Ruedy, R., Sato, M., and Lo, K. (2010b). Global Surface Temperature Change. *Reviews of Geophysics* **48**, 1-29.

Hatfield, J. L., and Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes* **10**, 4-10.

Iizumi, T., Nishimori, M., and Yokozawa, M. (2010a). Diagnostics of Climate Model Biases in Summer Temperature and Warm-Season Insolation for the Simulation of Regional Paddy Rice Yield in Japan. *Journal of Applied Meteorology and Climatology* **49**, 574-591.

Iizumi, T., Yokozawa, M., and Nishimori, M. (2010b). Probabilistic evaluation of climate change impacts on paddy rice productivity in Japan. *Climatic Change* **107**, 391-415.

Iqbal, T. (2016). Rice straw amendment ameliorates harmful effect of salinity and increases nitrogen availability in a saline paddy soil. *Journal of the Saudi Society of Agricultural Sciences*.

Ishimaru, T., Hasegawa, T., Kondo, M., Kuwagata, T., Yoshimoto, M., and Fukuoka, M. (2011a). Spikelet sterility of rice observed in the record hot summer of 2007 and the factors associated with its variation. *Agric. Meteorol* **67**, 225-232.

Ishimaru, T., Hasegawa, T., Kondo, M., Kuwagata, T., Yoshimoto, M., and Fukuoka, M. (2011b). Spikelet sterility of rice observed in the record hot summer of 2007 and the factors associated with its variation. *Agric. Meteorol* **67**, 8.

Ishimaru, T., Hirabayashi, H., Sasaki, K., Ye, C., and Kobayashi, A. (2016). Breeding efforts to mitigate damage by heat stress to spikelet sterility and grain quality. *Plant Production Science* **19**, 12-21.

Jing, L., and Jichao, Y. (2012). Research Progress in Effects of Different Altitude on Rice Yield and Quality in China. *Greener Journal of Agricultural Sciences* **2**, 340-344.

Körschens, M. (2006). The importance of long-term field experiments for soil science and environmental research – a review. *PLANT SOIL ENVIRON* **52**, 1–8.

Kim, H. Y., Ko, J., Kang, S., and Tenhunen, J. (2013). Impacts of climate change on paddy rice yield in a temperate climate. *Glob Chang Biol* **19**, 548-62.

Koyama, S., and Hayashi, H. (2017). Rice yield and soil carbon dynamics over three years of applying rice husk charcoal to an Andosol paddy field. *Plant Production Science* **20**, 176-182.

Kurihara, K., Takayabu, I., Ishihara, K., Murazaki, K., Sasaki, H., Sato, Y., Fukuyama, Y., Saitou, H., Yukimoto, S., and Noda, A. (2005a). Projection of Climatic Change over Japan Due to Global Warming by High-Resolution Regional Climate Model in MRI. *SOLA,* **Vol. 1**, 4.

Kurihara, K., Takayabu, I., Ishihara, K., Murazaki, K., Sasaki, H., Sato, Y., Fukuyama, Y., Saitou, H., Yukimoto, S., and Noda, A. (2005b). Projection of Climatic Change over Japan Due to Global Warming by High-Resolution Regional Climate Model in MRI. *SOLA,* **Vol. 1**, 97-100.

Li, S., Li, J., Li, C., Huang, S., Li, X., Li, S., and Ma, Y. (2016). Testing the RothC and DNDC models against long-term dynamics of soil organic carbon stock observed at cropping field soils in North China. *Soil and Tillage Research* **163**, 290-297.

Lobell, D. B., and Field, C. B. (2007a). Global scale climate–crop yield relationships and the impacts of recent warming. *Environmental Research Letters* **2**, 1-8.

Lobell, D. B., and Field, C. B. (2007b). Global scale climate–crop yield relationships and the impacts of recent warming. *Environmental Research Letters* **2**, 014002.

Mahmoud, E., Ibrahim, M., Robin, P., Akkal-Corfini, N., and El-Saka, M. (2009). Rice Straw Composting and Its Effect on Soil Properties. *Compost Science & Utilization* **17**, 146-150.

Marschner, B., and Bredow, A. (2002). Temperature effects on release and ecologically relevant properties of dissolved organic carbon in sterilized and biologically active soil samples. *Soil Biol. Biochem* **34**, 459– 466.

Matsui, T., Kobayasi, K., Yoshimoto, M., and Hasegawa, T. (2015a). Stability of Rice Pollination in The Field Under Hot And Dry Conditions in The Riverina Region of New South Wales, Australia. *Plant Production Science* **10**, 57-63.

Matsui, T., Omasa, K., and Horie, T. (2015b). The Difference in Sterility due to High Temperatures during the Flowering Period among Japonica-Rice Varieties. *Plant Production Science* **4**, 90-93.

Moriizumi, M., and Matsunaga, T. (2011). Molecular weight separation of hot-water extractable soil organic matter using high-performance size exclusion chromatography with chemiluminescent nitrogen detection. *Soil Science and Plant Nutrition* **57**, 185-189.

Morita, S., Wada, H., and Matsue, Y. (2016). Countermeasures for heat damage in rice grain quality under climate change. *Plant Production Science* **19**, 1-11.

Nagai, T., and Makino, A. (2009). Differences between rice and wheat in temperature responses of photosynthesis and plant growth. *Plant Cell Physiol* **50**, 744-55.

Nakajima, M., Cheng, W., Tang, S., Hori, Y., Yaginuma, E., Hattori, S., Hanayama, S., Tawaraya, K., and Xu, X. (2016). Modeling aerobic decomposition of rice straw during the off-rice season in an Andisol paddy soil in a cold temperate region of Japan: Effects of soil temperature and moisture. *Soil Science and Plant Nutrition* **62**, 90-98.

Nakanishi, T., Atarashi-Andoh, M., Koarashi, J., Saito-Kokubu, Y., and Hirai, K. (2012). Carbon isotopes of water-extractable organic carbon in a depth profile of forest soil imply a dynamic relationship with soil carbon. *European Journal of Soil Science* **63**, 495-500.

Nishida, M., Iwaya, K., Sumida, H., and Kato, N. (2007). Changes in natural15N abundance in paddy soils under different, long-term soil management regimes in the Tohoku region of Japan. *Soil Science and Plant Nutrition* **53**, 310-317.

Nishida, M., and Sato, Y. (2015). Areal variation in the relationship between natural15N abundances of rice (Oryza sativaL.) and soil. *Soil Science and Plant Nutrition* **62**, 48-51.

Peltre, C., Christensen, B. T., Dragon, S., Icard, C., Kätterer, T., and Houot, S. (2012). RothC simulation of carbon accumulation in soil after repeated application of widely different organic amendments. *Soil Biology and Biochemistry* **52**, 49-60.

Rasmussen, P. E., Goulding, K. W. T., Brown, J. R., Grace, P. R., Janzen, H. H., and Korschens, M. (1998). Long-Term Agroecosystem Experiments: Assessing Agricultural Sustainability and Global Change. *SCIENCE* **282**, 893-896.

Richter, D. d., Hofmockel, M., Callaham, M. A., Powlson, D. S., and Smith, P. (2007). Long-Term Soil Experiments: Keys to Managing Earth's Rapidly Changing Ecosystems. *Soil Science Society of America Journal* **71**, 266.

Rillig, M. C., Caldwell, B. A., Wösten, H. A. B., and Sollins, P. (2007). Role of proteins in soil carbon and nitrogen storage: controls on persistence. *Biogeochemistry* **85**, 25-44.

Ros, G. H., Hoffland, E., van Kessel, C., and Temminghoff, E. J. M. (2009). Extractable and dissolved soil organic nitrogen – A quantitative assessment. *Soil Biology and Biochemistry* **41**, 1029-1039.

Senbayram, M., Dixon, L., Goulding, K. W., and Bol, R. (2008). Long-term influence of manure and mineral nitrogen applications on plant and soil 15N and 13C values from the Broadbalk Wheat Experiment. *Rapid Commun Mass Spectrom* **22**, 1735-40.

Shen, Y., and Kobayashi, K. (2017). Responses of spring phenology in a fruit tree species (Pyrussp. cv. Pingguoli) to the changes in surface air temperature in Northeast China. *International Journal of Climatology* **37**, 2757-2764.

Shirato, Y., Hakamata, T., and Taniyama, I. (2004). Modified rothamsted carbon model for andosols and its validation: changing humus decomposition rate constant with pyrophosphate-extractable Al. *Soil Science and Plant Nutrition* **50**, 149-158.

Shirato, Y., and Taniyama, I. (2003). Testing the suitability of the Rothamsted Carbon model for long-term experiments on Japanese non-volcanic upland soils. *Soil Science and Plant Nutrition* **49**, 921-925.

Shirato, Y., Yagasaki, Y., and Nishida, M. (2011). Using different versions of the Rothamsted Carbon model to simulate soil carbon in long-term experimental plots subjected to paddy–upland rotation in Japan. *Soil Science and Plant Nutrition* **57**, 597-606.

Soler, M., Ruiz-Raya, F., Carra, L. G., Medina-Molina, E., lamo, J. D. I.-A., and Martın-Galvez, D. (2014). A Long-Term Experimental Study Demonstrates the Costs of Begging That Were Not Found over the Short Term. *PLoS ONE* **9**.

Sparling, G., Vojvodic´-Vukovic´M, and LA, S. (1998). Hot-water-soluble C as a simple measure of labile soil organic matter: The relationship with microbial biomass C. *Soil Biol. Biochem*, 1469.

Thord Karlsson, L. O., Andrén, O., Kätterer, T., and Mattsson, L. (2003). Management effects on topsoil carbon and nitrogen in Swedish long-term field experiments—budget calculations with and without humus pool dynamics. *European Journal of Agronomy* **20**, 137-147.

Tsukaguchi, T., and Iida, Y. (2015). Effects of Assimilate Supply and High Temperature during Grain-Filling Period on the Occurrence of Various Types of Chalky Kernels in Rice Plants (Oryza sativaL.). *Plant Production Science* **11**, 203-210.

van Asten, P. J. A., van Bodegom, P. M., Mulder, L. M., and Kropff, M. J. (2005). Effect of Straw Application on Rice Yields and Nutrient Availability on an Alkaline and a pH-neutral Soil in a Sahelian Irrigation Scheme. *Nutrient Cycling in Agroecosystems* **72**, 255-266.

Wada, E. (1952). Studies on the response of heading to day-length and temperature in rice plants. *Japanese Journal of Breeding* **2**, 55-72.

Watanabe, T., Man, L. H., Vien, D. M., Khang, V. T., Ha, N. N., Linh, T. B., and Ito, O. (2009). Effects of continuous rice straw compost application on rice yield and soil properties in the Mekong Delta. *Soil Science and Plant Nutrition* **55**, 754-763.

Welch, J. R., Vincent, J. R., Auffhammer, M., Moyae, P. F., Dobermann, A., and Daweg, D. (2010). Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *PNAS* **107**, 14562–14567.

Weng, J. H., and Chen, C. Y. (1987). Differences between Indica and Japonica rice varieties in CO2 exchange rates in response to leaf nitrogen and temperature. *Photosynthesis Research*, 171-177.

Werth, M., and Kuzyakov, Y. (2010). 13C fractionation at the root–microorganisms–soil interface: A review and outlook for partitioning studies. *Soil Biology and Biochemistry* **42**, 1372-1384.

Yoneyama, T., Nakanishi, Y., Morita, A., and Liyanage, B. C. (2001). δ13C values of organic carbon in cropland and forest soils in Japan. *Soil Science and Plant Nutrition* **47**, 17-26.

Yoshida, S. (1973). Effects of temperature on growth of the rice plant (Oryza sativaL.) in a controlled environment. *Soil Science and Plant Nutrition* **19**, 299-310.

Zakaria, S., Matsuda, T., Tajima, S., and Nitta, Y. (2015). Effect of High Temperature at Ripening Stage on the Reserve Accumulation in Seed in Some Rice Cultivars. *Plant Production Science* **5**, 160-168.

Zhao, B., Maeda, M., and Ozaki, Y. (2002). Natural15N and13C abundance in Andisols influenced by long-term fertilization management in Japan. *Soil Science and Plant Nutrition* **48**, 555-562.