THE RESPONSES OF SOIL ORGANIC CARBON AND NITROGEN CONTENTS, AND THEIR MINERALIZATION TO GLOBAL WARMING IN RICE PADDY ECOSYSTEMS

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THE RESPONSES OF SOIL ORGANIC CARBON AND NITROGEN CONTENTS, AND THEIR MIENARLIZATION TO GLOBAL WARMING IN RICE PADDY ECOSYSTEMS

水田生態系における土壌炭素 窒素及びそれらの無機化に及ぼす地球温暖化の影響

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Contents

Abstract

Global warming is caused by increasing concentrations of greenhouse gases in the atmosphere, such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide by anthropogenic activities, including intensive agriculture. Rice paddy is worldwide considered as a major source of atmospheric CH_4. Nitrogen (N) is an essential element for plant growth and N cycling in paddy soil is also greatly affected by global warming. Soil organic carbon (SOC) decomposition and N mineralization are closely coupled with each other and would be influenced by climate change and in turn affect C and N cycling in the terrestrial ecosystems. However, so far, the impacts of climate change such as global warming and elevated atmospheric CO_2 concentration ([CO_2]) on SOC and total N (TN) contents, and their mineralization have still not been fully understood in rice paddy ecosystems. Therefore, three level experiments from indoor simulation (via aerobic and anaerobic incubation) to open-field manipulation were conducted in this study.

In the first experiment, Andisol and Inceptisol paddy soils collected from Morioka and Yamagata were firstly pre-incubated at 25 °C and 40% water-filled pore space (WFPS) for four weeks, then aerobically incubated under four temperature (±5, 5, 15 and 25 °C) and two soil moisture (60% and 100% WFPS) conditions (simulating field condition in Tohoku region, Japan during off-rice season) for 24 weeks, and finally anaerobically incubated at 30 °C and under waterlogged conditions (modeling

field condition during following rice growth season) for four weeks. The main objective of this experiment was to simulate the effects of soil temperature and moisture condition on SOC decomposition and N mineralization during off-rice season, and the subsequent CH_4 production during rice growth season in Tohoku region, Japan. The results showed that both aerobic and anaerobic SOC decomposition and N mineralization in Andisol and Inceptisol were significantly affected by soil temperature. However, the significantly stimulated effect of soil moisture on aerobic N mineralization was only found in Andisol probably owing to its higher inorganic N (NH_4^+-N + NO_3^--N) concentration compared with Inceptisol. After 24-week aerobic incubation, CH_4 production during the subsequently anaerobic incubation was quite low and decreased with the increase of previous soil temperature during aerobic incubation. This result implied that high soil temperature and medium moisture during off-rice season would favorable to mitigate CH_4 emission in the following rice growth season. Despite high SOC and TN contents in Andisol, it had less liable C and N contents observed by the ratios of decomposed C (CO_2 + CH_4) to SOC, and mineralized N (NH_4^+-N production) to TN, compared with Inceptisol. These results suggested that the C and N in Andisol were more stable than Inceptisol with less biodegradability.

In the second experiment, a 5-year continuous soil warming experiment during both rice growth (+2 °C above control temperature (CT)) and off-rice (+1 °C above CT) seasons was carried out in a single rice paddy field in Tsukuba, Japan to

investigate the effect of soil warming on rice biomass, SOC and TN contents, and their mineralization in single rice paddy ecosystem. Soil samples were collected bi-yearly in autumn after rice growth season, and in spring after fallow season, respectively. The 5-year results showed that rice aboveground biomass and root biomass were not significantly increased by soil warming probably due to the decreased N availability expressed by anaerobic NH_4^+-N production. However, soil warming significantly decreased SOC and TN contents, C decomposition and N mineralization potentials obtained from 4-week directly anaerobic incubation of air-dried soil samples at 30 °C and under submerged conditions. This result indicated that soil warming had a trend to decrease SOC and TN contents in rice paddy field, leading to a positive feedback of soil organic matter (SOM) decomposition to future climate change. The annual decreased trend of SOC and TN contents in this study might be mainly ascribed to yearly removal of rice straw after rice harvest. The seasonal variations of SOC and TN contents could be explained by the enhanced C input via plant photosynthesis during rice growth season and accelerated rate of SOM decomposition during off-rice season. After the annual and seasonal soil samples were pre-incubated at 25 °C and 40% WFPS for four weeks, CH_4 production during subsequently anaerobic incubation could be ignored compared with CO_2 production, which might be explained by rapid depletion of labile substrates and altered soil microbial communities. Also, the effect of soil warming on C and N mineralization potentials was significantly eliminated by pre-incubation.

In the third experiment, an open-field 5-year experiment combined with elevated [CO_2] (+200 ppm above the ambient [CO_2] treatment) and elevated soil temperature (+2 °C above CT) during rice growth season was conducted in a single rice paddy field in Tsukuba-FACE site, Japan since 2010 to estimate the interactive effects of elevated soil temperature and atmospheric [CO_2] on amount and components of SOM in rice paddy soil. Due to large variations in soil layers and replicate soil sampling sites, the really combined effects of elevated [CO_2] and soil temperature on C decomposition and N mineralization could not be statistically qualified in this study. As a whole, the increased trend of averaged SOC and TN contents induced by elevated [CO_2] was observed in the first soil layer (0-10 cm). Contrary to elevated [CO_2], soil temperature had a trend to decrease SOC and TN contents in the first soil layer. SOC decomposition and N mineralization potentials were also increased by elevated [CO_2] but decreased by elevated soil temperature. The fraction of new plant-derived C into paddy soil due to elevated [CO_2] under control and elevated soil temperature conditions, calculated by $\delta^{13}C$ values of decomposition C (CO_2+CH_4 productions) was both 34.0% in first soil layer under two temperature conditions. In the second soil layer (10-20 cm), it was 18.1% in control soil temperature and 16.4% in elevated soil temperature. This result indicated that plant-derived decomposable C in the soils was not affected by elevated soil temperature.

In conclusion, these results in this study showed that both aerobic and subsequently anaerobic SOC decomposition and N mineralization were significantly

affected by soil temperature and moisture under aerobic incubation conditions. Soil warming significantly decreased SOC and TN contents in plow soil layer observed by stimulated C decomposition and N mineralization potentials, and insignificantly increased rice biomass in rice paddy ecosystem, thus, probably leading to a positive feedback of SOM decomposition to future climate change. Elevated [CO_2] had a trend to enhance SOC and TN contents larger than their mineralization resulting in an increased SOC stocks and sequestration in rice paddy ecosystem. The combined effects of soil warming and [CO_2] on SOM amount and components should be further studied under different rice paddy field conditions.

日本語要旨

集約農業を含む人為活動によって大気中における二酸化炭素（CO_2）、メタン（CH_4）、亜酸化窒素などの温室効果ガスの濃度上昇が地球温暖化を引き起こしている。水田は、CH_4 の主要な放出源である。窒素は植物成長のために必要な元素であり、また土壌中の窒素循環も地球温暖化に影響される。土壌中における有機態炭素（SOC）の分解と窒素無機化は、互いに密接に連動し、気候変動に影響されて、陸域生態系における炭素・窒素の循環に影響を与える。しかしながら、地球温暖化と大気中 CO_2 濃度上昇が、水田生態系における炭素・窒素およびそれらの無機化にどのように影響を与えるかに関してはまだ不明である。従って、本研究では、室内のモデル実験、開放的な水田で温度上昇ならびに温度と CO_2 濃度の同時上昇の野外圃場において、水田生態系における土壌炭素・窒素およびそれらの無機化に及ぼす地球温暖化の影響に関する実験を行った。

まず、室内のモデル実験においては、東北地方の盛岡市と山形市にある黒ボク土と灰色低地土の水田圃場から採取した土壌を、25℃と WFPS 40%の水分条件で 4 週間前培養した後、それぞれ温度 4 段階（±5, 5, 15, 25℃）と水分 2 段階（WFPS の 60, 100%）の異なる処理を行い、24 週間好気的に培養し、その後それらのサンプルを 30℃、湛水条件下で 4 週間嫌気的に培養した。東北地方の水田圃場において、休耕期間における土壌有機物の分解とイネ生育期間中における CH_4 生成が、土壌温度と水分にどのように影響されるかをモデル

実験で明らかにするのは、本室内実験の主要な目的であった。その結果、土壌温度と水分は、好気条件とそれに続いての嫌気条件でも、２種類土壌の SOC 分解に与える影響は有意であったが、窒素無機化に与える影響は、黒ボク土と灰色低地土の間で異なった。好気培養後の嫌気的な培養では、好気培養時の温度が高ければ高いほど、有機物分解からの CH_4 生成量が低かった。このことにより、休耕期の高気温と適切な土壌水分は、稲生育期間中に土壌有機物由来の CH_4 排出量を抑制することが示唆される。また、炭素分解量と SOC 量および窒素無機化量と全窒素量（TN）の割合は、灰色低地土より黒ボク土のほうが低かったことから、黒ボク土の土壌有機物の安定性が灰色低地土より高かった。

次に、つくば市にある農業環境研究所内水田に、4 反復の温暖化区と対照区を設け、単作稲作水田生態系における稲の生育、土壌 SOC と窒素およびそれらの無機化に及ぼす土壌温暖化の影響を調査するために、水田生育期間（湛水期、対照温度（CT）+ 2℃以上）と休耕期間（落水期、CT + 1℃以上）に土壌加温実験を 5 年間継続して行った。土壌サンプルは毎年に水稲の収穫後の秋と休耕期の後の春に 2 度採集した。その結果、稲の地上部バイオマスと根バイオマスは、温暖化の影響が見られなかった。しかし、温暖化は SOC と TN を有意に減少させ、また、30℃・湛水条件、4 週間で測定した炭素分解量および窒素無機量も有意に減少させた。また、SOC と TN は、年々減少傾向にあり、収獲後の稲わらをすべて持ち出すことによるものだと考えられる。季節性の SOC と TN 含量の変動は、水稲生育期間中における植物光合成を介した炭素の

インプットと、休耕期間中における SOC の分解を介した炭素のアウトプットによるものと考えられる。さらに 25℃・40% WFPS で 4 週間前培養を行った風乾土壌サンプルは、前培養を行わない土壌サンプルと比べると、土壌炭素分解量と窒素無機量に及ぼす温暖化の影響は、前培養より消失されたことを明らかにした。

そして、実験 3 では、つくばみらい FACE 実験施設において、土壌温度と大気 CO_2 濃度の同時上昇が、水田土壌中有機物の量と質にどのように影響を与えるかを調べるため、実施 5 年後の各処理区の跡土壌を採集し分析を行った。イネ生育期間中の土壌温度と大気 CO_2 濃度の上昇幅は、対照区より 2℃と 200ppm であった。土壌層および土壌のサンプリング場所変動が大きかったため、本研究において炭素の分解と窒素無機化に対する CO_2 と土壌温度の上昇の実際の複合効果が統計的に特定できなかった。その結果、全体傾向としては、土壌表層（0-10cm）の SOC と TN 含量は CO_2 の濃度上昇によって増加し、土壌温度上昇によって減少した。炭素分解量と窒素無機化量に及ぼす温度と CO_2 の濃度上昇の影響は、SOC と TN 含量と同様であった。大気 CO_2 濃度上昇処理区においては、$\delta^{13}C$ 値を用いて計算した易分解炭素量（嫌気培養実験から生成された CO_2 と CH_4 の総量）のうち、新しい植物由来の炭素画分は二つの温度条件下でどちらも 0-10cm の表層で 34.0%であった。10-20 cm の表下層においては、対照温度区は 18.1%、温度上昇区は 16.4%であった。この結果より、稲植物から土壌への炭素転流は、温度上昇に及ぼす影響がなかったことが示唆された。

以上３つの実験結果をまとめると、好気培養をする時の土壌温度と水分は、好気的及びその後の嫌気的な SOC の分解に有意に影響を与えることを示唆した。温度上昇は、単作稲作水水田土壌中の SOC と TN 含量および炭素分解量と窒素無機化量を有意に減少させ、将来の気候変動に正のフィードバックをもたらす恐れがあり、一方、大気 CO_2 の濃度上昇は、炭素の分解と窒素無機化より、SOC と TN 含量を増加させる傾向があり、水田生態系における炭素の貯蔵を増加させると考えられる。単作稲作水田と異なる水田生態系における土壌有機物に及ぼす温度と大気 CO_2 濃度の上昇の影響は、今後更なる研究が必要である。

中文摘要

包括密集农业在内的人类活动所导致大气中，二氧化碳（CO_2）、甲烷（CH_4）和氧化亚氮等温室气体浓度的不断上升，是当今全球变暖的重要原因。稻田生态系统是大气中 CH_4 排放的重要来源之一。氮素（N）是作物生长的必需元素之一。稻田生态系统中，氮素的循环也会受到全球变暖的很大影响。土壤有机碳的分解与氮素的矿化过程，两者往往紧密联系，且相互影响；同时，也易受全球气候变化的影响。全球气候变化对氮素矿化的影响会进一步影响到陆地生态系统中碳、氮循环。然而，关于以全球变暖和大气中 CO_2 浓度升高为典型的气候变化对稻田土壤有机碳、氮的含量及其矿化过程影响的研究，目前还不是十分的明确。为此，本研究设计了从室内好氧和厌氧培养到田间模拟的不同层次的三个实验，来探讨水田生态系统中土壤有机碳、氮含量及其矿化过程对气候变化的响应规律。

在第一个实验中，火山灰土和始成土发育的两种水稻土（以下简称火山灰土和始成土）分别采集于日本岩手县盛岗市和山形县山形市。风干后的两种水稻土先在 25°C 和 40% 土壤充水孔隙度（WFPS）的条件下预培养 4 周，然后在四种培养温度（±5、5、15 和 25°C）和两种水分（60% 和 100% WFPS）条件下好氧培养 24 周，最后在淹水和 30°C 的条件下厌氧培养 4 周。好氧和厌氧培养下的温度和水分条件分别是模拟日本东北地区水稻休闲期和生长期间的温度和水分条件。本研究的主要目的在于模拟土壤温度和水分对休闲期间稻田土壤有机碳的分解和氮素的矿化过程以及对后续生长期间 CH_4 产生的影响。本研究的结果表明，

在好氧和厌氧培养期间，有机碳的分解和氮素的矿化均受到土壤温度的显著影响。然而，土壤水分只对火山灰土中氮素的好氧矿化有显著的促进作用，原因可能为火山灰土中无机态氮素（铵态氮和硝态氮）含量比始成土中的要高。后续厌氧培养过程中所产生的 CH_4 含量很低，且随前期 24 周好氧培养中土壤温度的升高而显著下降。该结果暗示了休闲期间，升高土壤温度有助于减少来年水稻生长期间稻田土壤中 CH_4 的排放。尽管火山灰土中有机碳、氮的含量比始成土的要高，但是易分解（矿化）碳、氮的含量（分别以 CO_2 和 CH_4 生成量和铵态氮的生成量表示）占整个有机碳和总氮的比例却比始成土中的要低。这表明火山灰土中有机碳、氮要比始成土中的要更稳定，具备更低的生物降解有效性。

在第二个实验中，我们在日本的筑波市开展了长达 5 年的单一种植水稻的生长期和休闲期间，持续加温稻田土壤的田间实验。与常规土壤温度处理相比，加温处理中，土壤温度分别在水稻生长期和休闲期间升高了 2°C 和 1°C。本研究的目的在于探讨稻田土壤温暖化对水稻生物量（地上和根部（地下））、有机碳、氮含量以及其矿化潜势的影响。每年，稻田土壤分别于水稻生长期后的秋季和休闲期的春季采集。5 年土壤温暖化的实验结果显示，水稻地上和根部的生物量均不受土壤温暖化的显著影响，原因可能为土壤温度的升高使得厌氧培养过程中，以铵态氮生成量为标志的土壤氮素的生物有效性降低。然而，土壤温暖化却显著地降低了有机碳、氮的含量。同时，土壤温暖化也使得在淹水和 30°C 的条件下，直接用风干土进行 4 周厌氧培养所观测到的矿化潜势降低。该结果表明，土壤温暖化使稻田土壤有机碳、氮含量有降低的趋势，从而会造成有机质的分解对未来

的气候变化产生正的反馈效应。水稻收获后，水稻秸秆直接从田间移除，这可能是造成稻田土壤有机碳、氮含量的年际变化不断降低的原因。有机碳、氮的季节性变化可以通过土壤温暖化，一方面使在水稻生长期间植物光合作用的加强，使得土壤有机质的含量升高；另一方面使水稻休闲期有机质分解速率的加快，这两方面来进行解释。此外，我们还对稻田土壤开展了，先在 25°C 和 40%条件下进行预培养，然后进行厌氧培养的实验。结果显示，与直接进行的厌氧培养实验相比，预培养结束后再进行厌氧培养过程后的稻田土壤中，所产生的 CH_4 含量极低，可以被忽略不计。原因可能为预培养过程使得易分解的底物被大量的消耗，同时也会使土壤微生物的群落发生相应的改变。预培养过程也可能会抵消土壤温暖化对稻田有机碳、氮矿化过程造成的显著影响。

在第三个实验中，我们在日本的筑波市开展了从 2010 年开始连续五年的在水稻生长期间，增加 CO_2 浓度（比常规处理高 200 ppm）和土壤温度（比常规处理高 2°C）的田间实验。该实验的目的在于探讨同时升高土壤温度和 CO_2 浓度对稻田土壤中有机质含量以及组成的影响。由于土层和采样地点之间的较大差异，使得 CO_2 浓度和土壤温度的升高对有机碳分解以及氮素矿化的实际影响不能从统计上加以定性。但从总体上来说，CO_2 浓度的升高使得 0-10 cm 土层中有机碳、氮的含量具有升高的趋势。与此相反，土壤温度的升高使得第一层（0-10 cm）土样中有机碳、氮的含量有减少的趋势。有机碳的分解潜势和氮素的矿化潜势随 CO_2 浓度的升高而增大，却随土壤温度的升高而降低。我们用分解碳（CO_2 与 CH_4 生成量的总和）中的 $\delta^{13}C$ 值，计算了常规土壤温度和升高土壤温度的两种情

况下，因 CO_2 浓度的升高使得土壤获得从植物由来的、新的有机碳占总有机碳的比例。结果发现，两种土壤温度条件下，在第一层土样中该比例均为 34%；而在第二层（10-20 cm）土样中，该比例在常规土壤温度和对照土壤温度处理下分别为 18.1% 和 16.4%。该结果表明，稻田土壤中植物由来的土壤易分解有机碳含量不受土壤温度升高的影响。

总而言之，本研究的实验结果表明有机碳、氮的好氧和厌氧矿化过程均会受到好氧培养期间土壤温度和水分的显著影响。土壤温暖化会促进有机碳、氮的矿化潜势，但不会增加水稻的生物量，从而会使稻田土壤中有机碳、氮的含量显著下降。土壤温暖化会造成稻田土壤中有机质的分解对未来的气候变化产生正的反馈效应。CO_2 浓度的升高有促进稻田土壤有机碳、氮的含量及其矿化潜势的趋势。但对其含量的促进作用会比对其矿化的促进作用要大，因而会造成稻田生态系统中有机碳库的增加和对其截存能力的增强。土壤温度和 CO_2 浓度的升高对稻田生态生态系统中有机质含量和组成的交互作用还有待于在不同田间条件下进行更进一步的研究。

Abbreviations

Abbreviation	Full name
CH_4	Methane
CO_2	Carbon oxide
$[CO_2]$	Carbon oxide concentration
DOC	Dissolve organic carbon
EC	Electronic conductivity
FACE	Free-air CO_2 enrichment
F_{plant}	Fraction of new soil carbon input derived from plant
GHG	Greenhouse gas
GWP	Global warming potential
IPCC	Inter-governmental Panel on Climate Change
NH_4^+-N	Ammonium nitrogen
NO_3^--N	Nitrate nitrogen
N_2O	Nitrous oxide
NPP	Net primary production
ppm	Parts per million
Q_{10}	Temperature coefficient at 10 °C
SOC	Soil organic carbon
SOM	Soil organic matter
T-FACE	Elevated temperature and free-air CO_2 enrichment
TN	Total nitrogen
WFPS	Water-filled pore space
$\delta^{13}C$	The abundance of ^{13}C in per mil (‰) relative to stand sample (VPDB)
$\delta^{15}N$	The abundance of ^{15}N in per mil (‰) relative to stand sample (N_2)

Chapter Ⅰ General Introduction

1.1. Rice and paddy soil

Rice (*Oryza sativa* L.) is one of three kinds of primary food crops (wheat, rice and maize) in the world, supporting more than half of the world's population (IRRI, 2006). It is an annual grass that evolved from a semi-aquatic ancestor. Therefore, it is mostly grown under flooded lowland conditions throughout the growth season and approximately 90% is produced in Asian countries (e.g., China, India, and Indonesia). Rice growth is extremely sensitive to water shortage. It has been estimated that approximately 70% of water used in agriculture was consumed by rice production (Zhang, 2007). According to the data base in Food and Agriculture Organization of the United Nations (FAO), the global rice harvest area and yield during the period from 1961 to 2014 has increased from 115 to 162.7 million ha and from 1869.3 to 4556.9 kg ha^{-1}, respectively (Fig.1.1). Rice production has increased from 215.6 to 741.5 million tonnes in the world and from 198.8 to 667.0 million tonnes in Asia during the period from 1961 to 2014 (Fig. 1.2). It is evident that China and India are two countries having the largest rice plant areas, the most grain production, and highest amount of rice consumption mainly due to their huge population in the world. The percentage of rice produced in Japan in global rice production has decreased year by year from 2.5% in 1961 to 1.4% in 2014 (Fig. 1.2). Compared with other food

crops, rice is still a relatively attractive production option due to government encouragement, high yield and profitable income in despite of the recently stable production and area of rice in the world.

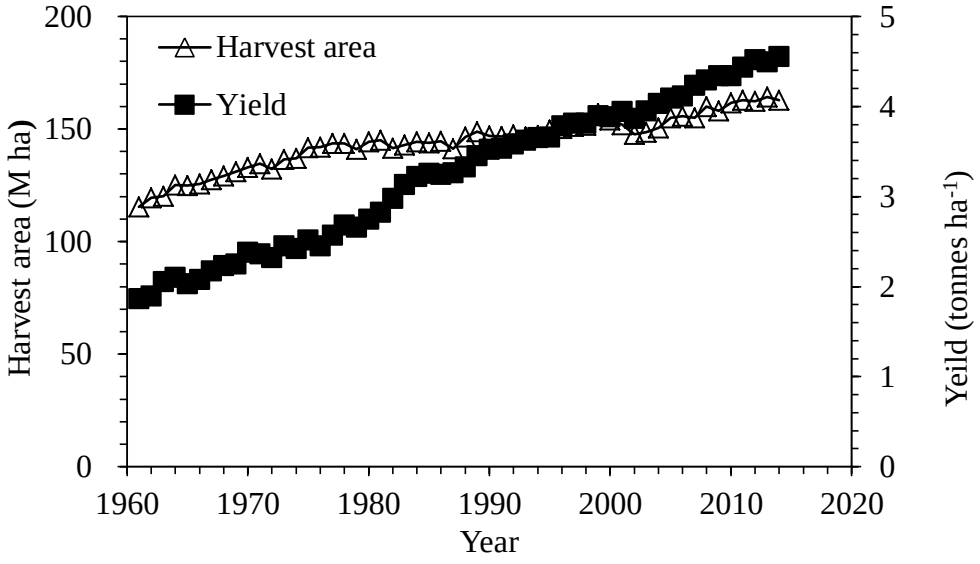

Fig.1.1. Global paddy harvest area and yield from 1964 to 2014 (accessed FAO data base in Dec. 2016).

According to local precipitation and annual air temperature, rice can be cultivated in single, double and even three seasons every year. For example, single rice growth is a common cultivation system in Tohoku and Hokkaido regions in Japan which are located in cold temperate region of Japan characterized by cold air temperature and heavy snow cover in winter while rice can be grown twice a year in Kyushu region of Japan under high temperature and abundant rainfall conditions. In Hainan province, China, rice can even be grown three seasons a year because of tropical monsoon climate. Recently, A new study investigated annual dynamics of paddy rice areas in northern Asia (China, Japan, South Korea and North Korea) from

2000 to 2014 and found that paddy rice centroid shifted northward from 41.16°N to 43.70°N (about 310 km) in northeastern China with the promotion of market, technology, policy and climate (Dong et al., 2016).

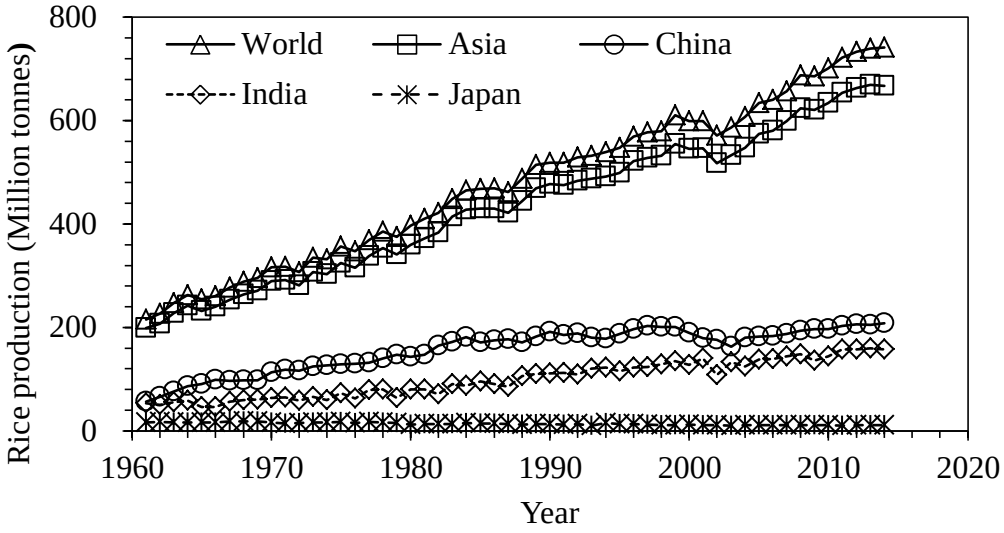

Fig.1.2. Rice production in the word and in parts of Asian countries from 1961 to 2014 (Accessed FAO data base in Dec. 2016).

Paddy soil is the largest anthropogenic wetland ecosystem and can originate from any type of soil in pedological terms. The development of paddy soil is distinctly driven by many soil management practices which can greatly change the soil's original character (Kirk, 2004). Specifically, these soil managements consist of alternative submergence and drainage, ploughing and puddling, liming and fertilization (organic manure, rice straw and other crop residues, and chemical fertilizer). Since rice growth is highly dependent on water irrigation, paddy soil is nearly submerged throughout the growing season. It is often drained one or two weeks during mid-season and before harvest and across the whole fallow season. Therefore,

the change in oxic and anoxic conditions induced by water regimes leads to temporal and spatial (vertical and horizontal) variations in reduction and oxidation reactions thus, affecting SOM decomposition and stabilization (Cheng et al., 2009b; Kögel-Knabner et al., 2010). A typical horizon of paddy soil is shown in Fig.1.3 as described in FAO (2006). The plough layer of rice paddy soil with the depth of approximately 20 cm can be roughly divided into oxidation layer and reduction layer. The thickness of the oxidation layer may range from several mm after flooding, to several cm when the rice plant are fully grown and start to release oxygen from their roots (Frenzel et al., 1992). Reduction layer is characterized by the absence of free oxygen in the soil solution, lower redox potential (Eh) and hydraulic conductivity (Chen and Liu, 2002).

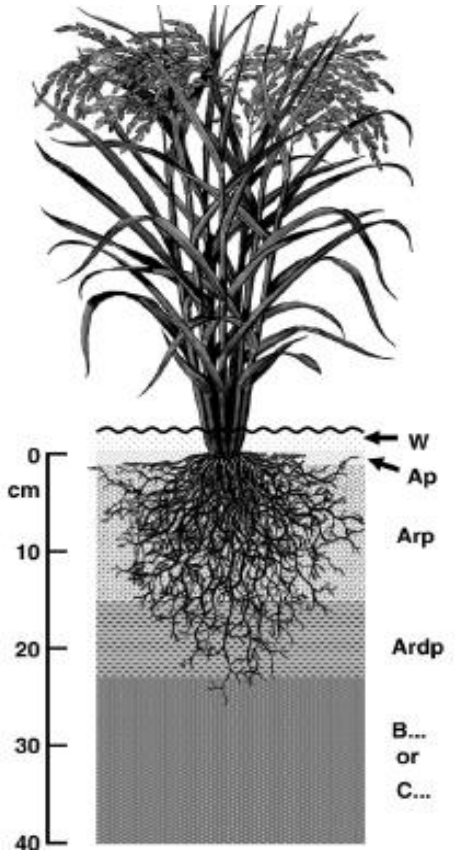

Fig.1.3. Typical horizon sequence of a rice paddy soil (horizon designation according to FAO, 2006).

The amount of organic carbon stored in paddy soils was reported to be greater than in upland soils because of different biochemical processes and mechanisms specifically caused by the presence of flooded water in paddy soils (Guo and Lin, 2001). Over past several decades, many studies on methane (CH_4) and nitrous oxide (N_2O) emissions have been done in rice paddy fields around the world (Kimura et al., 2004, Cheng et al., 2006; Cai et al., 2016). With the rapid decrease of Eh, alternative electron acceptors in the order of NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-} are reduced by various microorganisms in the flooded rice paddy soil resulting in substantial N_2O emission from soil to the atmosphere. Only when the Eh decreased to a certain threshold, the

production of CH_4 will start by methanogenesis. Although great progress has been made on the mechanisms of CH_4 production, oxidation and transportation over several decades, the questions on how to the mitigation of CH_4 emission via effective practices in rice paddy field is still one of current hotpots in soil sciences and global climate change.

1.2. Global climate change

Due to increased fossil fuel consumption and intensive anthropogenic activities, atmospheric concentrations of greenhouse gases (GHG) such as carbon oxides (CO_2), CH_4 and N_2O are increasing since the industrial revolution. Consequently, the increases in GHG emissions in various terrestrial ecosystems lead to large global climatic changes such as increasing global temperatures, uneven distribution of global water cycling (infrequency of rain fall and occurrence of drought), accelerated loss of soil organic carbon (SOC) storage, melting glaciers and rising sea level, and increased nitrogen deposition. Based on our research topic in this study, we mainly emphasize on global warming, increased atmospheric [CO_2] and imbalanced hydrological cycle as below.

1.2.1. Global warming

Recently, it is widely realized that global temperature are increasing year by year which are supported by substantial data obtained from various model prediction and in situ observations. For example, according to the 5th report in Intergovernmental

Panel on Climate Change (IPCC, 2013), a linear trend showed that the globally averaged surface temperature has elevated by 0.85 °C (0.65-1.06 °C) over the period from 1880 to 2012, and by 0.72 °C (0.49-0.89 °C) over the period from 1951 to 2012, respectively. Moreover, it is predicted to increase by 0.3-4.8 °C by the end of 21st century (IPCC, 2013). Both the daily minimum (i.e., night) and maximum (i.e., daytime) temperatures have been increasing in the past 100 years and their increasing trends are still lasting in a linear association with current atmospheric GHG concentrations. The night temperature was found to increase faster than the daytime temperature (Kukla and Karl, 1993; Easterling et al., 1997; IPCC, 2007). It has been predicted that global averaged maximum and minimum temperatures over land have increased at rate of more than 0.1 °C per decade since 1950 (Lobell et al., 2011).

According to the annual air temperature database from Japan Meteorological Agency, the air temperature has increased by 1.16 °C over 100 years from 1890 to 2015 (Fig.1.4). Many studies suggested global warming has a great impact on plant growth and C storage in forest, grassland and rice paddy ecosystems (Cheng et al., 2010; Keenan et al., 2014; Laza et al., 2015; Suzuki et al., 2016). Generally, experimental warming can stimulate plant growth and improve the net primary production (NPP) resulting in an increase in plant-derived C input into the soil. On the other hand, it can also accelerate soil organic matter (SOM) mineralization by stimulating soil microbial activities leading to SOC loss. So far, the feedback of SOM decomposition and sequestration to global warming has not yet been fully understood

since no consistent conclusions with regard to the effect of experimental warming on plant growth and SOC storage are drawn due to the high spatial and temporal variations in ecosystems, plant species, and microbial availability of SOC.

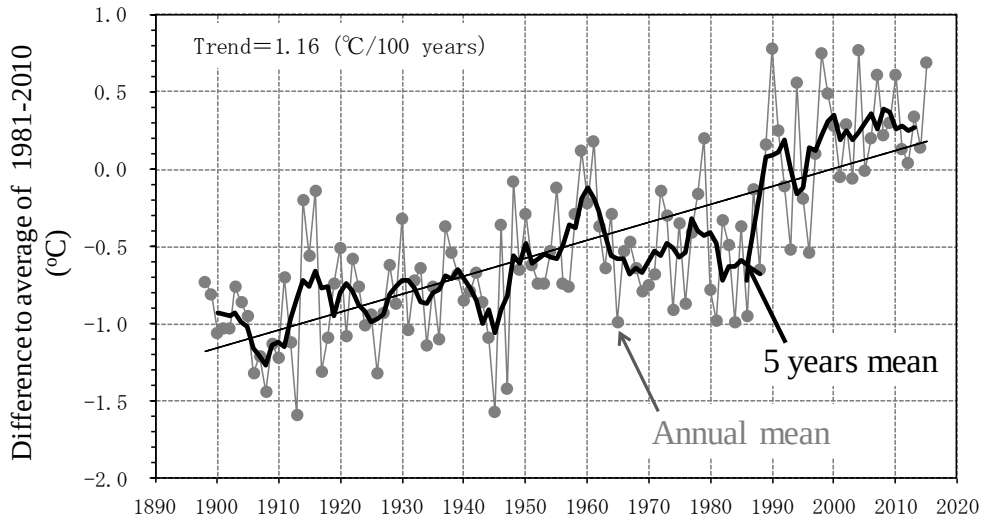

Fig.1.4. The annual air temperature in Japan from 1898 to 2015. Data was downloaded from Japan Metrological Agency, 2016.

1.2.2. Elevated atmospheric [CO_2]

The elevation of atmospheric [CO_2] is a very crucial form of current global climate change. Based on the in situ observation in the atmosphere around the world over long-time scale, [CO_2] induced by fossil fuel consumption and land use change is rapidly increasing with the time. It has been reported that the atmospheric [CO_2] has continuously risen from 280 to 400 ppm during the period from 1850 to 2012, and it is projected to rise to up to 900 ppm by the end of 21st century (IPCC, 2013). According to the in situ observation at Maunna Loa, Hawaii, USA (19.5362°N, 155.5763°W) since 1958, the annual atmospheric [CO_2] exceeded 400 ppm in 2015

(Fig.1.5). It is no doubtful that the elevated [CO_2] under future climate change is associated with an increase in air temperature since it is the cause of rising atmospheric [CO_2] nowadays.

As the key substrate for plant photosynthesis, elevated [CO_2] has almost certainly influence on physiological metabolism, yield, and quality in crops (Wang et al., 2016). In order to simulate the response of plant growth to the increased [CO_2] in the future, free-air CO_2 enrichment (FACE) experiments are conducted in grassland, forest and rice paddy ecosystems around the world (Shaw et al., 2002; Asshoff et al., 2006; Cheng et al., 2008; Cheng et al., 2010; Cai et al., 2016). For example, Cheng et al. (2010) conducted an interactive effect of elevated [CO_2] and night temperature on rice growth and CH_4 emission in Japanese paddy soils. Their results suggested that elevated [CO_2] increased the net dry weight of rice plants by 12.7% and 38.4% under high and low night temperature conditions, respectively because of the stimulation of rice photosynthesis by elevated [CO_2]. Reich et al. (2014) reported that elevated [CO_2] enhanced more than 33% plant biomass in perennial grassland when both summer rainfall and nitrogen supply were at higher levels. But the significant effect of elevated [CO_2] on plant biomass was eliminated by lower rainfall and nitrogen. These results indicate that elevated [CO_2] can increase NPP but its stimulation of plant growth is closely interacted with other environmental variables such as air temperature, moisture content and nutrient supply.

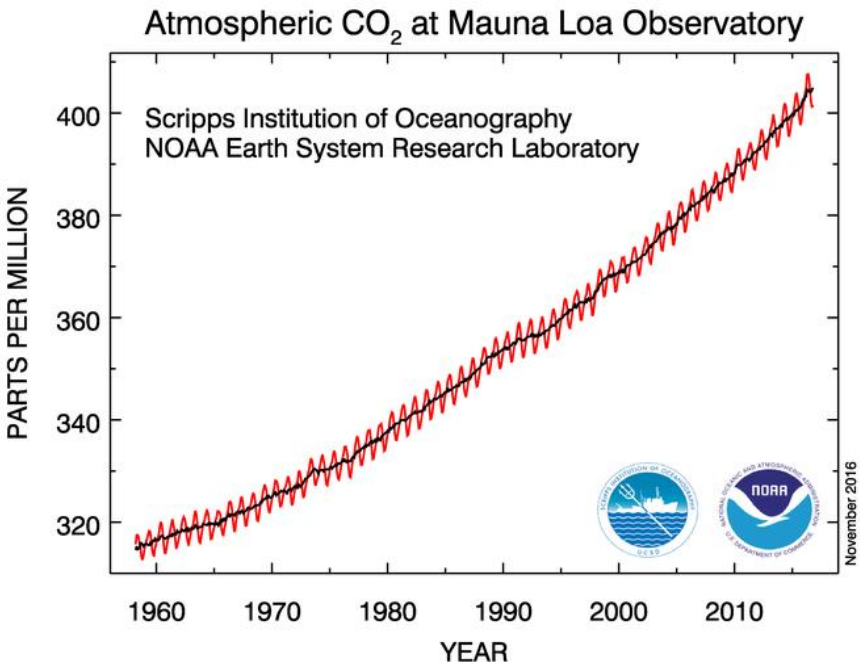

Fig.1.5. The atmospheric [CO_2] expressed as the mole fraction in dry air, at Mauna Loa Observatory, USA since 1958 to 2015 (The red and black lines represent the seasonally and yearly corrected [CO_2], respectively).

1.2.3. The imbalance of hydrological cycle

Climate change can evidently alter energy budgets between the atmosphere and surface, thus affect hydrological cycling between the atmosphere and pedosphere. The infrequent drought and enhanced precipitation are two representatives of the imbalance of hydrological cycle caused by global warming. Currently increased temperature will make the dry areas much drier (generally throughout the subtropics) and wet areas much wetter, especially in the mid to high attitudes because global warming makes the snow melt earlier as result of the increased risk of flooding in the early spring, but an increased risk of drought in summer (Trenberth, 2011). On the other hand, global warming can also promote water evaporation from ocean and river,

leading to an increase in water demand for crop production. It has been reported that the water holding capacity of air increased by about 7% per 1 °C warming, which would cause an enhancement of water vapor in the atmosphere (Trenberth, 2011). Based on long-term observation in four sites, IPCC (2013) reported that precipitation has generally increased at the attitudes more than 30°N during the period from 1900 to 2005 but decreasing trend of precipitation has been found in tropic land areas since the 1970s. Although a lot of work have been involved in observation of precipitation and drought in various terrestrial and ocean ecosystems around the world, due to high temporal and spatial variations in hydrological cycling, its response to climate change is needed to further study.

1.3. Plant growth responses to climate change

1.3.1. Response to global warming

The global warming effect on plant growth has been studied in forest, grassland and rice paddy ecosystems with various experimental warming methods (overhead infrared lamps, and passive night-time warming, wire heating) over several decades (Rustad et al., 2001; Hoeppner and Dukes, 2012; Natali et al., 2012; Usui et al., 2016) An early meta-analysis in 2001 showed that, across all sites and years, 2-9 years of experimental warming in the range of 0.3-6.0 ℃ significantly increased plant productivity by 19%. Response of plant productivity was generally larger in low tundra ecosystems than in forest and grassland ecosystems (Rustad et al., 2001). Due

to large temporal and spatial variations in plant growth response to experimental warming, so far, no consensus has been reached on the response of rice biomass to experimental warming (Ziska et al., 1998; Cheng et al., 2009a; Kim et al., 2011; Usui et al., 2016). Usui et al. (2016) conducted a 3-year T-FACE (elevated soil temperature and [CO_2]) in Tsukuba, Japan and suggested that elevated soil and water temperatures during rice growth season significantly increased aboveground biomass by 3.6%. The elevation of air temperature is the most direct performance of global warming. It has been reported that high temperature-induced spikelet sterility is a major factor increasing rice biomass but reducing rice yield with low harvest index under future climate conditions (Peng et al., 2004; Gaihre et al., 2013; Jung et al., 2015; Laza et al., 2015).

1.3.2. Response to elevated [CO_2]

CO_2 is the substrate for photosynthesis and thus, it can accelerate plant growth. The response of plant growth to elevated [CO_2] is arousing great concern. Many previous studies have evidenced that elevated [CO_2] can stimulate plant growth in various ecosystems such as forest, grassland and paddy filed (Ellsworth et al., 2004; Tokida et al., 2010; Xu et al., 2014; Usui et al., 2016). Since plant growth is the result of multiple effects of biotic and abiotic factors, the elevated [CO_2] as an important environmental variable, will largely interact with other variables (e.g., soil water content and nutrient supply) to affect plant growth. It is probable that the stimulated effect of elevated [CO_2] on plant growth may be weaker under the limited nutrient

availability condition. For example, it has been reported that elevated [CO_2] led to an increase in plant biomass of more than 33% when summer rainfall, nitrogen supply, or both were at the higher levels. But no increase in plant biomass due to elevated [CO_2] was observed when both rainfall and nitrogen were at their lower level (Reich et al., 2014). In addition, the stimulation of plant growth and altered plant physiology would vary with plant spices, growth stages and environmental conditions. A meta-analysis showed that the effect of elevated [CO_2] on plant physiology and growth varied with temperature regimes, functional groups, photosynthetic pathways (Wang et al., 2012).

1.4. SOM decomposition to climate change

1.4.1. Response to global warming

The decomposition of SOM, which is a primary source of soil respiration, is significantly influenced by temperature since global warming can stimulate soil microbial activities, thus accelerate SOM decomposition, resulting in C loss in terrestrial ecosystems. (Davidson and Janssens, 2006; Zhang et al., 2006; He et al., 2013; Benbi et al., 2014). So far, the response of SOM decomposition to global warming, which is generally expressed as Q_{10}- a quotient of the change in respiration caused by a 10 ℃ temperature change, is still not well understood due to temporal and spatial variations in SOM decomposition in various ecosystems. Since SOM consists of various organic components with different temperature sensitivities, accurately estimating their decomposition responses to global warming is one of

current hotspot in environmental and soil sciences (Conen et al., 2006; Vanhala et al., 2007; Benbi et al., 2014). An early previous meta-analysis showed that, across all sites and years, 2-9 years of experimental warming in the range of 0.3-6.0 ℃ significantly increased soil respiration rates by 20% (with a 95% confidence interval of 18-22%), net N mineralization rates by 46% (with a 95% confidence interval of 30-64%). Moreover, the response of soil respiration to warming was generally larger in forest ecosystem compared to low tundra and grassland ecosystems (Rustad et al., 2001).

Compared with forest and grassland, simulated warming experiments are few conducted in rice paddy ecosystem which is characterized by intensive cultivation and high amount of fertilizer input. A T-FACE experiment conducted in China suggested that the temperature sensitivity of SOM decomposition increased with increasing SOM recalcitrance in rice paddy soil (Chen et al., 2015). A study which was conducted in growth chamber with controlled and elevated day and night temperatures throughout rice growth season also indicated that elevation in temperatures increased CH_4 emission rates, with a more pronounced effect from flowering to maturity. However, N_2O emissions were negligible during the rice-growing season (Gaihre et al., 2013).

1.4.2. Response to elevated [CO_2]

Many previous studies have indicated that SOC and total (TN) contents would increase mainly due to the enhanced SOM input derived from plant by elevated [CO_2] (Zak et al., 1993; Jastrow et al., 2005; Bhattacharyya et al., 2013). However, the large

spatial heterogeneity of SOM may prevent the detection of small change in soil C content under elevated [CO_2] (Jastrow et al., 2005). It has been reported that elevated [CO_2] stimulated the temperature sensitivity of recalcitrant SOM but had no effect on labile SOM in rice paddy field (Chen et al., 2015). The activities of C-transforming enzyme such as β-glucosidase and microbial biomass carbon were also significantly increased by elevation of [CO_2] in tropical flooded rice paddy field (Bhattacharyya et al., 2013). A FACE experiment conducted in China suggested that elevated [CO_2] facilitated C and N accumulation in rice paddy ecosystem (Guo et al., 2015). On the other hand, elevated [CO_2] can promote C outputs from wetlands through gaseous (Cheng et al., 2008; Tokida et al., 2010) and dissolved (Freeman et al., 2004 and Guo et al., 2011) pathways. Since C and N cycles are strongly coupled with each other, the enhanced C exports may also increase N loss (Guo et al., 2011) from wetlands.

1.5. SOM turnover as C and N cycling in rice-soil ecosystems

It is well known that SOM decomposition and humification in soils are series of biochemical processes regulated by various soil microorganisms. The turnover of SOM in rice-soil ecosystems is very complex because of the close interactions of rice and soil under different environmental conditions. Rice growth is largely dependent on N supply from soil. Since great proportion of organic N can't be absorbed by rice directly, soil organic N is mineralized into active inorganic N such as ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) which are available to

rice growth. Also, some mineralized N will be assimilated as organic N by soil microorganism. On the other hand, the rice root exudates can increase dissolved organic matter content and consequently stimulate soil microbial activities. The capacity of microbial reduction electron accepters in flood paddy soil is the order of O_2> NO_3^-> Fe^{3+}> Mn^{4+}> SO_4^{2-}> CO_2 in the presence of available SOC as energy source. In this case, N can be lost by denitrification and leaching in flooded rice paddy soil. The main form of mineral N is NH_4^+-N in flooded paddy soil.

As energy source, SOC can be composed into CO_2 throughout various biochemical processes mediated by microorganism. Only when Eh decreases to a certain threshold, methanogenesis occur in flooded paddy field. It has been suggested that the critical soil Eh for initiation of CH_4 production is generally observed approximately from -150 to -160 mV. Between -230 and -150 mV, the relationship of CH_4 production and soil Eh appeared to be negatively exponential (Wang et al., 1993). It should be mentioned that only a small fraction of SOC can be decomposed into CO_2 and CH_4, large amount of SOC is physically and biochemically protected by aggregates and soil minerals.

After rice harvest, rice paddy is often drained and rice straw is often incorporated into the soil as manure to maintain soil fertility. Many studies have suggested that rice straw return/application can greatly increase SOC content, improve soil structure and stimulate soil microbial activities (Lugato et al., 2006; Yan et al., 2009; Zhang et al., 2015). However, rice straw is also commonly identified as a main substrate for CH_4

production in paddy soil ecosystems (Zou et al., 2005; Yang et al., 2010; Zhang et al., 2015; Nakajima et al., 2016). During the drainage period, paddy soil condition changes from anoxic condition to oxic condition which will further affect the form of SOM decomposition. It has been reported that flooded paddy soil clearly harbored lower copy numbers of denitrifiers than dry paddy soil (Liu et al., 2012; Yang et al., 2016), implying that submerged soils might have low denitrifying activities. Therefore, the main processes for N loss are nitrification and leaching. Since the produced CH_4 will be rapidly oxided by O_2 in the presences of methanotrophs, CO_2 is the only gaseous product of C decomposition in aerobic paddy soil. To some extent, the drained paddy soil can be considered as upland soil.

1.6. CH_4 and N_2O emissions in rice paddy ecosystems

1.6.1. CH_4 emission

Atmospheric CH_4 is the second most important GHG after CO_2 since its global warming potential is 28-34 times higher than that of CO_2 on the 100-year time horizon. It has been reported that global averaged atmospheric CH_4 has increased from 722 ppb in 1750 to 1803 ppb in 2011 (IPCC, 2013). The estimates of CH_4 emission derived from paddy fields range from 31 to 112 Tg yr^{-1}, equivalent to 19% of global total emissions (IPCC, 2007). It is well known that CH_4 production is the terminal step of anaerobic SOM decomposition, which requires sequential cooperation of multiple groups of microorganisms (Conrad, 1999; Megonigal et al.,

2004). Both CO_2 and CH_4 are end products of anaerobic decomposition of SOM. The flooded environment, created during rice cultivation, provides anaerobic condition favoring CH_4 production by metahnogens. However, only small part of the produced CH_4 is emitted to the atmosphere through soil- or water-atmosphere and by the rice aerenchyma (Hou et al., 2000). While a greatly large fraction of produced CH_4 are oxidized at oxic-anoxic interfaces (Kimura et al., 2004). Previous studies have suggested that the substrates for CH_4 production can be derived from three sources: (1) SOM, (2) root organic carbon (ROC) including root exudates and sloughed-off dead root, and (3) incorporated organic matter such as rice straw (Chidthaisong and Watanabe, 1997; Watanabe et al., 1999; Yuan et al., 2014). Yuan et al. (2014) suggested that rice straw addition stimulated CH_4 production from SOM and ROC, leading to a positive feedback on CH_4 production from paddy field. The probable reasons could be attributed to the significant increase of the abundance of methanogenic archaea induced by rice straw addition.

1.6.2. N_2O emission

N_2O is a greenhouse gas with a great impact on human life and environment. It is also called “laughing gas” because of it being used as an anesthetic in medicine sciences. It has been reported that the100-year global warming potential (GWP) of N_2O is about 300 times larger than that of CO_2 and its lifetime is considered to 131 years. The concentration of atmospheric N_2O has been reported to increase by 20% from 270 ppb in 1750 to 324 ppb in 2011 (IPCC, 2013). As shown in Fig.1.6, N_2O is

produced from microbial mediated nitrification and denitrification in soils and its emission can be easily modified by diverse climate, soil, and vegetative conditions with large temporal and spatial variations (Wrage et al., 2001; Stehfest and Bouwman, 2006; Philibert et al., 2012). Previous studies suggested that agriculture contributes about 60% of the total anthropogenic emission of N_2O (IPCC, 2013). The major natural sources of N_2O are the oceans and forest. Paddy soil is considered to be one of important source of N_2O emission as a result of the mid-season drainage and dry-wet episodes, and high amount of N fertilization (Cai et al., 1997; Zou et al., 2005). How to mitigate N_2O emission in rice paddy field has roused a lot of interests in the world. Using nitrification inhibitors may be a potential management strategy to reduce N_2O emissions in irrigated rice. For example, a study was conducted in intensive irrigated rice-upland crop rotation system with application of dicyandiamide (DCD), a nitrification inhibitor, to investigate its effect on nitrogen transformation and N_2O emission. It showed that N_2O emission was significantly lower in DCD applied soil sample and the reasons could be attributed to its inhibition in both nitrification rates and the fraction of N_2O in nitrification products (Lan et al., 2013).

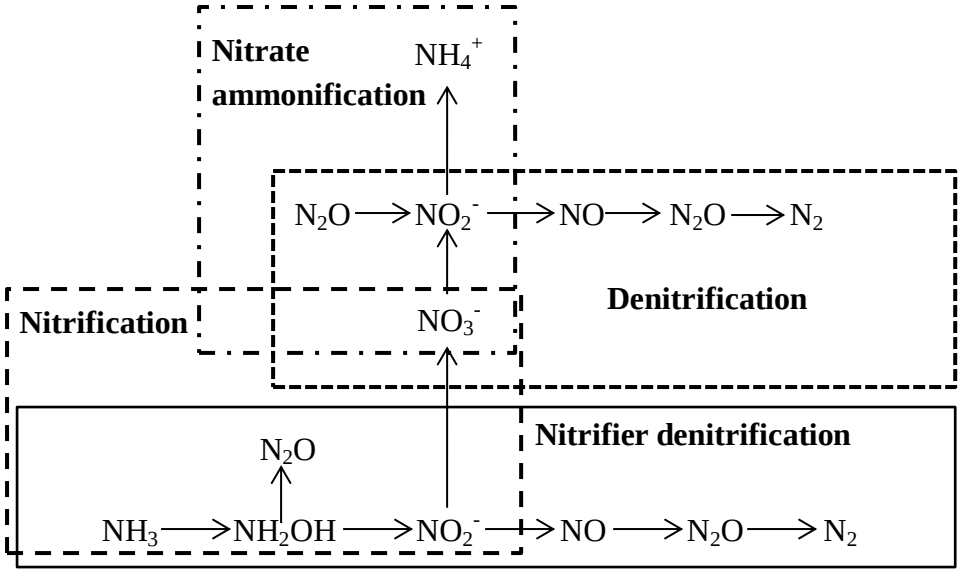

Fig.1.6. Microbial sources of N_2O production in soil (cited from Wrage et al. (2001)).

1.7. Objectives and outlines of this study

Global climate change is the result of increasing GHGs mainly induced by intensified human activities in various ecosystems. Rice paddy ecosystem is a very important component of terrestrial ecosystems and can be easily affected by climate change. So far, it has still not been fully understood the responses of crucial processes associated C and N cycling in plant-rice paddy systems to future climate change. Therefore, three level experiments were conducted to study the effects of soil moisture, temperature and elevated [CO_2] on amounts and components of SOC and TN in rice paddy ecosystems.

First, according to the local moisture and temperature conditions during off-rice season and rice growth season in Tohoku region, Japan, a laboratory incubation experiment with two moisture and four temperature levels was conducted in Inceptisol

and Anidisol paddy soils to investigate the responses of SOC and TN contents, and decomposition potentials in two kinds of paddy soils with different physical and chemical properties to increases of soil temperature and moisture (Chapter Ⅱ).

The second experiment is a 5-year soil warming experiment which was carried out in a rice paddy field in National Institute of Agro-Environmental Science (NIAES), Tsukuba, Japan during rice growth season (+2 °C above ambient temperature (AT)) and off-rice season (+1 °C above AT). The rice biomass, SOC and TN contents, C and N mineralization potentials in air-dried soil samples were measured. In addition, the effect of pre-incubation on the responses of C and N mineralization potentials to soil warming was also studied (Ⅲ).

Finally, we participated in the T-FACE project located in Mirai City, Tsukuba, Japan which was designed by NIAES since 2010. In this experiment, the main factor was the elevation of [CO_2] which was 200 ppm higher than ambient [CO_2] during rice growth season. The split factor was elevated soil temperature which was 2 °C higher than ambient soil temperature. After 5 years, we collected soil samples in soil layers from 0-30 cm in October, 2014 (every 10 cm as a layer). The amounts of SOC and TN, $\delta^{13}C$ values in SOC and decomposed C (CO_2-C+CH_4-C), $\delta^{15}N$ value, C and N mineralization potentials were determined to investigate the interactive effects of elevated soil temperature and [CO_2] on amounts and components of SOC and N in a single rice paddy ecosystem (Chapter Ⅳ).

Chapter Ⅱ Soil organic carbon decomposition and nitrogen mineralization influenced by soil temperature and moisture in Andisol and Inceptisol paddy soils in a cold temperate region of Japan

2.1. Introduction

Soils store twice as much carbon (C) as the atmosphere and about four times as much as plant biomass (Batjes, 1996; IPCC, 2013). The changes in stock of SOC in terrestrial ecosystems in response to climate change depend on the balance between C inputs to soil by NPP and C outputs into the atmosphere by microbial decomposition (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009). A small change in soil respiration resulting from natural processes or anthropogenic activities could significantly intensify or mitigate atmospheric CO_2 emission (Baveye, 2007; Smith et al., 2008). Soil temperature and moisture have been identified as two key environmental factors regulating the C decomposition and N mineralization in agroecosystems (Davidson and Janssens, 2006; Xu et al., 2012; Zhou et al., 2014b; Huang et al., 2015). An increase in soil temperature accelerates SOC decomposition because temperature-dependent reactions performed by microorganisms result in more rapid CO_2 emissions from soil to the atmosphere (Trumbore and Czimczik, 2008;

Karhu et al., 2014). Soil moisture can have a great impact on SOC decomposition by affecting the oxygen diffusion into the soil and the substrate availability for soil microorganisms (Linn and Doran, 1984; Suseela et al., 2012; Wang et al., 2014; Zhou et al., 2014b; Sierra et al., 2015). Many studies about the response of SOC decomposition to temperature change are mainly conducted in forest, grassland and upland ecosystems (Luo et al., 2001; Conen et al., 2006; Vanhala et al., 2007; He et al., 2013; Zhou et al., 2014b; Xu et al., 2016a). However, relatively few studies have examined the combined effects of soil temperature and moisture on SOC decomposition in paddy soils.

Rice paddies account for a large fraction of the wetland ecosystem with most of them in Asian countries. Single rice cropping is a common system in cold temperate region like the northeastern Japan where there is a long snow cover period during the winter season. Andisols and Inceptisols are commonly used for rice cultivation in Japan (Shoji et al., 1994; Cheng et al., 2007). Andisols are characterized by specific physico-chemical properties like low bulk density, high SOC content, high porosity, and stable soil aggregates (Shoji et al., 1994; Dorel et al., 2000; Hoyos and Comeford, 2005). On the other hand, Inceptions are considered as the most widely distributed soils, which were formed through the alteration of parent material. They are characterized by weak pedogenesis, no accumulation of clays, low cation exchange capacity and poor soil fertility (Foss et al., 1983). Besides the chemical recalcitrance, the formation of organo-mineral associations, resulting in the physical protection of the soil organic

matter, has been reported as a major process for C stability in soils (Saggar et al. 1996; Müller and Höper, 2004; Davidon and Janssens, 2006). For example, Frøseth and Bleken (2015) studied SOC decomposition in a clay soil and a sandy soil at low temperature (0-15 °C) and found that temperature sensitivity of SOC was the same in both soils although SOC decomposition was twice as fast in the sandy soil as in the clay soil. On the basis of evident differences in physical and chemical properties between Andisol and Inceptisol, the response of SOC decomposition to soil temperature and moisture might vary between these two soils.

In northeastern Japan, the single rice is generally grown from early May to mid-October under mostly submerged soil conditions throughout the rice growth season (Fig. 2.1 and Fig. 2.2). The short periods of drainage are conducted in the middle of rice growth before booting stage and about 2 weeks prior to rice harvest. The rice paddies are then left under aerobic condition for the off-rice season, usually lasting from late October to late April (Nakajima et al., 2016). Due to the low soil temperatures as a result of the long winter season with heavy snow in northeastern Japan (Fig. 2.1 and Fig. 2.2), the decomposition of SOC is slow during the off-rice season and a considerable amount remains in the soil at the beginning of the subsequent rice growth season. However, it is still not fully understood whether the undecomposed SOC and unmineralized N during the off-rice season will lead to a remarkable CH_4 production and NH_4^+-N accumulation in the following rice growth season. Therefore, we conducted an incubation experiment to simulate the effects of soil temperature and

moisture during off-rice season on SOC decomposition and N mineralization in two contrasting paddy soils. The objectives of this study were (1) to investigate the effects of soil temperature and moisture conditions during the off-rice season on SOC decomposition and N mineralization in Andisol and Inceptisol paddy soils, and (2) to compare the SOC decomposition and N mineralization between these two soils during both aerobic and anaerobic incubations.

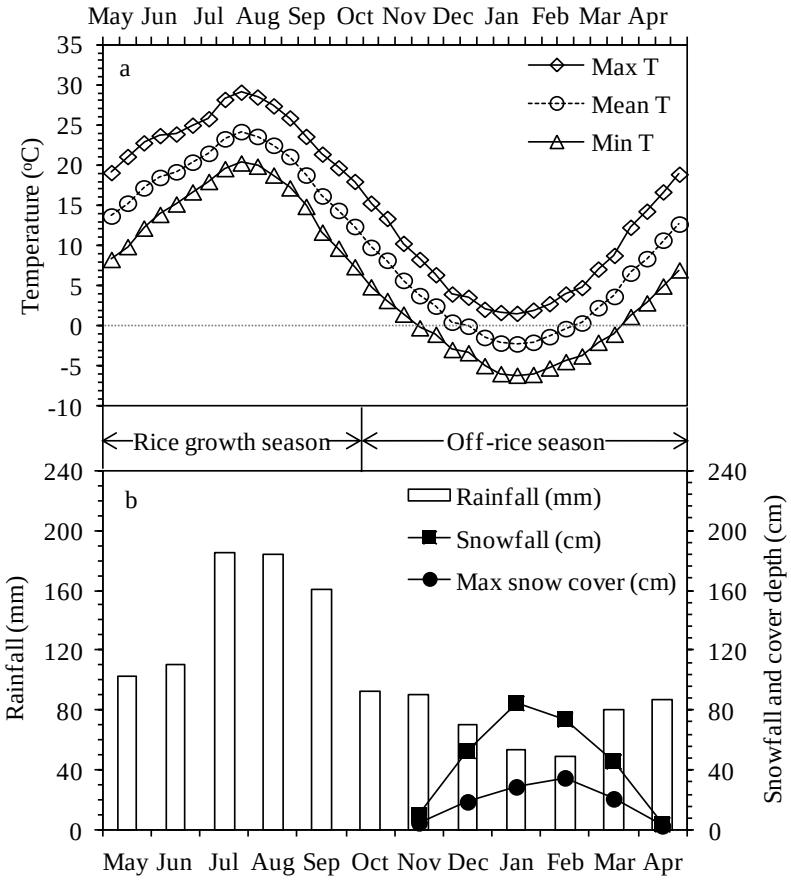

Fig.2.1. The averages of maximum, mean and minimum air temperatures for each ten days from May to April (a); the monthly rainfall, snowfall and maximum snow cover in the off-rice season (b) in Morioka City, Iwate Prefecture, Japan. All data were the average values from 1981 to 2010 (30 years). Data were originated from the database in Japan Meteorological Agency.

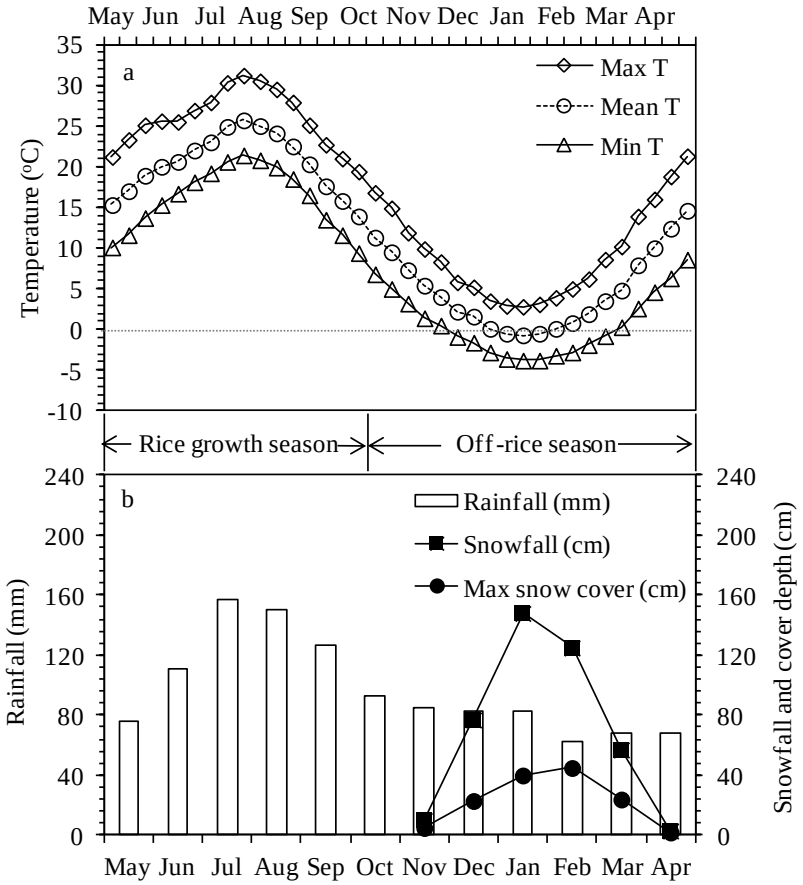

Fig.2.2. The averages of maximum, mean and minimum air temperatures for each ten days from May to April (a); the monthly rainfall, snowfall and maximum snow cover in the off-rice season (b) in Yamagata City, Yamagata Prefecture, Japan. All data were the average values from 1981 to 2010 (30 years). Data were originated from the database in Japan Meteorological Agency.

2.2. Material and Methods

2.2.1. Soil sampling, soil property analysis and pre-incubation

Soil samples were taken from 0-10 cm depth at Tohoku Agricultural Research Center (39°42′N, 141°09′E), National Agriculture and Food Research Organization, Iwate Prefecture, and from 0-15 cm depth at Yamagata Integrated Agricultural

Research Center (38°16′N, 140°19′E), Yamagata Prefecture, Japan, respectively. Both sites are located in northeastern Japan (Fig. 2.3). The samples taken from Iwate and Yamagata were respectively classified as Andisol and Inceptisol (Soil Survey Staff 1999). These two paddy soils are typical in cold temperate region of northeastern Japan. After the manual removal of the visible roots and small stones, soil samples were air dried, sieved through a 2-mm sieve and finally stored at room temperature to further analysis.

Table 2.1 Initial properties of Andisol and Inceptisol paddy soils taken in Iwate and Yamagata Prefectures, Japan.

	Andisol	Inceptisol
Site	Iwate (39°42 N, 141°09 E)	Yamagata (38 16 N, 140° 19 E)
pH (H_2O)	5.84	5.46
EC (μS cm^{-1})	101.7	89.10
SOC (mg C kg^{-1})	89.0	13.62
TN (mg N kg^{-1})	6.70	1.15
C/N	13.29	11.84
$\delta^{13}C$ (‰)	-22.20	-24.87

The SOC and TN in two air-dried soils were measured by dry combustion using an NC analyzer (SUIGRAPH NC-220F; Sumika Chemical Anaysis Service Ltd., Tokyo, Japan). Gravimetric moisture content was measured via mass loss after oven-dried at 105 °C for 24 h. Soil pH and electron conductivity (EC) in a 1:2.5 (soil/water ratio, w/v) mixture were measured by pH meter (D-51, Horiba, Kyoto, Japan) and EC meter (Cond-meter DS-51, Horiba, Kyoto, Japan), respectively. The

$\delta^{13}C$ value in two soils was measured by an isotope ratio mass spectrometer (IR-MS; Flash 2000, Delta V Plus; Thermo Scientific, Gemanry). The basic properties of Andisol and Inceptisol paddy soils are shown in Table 2.1.

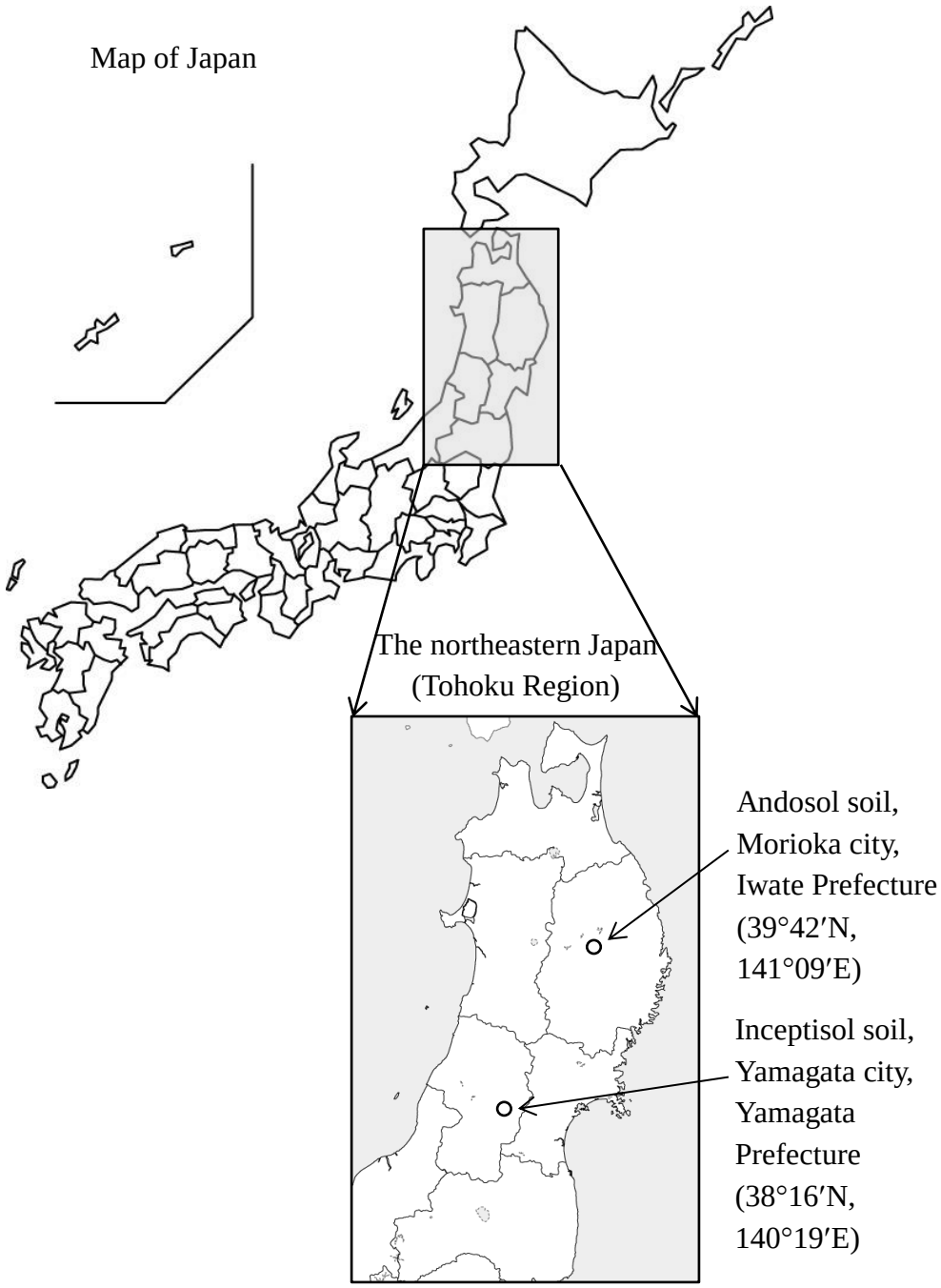

Fig.2.3. The map of the northeastern Japan (Tohoku region) and the experimental sites where the soil samples were collected.

Before the aerobic incubation experiment, soils samples were pre-incubated at 25 °C for four weeks in the dark to restore microbial activity. Soil moisture was maintained at 40% WFPS by periodic addition of deionized water. The WFPS was calculated from equation (1):

$$WFPS = \frac{\theta_V}{1-\left(\frac{BD}{PD}\right)} \times 100 \qquad (1)$$

where θ_v, BD and PD are volumetric soil moisture, soil bulk density and soil particle density (2.65 g cm^{-3} as a fixed constant), respectively (Aulakh et al., 1991; Cheng et al., 2004).

2.2.2. Aerobic incubation

Based on temperature and moisture conditions during the off-rice season in northeastern Japan (Fig. 2.1 and Fig. 2.2), an aerobic incubation experiment was conducted under four temperature levels (-5 to 5 (noted by ±5), 5, 15, 25 °C) and two moisture levels (60% and 100% WFPS; abbreviated L and H). Temperature level at ±5 ℃ was used to simulate diurnal variation of soil temperature between night (-5 °C, 12 h) and day (5 °C, 12 h) and the corresponding freeze-thaw cycles occurring during the off-rice season in Tohoku region, Japan. Hence, there were eight treatments in this experiment for each soil type, namely L±5, L5, L15, L25, H±5, H5, H15 and H25.

After the 4-week pre-incubation, 5 g subsamples (dry-weight basis) were placed into 68-mL serum bottles (total 48 samples). Soil moisture in half of serum bottles were adjusted to 60% and 100% WFPS, respectively, by adding deionized water with a

mini-pipette. Then, all serum bottles were purged with pure air (80% N_2 + 20% O_2) and capped with a butyl rubber stopper with aluminum seal. At the interval of every two weeks through the incubation, the headspace of each serum bottle was sampled to measure CO_2 and CH_4 concentrations. After each gas sampling, the headspace was purged with pure air and the butyl rubber stopper and aluminum seal were replaced with new ones.

In parallel, 10 g (on an oven-dried basis) of pre-incubated soil was placed into 100-mL plastic bottles (total 192 bottles) to measure the changes in SOC and TN contents, $\delta^{13}C$ value, NH_4^+-N and NO_3^--N concentrations. All bottles were covered with the caps loosely to maintain aerobic conditions and then incubated under the four temperature and the two moisture conditions as described above. During the aerobic incubation, loss of water was corrected every 3 or 4 days by using a mini-pipette. Three replications in each treatment were taken out from the incubators after 6, 12, 18, and 24 weeks; oven-dried at 70 ℃ for 48 h; and finely ground into powder for SOC, TN and $\delta^{13}C$ value measurements. A part of air-dried soil samples were extracted by shaking with 30 ml of 10% KCl solution for 30 min. The extracted solutions were filtered and stored in a deep freezer (-18 °C) for measuring NH_4^+-N and NO_3^--N concentrations.

2.2.3. Anaerobic incubation

After the 24-week aerobic incubation, all soil samples in the serum bottles were submerged with 10 ml of deionized water, purged with pure N_2 gas for 5 min and capped with a butyl rubber stopper and an aluminum seal. They were later incubated at

30 °C for 4 weeks. Then, the difference we aimed to investigate between our samples during this anaerobic incubation would only be derived from the effect of soil temperature and moisture during the precedent aerobic incubation step. The three replicates within each treatment were used to measure the headspace concentrations of CO_2 and CH_4 as same as for the aerobic incubation. After anaerobic incubation, soil samples in serum bottles were air-dried at 70 °C for 48 h; and finely ground into powder for SOC, TN, NH_4^+-N, NO_3^--N, $\delta^{13}C$ value measurements as described in section 2.2.2.

2.2.4. Q_{10} and $\delta^{13}C$ calculation

In our study, temperature coefficient (Q_{10}) was calculated to assess the increase in cumulative CO_2 production as a result of a temperature increase of 10 °C during the 24-week aerobic incubation. It was calculated from equation (2):

$$Q_{10} = \left(K_2 / K_1\right)^{10/(T_2 - T_1)} \quad (2)$$

where k was the cumulative CO_2 production in aerobic incubation and T was the temperature level of ±5, 5, 15 and 25 °C, respectively. The average temperature change at ±5 °C was regarded as 0 °C.

The $\delta^{13}C$ value was expressed in part per thousand (‰) relative to the Vienna Pee Dee Belemnite (VPDB) international standard and calculated as following equation (3):

$$\delta^{13}C = \left[\left(R_{soil} - R_{standard}\right) / R_{standard}\right] \times 1000 \quad (3)$$

Where R_{soil} and $R_{standard}$ were $^{13}C/^{12}C$ isotopic ratio in sample and VPDB,

respectively.

2.2.5. CO_2, CH_4, NO_3^--N and NH_4^+-N measurements

The CO_2 and CH_4 in headspace after aerobic and anaerobic incubation phrases were measured by gas chromatography (GC-8A; Shimadzu, Kyoto, Japan) equipped with thermal conductivity detector (TCD) and flame ionization detector (FID). NO_3^--N and NH_4^+-N concentrations in soil extracts with 30 ml of 10% KCl solution after pre-incubation, aerobic and anaerobic incubation were determined by the nitroprusside method (Anderson and Ingram, 1989) and by the hydrazine reduction method (Sawicki and Scaringelli, 1971; Hayashi et al., 1997), respectively.

NO_3^--N production during aerobic incubation was calculation with the difference in NO_3^--N concentration between soil samples after aerobic incubation and pre-incubation. Similarly, NH_4^+-N production during anaerobic incubation was the deduction of NH_4^+-N concentration between soil samples after anaerobic incubation and 24-week aerobic incubation. It should be noted that NO_3^--N concentration in the anaerobic incubated soil samples was below the detection limit.

2.2.6. Statistical analysis

Two-way analyses of variance (ANOVA) were performed to evaluate the effects of soil temperature, moisture, and their interaction on CO_2 and CH_4 productions, total decomposed C, mineralized N and the ratios of total decomposed C to SOC for two paddy soils. The statistical analysis was computed using SPSS statistics version 21

(SPSS Inc., Chicago, IL, USA).

2.3. Results

2.3.1. Changes in SOC content during the aerobic incubation

Changes in SOC content between Andisol and Inceptisol after every 6 weeks of aerobic incubation is shown in Fig.2.4. SOC content varied from 13.00 to 13.88 g C kg^{-1} in Inceptisol and from 86.25 to 88.40 g C kg^{-1} in Andisol, respectively. The decreases in SOC content in both two soils under low temperatures (±5 and 5 °C) were relatively stable and slightly decreased with incubation time. While, the distinct decreasing trend of SOC content in both soils was observed in high temperatures (15 and 25 °C). On the whole, the main trends of SOC content in both soils decreased with incubation time. ANOVA results showed that SOC contents in both two soils after 24-week aerobic incubation were significantly decreased by temperature (both $P<0.01$) and soil moisture (both $P<0.01$). However, no significantly interactive effects of temperature and moisture on SOC content after 24-week aerobic incubation was observed in both Andisol and Inceptisol paddy soils.

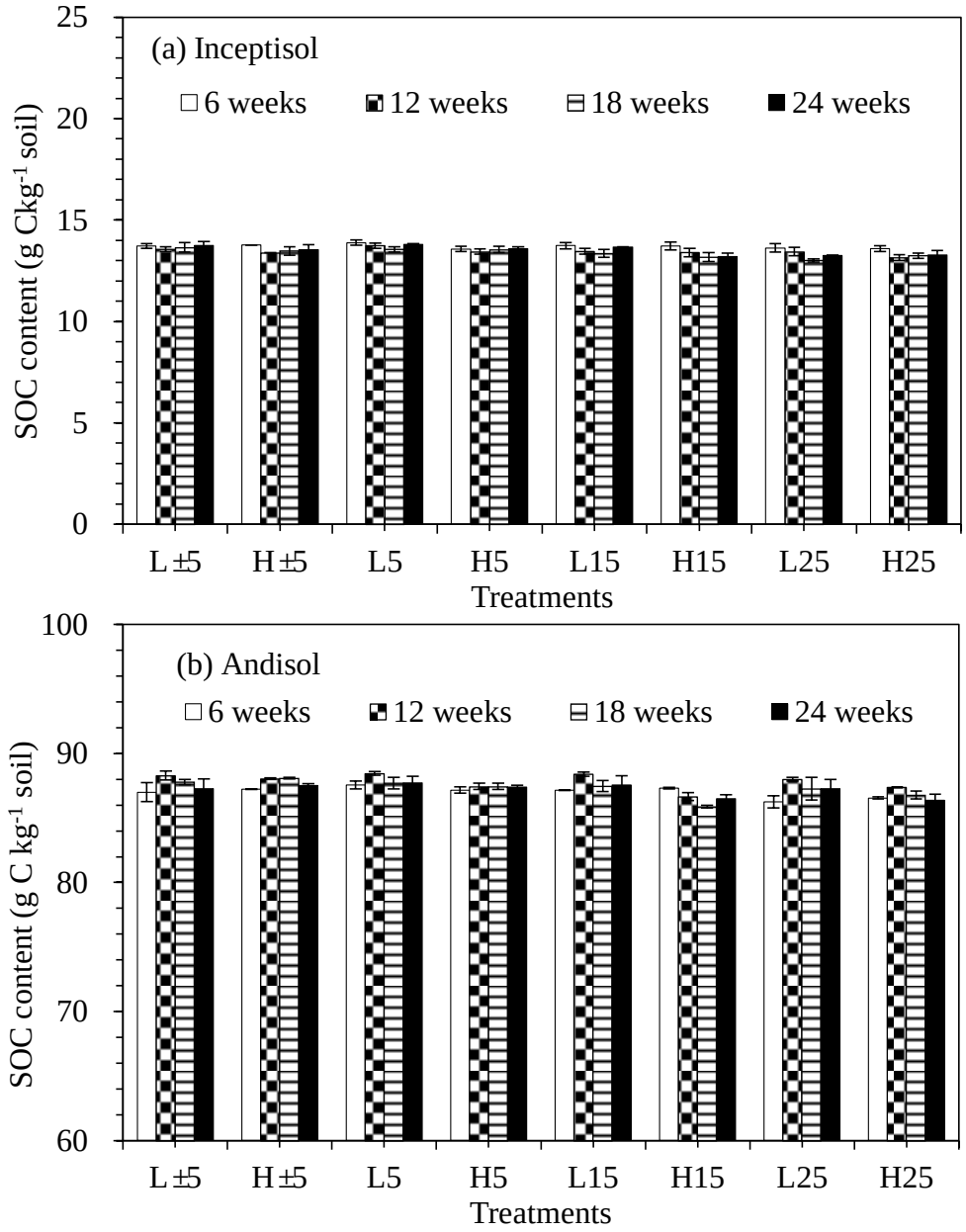

Fig.2.4. Changes in SOC content in Inceptisol (a) and Andisol (b) paddy soils after 6, 12, 18 and 24 weeks of aerobic incubation under four soil temperature and two moisture conditions. Low (60% WFPS) and high (100% WFPS) moistures were abbreviated to L and H, respectively. The error bars were the standard deviations (n=3).

2.3.2. Changes in TN content during the aerobic incubation

Changes in TN content in two paddy soils after 24-week aerobic incubation under four temperature and two moisture conditions are shown in Fig 2.5. TN content ranged between 1.02 and 1.23 g N kg^{-1} in Inceptisol and between 6.27 and 6.69 g N kg^{-1} in Andisol, respectively. TN contents in both soils among all treatments flocculated with time with large standard deviation. Overall, TN was slightly decreased with increasing soil temperature and moisture. ANOVA results showed that soil temperature and moisture had no significant effect on TN content in two paddy soils after 24-week aerobic incubation.

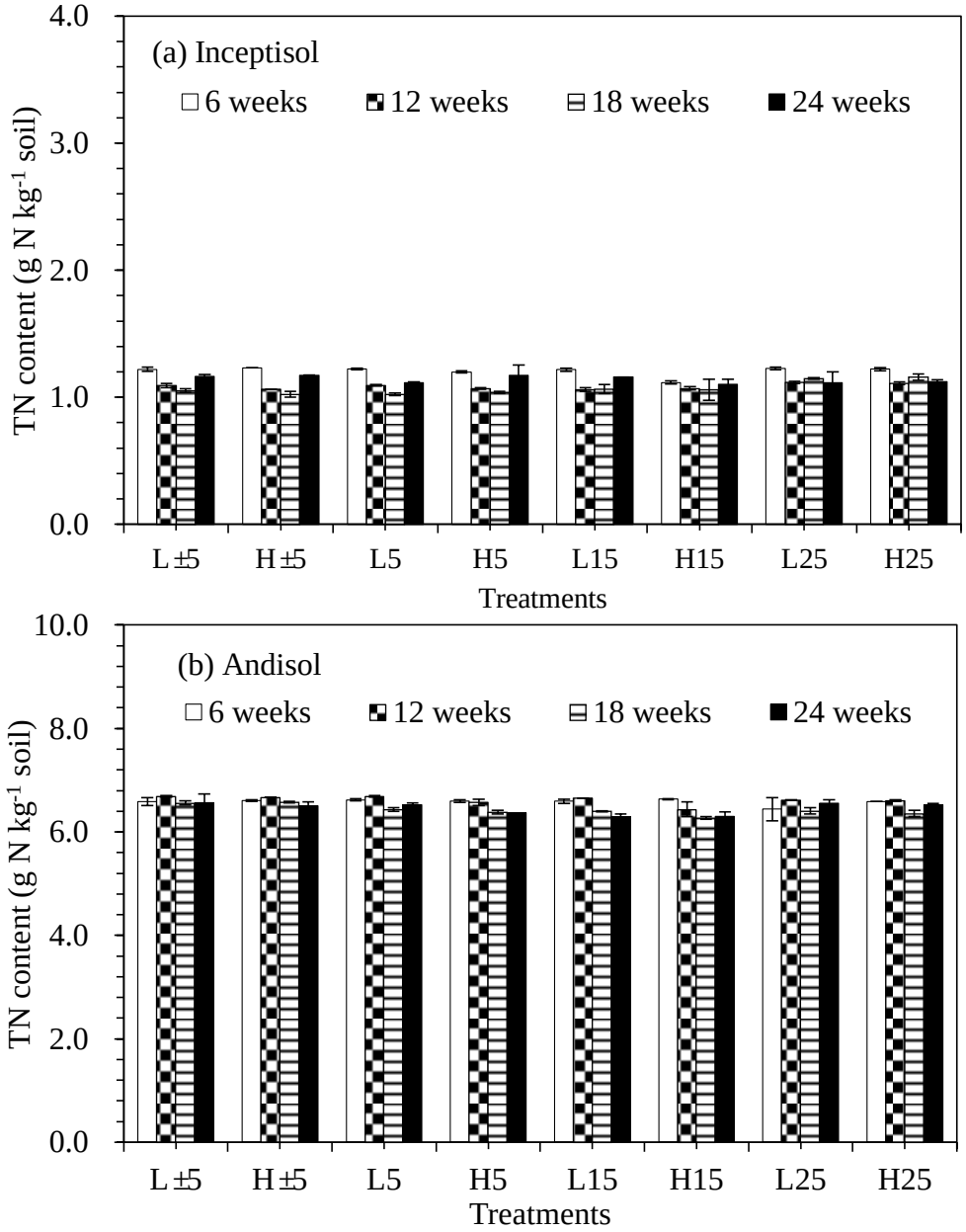

Fig.2.5. Changes in TN content in Inceptisol (a) and Andisol (b) paddy soils after 6, 12, 18 and 24 weeks of aerobic incubation under four soil temperature and two moisture conditions. Low (60% WFPS) and high (100% WFPS) moistures were abbreviated to L and H, respectively. The error bars were the standard deviations (n=3).

2.3.3. Changes in $\delta^{13}C$ value during the aerobic incubation

Changes in $\delta^{13}C$ value in Inceptisol and Andisol after 6, 12, 18 and 24 weeks of aerobic incubation are shown in Fig. 2.6. The $\delta^{13}C$ value varied from -24.48 to -24.21‰ in Inceptisol and from -21.96 to -22.17‰ in Andisol, respectively. The means of $\delta^{13}C$ value in both soils under different temperature and moisture conditions were higher than the $\delta^{13}C$ values in each original soil before incubation. The temporal changes in $\delta^{13}C$ value in both soils under high soil temperatures (15 and 25 °C) were larger than under low temperatures (±5 and 5 °C). ANOVA results indicated that only soil temperature had a significant effect on $\delta^{13}C$ value in Inceptisol after 24-week incubation. However, no significant effect of soil temperature and moisture was found on $\delta^{13}C$ value in Andisol after 24-week aerobic incubation.

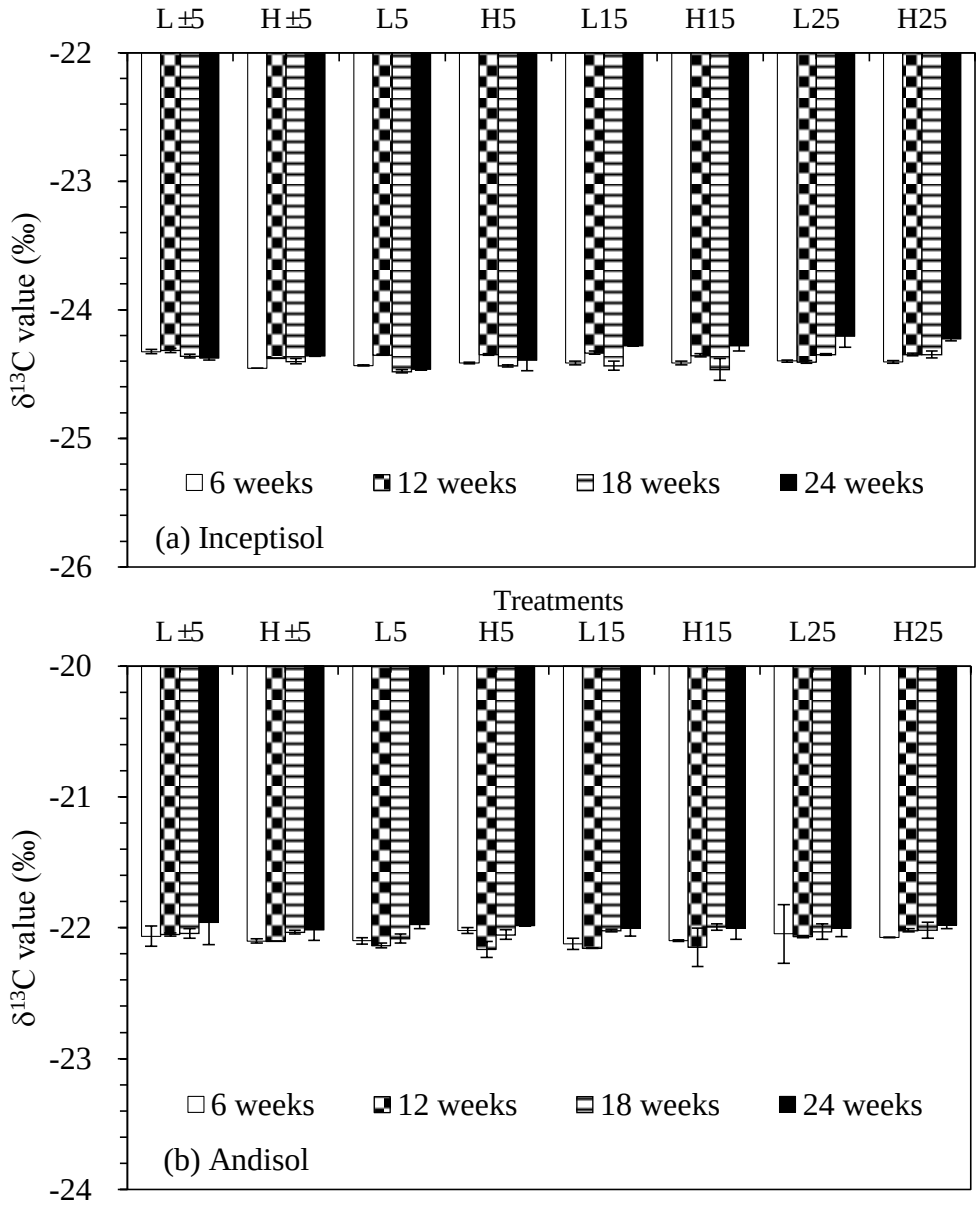

Fig.2.6. Changes in $\delta^{13}C$ value in Inceptisol (a) and Andisol (b) paddy soils after 6, 12, 18 and 24 weeks of aerobic incubation under four soil temperature and two moisture conditions. Low (60% WFPS) and high (100% WFPS) moistures were abbreviated to L and H, respectively. The error bars were the standard deviations (n=3).

2.3.4. Changes in NO_3^--N concentration during the aerobic incubation

The NO_3^--N concentration varied from 14.9 to 48.3 mg N kg^{-1} in Inceptisol which were lower than NO_3^--N concentration in Andisol ranged between 19.7 and 131.7 mg N kg^{-1} (Fig. 2.7). The NO_3^--N concentration in both soils under all soil temperature and moisture treatments significantly increased with incubation time. The average of NO_3^--N concentration in both soils was also larger than NO_3^--N concentration in each initial soil before incubation. ANOVA results showed soil temperature and moisture significantly increased NO_3^--N concentration in both soils (P<0.05). The increasing trend of NO_3^--N concentration under low temperatures was not as large as those under high temperatures. Also, there was significant interaction of soil temperature and moisture on NO_3^--N concentration in two kinds of paddy soils (P<0.05).

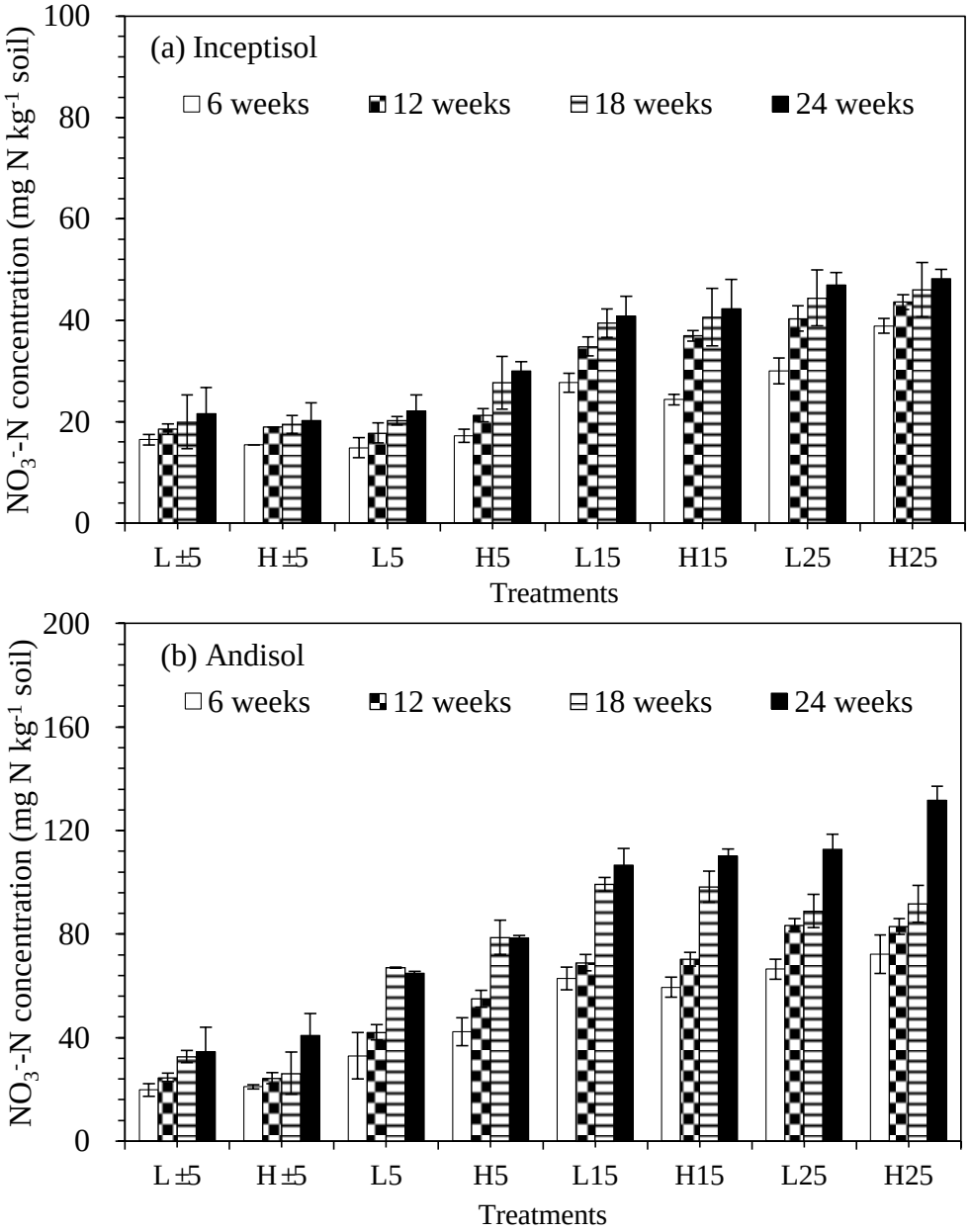

Fig.2.7. Changes in NO_3^--N concentration in Inceptisol (a) and Andisol (b) paddy soils after 6, 12, 18 and 24 weeks of aerobic incubation under four soil temperature and two moisture conditions. Low (60% WFPS) and high (100% WFPS) moistures were abbreviated to L and H, respectively. The error bars were the standard deviations (n=3).

2.3.5. Changes in NH_4^+-N concentration during the aerobic incubation

The temporal decreasing trends of NH_4^+-N concentration in both paddy soils under four soil temperature and moisture conditions are shown in Fig.2.8. The NH_4^+-N concentration varied from 8.92 to 30.01 mg N kg^{-1} in Inceptisol which were lower than those in Andisol ranging from 24.33 to 80.58 mg N kg^{-1}. NH_4^+-N concentration decreased with increasing soil temperature and moisture. However, the decrease in NH_4^+-N concentrations in both soils under low temperature treatments were lower than those under high moisture treatments. Statistical analysis indicated that soil temperature had significant impact on NH_4^+-N concentration in both soils. While, no significant effect of soil moisture on NH_4^+-N concentration was not found in both soils.

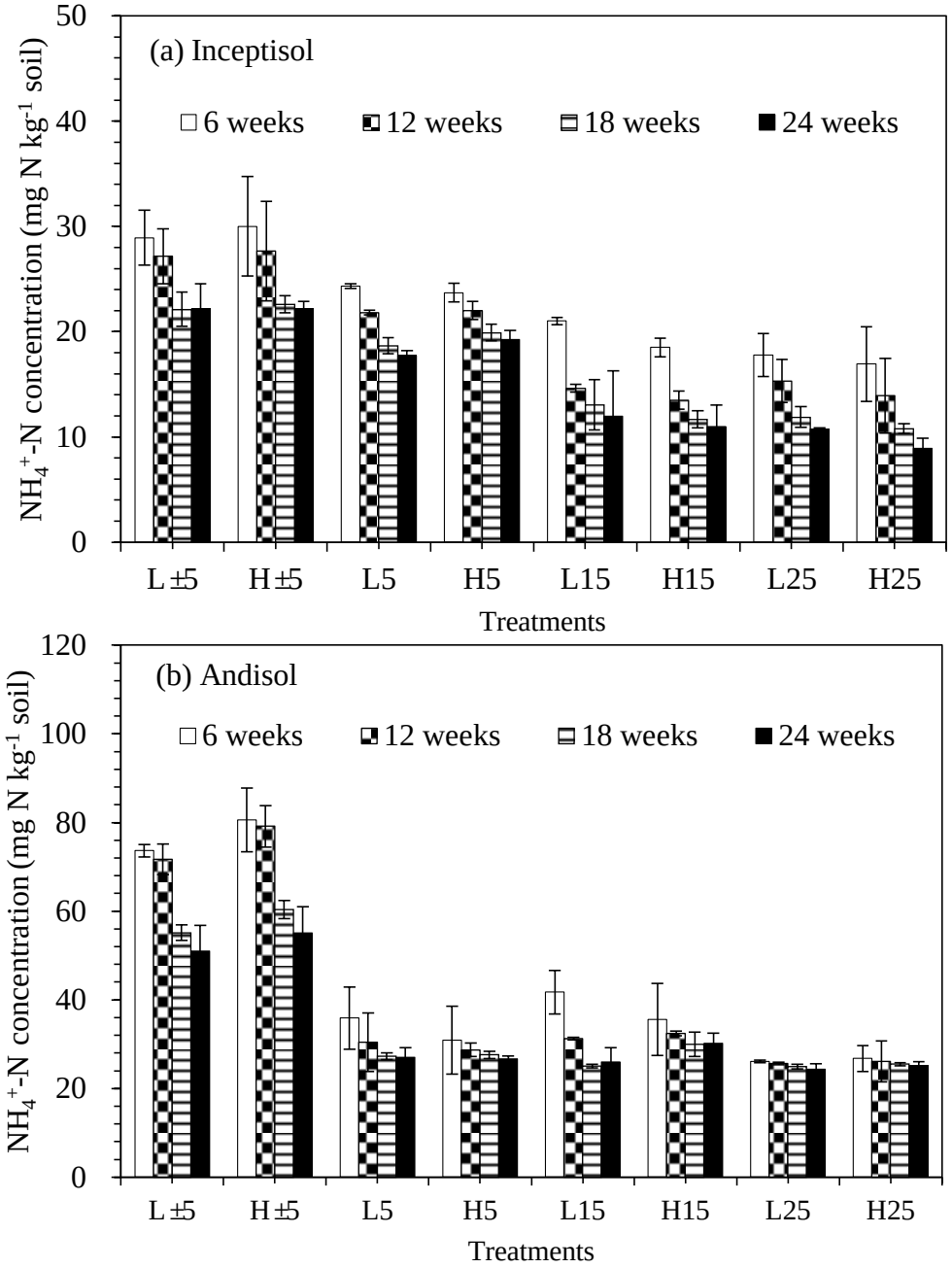

Fig.2.8. Changes in NH_4^+-N concentration in Inceptisol (a) and Andisol (b) paddy soils after 6, 12, 18 and 24 weeks of aerobic incubation under four soil temperature and two moisture conditions. Low (60% WFPS) and high (100% WFPS) moistures were abbreviated to L and H, respectively. The error bars were the standard deviations (n=3).

2.3.6. CO_2 production during the aerobic incubation

The cumulative CO_2 productions during the 24-week aerobic incubation in the Andisol and Inceptisol paddy soils are shown in Fig 2.9. The cumulative CO_2 production increased with soil temperature for both paddy soils. The total CO_2 productions ranged from 72.2 to 1018.6 mg C kg^{-1} in Andisol and from 64.9 to 599.8 mg C kg^{-1} in Inceptisol, respectively (Table 2.2). Soil temperature and moisture had positive effects on CO_2 production in all soil samples (Table 2.2, $P<0.01$). For the same temperature and moisture conditions, the cumulative CO_2 productions in Andisol were always higher than in Inceptisol. However, at low temperatures, the difference between the two soils was not as distinct as that at high temperatures. Besides, an interactive effect of soil temperature and moisture was observed for both paddy soils.

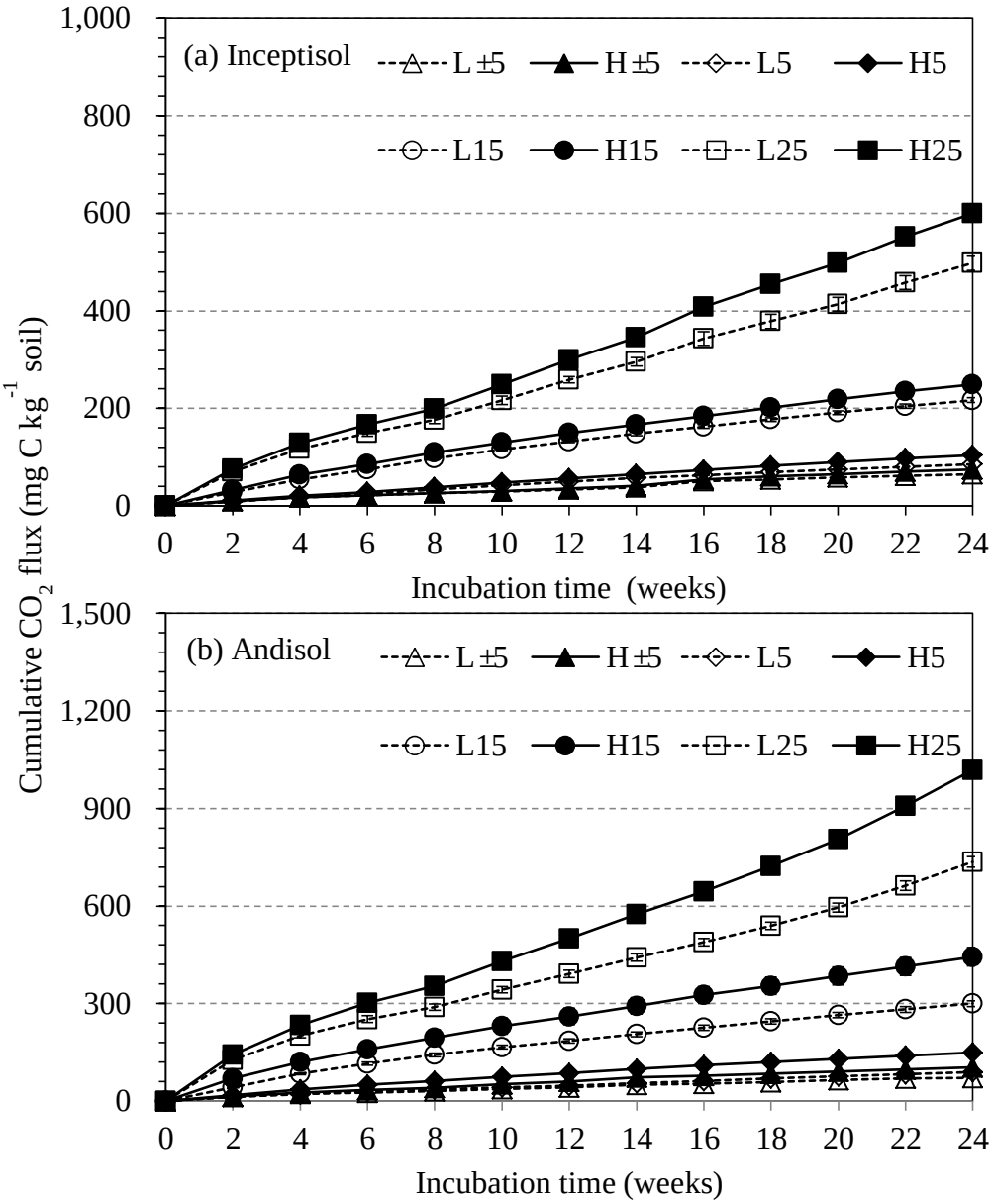

Fig.2.9. Cumulative CO_2 production from Inceptisol (a) and Andisol (b) soil samples during the 24-week aerobic incubation under four temperature (±5, 5, 15 and 25 °C) and two moisture (60% and 100% WFPS) conditions. Low (60% WFPS) and high (100% WFPS) moistures were abbreviated to L and H, respectively. The error bars were the standard deviations (n=3).

Table 2.2 The cumulative CO_2 productions during the 24-week aerobic incubation under four temperature and two moisture conditions, CO_2 and CH_4 productions (CO_2 + CH_4) during the subsequent 4-week anaerobic incubation at 30 °C under submerged conditions, and the total decomposed C during both aerobic and anaerobic incubations of Andisol and Inceptisol soil samples, respectively. Each data was presented as mean and standard deviation (n=3). The *P* values were obtained from the two-way ANOVA for the effects of temperature, moisture and their interaction on each tested parameter.

Temperature	Moisture (WFPS)	Code	Aerobic incubation (CO_2) (mg C kg^{-1} soil)		Anaerobic incubation (CO_2+CH_4) (mg C kg^{-1} soil)		Total decomposed C (CO_2+CH_4) (mg C kg^{-1} soil)	
			Andisol	Inceptisol	Andisol	Inceptisol	Andisol	Inceptisol
±5 ℃	60%	L±5	72.2±6.3	64.9±3.4	184.7±4.7	248.3±21.0	256.9±6.1	313.2±21.2
	100%	H±5	103.3±1.6	74.5±4.6	177.5±7.4	226.8±4.8	280.8±9.0	301.3±9.4
5 ℃	60%	L5	89.1±4.9	85.6±4.4	174.5±6.5	238.7±18.9	263.6±11.3	324.2±19.1
	100%	H5	149.5±2.9	103.7±7.7	165.6±14.1	233.9±22.9	315.1±16.4	337.7±27.5
15 ℃	60%	L15	299.8±9.1	216.8±4.7	139.6±2.0	98.0±0.4	439.4±8.2	314.8±5.1
	100%	H15	443.8±28.4	249.0±13.1	144.5±17.5	87.5±1.8	588.3±34.2	336.5±14.3
25 ℃	60%	L25	736.0±15.9	497.6±14.7	124.1±2.1	76.7±1.9	860.1±17.8	574.4±12.9
	100%	H25	1018.6±12.7	599.8±14.0	127.5±0.8	69.0±1.9	1146.1±13.4	668.8±12.9
ANOVA results (*P* value)								
Temperature			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Moisture			<0.01	<0.01	0.60	0.05	<0.01	<0.01
Temperature × Moisture			<0.01	<0.01	0.44	0.71	<0.01	0.06

2.3.7. CO_2 and CH_4 productions during the anaerobic incubation

During the 4-week anaerobic incubation, soil temperature and moisture conditions were strictly the same for all soil samples (30 °C and submerged conditions). Therefore, the measured differences were resulted from the effect applied during the previous aerobic incubation phase. In the following sections, we named previous soil temperature and moisture conditions during the aerobic phase of the incubation. The CO_2 and CH_4 productions during the 4-week anaerobic incubation are shown in Table 2.3. The CO_2 production ranged from 124.1 to 184.6 mg C kg^{-1} in Andisol and from 68.9 to 248.1 mg C kg^{-1} in Inceptisol, respectively. The CO_2 production in both paddy soils was higher for previous temperatures at ±5 and 5°C and lower for previous temperatures at 15 and 25 °C. The difference in CO_2 production between ±5 °C and 25 °C for previous temperature treatment was larger in the Inceptisol than in the Andisol soil samples. A significant effect of previous temperature on CO_2 production was found for each soil (Table 2.3, $P<0.01$). The CH_4 production ranged from 7.4 to 110.8 μg C kg^{-1} in Andisol and from 9.0 to 243.9 μg C kg^{-1} in Inceptisol. The CH_4 productions were distinctly lower than CO_2 production for both paddy soils. There was no significant effect of previous soil moisture on CO_2 and CH_4 productions during the subsequently 4-week anaerobic incubation (Table 2.3).

Table 2.3 The CO_2 and CH_4 productions from Andisol and Inceptisol soil samples during the 4-week anaerobic incubation at 30 °C and under submerged conditions. Each data was presented as mean and standard deviation (n=3). The *P* values were obtained from the two-way ANOVA for the effects of temperature, moisture and their interaction on each tested parameter.

Tempe-rature	Moisture (WFPS)	Code	CO_2 production (mg C kg^{-1} soil)		CH_4 production (μg C kg^{-1} soil)	
			Andisol	Inceptisol	Andisol	Inceptisol
±5 ℃	60%	L±5	184.6±4.8	248.1±21.0	99.9±58.2	192.4±21.6
	100%	H±5	177.4±7.4	226.6±4.8	74.2±21.6	243.9±14.6
5 ℃	60%	L5	174.3±6.5	238.5±18.9	110.8±77.2	170.6±31.0
	100%	H5	165.5±14.1	233.8±22.9	70.3±25.5	154.0±0.0
15 ℃	60%	L15	139.6±2.0	98.0±0.4	15.6±4.5	21.6±12.2
	100%	H15	144.5±17.5	87.5±1.8	7.4±0.8	11.6±1.6
25 ℃	60%	L25	124.1±2.1	76.7±1.9	7.8±2.0	15. 9±6.0
	100%	H25	127.5±0.8	68.9±1,9	8.8±4.1	9.0±1.3
ANOVA results (*P* value)						
Temperature			<0.01	<0.01	<0.01	<0.01
Moisture			0.60	0.05	0.23	0.47
Temperature × Moisture			0.44	0.71	0.76	<0.01

2.3.8. Total decomposed C and the ratios of total decomposed C to SOC

The total decomposed C at the end of the whole incubation (aerobic + anaerobic) ranged from 256.9 to 1146.1 mg C kg^{-1} in Andisol and from 301.3 to 668.8 mg C kg^{-1} in Inceptisol, respectively. Under low temperature conditions (±5 and 5 °C), the total decomposed C was higher for the Inceptisol than for the Andisol while the situation was inverted under high temperature conditions (15 and 25 °C) (Table 2.2). Both the previous soil temperature and moisture had significant effects on total decomposed C for each soil. The ratios of anaerobically decomposed C (CO_2-C+CH_4-C) to total decomposed C varied from 11.1 to 71.9% in Andisol and from 10.3 to 79.2% in

Inceptisol (Table 2.4), respectively. Moreover, for two paddy soils, the ratios of anaerobically decomposed C to total decomposed C decreased with the increases of previous soil temperature and moisture (Table 2.4). The ratios of anaerobically decomposed C to SOC varied from 0.14 to 0.21% in Andisol and from 0.51 to 1.82% in Inceptisol, respectively. Only the effect of previous temperature on the ratios of anaerobically decomposed C to SOC was found significant for both paddy soils (Table 2.4). Total decomposed C/SOC ranged from 2.21 to 4.91% in Inceptisol, clearly higher than that in Andisol with values ranging from 0.29 to 1.29%. The effect of previous soil temperature and moisture, and their interaction on total decomposed C/SOC were significant for both paddy soils (Table 2.4).

Table 2.4 Ratios of anaerobically decomposed C to total decomposed C (aerobic + anaerobic), anaerobically decomposed C to soil organic C (SOC), and total decomposed C to SOC in Andisol and Inceptisol soil samples, respectively. The *P* values were obtained from the two-way ANOVA for the effects of temperature, moisture and their interaction on each tested parameter. The data was presented as mean and standard deviation in each treatment with 3 replications (n=3).

Temperature	Moisture (WFPS)	Code	Anaerobic decomposed C / total decomposed C (%)		Anaerobic decomposed C / SOC (%)		Total decomposed C / SOC (%)	
			Andisol	Inceptisol	Andisol	Inceptisol	Andisol	Inceptisol
±5 ℃	60%	L±5	71.9±2.0	79.2±1.6	0.21±0.01	1.82±0.15	0.29±0.01	2.30±0.16
	100%	H±5	63.2±0.6	75.3±0.8	0.20±0.01	1.67±0.04	0.32±0.01	2.21±0.07
5 ℃	60%	L5	66.2±0.5	73.6±1.9	0.20±0.01	1.75±0.17	0.30±0.01	2.38±0.14
	100%	H5	52.5±1.7	69.2±1.9	0.19±0.02	1.72±0.00	0.35±0.02	2.48±0.20
15 ℃	60%	L15	31.8±0.9	31.1±0.4	0.16±0.00	0.72±0.01	0.49±0.01	2.31±0.04
	100%	H15	24.6±2.4	26.0±0.8	0.16±0.02	0.64±0.01	0.66±0.04	2.47±0.11
25 ℃	60%	L25	14.4±0.1	13.4±0.6	0.14±0.00	0.56±0.01	0.97±0.02	4.22±0.09
	100%	H25	11.1±0.1	10.3±0.4	0.14±0.00	0.51±0.01	1.29±0.02	4.91±0.09
ANOVA results (*P* value)								
Temperature			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Moisture			<0.01	<0.01	0.60	0.05	<0.01	<0.01
Temperature × Moisture			<0.01	0.53	0.44	0.71	<0.01	<0.01

2.3.9. Changes in the Q_{10} of aerobic decomposition of SOC

The changes in Q_{10} values for Andisol and Inceptisol during the 24-week aerobic incubation are shown in Fig. 2.10. The Q_{10} values between 5 °C and 15 °C were always larger than those from 15 °C to 25 °C and from ±5 °C to 5 °C in both soils. The Q_{10} values were larger under low moisture condition than those under high moisture condition from 5 °C to 15 °C in the Andisol. Nevertheless, the Q_{10} values were smaller under low moisture condition than those under high moisture between ±5 °C and 5 °C in both soils (Fig. 2.10). The mean Q_{10} values of SOC decomposition rates under the four temperature and two moisture treatments calculated from 12-time measurements during the 24-week aerobic incubation are shown in Table 2.5. The averaged Q_{10} value in two soils was highest at temperature increases from 5 to 15 °C, and lowest at temperature changes from ±5 to 5 °C.

Table 2.5 The mean values of Q_{10} for SOC decomposition as cumulative CO_2 production obtained from 24-week aerobic incubation of Andisol and Inceptisol soil samples under the four soil temperature (±5, 5, 15 and 25 °C) and two moisture (60% and100% WFPS) conditions. Low (60% WFPS) and high (100% WFPS) moistures are abbreviated to Low M. and High M., respectively. Temperature difference at ±5 °C to 5 °C was considered as 5 °C.

	from ±5 to 5 °C		from 5 to 15 °C		from 15 to 25 °C	
	Low M.	High M.	Low M.	High M.	Low M.	High M.
Andisol	1.5	2.1	3.4	3.0	2.5	2.3
Inceptisol	1.7	1.9	2.5	2.4	2.3	2.4

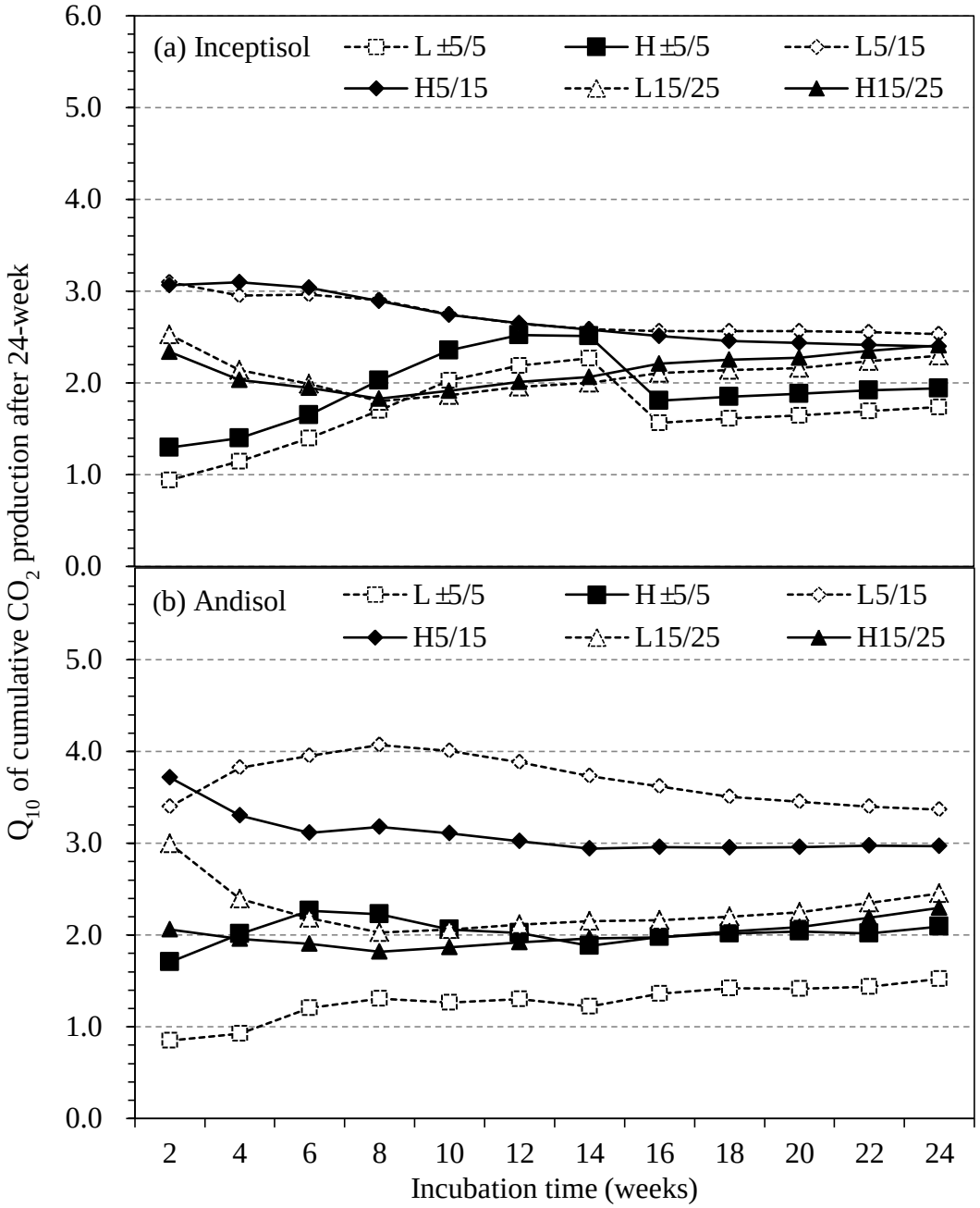

Fig.2.10. Values of Q_{10} for SOC decomposition as cumulative CO_2 production in Inceptisol (a) and Andisol (b) soil samples during the 24-week aerobic incubation under four soil temperature levels (±5, 5, 15 and 25 °C) for both low and high moisture conditions (60 and 100% WFPS). Low (60% WFPS) and high (100% WFPS) moistures are abbreviated to L and H, respectively.

2.3.10. Changes in SOC content, $\delta^{13}C$ value and NH_4^+-N concentration after 4-week anaerobic incubation

During the submerged condition, all soil samples were anaerobically incubated at 30 °C. Changes in SOC and TN contents, $\delta^{13}C$ value, and NH_4^+-N concentration after anaerobic incubation were the results of whole incubation (24-week aerobic + 4-week anaerobic incubation) (Table 2.6). Compared with 24-week aerobic incubation, the amounts of SOC and TN in both soils under previous four temperature and two moisture conditions only slightly decreased after anaerobic incubation. However, $\delta^{13}C$ value and NH_4^+-N concentration increased after anaerobic incubation. The NO_3^--N concentration in both soils were lower than detection limit. ANOVA results showed that SOC content were not significantly affected by soil temperature and moisture in both soils. While, the significant effect of previous temperature during aerobic incubation TN content was only found in Inceptisol rather than Anidisol. $\delta^{13}C$ value and NH_4^+-N concentration in both soils after anaerobic incubation were still significantly affected by previous soil temperature and moisture. No significant differences in SOC and TN contents, $\delta^{13}C$ value and NH_4^+-N concentration were observed in both soils after 4-week anaerobic incubation.

Table 2.6 Changes in SOC and TN contents, $\delta^{13}C$ value and NH_4^+-N concentration after subsequent 4-week anaerobic incubation under submerged and 30 °C conditions. The data was presented as mean and stand deviation in each treatment with 3 replications (n=3).

Temperature	Moisture (WFPS)	Code	SOC content (g C kg^{-1})		TN content (g N kg^{-1})		$\delta^{13}C$ value (‰)		NH_4^+-N concentration (mg N kg^{-1})	
			Inceptisol	Andisol	Inceptisol	Andisol	Inceptisol	Andisol	Inceptisol	Andisol
±5 ℃	60%	L±5	13.12±0.11	86.56±0.67	1.13± 0.01	6.49±0.05	-24.29±0.01	-22.07±0.05	86.3±0.9	111.5±2.8
	100%	H±5	13.17±0.66	85.93±1.60	1.13±0.03	6.44±0.12	-24.26±0.07	-22.04±0.03	86.9±4.1	110.7±1.6
5 ℃	60%	L5	13.03±0.04	87.10±0.81	1.13±0.01	6.38±0.04	-24.24±0.02	-21.99±0.02	90.0±3.8	91.1±1.1
	100%	H5	13.21±0.18	88.10±1.40	1.17±0.02	6.52±0.16	-24.31±0.03	-21.98±0.02	85.0±2.6	90.7±3.1
15 ℃	60%	L15	12.95±0.19	86.91±4.85	1.10±0.01	6.39±0.33	-24.27±0.08	-22.02±0.06	35.7±6.6	71.7±2.4
	100%	H15	12.92±0.19	83.59±2.45	1.10±0.02	6.18±0.15	-24.23±0.10	-21.96±0.03	30.8±3.1	72.9±6.4
25 ℃	60%	L25	12.98±0.27	85.59±0.37	1.12±0.02	6.32±0.04	-24.20±0.03	-21.93±0.03	33.0±3.8	63.1±3.3
	100%	H25	12.92±0.26	85.20±0.94	1.11±0.02	6.31±0.07	-24.12±0.03	-21.94±0.06	29.1±4.3	65.6±3.5
ANOVA results (*P* value)										
Temperature			0.48	0.24	<0.01	0.13	<0.01	<0.01	<0.01	<0.01
Moisture			0.78	0.35	0.41	0.59	0.47	0.21	0.06	0.66
Temperature × Moisture			0.90	0.39	0.10	0.27	0.18	0.55	0.59	0.82

2.4. Discussion

2.4.1. The amounts of SOC and TN affected by soil temperature and moisture

The SOC and TN contents in Andisol were remarkably larger than those in Inceptisol. Both soils had similar temporal trends of SOC contents during the aerobic and subsequently 4-week anaerobic incubation. Moreover, SOC contents in both soils were significantly affected by soil temperature and moisture. This result indicated that the elevation of soil temperature and moisture would lead to C loss in paddy soil due to the enhanced C decomposition which was line with previous studies (Zhou et al., 2014b; Huang et al., 2015). The temporal trend of TN content throughout 24-week aerobic incubation was not consistent in two soils. Specifically, TN content in Inceptisol rapidly decreased after 6-week incubation, then remained stable after 12-week incubation and unexpectedly increased after 18-week incubation However, TN content in Andisol increased after 6-week aerobic incubation in most treatments (except H5 and H15) and then rapidly decreased after 12-week incubation. The difference in dynamic of TN content between two soils probably could be mainly attributed to measurement accuracy and N loss during the procedures of soil oven-drying. In addition, contrary to SOC, TN content between two soils was not significantly affected by soil moisture throughout the aerobic incubation and after anaerobic incubation (Fig. 2.5 and Table 2.6). This result could be explained by the diminished effects of drying process of aerobic incubated soil samples prior to TN measurement. Further studies on the response of TN contents to soil temperature and moisture should be continued.

2.4.2. Effects of soil temperature and moisture on aerobic SOC decomposition

In this study, we simulated various climatic conditions (four soil temperature and two moisture conditions) for the off-rice season in the cold temperate region of northeastern Japan, in order to study the effect of soil temperature and moisture on SOC decomposition and N mineralization. The cumulative CO_2 production in both soil samples increased distinctly with the increase of soil temperature during the 24-week aerobic incubation (Fig.2.9 and Table 2.2). This result was consistent with Zhou et al. (2014a) who incubated three paddy soils at five temperatures (10, 15, 20, 25 and 30 °C) and 90% water content for 160 days. The positive feedback of SOC decomposition to increasing temperature could be attributed to the acceleration of microbial activities and uptake of soluble substrates resulting in increased soil respiration rates. Several studies reported that soil moisture had a significant impact on SOC decomposition mainly by affecting substrate availability and oxygen diffusion (Linn and Doran, 1984; Suseela et al., 2012; Wang et al., 2014; Zhou et al., 2014b; Sierra et al., 2015). The optimum soil moisture for SOC decomposition was usually found at intermediate level, (e.g., 60%WFPS) (Linn and Doran 1984; Oberbauer et al., 1992; Jassal et al., 2008). However, in this study, the cumulative CO_2 productions were higher at high moisture (100% WFPS) than those at low moisture (60% WFPS) (Fig. 2.9). The CO_2 production was not limited by high moisture in this study. This result might be due to enough oxygen availability by the shallow soil layer (about 1cm) and to the more soluble substrate for microbial activities.

The parameter Q_{10} is usually used to predict the response of SOC decomposition and sequestration in terrestrial ecosystems to future global warming (Fang et al., 2005; Xu et al., 2010; He et al., 2013; Zhou et al., 2014b). The mean values of Q_{10} for SOC decomposition as cumulative CO_2 production from the Andisol and the Inceptisol

computed from 12-time measurements during the 24-week aerobic incubation ranged from 1.7 to 2.4 in Inceptisol and from 1.5 to 3.4 in Andisol, respectively (Table 2.5). For both soils, the highest Q_{10} was found under low moisture (60% WFPS) between 5 and 15 °C (L5/15) and lowest at low moisture between ±5 and 5 °C (L±5/5) (Fig. 2.10 and Table 2.5). Moreover, Andisol had higher Q_{10} than Inceptisol at the temperature change from 5 to 15 °C under the two moisture regimes. This finding indicated that the decomposition of SOC in Andisol was more sensitive to temperature increase from 5 to 15 °C. Temperature at ±5 °C was used to simulate the freeze-thaw cycles during off-rice season in Tohoku region of Japan. Soil moisture only enhanced the Q_{10} at the temperature increase from ±5 to 5 °C in Andisol and Inceptisol. This result suggested that the freeze-thaw cycles enhanced the microbial activities at high moisture than that at low moisture. Previous studies have shown that freeze-thaw cycles can affect soil and plant residues decomposition, resulting in great changes in C cycles in terrestrial ecosystems mainly owing to the increase of nutrient availability (Yanai et al., 2004; Xu et al., 2016b). However, in our study, the total CO_2 productions in both soils at ±5 °C were lower than those at 5 °C. It could be attributed to relatively small temperature change between ±5 and 5 °C compared with those in previous studies (Yanai et al., 2004; Xu et al., 2016b).

2.4.3. CH_4 production during the anaerobic incubation

The decomposition of SOC in flooded paddy soils is different from that of aerobic soils because under submerged conditions with low redox potential, a significant fraction of SOC is fermented to CH_4 during rice growth season with high temperature and anaerobic condition (Kimura et al., 2004). Here, the rice growth season was simulated by the 4-week anaerobic incubation at 30 °C and under submerged

conditions. Compared with CO_2 production (more than 68.9 mg C kg^{-1}) during the 4-week anaerobic incubation, the CH_4 productions were very low (less than 243.9 μg C kg^{-1}) in all soil samples (Table 2.3). Furthermore, anaerobic CH_4 productions in soil samples at previous low temperatures (±5 and 5 °C) were distinctly larger than those at high temperatures (15 and 25 °C). This suggested that labile organic carbon was more beneficial to CH_4 production during the following anaerobic incubation.

The low CH_4 production during the 4-week anaerobic incubation in both soils could be explained in two reasons. Firstly, it was due to the fact that large part of labile substrate was decomposed during the previous aerobic incubation. Secondly, various electron acceptors in the soil samples were not completely reduced during the 4-week anaerobic incubation (Cheng et al., 2007). It has been previously reported that CH_4 was produced from the decomposition of SOC only after various electron acceptors (e.g., O_2, NO_3^-, $Fe^{3+,}$ Mn^{4+} and SO_4^{2-}) were completely reduced (Watanabe, 1984; Peters and Conrad, 1996; Yao et al., 1999; Cheng et al., 2007; Tokida et al., 2010). Substantial accumulation of nitrate was found at the end of aerobic incubation, which would also inhibit CH_4 production in subsequently anaerobic incubation. A significant negative correlation (P<0.01) between CH_4 production under anaerobic condition and NO_3^--N concentration after aerobic incubation was found in two soils in our study (Fig.2.11). Denitrification occurred during the following anaerobic incubation undoubtedly resulted in N_2O production which had much greater global warming potential than CH_4. In addition, we found a negative effect of the previous soil temperature on the combined CO_2 and CH_4 productions during subsequent anaerobic incubation (Table 2.2), and significant effects of previous soil temperature and moisture on ratios of anaerobic decomposed C to total decomposed C during both aerobic and anaerobic incubations (Table 2.4). This revealed that early stage of aerobic SOC decomposition

would affect the following anaerobic decomposition of SOC. On the basis of this finding in our incubation experiment, it implied that to some extent, increasing temperature and moisture in rice paddy field during off-rice season would decrease CH_4 emission during rice growth season.

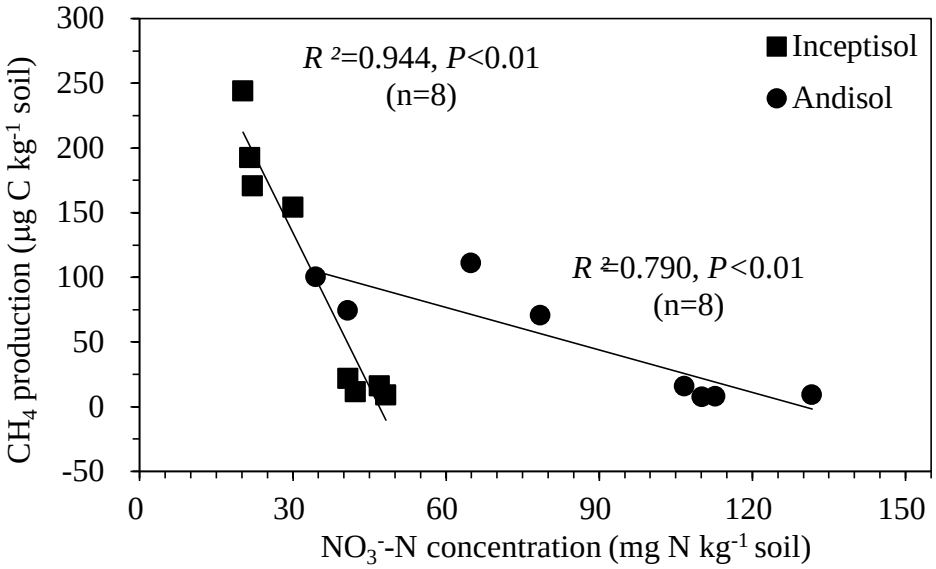

Fig.2.11. The correlation ship between CH_4 production during 4-week anaerobic incubation and NO_3^--N concentration after 24-week aerobic incubation in Inceptisol and Andisol paddy soils.

2.4.4. Comparison of SOC decomposition between the Andisol and the Inceptisol

The Andisol had higher aerobic CO_2 production than Inceptisol during the 24-week aerobic incubation (Fig. 2.9 and Table 2.2). This can be attributed to differences in SOC contents between Andisol (89.0 g C kg^{-1}) and Inceptisol (13.6 g C kg^{-1}). However, the aerobic CO_2 production from Andisol was not greatly larger than those of Inceptisol, despite the 6.5 times difference in SOC content between the two soils (Table 2.1). Moreover, the ratios of anaerobic decomposed C to SOC and the ratios of total decomposed C to SOC were largely different between Andisol and Inceptisol (Table 2.4). These results indicated that a greater fraction of SOC in Andisol was more stabilized than in Inceptisol. Our observations are consistent with previous

studies. For example, Parfitt et al. (1996) reported that SOC was more stable in the Andisol than in the Inceptisol under two contrasting land uses namely, pasture and cropland. It has been reported that Andisol contains abundant non-crystalline and poorly crystalline minerals and oxides which are chemically and physically associated with SOM to form soil organo-mineral complexes (Shoji et al., 1994; Matus et al., 2006; Chevallier et al., 2010). Tate et al. (1990) reported that SOM content in well-developed Andisols could reach up to 20%, and the degree of humification of humic acids in Andisol was higher than that of non-Andisols (Inceptisols, Histosols and Oxisols). This can also be used to explain why the ratio of total decomposed C to SOC was lower in the Andisol than in the Inceptisol in our study.

2.4.5. Comparison of N mineralization between Andisol and Inceptisol

During 4-week aerobic incubation, N mineralization from organic N to inorganic N (NO_3^--N and NH_4^+-N) was the main process of N transformation in rice paddy soils. During the subsequent anaerobic incubation, NO_3^--N can be consumed by denitrification and organic N will convert into NH_4^+-N. Therefore, the sum of NH_4^+-N and NO_3^--N productions can be considered as aerobic N mineralization. Similarly, NH_4^+-N production during anaerobic incubation can be regarded as anaerobic N mineralization (JSSPN, 1986; Cheng et al., 2016). Our results showed that soil temperature and moisture had great impacts on NO_3^--N and NH_4^+-N concentrations after 24-week aerobic incubation (Fig 2.7 and 2.8). The N mineralization during aerobic incubation was significantly affected by soil temperature in both soils. It indicated that soil temperature not only affected C decomposition but also had great impact on N mineralization. However, the significant soil moisture on aerobic N mineralization was only observed in Andisol. This result could be ascribed to the low

TN content in Inceptisol compared with Andisol. Although high TN content in Andisol, the ratios of aerobic mineralized N and anaerobic mineralized N to TN were lower than those in Inceptisol. This result suggested that the N in Inceptisol was much available than that in Andisiol (Table 2.7).

During the anaerobic incubation, the mineralized N in both soils was not significant affected by soil moisture since all soil samples had been submerged at the same water level. However, the mineralized N in both soils was still significantly affected by soil temperature. This finding suggested that soil temperature had much greater impacts on anaerobic N mineralization than moisture. In addition, the temperature trend of anaerobic N mineralization was different to that of aerobic N mineralization. It could be explained by the rapid depletion of labile N during aerobic incubation at high temperature.

Table 2.7 Aerobically mineralized N and anaerobically mineralized N and their ratios to TN in Inceptisol and Andisol. The parameters below were calculated by the mean values of mineralized N during aerobic and anaerobic incubations in each treatment with 3 replications (n=3).

Temperature	Moisture (WFPS)	Code	Aerobic N mineralization (mg N kg^{-1} soil)		Anaerobic N mineralization (mg C kg^{-1} soil)		Aerobic N mineralization/TN (%)		Anaerobic N mineralization/TN (%)	
			Inceptisol	Andisol	Inceptisol	Andisol	Inceptisol	Andisol	Inceptisol	Andisol
±5 ℃	60%	L±5	8.5	9.1	64.2	60.4	0.7	0.1	5.6	0.9
	100%	H±5	7.8	19.5	64.0	55.6	0.7	0.3	5.6	0.8
5 ℃	60%	L5	4.6	15.5	72.2	64.0	0.4	0.2	6.3	1.0
	100%	H5	14.1	28.8	65.7	64.0	1.2	0.4	5.7	1.0
15 ℃	60%	L15	17.7	56.2	23.7	45.8	1.5	0.8	2.1	0.7
	100%	H15	18.1	63.8	19.8	42.7	1.6	1.0	1.7	0.6
25 ℃	60%	L25	22.6	60.7	22.2	38.7	2.0	0.9	1.9	0.6
	100%	H25	22.0	80.4	20.2	40.4	1.9	1.2	1.8	0.6
ANOVA results (*P* value)										
Temperature			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Moisture			0.28	<0.01	0.06	0.42	0.28	<0.01	0.06	0.42
Temperature × Moisture			0.22	0.31	0.55	0.62	0.22	0.31	0.55	0.62

2.5. Conclusion

Significant enhancements of aerobic SOC decomposition and N mineralization by soil temperature were found in both Andisol and Inceptisol soil samples in this study. Soil moisture had significant impact on aerobic N mineralization only in Andisol. The aerobic and anaerobic N mineralization in both soils was significantly affected by soil temperature. Overall, the effect of soil temperature on C decomposition and N mineralization was greater than that of soil moisture. The total anaerobically decomposed C was decreased significantly with the increase of soil temperature applied during previous aerobic incubation. The rapid depletion of labile substrates and inhibition due to substantial amount of nitrate after aerobic incubation could be responsible for the extremely low CH_4 production during the subsequent anaerobic incubation. These results implied that acceleration of SOC decomposition during off-rice season by increasing soil temperature and moisture might be favorable to mitigate CH_4 production in rice growth season. Despite its high amounts of SOC and TN, the fractions of labile C and N expressed by decomposed C and mineralized N in Andisol were lower than those in Inceptisol, suggesting that SOC and TN in Andisol was more stable than Inceptisol. Since this research was conducted on the establishment of incubation experiments, further studies are needed to estimate the effect of soil temperature and moisture on SOC decomposition and N mineralization in rice paddy field during both off-rice and rice growth seasons.

Chapter Ⅲ Soil warming effects on soil C and N contents, and their mineralization potentials during both growth and fallow seasons in a single rice paddy field

3.1. Introduction

The global mean land surface temperature has increased by 0.85 °C over the period 1885-2012, and is predicted to substantially arise by 0.3-4.8 °C by the end of the 21st century depending on population growth and energy use scenarios, leading to increased frequencies of extreme meteorological events (IPCC, 2013). It has been shown that global warming can alter the terrestrial plant growth as well as C and N cycling in various ecosystems, such as forest, shrubland, grassland, tundra, wetland and cropland (Rustad et al., 2001; Frey et al., 2008; Cheng et al., 2010b; Lin et al., 2010; Lu et al., 2013, Gaihre et al., 2014; Karhu et al., 2016). Soils store the largest terrestrial C pool, approximately 2 times as much as the amount in the atmosphere and 4 times the amount of C in terrestrial vegetation (Lal, 2004). Little changes in SOC stocks may have a great impact on atmospheric [CO_2] (Smith et al., 2008; Stockmann et al., 2013). N is an essential element that most often limits plant growth and crop yield in soil-plant ecosystems. Progressive available N limitation can affect ecosystem responses to global warming with increased temperature and atmospheric [CO_2]. SOC and TN are heterogeneous mixtures of organic substances derived from plant photosynthesis or from organic matter application as fertilizer in agricultural lands. C decomposition and N mineralization always occur simultaneously into the soils (Cheng et al., 2016), and

can be easily affected by environmental factors such as soil temperature and moisture (Sierra, 1997; Wang et al., 2006; Tang et al., 2016). Although rice paddy field contains large stocks of C (Pan et al., 2004) and soil temperature is closely related to global warming, it is still not well understood how soil warming affects C decomposition and N mineralization during both rice growth and fallow seasons in rice paddy ecosystems.

Rice paddies are a key component of global wetland ecosystems. They not only provide a staple food for a large portion of the world's population, especially in Asian countries, but are also considered as one of the largest sources of atmospheric methane CH_4 which has about 28-34 times higher potential for thermal absorption than CO_2 to contribute to global warming on the 100-year time horizon (IPCC, 2013). The decomposition of SOC in submerged rice soils is different from that of aerobic soils in that CH_4 is also an end product of decomposition of SOC in addition to CO_2 (Kimura et al., 2004, Cheng et al., 2016). To accurately simulate land feedbacks to climate change, we need to more thoroughly understand the responses of C and N cycling including C storage and N availability to global warming in terrestrial ecosystems. For this reason, many soil warming experiments for various ecosystems have been carried out around the world over the last several decades (Edwards, 1975; Van Cleve et al., 1990; Hartley et al., 1999; Rustad et al., 2001; Frey et al., 2008; Lin et al., 2010; Patil et al., 2010; Bai et al., 2013; Lu et al., 2013). A meta-analysis suggested that experimental warming increased plant-derived C influx by 4.4%, but that the stimulation was basically offset by the increase in warming induced efflux, resulting in insignificant changes in litter and soil C contents (Lu et al., 2013). Similarly, the net N mineralization and net nitrification rates were largely increased, but soil TN and microbial N contents were not affected by experimental warming (Bai et al., 2013). However, most experimental results used in these meta-analyses were obtained from natural

ecosystems, like forests, shrublands, grasslands, tundra and wetlands. Relatively few studies were conducted in croplands and submerged rice paddies which are characterized by high degrees of human disturbance and large input of fertilizers (Bai et al., 2013; Lu et al., 2013).

There are many experimental warming methods for simulating global warming in various terrestrial ecosystems (e.g., forests and grassland) with greenhouse and/or closed chamber, open top chamber (OTC), infrared heater, soil heating cable, reflective curtain (Bai et al., 2013; Lu et al., 2013; Liu et al., 2016). Similarly, various experimental warming experiments were carried out in rice paddy ecosystems by OTC (Ziska et al., 1998), controlled-environment chamber (Sakai et al., 2001; Cheng et al., 2008), natural sunlight temperature gradient chamber (TGC, Kim et al., 2011), and T-FACE by ceramic infrared heaters (Gaihre et al., 2014, Wang et al., 2016), and by combination of soil temperature with elevated [CO_2] under the open field conditions (Tokida et al., 2010; Adachi et al., 2014; Usui et al., 2016). A common major purpose of the researches above was to study the responses of rice plant physiology, growth and yield to climate change with elevation of temperature and/or atmospheric [CO_2] during rice growth seasons, especially in Asian countries (China, Korea, Japan and the Philippines). It is well known that rice paddy fields are characterized mainly by waterlogged and drainage conditions during rice growth and fallow seasons, respectively. To our best knowledge, the effects of soil warming on paddy fields covering both seasons have not been reported previously, despite the fact that many rice paddies are used for single rice production with about seven months of fallow period per year in temperate zone countries such as China, Korea and Japan.

The response of rice growth as well as C decomposition and N mineralization to continually increasing global warming can further affect soil C stocks and

sequestration in rice paddy ecosystems. In the present study, we hypothesized that during rice growth season, the increase of plant-derived C input would surpass the enhanced soil C output as soil respiration due to soil warming, resulting in a net increase of C stocks in rice paddy ecosystems. While in the fallow season, the acceleration of soil respiration induced by soil warming would cause C loss in paddy field owing to no plant-derived C input. To test our hypotheses, a 5-year soil warming experiment was conducted in a Japanese rice paddy field from 2007 to 2011. In addition, we carried out anaerobic incubation to assess the C decomposition and N mineralization potentials. Therefore, the objective of this study was to investigate the direct effect of soil warming on plant biomass, SOC and TN contents, dissolved organic carbon (DOC) content (extracted by hot water, cool water and KCl solution, respectively), decomposed C and mineralized N potentials in a rice paddy field at the seasonal and annual time scales.

3.2. Material and Methods

3.2.1. Site description and field management

A soil warming experiment was conducted in the experimental paddy fields of NIAES, Tsukuba, Japan (36°01′N, 140°07′E) from May 2007 to December 2011 for both rice growth season and fallow season. Four 40 m^2 (10 m × 4 m) fields were used as four blocks (replicates). Within each block, we randomly assigned two treatment plots (5 m × 4 m); one for ambient temperature (AT) and the other for elevated temperature (ET). The soil in the top 55 cm layer is a Gray lowland soil (Fluvisols), which was moved several decades ago from alluvial rice paddies located about 10 km away from the experimental field. The soil below 55 cm was an Allophanic Andisol, corresponding to the original soil in this area (Fukuoka et al., 2012). Single rice

production is a typical cropping system for paddy fields in this region, where rice is generally grown from May to September. After rice harvest, the paddy field is left under drained conditions for a fallow season of about seven months, from October-November to April-May (Fig. 3.1).

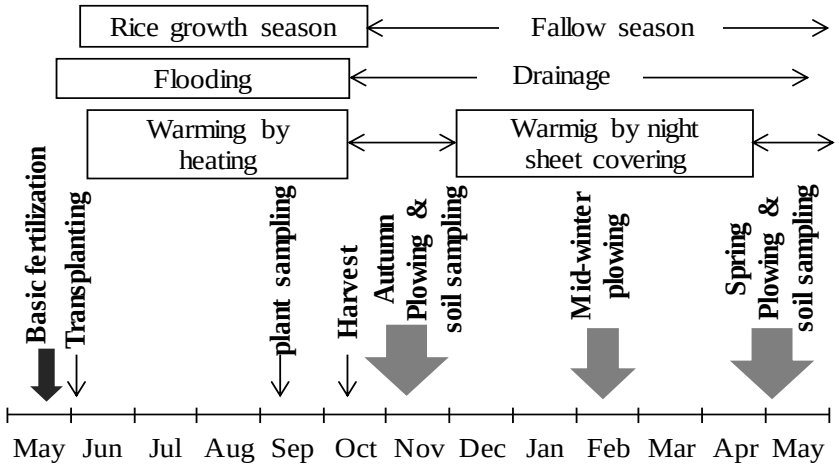

Fig.3.1. The annual experimental schedule and field management for soil warming experiment in a Japanese single rice paddy field from 2007 to 2011.

For rice growing, the field was submerged mid-May and puddled about 4-5 days prior to transplanting in early to mid-June (Fig. 3.1). Just prior to puddling, N, phosphorous and potassium fertilizers were applied at a rate of 7.5 g m^{-2} for N, P_2O_5 and K_2O, respectively, in the form of coated urea for N, superphosphate for P_2O_5 and KCl for K_2O. For N application, three types of coated urea (LP40, LP70 and LPS100 (JCAM AGRI Co., Ltd., Tokyo, Japan)) were applied at an equal rate (2.5 g m^{-2}) according to N demand for rice growth at different stages. Rice seedlings (three seedlings per hill) were transplanted into each block at a density of 23.8 hill m^{-2} (28 × 15 cm (hill × row)). Three cultivars differing in maturity groups were planted (line by line) in each treatment plot except in 2007, when we grew six cultivars. In this study, we just focused on one representative cultivar, Koshihikari (intermediate maturing

variety) because of its good quality and intensive plant area in Japan.

3.2.2. Water and soil warming for both rice growth and fallow seasons

The two treatments of AT and ET laid out randomly in four blocks were not changed over five years since 2007. Two soil warming methods were adopted in the ET plot for submerged growing seasons and drained fallow seasons. During the rice growth seasons, the soil/water temperatures were elevated by 2°C above AT by on-off control of the heating wires (Fig. 3.2), placed on the soil surface between the rice rows, with the water temperature of all blocks being continuously measured, as was reported elsewhere (Tokida et al., 2010; Adachi et al., 2014; Usui et al., 2016) . Water and soil temperatures in plow-layer were nearly uniformly elevated (Fig. 3.2). Seasonal mean temperatures were raised by 1.9-2.0 ℃ for water and by 1.9-2.2 ℃ for soil (Table 3. 1), respectively. The soil/water warming treatment was continued at least until harvest of Koshihikari, except in 2007 when it was terminated 6 days before the harvest. During the submerged period, depth of water was kept approximately 6 cm. Mid-season drainage or intermittent irrigation was not carried out. After rice harvest, rice straw was removed from the experimental field. The top 15 cm soil layer in both AT and ET plots was tilled and left to drain for the fallow season (Fig.3.2).

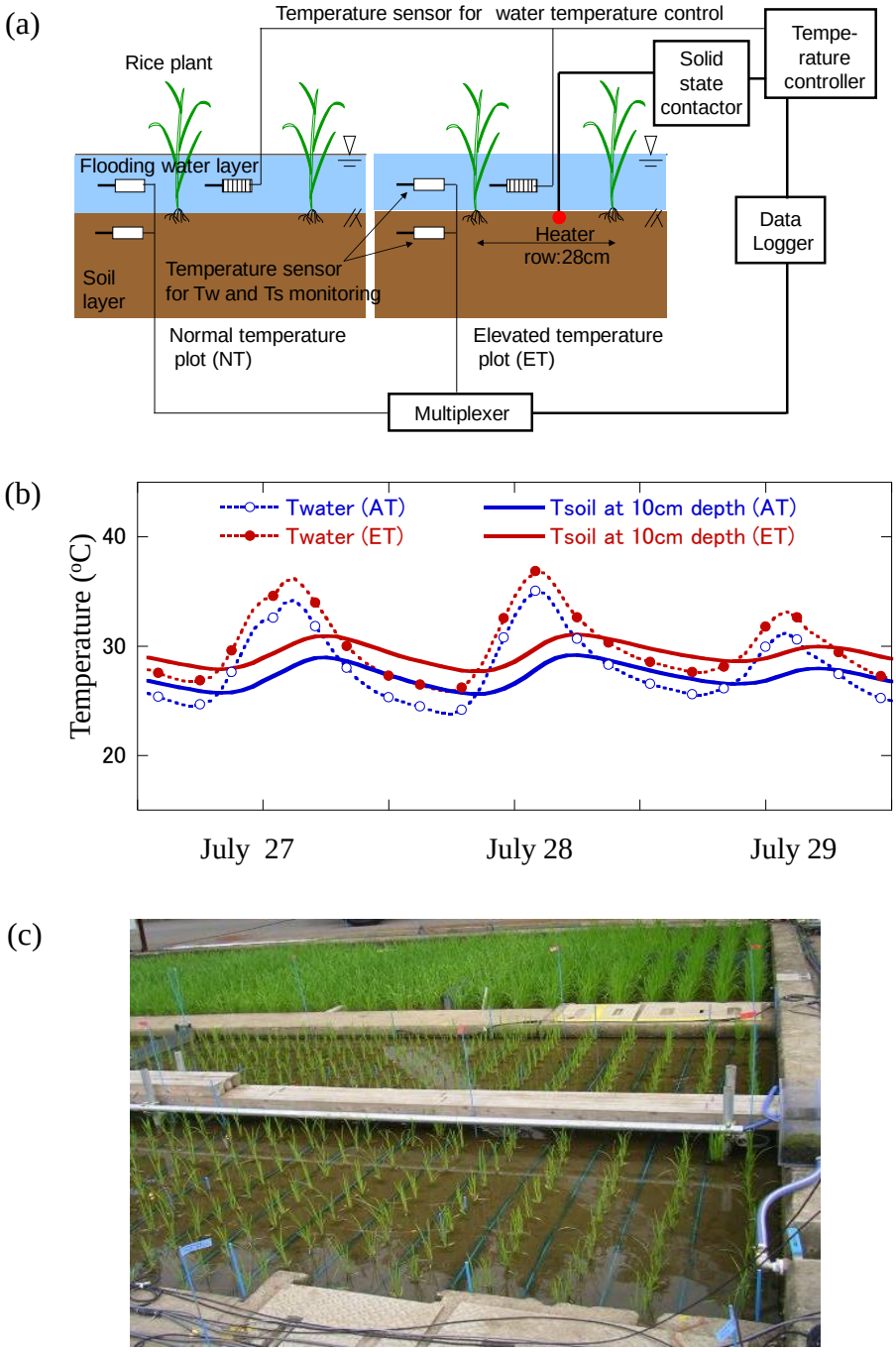

Fig.3.2. Schematic diagram of the system for the controlling the soil/water temperature and measurement of water temperature (Tw) and soil temperature (Ts) (a); the performance of Tw and Ts over 72 hours in the rice growing season on both ambient T (AT) and elevated T (ET) treatments (b); the heater wires on the rows of rice (c).

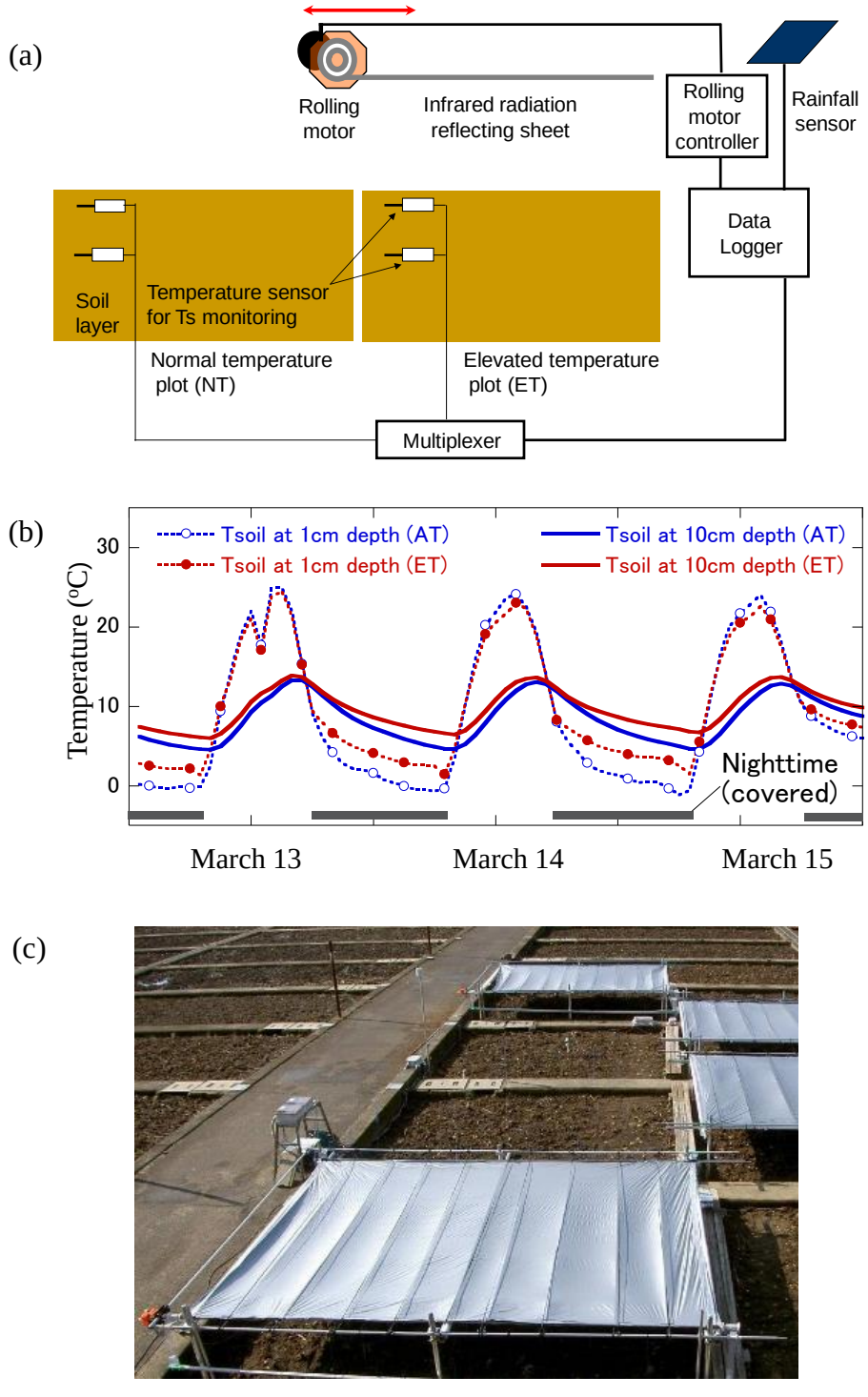

Fig.3.3. Schematic diagram of the system for the controlling infrared radiation reflecting sheet and modeling the soil temperature soil temperatures (Ts) at 1 and 10 cm depths (a); the Ts over 72 hours in the fallow season on both ambient T (AT) and elevated T (ET) treatments (b); the infrared radiation reflecting sheet covering for nighttime warming (c).

Table 3.1 Temperature differences between ambient and elevated soil and/or water temperature treatments during the rice growth and fallow seasons from 2007 to 2011.

Rice growth season									
Year	Month	Solar radiation	Air temperature	Ambient T		Elevated T		Elevated T – Ambient T	
				Tw (+1)[b]	Ts (-10)[c]	Tw (+1)	Ts (-10)	Tw (+1)	Ts (-10)
		(MJ m^{-2} d^{-1})	(℃)	(℃)	(℃)	(℃)	(℃)	(℃)	(℃)
2007	Jun-Sep[a]	15.3	24.4	26.2	25.9	28.0	27.8	1.9	1.9
2008	Jun-Sep	15.5	23.4	25.0	24.7	27.0	26.9	2.0	2.2
2009	Jun-Sep	15.1	23.0	23.9	23.6	25.8	25.7	2.0	2.1
2010	Jun-Sep	17.6	25.7	26.2	25.9	28.3	28.1	2.0	2.2
2011	Jun-Sep	16.9	24.6	25.4	25.1	27.3	27.1	1.9	2.0
Fallow season									
				Ts (-1)	Ts (-10)	Ts (-1)[d]	Ts (-10)	Ts (-1)	Ts (-10)
2007-2008	Nov-Mar[e]	10.8	6.4	6.9	7.3	8.0	8.3	1.1	1.0
2008-2009	Nov-Mar	10.4	6.3	6.2	6.8	7.3	7.8	1.0	1.0
2009-2010	Nov-Mar	10.1	5.9	5.5	5.9	6.5	6.8	1.1	0.9
2010-2011	Nov-Mar	11.5	6.3	6.2	6.7	7.2	7.7	1.0	1.0
2011-2012	Nov-Mar	10.2	5.9	5.9	6.4	7.0	7.5	1.1	1.0

[a] from heating to September, 30th; [b] water temperature at 1cm above the soil surface; [c] soil temperature at 10 cm depth below the soil surface; [d] soil temperature at 1 cm depth below the soil surface; [e] from heating to March, 31th .

3.2.3. Plant biomass measurement

At physiological maturity, we collected aboveground plant parts of 21 hills, equivalent to an area of 0.882 m^2 in the center of each plot at a height of about 10 cm above the soil surface for biomass and yield determination. After rice harvest, about 10 cm stems were left in the field as stubble and later plowed to soil during fallow season. After air-drying the rice plants under a rain shelter, the total weights were determined and a part of the samples was used to measure moisture content to further estimate aboveground biomass. At maturity, we sampled four hills in each plot and separated them by plant organs. One of the four hills was dug up with a monolith 28 × 15 cm by size to a depth of about 15 cm, and then soils were washed away to determine root dry mass after oven-drying at 80 °C for three days (Cheng et al., 2009a; Hasegawa et al., 2013).

3.2.4. Soil sampling and property analysis

Soil samples were collected from the plow layer (0-15 cm) in each plot, twice a year, before rice transplanting in April or May (hereafter, spring) and after rice harvest in Oct. or Nov. (hereafter, autumn). According to local conventional practice, the top layer was plowed for rice transplanting in spring and fallowed after rice harvest in autumn, respectively. The initial soil samples were collected in February 2007 prior to soil warming. Approximately 500 g soil samples were taken in a zigzag pattern and composited for each block, air-dried and sieved to 2 mm in the laboratory.

The measurements of pH, EC, SOC, TN contents was as the same as those described in section 2.2.2 in Chapter II. Each 5 g air-dried soil samples taken in soil warming experiment was extracted by 25 ml hot water (70 °C) for 12h, cool water and 20 ml of 15% KCl solution for 30 mins. The soil extraction was then filtered and

stored to measure DOC concentration by TOC analyzer (Shimadzu).

3.2.5. Pre-incubation and anaerobic incubation experiments

The available N in rice paddy is generally considered as the amounts of mineralized N from air-dried soil incubated at 30 °C and under submerged condition for four weeks (JSSSPN, 1986). Similarly, the CO_2 and CH_4 productions derived from the anaerobic decomposition of SOC can be considered as readily decomposable C derived from SOM (Yagi and Minami, 1990). It is well known that there is distinct difference in soil microbial activities before and after pre-incubation of air-dried soil samples. However, there are no comparative studies on the responses of pre-incubated and air-dried paddy soils to soil warming. Based on these two points, an anaerobic incubation experiment of pre-incubated soil samples and air-dried soil samples was carried out to investigate how soil available N and decomposable C potentials were simultaneously affected by 5-year soil warming. The procedures for 4-week pre-incubation air-dried soil samples at 25°C and under submerged condition were similar to the section 2.2.2 in Chapter II.

After pre-incubation, 5 g of pre-incubated soil samples and air-dried soil samples were immediately extracted by with 20 ml of 15% KCl solution by shaking for 30 min. The DOC concentration in the extracts was determined by TOC analyzer (Shimadzu). Each 5 g of pre-incubated and air-dried soil samples were anaerobically incubated at 30 °C and under submerged condition for 4 week as identically as the description in section 2.2.3 in Chapter II. After gas measurements during 4-week anaerobic incubation, pre-incubated and air-dried soil samples were immediately extracted with 20 ml of 15% KCl solution by shaking for 30 min. The concentration of NH_4^+-N in extraction from initial pre-incubated and air-dried soil samples was measured as

described above. The N mineralization was calculated from the difference in amount of NH_4^+-N between the anaerobically incubated and the bulk soil samples. The NO_3^--N concentration was below the detection limit in the submerged soil samples after the 4-week anaerobic incubation.

3.2.6. Statistical analysis

An analysis of variance (ANOVA) was performed by applying a split-split-plot design, in which year was treated as the main factor, season as the split-plot factor, and temperature as the split-split factor with four replications. The statistical significance was indicated at the level of $P<0.05$. All statistical analyses were conducted using SPSS 21 (SPSS Inc., Chicago, IL, USA).

3.3. Results

3.3.1. Plant biomass

The aboveground biomass and root biomass in AT and ET plots at rice harvest period are shown in Fig. 3.4. Aboveground biomass ranged between 1.137and1.439 kg m^{-2} in AT treatment, and between 1.091 and1.502 kg m^{-2} in ET treatment, respectively. The aboveground biomass for both two temperature treatments was lowest in 2007 and rapidly increased in 2008, then decreased after 2009 and remained relatively stable in last two years. The highest aboveground biomass in AT and ET treatments were found in 2008 and 2009, respectively. The root biomass varied from 0.070 to 0.112 kg m^{-2} in AT treatment and from 0.076 to 0.103 kg m^{-2} in ET treatment (Fig. 3.4). The highest root biomass was found in 2008 under both temperature treatments, while the lowest root biomass for AT treatment and ET treatments was observed in 2007 and in 2011, respectively. 5-year average total rice biomass was 1.366 kg m^{-2}in AT and 1.420 kg m^{-2}in ET. The effect of soil warming was not

significant for both aboveground biomass and root biomass (Fig. 3.4) although soil and water warming increased yearly average aboveground biomass and root biomass by 3.9% (P=0.17) and 4.0% (P=0.57), respectively. Besides, significant interannual variations of aboveground biomass and root biomass were observed in both AT and ET treatments.

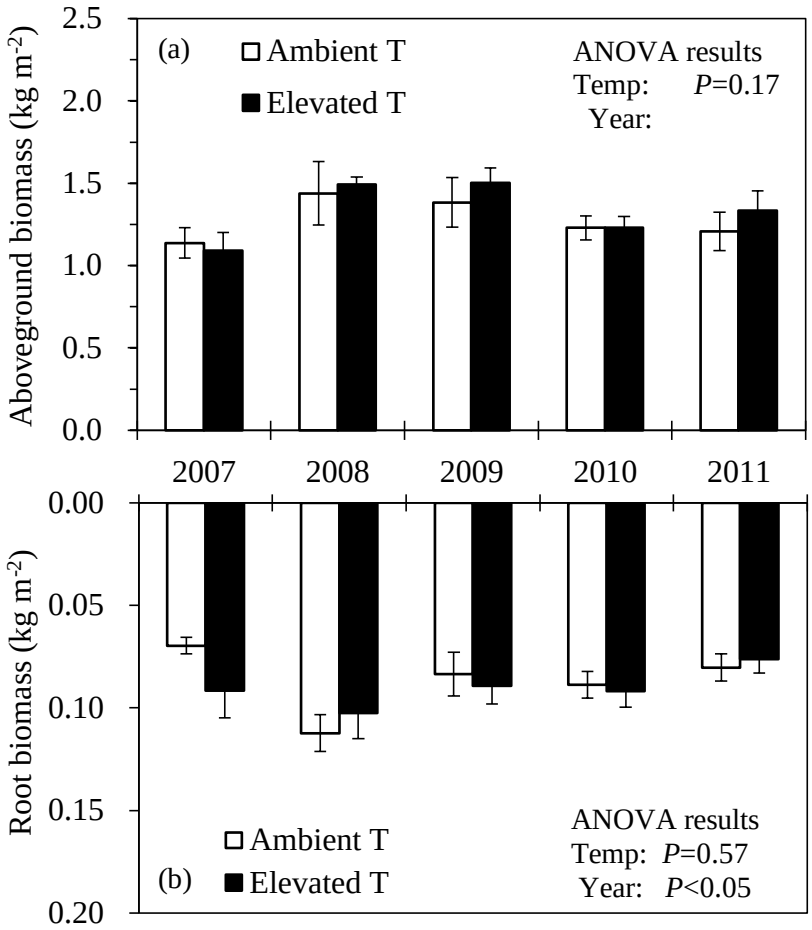

Fig.3.4. Rice aboveground biomass (a) and root biomass (b) after rice harvest under ambient (□) and elevated (■) soil temperature treatments from 2007 to 2011. Error bars were the standard errors (n=4). ANOVA results were embedded in the figure.

3.3.2. pH and EC

The dynamics of pH and EC in soil samples under two temperature treatments are shown in Fig.3.5. On the whole, the temporal trends of pH value in soil samples under two soil temperature trends were similar. The temporal trends of pH were

contrary to those of EC in all soil samples under two temperature treatments. ANOVA results showed there were no significant differences in pH and EC in soil samples under two temperature conditions. However, the yearly and seasonal variations of pH and EC were significant in soil samples under two temperature treatments. On average, both pH and EC in soil samples taken in autumn were higher than those in soil samples taken in spring (Fig.3.5).

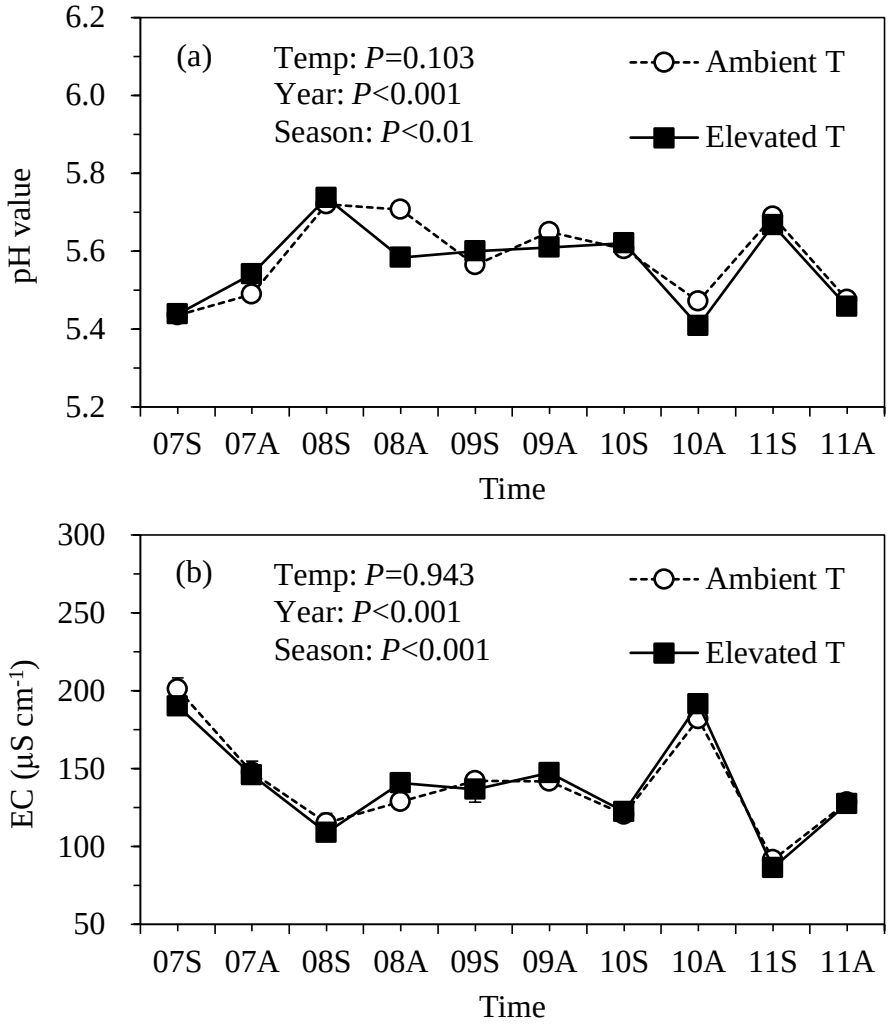

Fig.3.5. Dynamics of pH (a) and EC (b) in soil samples after ambient (○) and elevated (■) soil temperature treatments from spring (S) 2007 to autumn (A) 2011. Error bars were the standard errors (n=4). ANOVA results were embedded in the figure.

3.3.3. DOC concentrations extracted by hot water and cool water

DOC concentration extracted by hot water was much higher than that extracted

by cool water (Table 3.2). DOC concentrations in soil samples extracted by both hot water and cool water fluctuated under two soil temperature conditions. On average, DOC concentration extracted by hot water and cool water in AT treatment was higher in spring than in autumn. However, DOC concentration extracted by hot water in ET treatment was lower in spring than autumn. ANOVA results showed that elevated soil temperature had no significant effect on DOC concentration extracted by hot water and cool water. However, the yearly and seasonal variations of DOC concentrations extracted by hot water and cool water were significant (Table 3.2).

Table 3.2 Changes in DOC concentration extracted by hot water and cool water in soil samples under ambient and elevated soil temperatures from spring (S) 2007 to autumn (A) 2011. Data was represented as mean and standard error (n=4).

Year	Season	DOC (hot H_2O)		DOC (cool H_2O)	
		(mg C kg^{-1}soil)		(mg C kg^{-1}soil)	
		Ambient T	Elevated T	Ambient T	Elevated T
2007	Spring	859.8±44.9	721.1±132.0	106.6±4.9	101.2±4.7
	Autumn	809.7±23.1	710.8±34.0	97.8±6.1	78.2±8.1
2008	Spring	762.0±12.5	695.7±47.9	101.5±7.0	106.1±3.3
	Autumn	618.7±157.5	695.1±59.6	68.7±2.4	103.5±7.2
2009	Spring	943.9±79.3	886.0±45.3	163.8±14.3	106.6±8.9
	Autumn	942.2±33.4	943.0±41.9	105.3±8.8	100.4±4.3
2010	Spring	737.5±58.7	647.3±21.7	134.3±8.7	133.9±3.5
	Autumn	704.0±27.9	723.1±68.6	72.3±5.3	109.4±12.9
2011	Spring	498.2±35.8	503.9±42.1	152.9±7.2	66.2±2.3
	Autumn	534.8±5.9	701.6±144.8	72.3±5.3	46.52.8
Average	Spring	760.3	721.9	131.8	102.8
	Autumn	690.8	754.7	83.3	87.6
ANOVA results (*P* value)					
Year (Y)		<0.01		<0.01	
Season (S)		0.70		<0.01	
Temperature (T)		0.66		0.08	

3.3.4. SOC and TN contents

SOC contents at the beginning of this soil warming experiment were 19.5 and 19.3 g C kg^{-1} soil in AT and ET treatments without significant difference. During the 5-year soil warming experiment, the SOC contents varied from 19.0 to 20.1 g C kg^{-1} soil in AT treatment and from 18.6 to 19.8 g C kg^{-1} soil in ET treatment across all seasons and years (Fig. 3.6a). In autumn 2007, after the first rice growth season was over, the SOC content rapidly increased by 3.3% and 2.5% in AT and ET treatments, respectively. However, it subsequently decreased with time after first fallow season. Interestingly, SOC contents under both temperature treatments increased during following rice growth season, and gradually decreased in subsequent fallow season every year. ANOVA results showed the significant effects of soil warming (P<0.05), season sampling (P<0.01) and year (P<0.01) on the amounts of SOC (Fig. 3.6a). The SOC contents after rice harvest significantly decreased yearly for both AT and ET treatments (P<0.01, embedded graph in Fig. 3.6a). The TN contents at the beginning of this soil warming experiment were 1.47 and 1.46 g N kg^{-1} soil in AT and ET treatments without significant difference. During 5-year soil warming experiment, TN contents ranged from 1.40 to 1.47 g N kg^{-1} soil under AT condition and from 1.37 to 1.46 g N kg^{-1}soil in ET treatment (Fig. 3.6b). ANOVA results showed that the amounts of TN were significantly affected by soil warming (P<0.01), and year (P<0.01), but not by season sampling (P=0.11). Besides, TN contents in soil samples taken in autumn for two temperature treatments decreased with years but the trends were not significant (Both P =0.06, embedded graph in Fig. 3.6b).

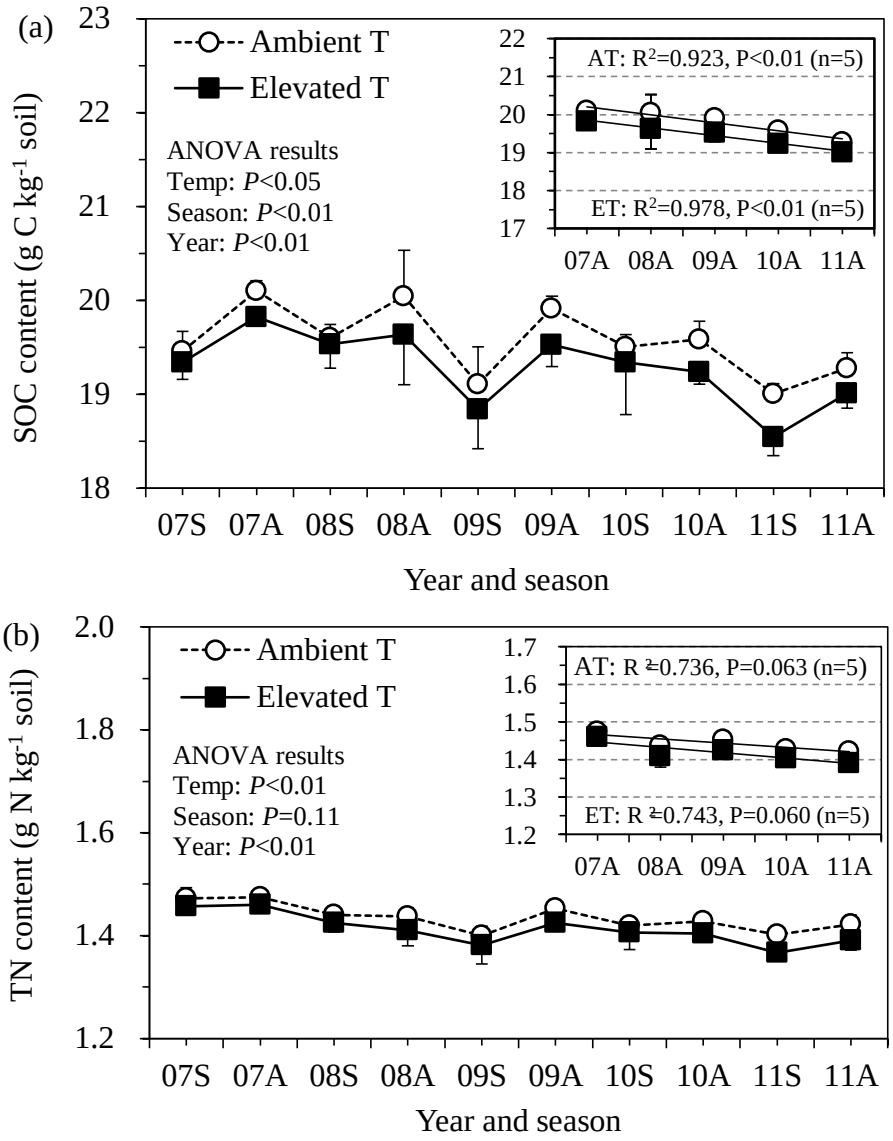

Fig.3.6. Changes in SOC (a) and TN (b) contents in soil samples after ambient (○) and elevated (■) soil temperature treatments from 2007, spring (S) to 2011, autumn (A). Error bars were standard errors (n=4). ANOVA results were shown in the figure. The embedded graphs in the right above showed the annual decreasing trend of SOC and TN contents in autumn samplings in both ambient and elevated soil temperature treatments, respectively.

3.3.5. C and N mineralization after the 4-week anaerobic incubation of air-dried soil samples

The productions of CO_2, CH_4, decomposed C and mineralized N (NH_4^+-N production) potentials after 4-week anaerobic incubation of air-dried soil samples are

shown in Table 3.3. The CO_2 production ranged from 551.9 to 739.0 mg C kg^{-1} in AT treatment and from 534.0 to 740.1 mg C kg^{-1} in ET treatment. While, the CH_4 productions ranged from 12.7 to 122.3 mg C kg^{-1} in AT treatment and from 7.1 to 121.5 mg C kg^{-1} in ET treatment, respectively. The variation range of CH_4 production was larger than that of CO_2 production in both temperature treatments (Table 3.3). The trends of decomposed C and mineralized N were similar to CO_2 and CH_4 productions for temperature treatments, seasonal and yearly samplings. The averages of CO_2 and CH_4 productions, and decomposed C potentials for the five years were higher in autumn than in spring. Moreover, CO_2 and CH_4 productions, decomposed C and mineralized N potentials were higher in AT treatment than in ET treatment. The ANOVA results showed that soil warming significantly affected CH_4 production (P<0.05), decomposed C (P<0.05) and mineralized N (P<0.01), and slightly affected CO_2 production (P=0.07) obtained from laboratory anaerobic incubation experiment (Table 3.3).

Table 3.3 The production potentials of CO_2, CH_4, decomposed C (CO_2-C + CH_4-C) and mineralized N (net NH_4^+-N production) after 4-week anaerobic incubation of air-dried soil samples under 5-year ambient temperature and elevated soil temperature conditions. Data was represented as mean and standard error (n=4).

Year	Season	CO_2 (mg C kg^{-1} soil)		CH_4 (mg C kg^{-1} soil)		Decomposed C (mg C kg^{-1} soil)		Mineralized N (mg N kg^{-1} soil)	
		Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T
2007	Spring	670.7±13.8	660.4±22.4	18.3±4.4	19.4±3.5	689.0±14.4	679.8±23.5	113.1±4.4	103.2±4.9
	Autumn	648.4±11.0	610.2±23.2	60.9±10.6	40.6±10.4	709.3±19.7	650.8±31.5	106.2±1.0	105.4±3.6
2008	Spring	617.2±16.2	587.5±24.7	40.0±3.2	41.0±6.8	657.3±18.5	628.5±29.1	99.3±3.8	99.0±2.9
	Autumn	588.2±17.9	593.3±25.3	12.7±4.0	7.1±2.6	600.9±21.6	600.4±27.1	92.5±3.1	95.2±3.7
2009	Spring	690.1±8.7	674.7±17.3	86.4±5.0	77.1±24.2	776.5±12.8	751.7±40.1	124.8±5.6	116.8±5.2
	Autumn	673.6±2.1	653.4±23.3	99.8±1.8	83.0±5.6	773.4±2.7	736.3±27.4	118.7±1.3	110.4±3.2
2010	Spring	662.2±8.5	664.7±31.2	92.2±6.6	88.8±5.3	754.4±13.1	753.5±35.9	119.4±3.0	117.9±4.0
	Autumn	739.0±8.8	740.1±25.0	122.3±8.6	121.5±7.1	861.3±15.7	861.6±29.2	117.4±2.4	110.0±0.9
2011	Spring	551.9±8.1	534.0±18.3	19.7±4.2	9.4±1.0	571.7±11.4	543.5±18.8	93.5±2.6	86.8±1.4
	Autumn	600.6±18.4	566.4±21.1	22.2±5.7	8.7±3.3	622.9±23.0	575.1±23.8	71.2±4.0	67.6±6.1
Average	Spring	638.4	624.3	51.3	47.1	689.8	671.4	110.0	104.8
	Autumn	650.0	632.7	63.6	52.2	713.5	684.8	101.2	97.7
ANOVA results (*P* value)									
Year (Y)		<0.01		<0.01		<0.01		<0.01	
Season (S)		0.25		0.01		0.06		<0.01	
Temperature (T)		0.07		0.02		0.02		0.01	

3.3.6. C and N mineralization after the 4-week anaerobic incubation of pre-incubated soil samples

The productions of CO_2, CH_4, decomposed C and mineralized N (NH_4^+-N production) potentials after 4-week anaerobic incubation of pre-incubated soil samples are shown in Table 3.4. The CO_2 production ranged from 246.2 to 339.9 mg C kg^{-1} in AT treatment and from 246.2 to 333.9 mg C kg^{-1} in ET treatment. While, the CH_4 productions ranged from 0.001 to 0.036 mg C kg^{-1} in AT treatment and from 0.001 to 0.021 mg C kg^{-1} in ET treatment, respectively (Table 3.4). The trend of decomposed C was similar to CO_2 for temperature treatments, seasonal and yearly samplings due to the negligible CH_4 production. The averages of CO_2 and CH_4 productions, and decomposed C potentials for the five years were higher in autumn than in spring. Moreover, CO_2 production and decomposed C potentials were higher in AT treatment than in ET treatment. The mineralized N production varied from 38.4 to 62.0 mg N kg^{-1} among soil samples under AT and ET treatments. The interannual variations in mineralized N potential were still observed in pre-incubated soil samples. However, no significantly seasonal differences in mineralized N potentials were found in pre-incubated soil samples under two temperature treatments. The ANOVA results showed that soil warming did not significantly affected CO_2 and CH_4 productions, decomposed C and mineralized N potentials (Table 3.4).

Table 3.4 The production potentials of CO_2, CH_4, decomposed C (CO_2-C and CH_4-C) and mineralized N (net NH_4^+-N production) after 4-week anaerobic incubation of pre-incubated soil samples under 5-year ambient temperature and elevated soil temperature conditions. Data was represented as mean and standard error (n=4).

Year	Season	CO_2 production (mg C kg^{-1} soil)		CH_4 production (mg C kg^{-1} soil)		Decomposed C (mg C kg^{-1} soil)		Mineralized N (mg N kg^{-1} soil)	
		Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T
2007	Spring	301.0±1.7	312.1±19.0	0.001±0.000	0.001±0.000	301.0±1.7	312.1±19.0	51.5±3.6	52.4±3.5
	Autumn	294.5±3.8	264.9±9.1	0.003±0.001	0.003±0.001	294.5±3.8	264.9±9.1	51.5±1.4	53.6±4.5
2008	Spring	246.2±6.0	266.1±15.1	0.006±0.001	0.007±0.001	246.2±6.0	266.1±15.1	50.1±2.3	45.0±1.6
	Autumn	246.2±23.2	264.2±9.6	0.004±0.001	0.003±0.001	246.2±23.2	264.2±9.6	43.7±1.8	51.3±3.8
2009	Spring	326.6±4.6	299.4±17.3	0.009±0.001	0.007±0.001	326.6±4.6	299.4±17.3	62.0±2.6	51.9±2.1
	Autumn	323.0±4.1	311.8±17.4	0.018±0.005	0.021±0.006	323.0±4.1	311.8±17.4	54.0±1.9	54.4±2.6
2010	Spring	295.0±8.7	295.4±18.5	0.008±0.001	0.010±0.004	295.0±8.7	295.5±18.5	49.0±3.4	55.7±3.2
	Autumn	339.9±12.4	333.9±17.3	0.036±0.009	0.018±0.004	339.9±12.4	334.0±17.3	55.9±3.4	51.4±1.5
2011	Spring	278.4±3.6	259.4±21.2	0.007±0.001	0.007±0.002	278.4±3.6	259.4±21.2	45.3±2.2	40.3±6.5
	Autumn	337.9±18.3	306.6±17.4	0.015±0.005	0.013±0.002	337.9±18.3	306.6±17.4	42.4±1.7	38.4±0.9
Average	Spring	289.4	286.5	0.006	0.006	289.4	286.5	51.6	49.0
	Autumn	308.3	296.3	0.015	0.012	308.3	296.3	49.5	49.8
ANOVA results (*P* value)									
Year (Y)		<0.01		<0.01		<0.01		<0.01	
Season (S)		0.06		<0.01		0.06		0.64	
Temperature (T)		0.31		0.22		0.31		0.44	

3.3.7. DOC concentration in air-dried and pre-incubated soil samples extracted by KCl solution

The changes in DOC concentration extracted by KCl solution in air-dried (without pre-incubation) and pre-incubated (with pre-incubation) soil samples under two temperature treatments are shown in Table 3.5. DOC concentrations varied from 148.4 to 310.5 mg C kg^{-1} in air-dried soil samples, which were distinctly higher than those in pre-incubated soil samples ranging from 97.2 to 194.3 mg C kg^{-1}. Consequently, the average seasonal DOC concentrations in air-dried soil samples were significantly higher than those in pre-incubated soil samples. The average DOC concentration in pre-incubated soil samples was less than 35.3% than that in air-dried soil samples. In this study, no significant effect of soil warming on DOC concentration extracted by KCl solution was found in air-dried and pre-incubated soil samples. However, the significantly seasonal difference in DOC concentration was observed in air-dried and pre-incubated soil samples. Soil samples taken in spring had higher DOC concentration than those in autumn ($P<0.05$, Table 3.5).

Table 3.5 DOC concentration extracted by KCl solution in air-dried and pre-incubated soil samples under 5-year ambient temperature and elevated temperature conditions. Data was represented as mean and standard error (n=4).

Year	Season	Without pre-incubation (mg C kg^{-1} soil)		With pre-incubation (mg C kg^{-1} soil)		Without-With (mg C kg^{-1} soil)		(Without-With)/Without (%)	
		Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T
2007	Spring	264.7±7.4	275.7±9.9	194.3±6.2	193.4±3.5	70.4±17.0	82.4±15.6	26.6±5.3	31.1±4.2
	Autumn	198.1±3.7	168.5±6.0	108.8 ±1.1	115.7±3.1	89.3±7.3	52.8±17.4	45.1±2.1	26.6±9.0
2008	Spring	225.9±7.5	226.0±8.4	122.8 ±0.5	141.4±4.1	103.1±13.8	84.7±11.1	45.7±3.1	37.5±2.5
	Autumn	154.4±2.8	140.5±5.5	106.5±4.0	99.4±1.5	47.9±4.3	41.1±9.2	31.0±3.1	26.6±4.1
2009	Spring	310.5±13.8	305.3±7.9	182.9±1.8	171.7±1.7	127.5±25.4	133.6±16.7	41.1±5.1	43.0±3.5
	Autumn	250.4±9.6	217.4±2.0	153.0±2.1	160.8±6.3	97.5±17.4	56.5±10.0	38.9±3.6	22.6±4.8
2010	Spring	237.7±4.9	233.0±3.8	145.0±4.6	154.9±2.5	92.7±3.4	78.1±4.8	39.0±1.6	32.9±1.3
	Autumn	253.6±2.4	269.9±4.8	139.2±0.5	150.8±2.4	114.4±5.1	119.1±5.8	45.1±1.1	47.0±0.9
2011	Spring	151.7±0.9	154.9±2.3	115.5±1.3	104.9±1.6	36.2±3.4	50.0±3.4	23.9±2.1	32.9±1.6
	Autumn	158.4±1.8	148.4±4.5	97.2±0.9	100.4±0.3	61.2±2.8	48.0±9.6	38.6±1.1	30.3±4.8
Average	Spring	238.1	239.0	152.1	153.2	86.0	85.7	35.2	35.5
	Autumn	203.0	188.9	120.9	125.4	82.0	63.5	39.7	30.6

ANOVA results (*P* value)				
Year (Y)	<0.01	<0.01	<0.01	<0.01
Season (S)	<0.01	<0.01	<0.05	0.63
Temperature (T)	0.30	0.28	0.11	<0.05

3.4. Discussion

3.4.1. Effect of water- and soil-warming on rice plant biomass

Meta-analysis results showed that experimental ecosystem warming significantly increased aboveground plant productivity by a mean of 19% (Rustad et al., 2001), and terrestrial plants biomass by 12.3% (Lin et al., 2010). The response of plant productivity to global warming is influenced by many factors such as ambient temperature in different latitudes, precipitation, plant species and soil nutrients. The warming-induced increase in plant productivity was probably responsible for direct increase in rate of photosynthesis at higher temperature, and indirect increase in nutrient availability from SOM decomposition (Rustad et al., 2001; Bai et al., 2013).

Previous studies have investigated the effects of elevated temperature on rice growth and yield. The response of rice biomass to elevated temperature was found to be highly variable across studies. Elevated temperatures have been reported to result in a decrease (Ziska et al., 1998; Jagadish et al., 2015), an increase (Kim et al., 2011) or have no effect (Gaihre et al., 2014) on the total rice biomass including aboveground biomass and root biomass. The discrepancy in these studies might be partly due to the differences in experimental conditions, soil types, environmental modifications and duration of observation. For example, a 4-year field T-FACE experiment carried out in Jiangsu, China for rice-wheat rotation ecosystem, indicated that warming decreased total biomass of rice in summer season larger than that of wheat in winter-spring seasons due to the large difference in ambient temperature between rice and wheat growth seasons (Wang et al., 2016). However, a FACE experiment with soil and water warming was carried out in a single rice paddy field in Iwate Prefecture (39°38’N, 140°57’E, 210 m above sea level), northeastern Japan during two growth seasons (2007

and 2008). It showed that elevation of soil and water temperatures (similar to this experiment) increased aboveground biomass by 9% (P<0.05) and decreased root biomass by 8% (P=0.07) (Tokida et al., 2010). After, similar single rice FACE experiment moved to Ibaraki Prefecture, Kanto region, central Japan (35°58'N, 139°60'E, 10 m above sea level), the 3-year (2010-2013) results showed that soil and water warming increased aboveground biomass only by 3.6% (P<0.05) (Usui et al., 2016). The different responses of the two single rice FACE experiments in Japan could be explained by the fact that, air temperature during rice growth season in Iwate was about 4 °C lower than that in Ibaraki (Hasegawa et al., 2013). In the present study, no significant difference was found in aboveground and root biomass of rice at mature stage under the two treatments across the years although soil and water warming increased yearly average aboveground biomass and root biomass by 3.9% (P=0.17) and 4.0% (P=0.57), respectively (Fig. 3.4a and b). Compared with rice-wheat rotation warming experiment in China (Wang et al., 2016) and single rice warming treatments in two FACE experiments in Japan (Hasegawa et al., 2013), our results implied that elevated temperature during the single rice season (in low latitude area under warm ambient temperature condition) could not significantly affect rice aboveground and root biomass. The decreased soil available N (expressed by mineralized N in Table 3.3) under elevated temperature condition could be a reason for the insignificant effect on rice aboveground and root biomass (Fig. 3.4), and rice yield (data not shown).

3.4.2. The effect of around-the-year soil warming on SOC and TN contents

It is well known that SOC and TN stocks are mediated by the balance between C and N inputs, and their outputs derived from plants and microorganisms through plant photosynthesis, N absorption and microbial decomposition of SOM (Pampolino et al., 2006; Reich et al., 2006; Cheng et al., 2016). Elevated temperature can stimulate C and

N inputs and outputs in terrestrial ecosystems simultaneously. A meta-analysis study suggested that soil respiration and DOC leaching were enhanced under warming condition while the stimulation of plant-derived C influx may offset the increase of warming-induced efflux, resulting in insignificant changes in litter and soil C content (Lu et al., 2013). Additionally, increased net N mineralization, nitrification and soil inorganic N as a result of warming can lead to higher N losses from soil (Bai al., 2013). However, the data for meta-analysis by Bai et al. (2013) and Lu et al. (2013) were almost collected from natural ecosystems, with a few from croplands. To our knowledge, we are the first to report the effect of soil warming on the SOC and TN changes in rice paddy field, especially focusing on both rice growth and fallow seasons in single rice ecosystem. In this study, seasonal (spring and autumn) and interannual variations of SOC and TN contents were observed (Fig. 3.6). ANOVA results showed that SOC and TN contents at AT were significantly higher than those at ET (Fig. 3.6a and Fig. 3.6b). The lower SOC content in ET treatment in both autumn sampling after rice growth season and spring sampling after winter fallow season can probably be attributed to two reasons. Firstly, rice biomass production was not affected by elevated soil temperature, causing no great plant-derived C input into soil. This finding was not in line with our previous hypothesis. Secondly, C output as SOC decomposition via microorganism was increased by elevated soil temperature during both rice growth and fallow seasons. The significantly lower decomposed C potential in soil samples from ET treatment after 4-week anaerobic incubation also evidenced that the readily decomposable C was lost during rice growth and fallow seasons (Table 3.4). Besides, the amounts of SOC under two temperature treatments in rice growth season were distinctly higher than those in fallow season, which was consistent to our hypothesis that SOC in rice growth season was increased by plant photosynthesis and decreased in

fallow season by microbial decomposition, respectively.

Many previous soil warming experiments suggested that the response of soil C efflux rates across a range of elevated temperatures was transitory, with greater increases over the first few years, but no significant increases in long-term studies in natural ecosystems (Luo et al., 2001; Rustad et al., 2001; Melillo et al., 2002; Knorr et al., 2005). In agroecosystem, N and other fertilizers were applied every season for crop growth, and the aboveground biomass except grains were left and plowed back into the soils after grains harvest. As a result, the transitory effect of elevated temperature on SOM decomposition was not easily observed in the field. It should be noted that the amounts of SOC significantly decreased across the years under both AT and ET treatments (both $P<0.01$, embedded graph in Fig. 3.6a). The interannual decreased trend of TN amounts was similar to that of SOC (both $P=0.06$, embedded graph in Fig. 3.6b). These results might be ascribed to the yearly removal of rice straw after rice harvest. Recently, a meta-analysis result has showed that rice straw incorporation largely increased SOC change rates in long-term experiments in Chinese rice paddies compared to only chemical fertilization (Tian et al., 2015).

3.4.3. Decomposed C and mineralized N potentials in air-dried affected by soil warming

Both CO_2 and CH_4 are products of the decomposition and fermentation of SOC in submerged paddy soils (Kimura et al., 2004). The productions of CO_2 and CH_4 during anaerobic incubation could be considered as the decomposable C in SOC. The ratio of CH_4 to decomposable C could be regarded as an index of CH_4 production potential from original SOC. CH_4 production derived from SOM decomposition differed to that during rice growth season (Yagi and Minami, 1990; Cheng et al., 2007). Similarly, the

NH_4^+-N production during anaerobic incubation could be regarded as mineralizable or available N as an index of soil fertility for rice production (Cheng et al., 2016). Previous studies have reported that elevated temperature could lead to decrease (Ziska et al., 1998), increase (Tokida et al., 2010) and no change (Yun et al., 2012; Gaihre et al., 2014) in CH_4 emission during rice growth season which largely depends on whether the total rice biomass is affected by warming treatment under different experimental conditions. According to Kimura et al. (2004), approximately 20% CH_4 emission is from SOC in bulk soil. However, there is no related report showing whether elevated temperature affects CH_4 production potential from decomposition of SOC in bulk soil. Though the actual CH_4 emission was not measured in this soil warming experiment for single rice paddy field, the ANOVA results showed that decomposed C and CH_4 production were significantly decreased by elevated temperature in both spring and autumn soil samples (Table 3.3). This implied that CH_4 emission from SOC in bulk soil could not be increased by water and soil warming in this single rice field. The rice aboveground biomass and root biomass were also not increased by water and soil warming (Fig. 3.4). These results suggested that the elevation of water and soil temperatures would not lead to an increase in CH_4 emission from both rice root exudates (new C from plant) and stable SOC (old C in bulk soil).

Similar to decomposed C potential, mineralized N potential was significantly decreased by elevated temperature in both spring and autumn air-dried soil samples (Table 3.3). Although we did not measure the real gross N mineralization under soil warming condition in the field, the mineralized N potential significantly decreased with elevated temperature, implying that the N fertility of single rice soil would be diminished by soil warming. Decomposed C and mineralized N are derived from the active fractions of SOM. Though the amounts of SOC and TN were notably lower in

ET treatment than in AT treatment for both seasons in the 5 years in most cases (Fig. 3.6), the ratios of decomposed C to mineralized N potentials were not significantly affected by soil warming (Table 3.3, P=0.68). Also, the ratios of decomposed C to SOC, and mineralized N to TN were not significantly affected by soil warming (Table 3.6, P=0.08 and 0.18, respectively). These results indicated that even if the SOC, TN, decomposed C and mineralized N potentials in single rice soil field were decreased by soil warming, the parts of active fractions (decomposed C and mineralized N potentials) in SOM were not affected.

Table 3.6 The ratios of decomposed C (CO_2-C+CH_4-C) and mineralized N (NH_4^+-N production) to SOC and TN after 4-week anaerobic incubation of air-dried soil samples under 5 year ambient temperature and elevated soil temperature conditions. All values were calculated by the average values of decomposed C and mineralized N in each treatment.

Year	Season	Decomposed C/Mineralized N		Decomposed C/SOC (%)		Mineralized N/TN (%)	
		Ambient T	Elevated T	Ambient T	Elevated T	Ambient T	Elevated T
2007	Spring	6.11	6.62	3.54	3.51	7.67	7.08
	Autumn	6.68	6.17	3.53	3.28	7.20	7.22
2008	Spring	6.63	6.34	3.35	3.22	6.89	6.95
	Autumn	6.50	6.32	3.00	3.05	6.43	6.75
2009	Spring	6.25	6.45	4.07	3.99	8.92	8.48
	Autumn	6.52	6.67	3.88	3.77	8.17	7.75
2010	Spring	6.34	6.40	3.87	3.89	8.41	8.39
	Autumn	7.34	7.84	4.40	4.48	8.22	7.84
2011	Spring	6.12	6.25	3.01	2.93	6.67	6.35
	Autumn	8.78	8.72	3.23	3.02	5.00	4.87
Average	Spring	6.29	6.41	3.57	3.51	7.71	7.45
	Autumn	7.16	7.14	3.61	3.52	7.01	6.89
ANOVA results (*P* value)							
Year (Y)		<0.01		<0.01		<0.01	
Season (S)		<0.01		0.51		<0.01	
Temperature (T)		0.70		0.08		0.18	

3.4.4. The effect of pre-incubation on the responses of C and N mineralization potentials to soil warming

In pre-incubated soil samples, the average decomposed C potentials in AT and ET were 298.8 and 291.4 mg C kg^{-1}, respectively. For air-dried and pre-incubated soil samples, the average decomposed C potentials in autumn were slightly higher than those in spring. Compared with air-dried soil samples, the loss of decomposed C potential caused by pre-incubation was 57.4% in spring and 56.3% in autumn. It could be ascribed to the rapid labile C depletion during the pre-incubation phase as mentioned above. Lagomarsino et al. (2016) presumed that pre-incubation could promote microbial growth and adaptation to incubation conditions, resulting in a substantial consumption of the most labile substrates. The amounts of decomposed C potentials were similar to those of CO_2 production due to lower CH_4 produced under the anaerobic conditions. Similar to CH_4 production, the decomposed C potentials in air-dried soil samples were significantly decreased by elevated soil temperature (P= 0.019), whereas this significant impact was eliminated in pre-incubated soil samples (P=0.31, Table 3.4). It might also be due to the depletion of labile C in pre-incubation phrase. Benbi et al. (2014) suggested that labile C was more sensitive to temperature than stable C. Apparently, pre-incubated soil samples had less labile C than air-dried soil samples. Consequently, a portion of stable organic C such as mineral associated or physical protected SOC by soil aggregates in pre-incubated soil samples would be decomposed in subsequently anaerobic incubation.

The dynamics of mineralized N production potential in soil samples with and without pre-incubation are shown in Table 3.3 and Table 3.4. The mineralized N potentials in air-soil samples varied from 71.2 to 124.8 mg N kg^{-1} in AT and from 67.6 to 116.8 mg N kg^{-1} in ET, respectively (Table 3.3). Nevertheless, in the pre-incubated

soil samples, the mineralized N potentials ranged between 42.4 and 62.9 mg N kg^{-1} in AT, and between 38.4 and 55.7 mg N kg^{-1} in ET, respectively (Table 3.4). Similar to decomposed C, soil warming significantly decreased the mineralized N potential in air-dried soil samples but not in pre-incubated soil samples. Pre-incubation leaded to an approximately 51.2% loss of average mineralized N at 25°C and 40% WFPS. These results indicated that pre-incubation would alter soil N availability, resulting in less mineralized N production during the subsequent anaerobic incubation.

3.4.5. Effect of soil warming in DOC concentration in paddy soil

It is well argued that DOC is considered as the most active fraction of SOM due to its high microbial avaiablitiy although it accounts for a relatively small fraction (0.04-0.2%) in SOC (Zsolanay, 1996). Generally, it is derived from plant exudates and partial decomposition of SOM (Billett et al., 2004; Freeman et al., 2004). The average DOC concentration in pre-incubated soil samples was less than 35.3% than that in air-dried soil samples. This finding was consistent with the observation by Villada et al. (2016) that concentrations of cold water extractable C in air-dried forest soil (in mineral horizon) was two-fold greater than those in field moist soils. One probable reason could be attributed to the increased microbial activities resulting in accelerated DOC decomposition. Akagi et al. (2007) compared amounts and characteristics of DOC in air-drying and pre-incubated soil samples, indicating that DOC extracted from pre-incubated soil samples was only 20% of that in air-dried soil samples. The labile compounds released during air-drying were decomposed during the pre-incubation. The main reason for the difference in DOC between air-dried and pre-incubated soil samples could be due to the impact of air-drying on the microbial activity. On the other hand, compared with moist soil, the release of microbial cell

lysis and intracellular solutes during air-drying of initial fresh soil could make great contribution to increased DOC concentration. Previous studies indicated that rewetting of soils leaded to enhancement of SOC mineralization and other nutrients release (Wu and Brookes, 2005; Sawada et al., 2016).

Soil samples taken in spring had higher DOC concentration extracted by KCl solution than those in autumn (P<0.05, Table 3.5). This result could be explained by the removal of rice straw after rice harvest and high soil temperature in autumn although SOC contents in air-dried soil samples were higher in autumn than those in spring (Fig.3.6a). In addition, the significantly interannual variation of DOC concentration was observed in air-dried and pre-incubated soil samples. It might be ascribed to the differences in soil moisture and duration of air drying of fresh samples across 5 years.

3.5. Conclusion

In this study, soil warming did not significantly affect aboveground and root biomass of rice at maturity stage, probably due to the decreased N availability (observed as NH_4^+-N production). Contrary to our hypothesis, elevation of soil temperature during rice growth season distinctly reduced SOC and TN contents in rice paddy field as a result of stimulation of SOM decomposition and insignificant enhancement of plant-derived C and N inputs. C decomposition and N mineralization potentials obtained from the 4-week anaerobic incubation at 30 °C and under submerged condition were also significantly decreased by soil warming. However, the significant decreased effect of soil warming on C decomposition and N mineralization would be offset by pre-incubation. Moreover, pre-incubation of air-dried soil samples significantly decreased DOC extracted by KCl solution, probably owing to the rapid

depletion of labile SOM and the stimulated soil microbial activity. The interannual decrease of SOC and TN contents in both soil temperature treatments might be due to the yearly removal of rice straw from the field. SOC content and average decomposed C potential under two temperature conditions were higher in autumn samples than those in spring samples due to the increased plant-derived C input via plant photosynthesis during rice growth season. Our study indicated that soil warming during both rice growth and fallow seasons would decrease SOC and N storages in single rice paddy and lead to a positive feedback to further global climate warming.

Chapter Ⅳ The amounts and compositions of soil organic C and N affected by 5-year elevated [CO_2] and soil temperature in rice paddy ecosystem

4.1. Introduction

The increase of atmospheric[CO_2]and air temperature are the main features of climate changes. It has been reported that the globally averaged surface temperature has increased by 0.85°C over the period 1880-2012 and it is expected to increase by 0.3-4.8 °C by the end of this century (IPCC, 2013). The monthly atmospheric [CO_2] observed in Mauna Loa, Hawaii, USA, has risen from 315.7 ppm in 1958, March to 404.5 ppm in 2016, October. The rate of increase in [CO_2] from an average of 0.85 ppm yr^{-1} in the 1960s to approximately 2 ppm yr^{-1} in last decade (2000 to 2010) (http://www.esrl.noaa.gov/gmd/ccgg/trends/gr.html, accessed 6, December, 2016). Many previous studies have indicated that elevated [CO_2] and/or soil warming have great impacts on plant growth, soil microbial community, C decomposition and N mineralization in forest, grassland, and (Jones and Donnelly, 2004; Wan et al., 2007; Natali et al., 2012; Schleppi et al., 2012; Sigurdsson et al., 2013; Yin et al., 2013; Zhou et al., 2016). The main findings for these previous studies are that elevated [CO_2] can enhance plant photosynthesis, resulting in soil C inputs. Soil warming can accelerate soil microbial activities, thus promote SOM turnover leading to C and N loss via many processes. It has been argued that soils contain the largest pool of terrestrial organic carbon and are a major source of atmospheric CO_2 (Van Groennigen

et al., 2014).

Compared with natural ecosystems such as forest and grassland, similar studies on the interactive effects of elevated [CO_2] and soil temperature on components and decomposition of SOM are few conducted in paddy ecosystem which is characterized by intensive cultivation and high amount of fertilizer inputs. Recently, the combined effects of elevated [CO_2] and experimental warming (air/soil) on plant growth and yield have been studied over several decades (Cheng et al., 2009; Tokida et al., 2010; Hasegawa et al., 2013, Usui et al., 2016, Wang et al., 2016). However, no consensus are reached on the enhanced rice growth and yield induced by elevation of [CO_2] and soil warming. For example, Tokida et al. (2010) reported that elevated soil and water temperature increased aboveground biomass of rice by 9% and decreased rice root biomass by 8%. However, Wang et al. (2016) analyzed 4-year rice yield data obtained from a similar T-FACE experiment in rice-wheat rotation field in China, suggesting that elevated [CO_2] (500 ppm) significantly increased grain yield of rice by 8%. Rice yield and biomass were slighted decreased by averaged 4.8% and 5.3% under canopy air warming. Evidently, SOC stock is the result of its input by plant photosynthesis and its output by decomposition. Since CO_2 is substrate of plant photosynthesis and soil temperature will affect the rates of C decomposition and N mineralization. Unfortunately, so far, the responses of C decomposition and N mineralization to both elevated [CO_2] and soil warming have not been largely conducted in paddy field.

The objectives of this study were to (1) investigate the effects of 5-year elevated soil temperature and [CO_2] on amounts and components of SOC and TN in soil layers from 0-50 cm; (2) quantify the fraction of C inputs from plant to paddy soil after 5-year elevated CO_2 and soil warming during rice growth season.

4.2. Material and Methods

4.2.1. FACE site and elevation of soil temperature and [CO_2]

A T-FACE experiment was conducted at Tsukuba FACE site located in Tsukubamiraishi, Ibaraki Prefecture, Japan (35°58′N, 139°60′E, 10 m above sea level) by the research group of Agro-Meteorology Division, NIAES, Japan, since 2010. The climate condition has been described by Usui et al. (2016). Briefly, climate was humid subtropical, with an average annual temperature of 13.8 °C and annual precipitation of 1280 mm. Paddy soil type in experimental site was a Fluvisol. The soil properties at the site have also been described by Hasegawa et al. (2013). In brief, the soil contained 21.4 mg C and 1.97 mg N kg^{-1}, has a bulk density of 0.87 g cm^{-3}, and is composed of 36% sand, 40% silt, and 23% clay in average.

The method for controlling [CO_2] in an open paddy field during rice growth season has been previously described by Nakamura et al. (2012). Briefly, the elevated [CO_2] (F, hereafter) treatments were imposed on four octagonal plots in a paddy field. Four blocks (replications) were established in paddy fields, each consisting of two octagonal plots (240 m^2, 17 m across): an ambient [CO_2] (A, hereafter) plot and an FACE plot. The FACE plots were equipped with polyethylene tubing (Kiriko type-R, MKV Dream Co., Ltd, Tokyo, Japan) installed horizontally on the edges of the FACE rings at approximately 30 cm above the rice canopy. The emission tubes had small holes of 0.3-0.5 mm in diameter at 40-mm intervals on the lower half of each tube. CO_2 was released from the windward sides of FACE rings. The [CO_2] measured at the central point at FACE plot was targeted to be 200 μmol mol^{-1} above that at the ambient [CO_2] (A, hereafter) plots during daylight hours (from the sunrise to sunset).

A soil and water warming split-plot factorial experiment with Ambient (C,

hereafter) and elevated temperature (T, hereafter) treatments as were also designed in each [CO_2] treatment. The on-off hearing method for water and soil warming during rice growth season has been described by Tokida et al. (2010) and Adachi et al. (2014). In brief, in each block, the elevated soil and water temperature area (3.0 m × 5.4 m) was corrugated by PVC boards. The on-off control of heaters with wires (type CRX; Tokyo Technological Labo Co., Ltd., Tokyo, Japan) was used to elevated soil and water temperatures by 2 °C compared with AT treatment. The elevation of soil and water temperatures started within approximately one week after transplanting and terminated approximately two weeks prior to harvest, when the surface water was drained for harvesting.

Therefore, there were four treatments with combination of [CO_2] and soil/water temperature and each treatment had four replications in this study. The ambient [CO_2] and ambient soil temperature were regarded as control treatments. Finally, they were abbreviated into FC, FT, AC, AT, respectively in this chapter.

4.2.2. Soil sampling and property analysis

In October, 2014 after 5-year elevated [CO_2] and soil temperature during rice growth season, soil cores with five replications were taken from 0-50 cm in each treatment in FACE experimental field. Each soil core was equivalently divided into five soil cores with a height of 10 cm. Then, all soil cores were moved to greenhouse, air-dried, ground into powder, and stored at room temperature for further analysis. In each soil sample, pH, EC, SOC and TN contents, $\delta^{13}C$ and $\delta^{15}N$ values, NO_3^--N, $NH4^+$-N concentrations were measured as described in section 2.2.1 and 2.2.5 in Chapter II.

4.2.3. Anaerobic incubation

Since our previous study in Chapter III showed that a great proportion of C was lost during the pre-incubation, we directly conducted anaerobic incubation of air-dried soil samples taken in four treatments in T-FACE experimental site. The major processes of 4-week and 8-week anaerobic incubations of air-dried soil samples were identical to those as described in section 2.2.3 in Chapter Ⅱ. It should be mentioned that after measuring CO_2 and CH_4 productions at the headspace of each serum bottle, approximately 30 ml gas was injected into 20-mL vacuum serum bottle to further measure $\delta^{13}C$ value in produced CO_2 and CH_4 during anaerobic incubation.

4.2.4. Measurement of $\delta^{13}C$ value in produced CO_2 and CH_4, and calculation of fraction of plant derived C in SOC

The $\delta^{13}C$ value in produced CO_2 and CH_4 ($\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2) after 4-week and 8-week anaerobic incubation was measured by a conventional continuous-flow (CF) gas chromatography-isotope ratio mass spectrometry (C-IRMS) instrument as described by Tokida et al. (2014). The $\delta^{13}C$ value in decomposed C was calculated by $\delta^{13}C$ values and productions of CO_2 and CH_4 according to law of conservation of ^{13}C mass. Similarly, on the basis of C balance between plant and soil, the fraction of C input derived from rice plant (F_{plant}) was calculated as following equation:

$$F_{plant} = \left(\delta^{13}C\_\mathrm{Dec}_{\text{-F}} - \delta^{13}C\_\mathrm{Dec}_{\text{-A}}\right) / \left(\delta^{13}C\_\mathrm{plant} - \delta^{13}C\_Dec_{\text{-A}}\right)$$

Where $\delta^{13}C$-$Dec_{\text{-F}}$, $\delta^{13}C$-$Dec_{\text{-A}}$ represented $\delta^{13}C$ value in decomposed C as the sum of CO_2 and CH_4 in F and A treatments, respectively. $\delta^{13}C$-plant stands for the $\delta^{13}C$ value in rice, -38.17‰.

4.3. Results

In this study, we found large spatial variations in SOC, TN, $\delta^{13}C$, $\delta^{15}N$ in paddy soil samples after 5-year FACE and elevated soil temperature treatments among four plots (replications). Moreover, there were large variations in those parameters above in each treatment at each plot in soil layers from 0-50 cm. The SOC content in 30-50 cm soil layer was larger than those in sublayer form 10-30 cm in some treatments. In this study, we just showed the results in three layers from 0-30 cm.

4.3.1. pH and EC

The pH and EC in soil samples from 0-30 cm after 5-year elevated [CO_2] and soil temperature conditions are shown in Fig 4.1. Soil pH in all soil samples ranged between 5.9 and 6.4 under four treatments. pH in soil samples rapidly increased with soil layers from 0-30 cm. EC varied from 67.5 to 142.8 μS cm^{-1} in all soil samples under four treatments. Different to pH，EC decreased with soil layers. Since there were large variations of pH and EC in soil samples under the same treatment among four replications, we could not employ statistical analysis to qualify the effects of elevated [CO_2] and soil temperature on pH and EC. However, the overage soil pH under AT treatment was higher than under ET treatment. The decrease in pH induced by F treatment was only observed in 20-30 cm soil layer.

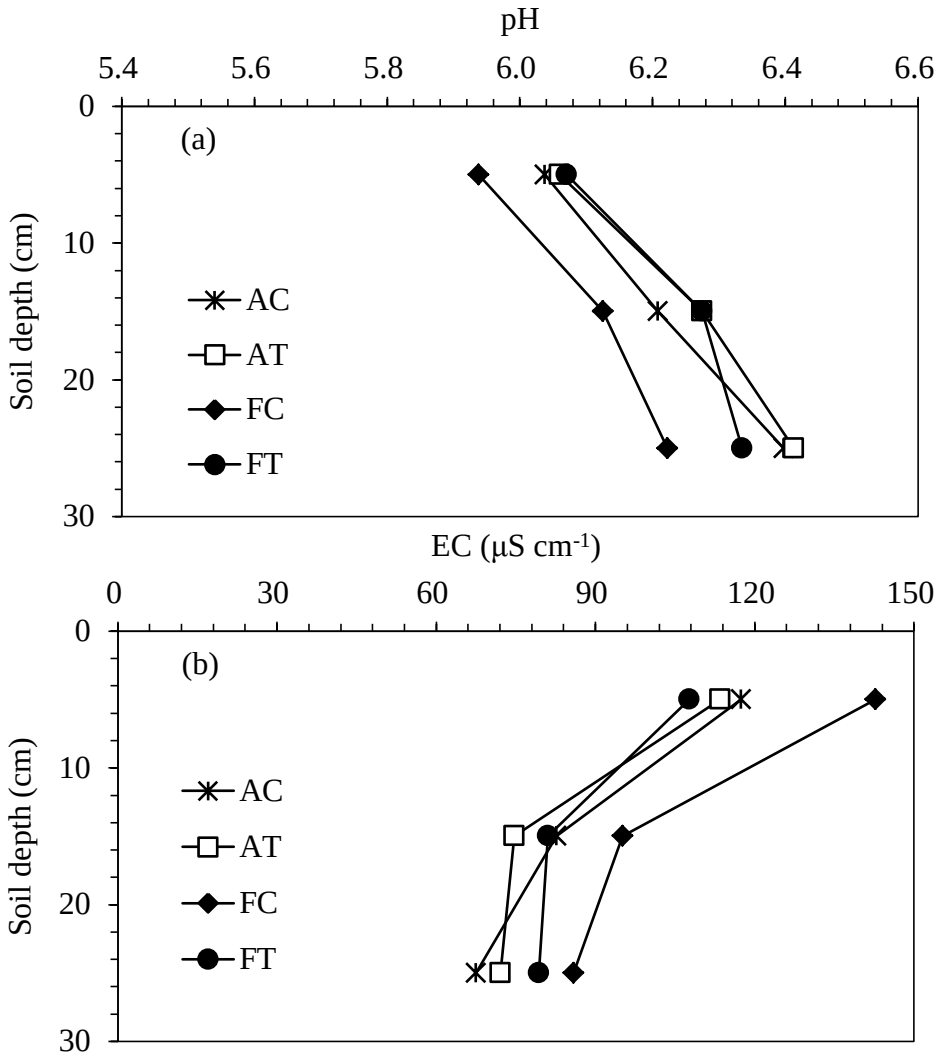

Fig.4.1. pH (a) and EC (b) in soil samples in 0-30 cm soil layer after 5-year elevated [CO_2] and soil temperature treatments.

4.3.2. SOC and TN contents

The dynamics of SOC and TN affected by 5-year elevated CO_2 and soil temperature in soil samples from 0-30 cm soil layer are shown in Fig. 4.2. SOC and TN varied from 8.8 and 20.7 g C kg^{-1} soil and from 0.74 to 1.82 g N kg^{-1} soil, respectively. Both SOC and TN contents decreased with soil layers in all treatments. However, the variations of SOC and TN contents in each treatment increased with soil layers. The trend of SOC content with soil layers in each treatment was similar to that of TN content. As a whole, SOC content in F treatment was larger than A treatment. T

treatment decreased SOC and TN contents in the first (0-10 cm) and third (20-30 cm) soil layers. Unexpectedly, FT treatment had higher SOC and TN contents than FA treatment in the second layer (Fig.4.2).

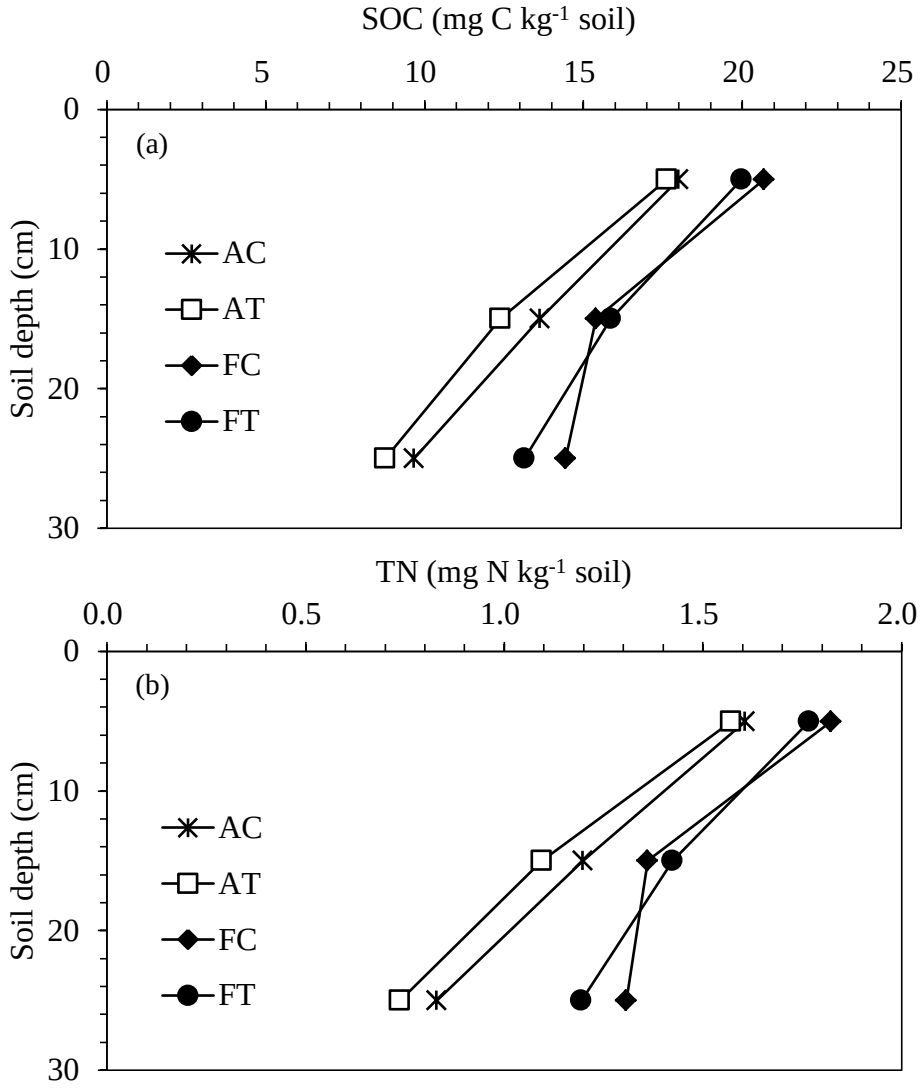

Fig.4.2. SOC (a) and TN (b) contents in soil samples in 0-30 cm soil layer after 5-year elevated [CO_2] and soil temperature treatments.

4.3.3. $\delta^{13}C$ and $\delta^{15}N$ values in bulk soil

Changes in $\delta^{13}C$ and $\delta^{15}N$ values in bulk soil samples in 0-30 cm soil layer under 5-year elevated [CO_2] and elevated soil temperature conditions are shown in Fig. 4.3. $\delta^{13}C$ and $\delta^{15}N$ values in all soil samples varied from -26.9 and -22.7‰, and from 3.3

to 6.9‰, respectively. Both $\delta^{13}C$ and $\delta^{15}N$ values increased with soil layers. Moreover, both $\delta^{13}C$ and $\delta^{15}N$ values in soil samples under F treatment were lower than A treatment in the first and third soil layers. In the second soil layer, $\delta^{13}C$ values were very close in four treatments. However, $\delta^{15}N$ value in AT treatment was higher than those in other three treatments (Fig. 4.3).

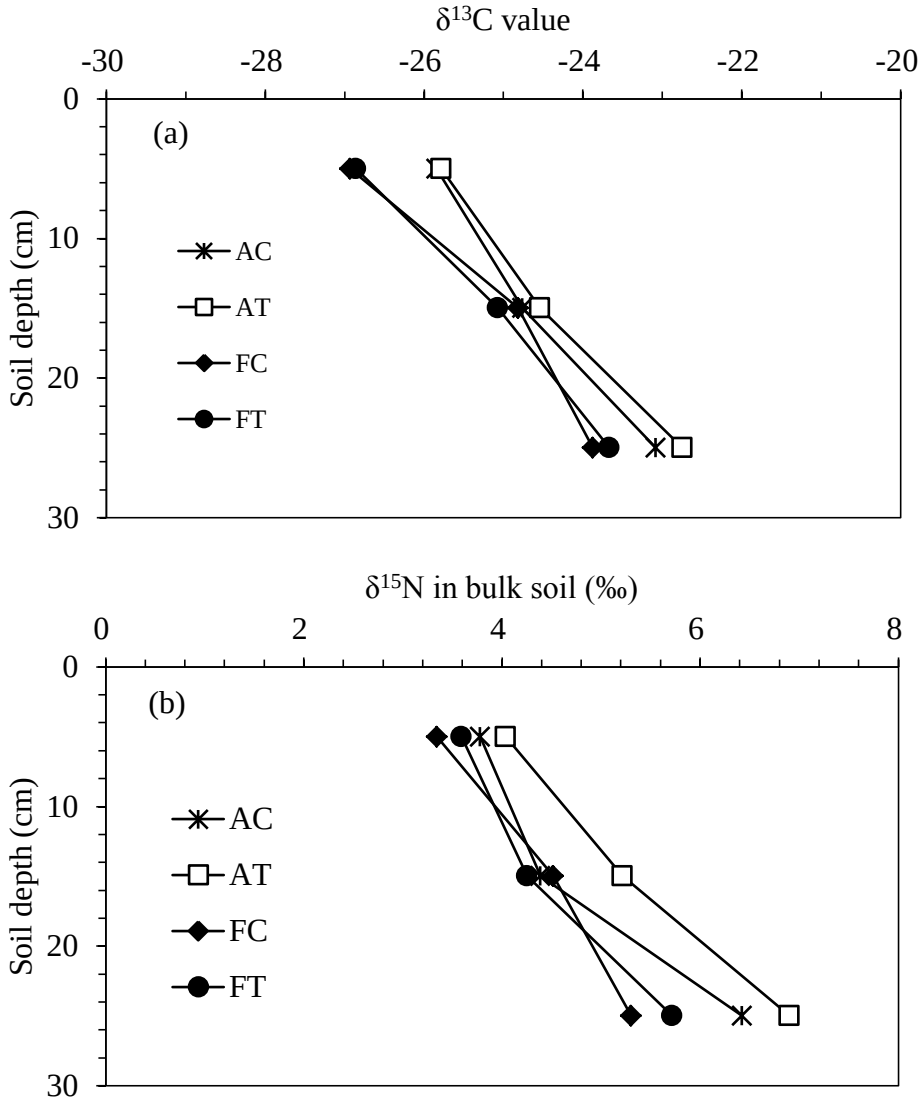

Fig.4.3. $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values in bulk soil samples in 0-30 cm soil layer under 5-year elevated [CO_2] and elevated soil temperature conditions.

4.3.4. DOC concentration

Changes in DOC concentration in soil layers from 0 to 30 cm under 5-year elevated [CO_2] and soil temperature treatments are shown in Fig. 4.4. DOC concentration in all soil samples ranged between 131.6 and 294.2 mg C kg^{-1} soil with the highest in the first soil layer and the lowest in the third soil layer. In the first soil layer, DOC concentration was in the order followed by FC> AC> AT> FT. DOC concentrations under F treatment were higher than those under A treatment in the second and third soil layers. Soil samples in AC treatment had higher DOC concentration than AT treatment in all soil layers. However, DOC concentrations in FT treatment were higher than those in FC treatment in the third soil layer.

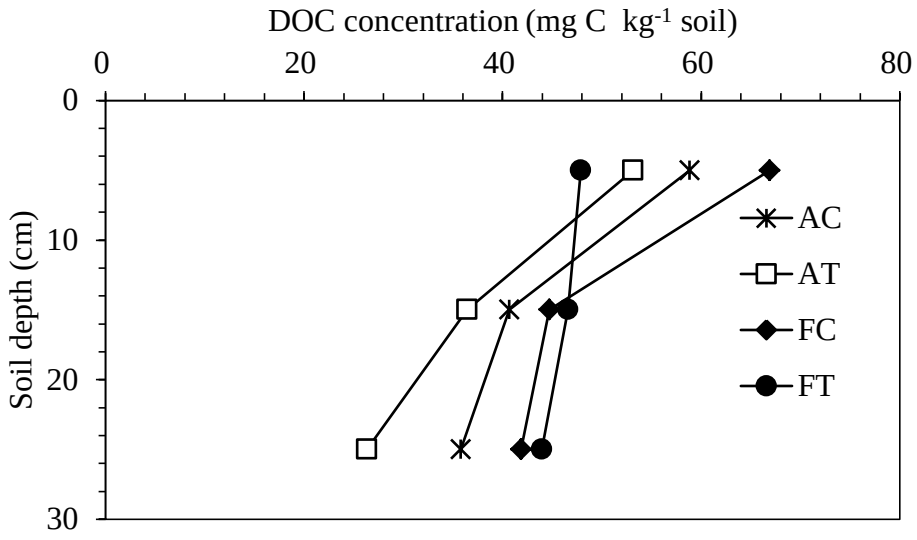

Fig.4.4. DOC concentration in bulk soil samples in 0-30 cm soil layer under 5-year elevated [CO_2] and soil temperature conditions.

4.3.5. CO_2 and CH_4 productions after 4-week anaerobic incubation

The productions of CO_2 and CH_4 after 4-week anaerobic incubation of soil samples under 5-year elevated [CO_2] and soil temperature conditions are shown in Fig. 4.5. CO_2 production in all soil samples during anaerobic incubation varied from 42.2 to 456.3 mg C kg^{-1} soil in three soil layers. The average CO_2 production under four treatments in three soil layers was 421.7 (0-10 cm), 200.3 (10-20 cm) and 79.3 (20-30 cm) mg C kg^{-1} soil, respectively. In the first soil layer, CO_2 production was highest under FC treatment. There was no difference in CO_2 production in other three treatments. Compared with AT and ET treatments, both FC and FA increased CO_2 production only in the third soil layer. CH_4 production in all soil samples ranged between 8.5 and 50.5 mg C kg^{-1} soil in the first layer. The CH_4 production under four treatments in the first layer was in the order followed by FC > FT >AC >AT. However, the CH_4 production was quite low in the second layer and no CH_4 production was found in the third layer.

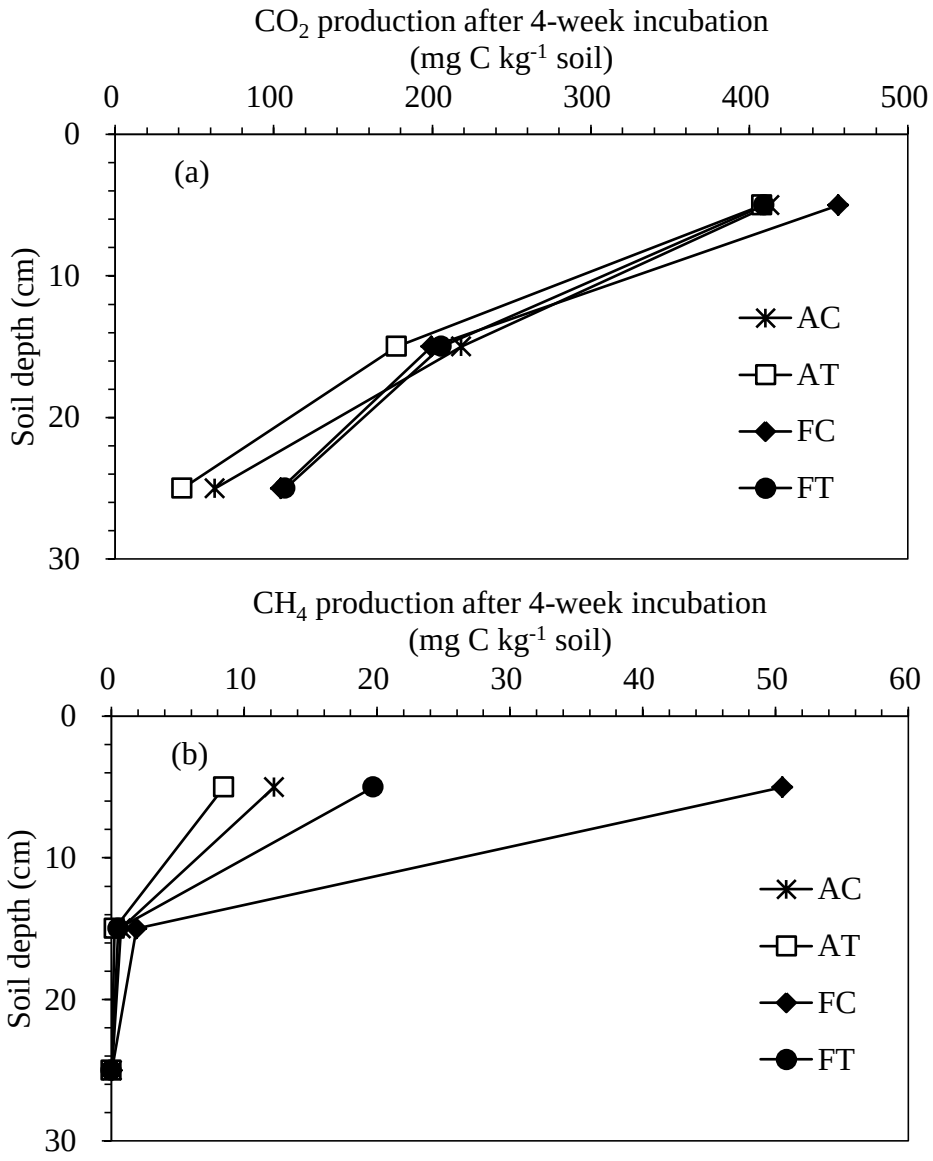

Fig.4.5. The productions of CO_2 (a) and CH_4 (b) after 4-week anaerobic incubation of soil samples from 0-30 cm soil layer under 5-year elevated [CO_2] and soil temperature conditions.

4.3.6. CO_2 and CH_4 productions after 8-week anaerobic incubation

The CO_2 and CH_4 productions after 8-week anaerobic incubation are shown in Fig.4.6. CO_2 production after 8-week anaerobic incubation ranged between 67.2 and 616.2 mg C kg^{-1} soil. It decreased with soil layers and obviously higher than those after 4-week anaerobic incubation. Compared with A treatment, F treatment had distinctly higher CO_2 production after 8-week anaerobic incubation of soil samples taken in first (0-10 cm) and third (10-20 cm) soil layers. The T treatment decreased CO_2 production in soil samples taken in first and third soil layers. The produced CH_4 during 8-week anaerobic incubation was nearly observed in first soil layer and varied from 61.7 to 125.7 mg C kg^{-1} soil. Similar to CO_2 production, CH_4 production in elevated [CO_2] was higher than those in A treatment. The T treatment also leaded to lower CH_4 production compared with C treatment.

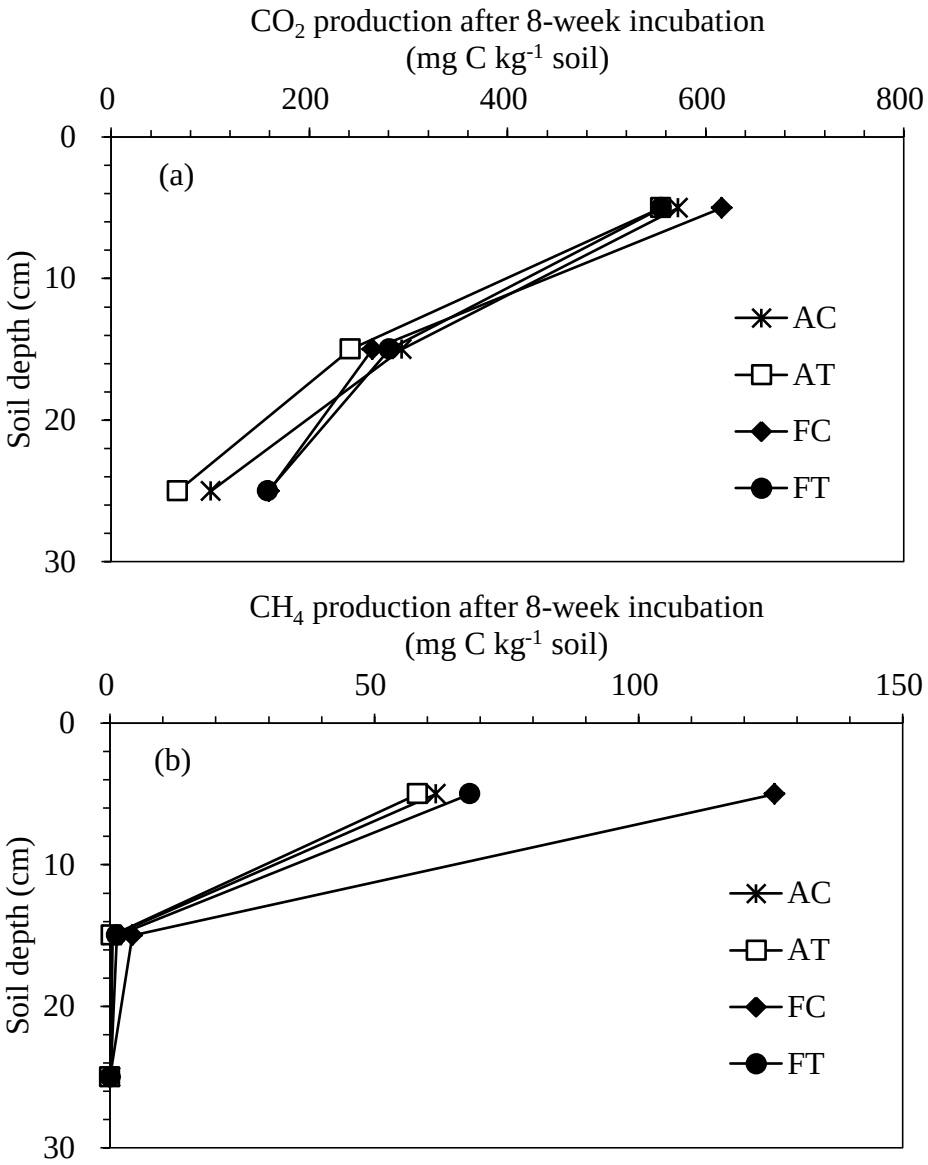

Fig.4.6. The productions of CO_2 (a) and CH_4 (b) after 8-week anaerobic incubation of soil samples from 0-30 cm soil layer under 5-year elevated [CO_2] and soil temperature conditions.

4.3.7. $\delta^{13}C$ value in CO_2, CH_4 productions and decomposed C after 4-week anaerobic incubation

The $\delta^{13}C$ value in CO_2 production obtained after 4-week anaerobic incubation varied from -30.6 to -25.1‰ and it decreased in second soil layer and then increased in third soil layer. F treatment had remarkable lower $\delta^{13}C$ value in CO_2 production than A treatment in all three soil layers. Elevated soil temperature decreased the $\delta^{13}C$ value in CO_2 production only in the first soil layer and no consistently decreased trend of $\delta^{13}C$ value in CO_2 production was found in the second and third soil layers. The $\delta^{13}C$ value in CH_4 production after 4-week anaerobic incubation varied from -82.4 to 62.5‰ which was distinctly lower than that in CO_2 production. No consistent trend of $\delta^{13}C$ value in CH_4 production was found in three soil layers and four treatments. The $\delta^{13}C$ value in the decomposed C after 4-week anaerobic incubation varied from -32.0 to -25.1‰. In the first layer, $\delta^{13}C$ value in the decomposed C was lower than $\delta^{13}C$ value in produced CO_2. However, in the second and third soil layers, $\delta^{13}C$ value in decomposed C and produced CO_2 were similar because of low CH_4 production. In F treatment, the $\delta^{13}C$ value in the decomposed C increased with soil layers. However, in C treatment, it decreased in second layer but increased in the third layer. Similar $\delta^{13}C$ values in the decomposed C were found in soil samples under two temperature treatments.

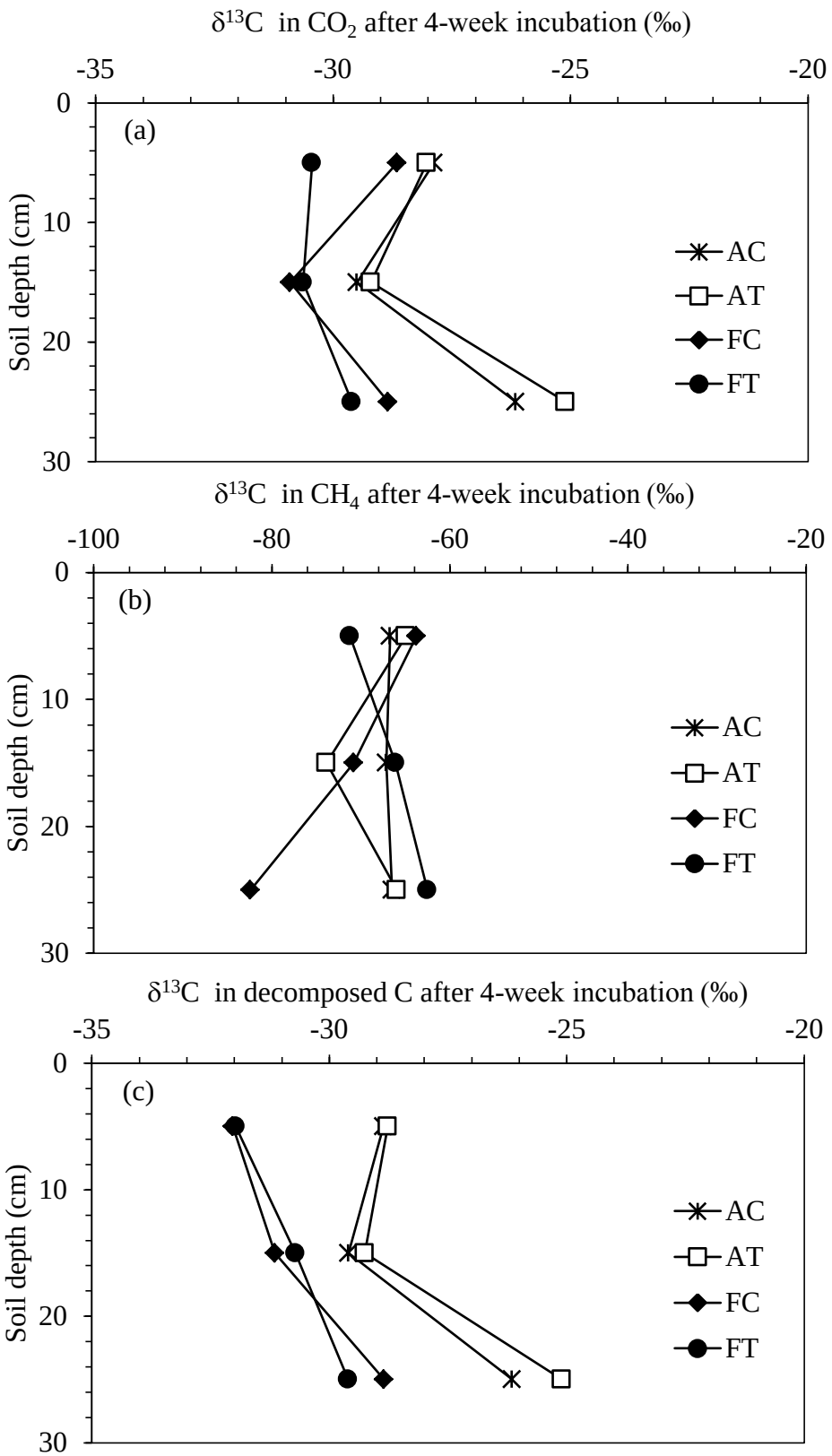

Fig.4.7. $\delta^{13}C$ value in productions of CO_2 (a), CH_4 (b) and decomposed C (c) after 4-week anaerobic incubation of soil samples in 0-30 cm soil layer under 5-year elevated [CO_2] and soil temperature conditions.

4.3.8. $\delta^{13}C$ value in CO_2, CH_4 productions and decomposed C after 8-week anaerobic incubation

As shown in Fig 4.8, $\delta^{13}C$ value in CO_2 production after 8-week anaerobic incubation varied from -31.3 to -25.4‰. The trend of $\delta^{13}C$ value in CO_2 production after 8-week anaerobic incubation with soil layers was similar to that in CO_2 production after 4-week anaerobic incubation. Under F condition, T treatment decreased $\delta^{13}C$ value in CO_2 production in the first and second layers. Under A condition, $\delta^{13}C$ value in CO_2 production had no big difference between two soil temperature treatments in the first and second layers. Similar to $\delta^{13}C$ value in 4-week CO_2 production, the $\delta^{13}C$ value in 8-week CO_2 production showed big variations in three soil layers and four treatments. The $\delta^{13}C$ value in decomposed C production after 8-week anaerobic incubation varied from -32.2 to -25.7‰. Its trend with soil layers was similar to that of the $\delta^{13}C$ value in decomposed C production. Soil samples under F condition had lower $\delta^{13}C$ value in decomposed C production than those under A condition. Under F condition, T treatment decreased the $\delta^{13}C$ value in decomposed C production in the first and second layer but had no change in the $\delta^{13}C$ value in decomposed C production in the third soil layer.

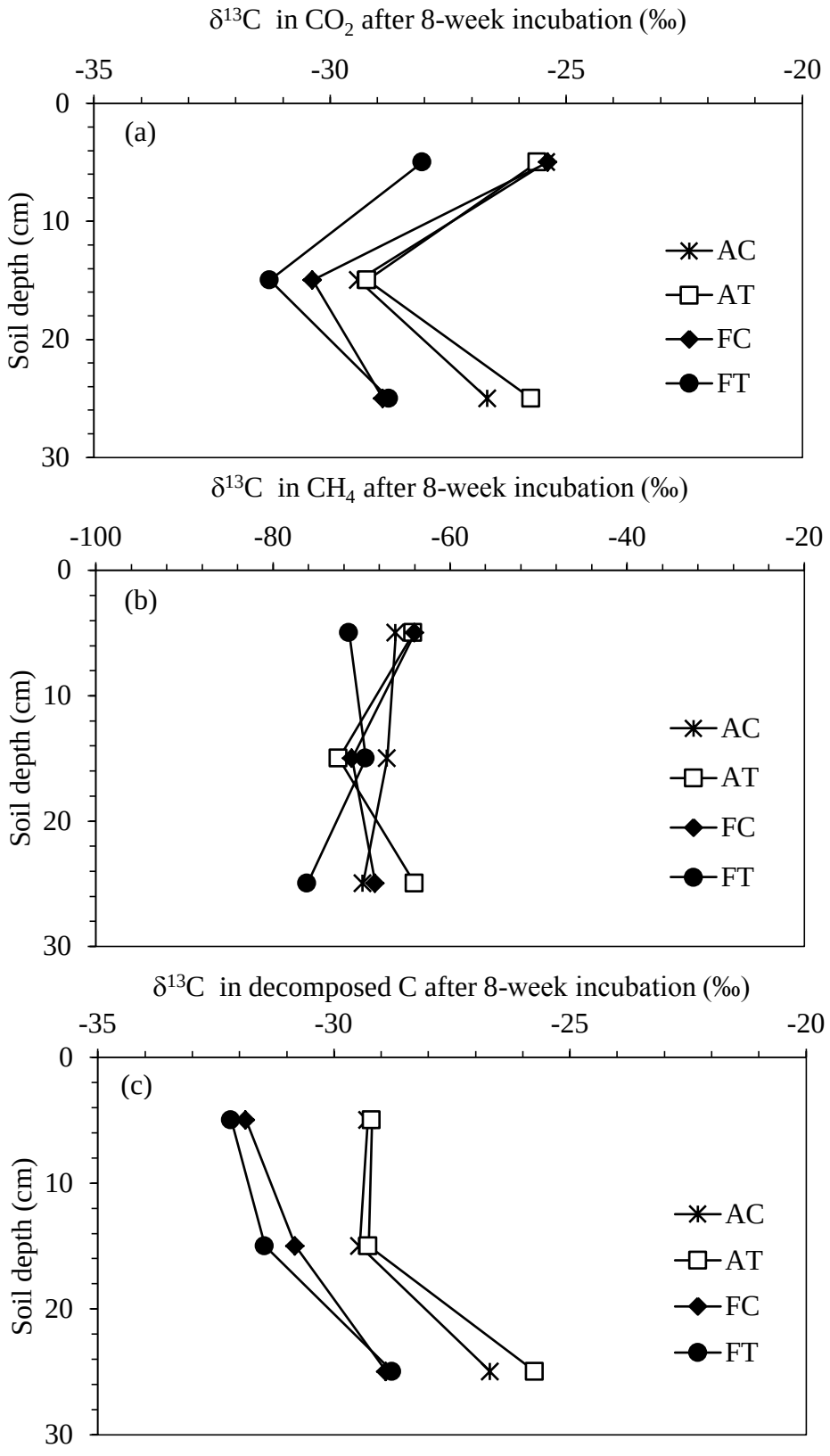

Fig.4.8. $\delta^{13}C$ value in productions of CO_2 (a), CH_4 (b) and decomposed C (c) after 8-week anaerobic incubation of soil samples from 0-30 cm soil layer under 5-year elevated [CO_2] and soil temperature conditions.

4.3.9. NH_4^+-N production during 4-week and 8-week anaerobic incubation

Changes in NH_4^+-N production in soil samples during 4-week and 8-week incubation are shown in Fig. 4.9. NH_4^+-N production after 4-week anaerobic incubation ranged between 12.9 and 98.9 mg N kg^{-1} soil in all soil samples and it obviously decreased with soil layers. In all soil layers, T treatment had a trend to decrease 4-week NH_4^+-N production. However, only in the third layer, F treatment increased 4-week NH_4^+-N production, compared with A treatment.

The 8-week NH_4^+-N production varied from 17.2 to 111.0 mg N kg^{-1} soil in all soil layers and four treatments. The decreased trend of NH_4^+-N production after 8-week incubation with soil layers was similar to that after 4-week incubation. Unexpectedly, 8-week NH_4^+-N production was not much higher than 4-week NH_4^+-N production. In the first layer, under F condition, T treatment remarkably decreased 8-week NH_4^+-N production. However similar decrease of NH_4^+-N production caused by elevated soil temperature was not observed under A condition. There was no change in 8-week NH_4^+-N production between two temperature treatments in the second and third soil layers.

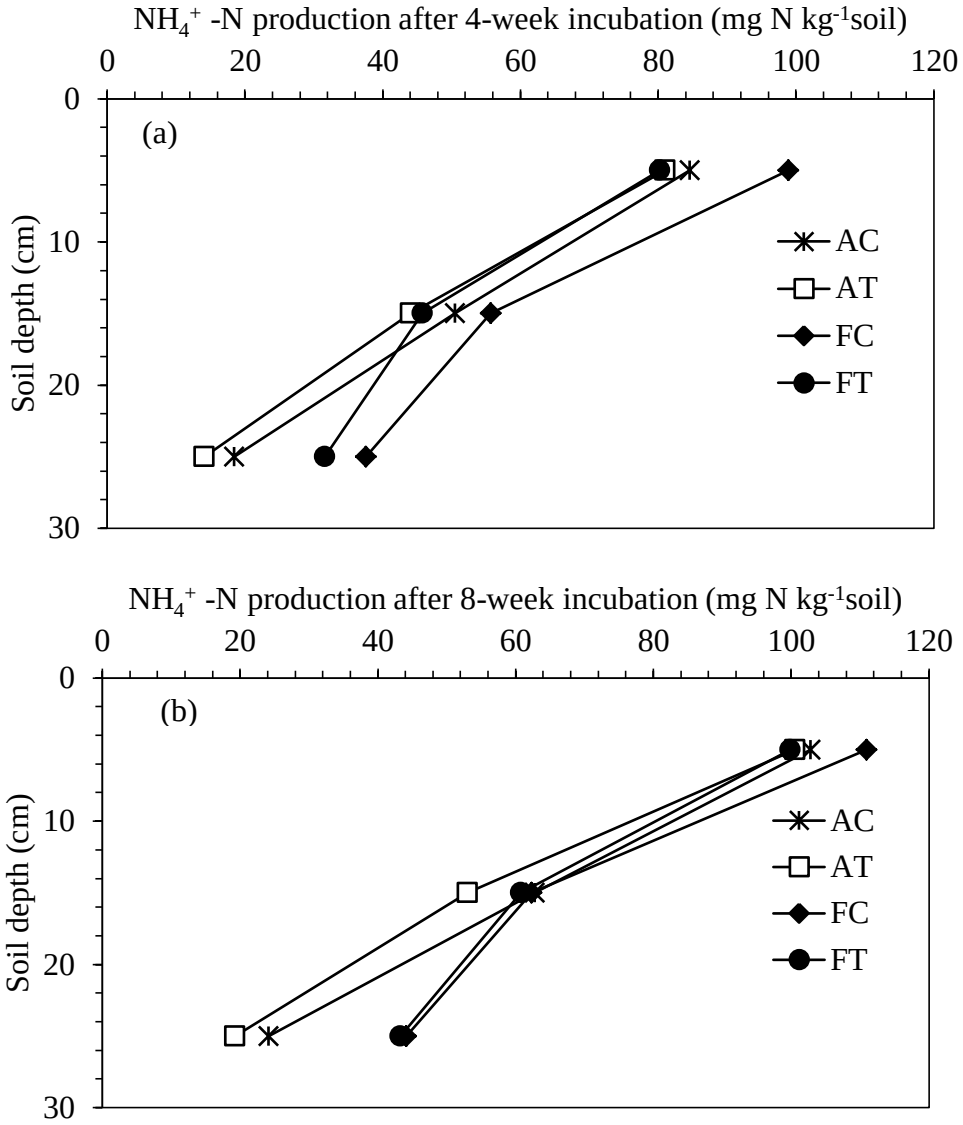

Fig.4.9. NH_4^+-N production after 4-week (a) and 8-week (b) anaerobic incubation of soil samples in 0-30 cm soil layer under 5-year elevated [CO_2] and soil temperature conditions.

4.3.10. NO_3^--N concentration in bulk soils and anaerobically incubated soils

NO_3^--N concentrations in bulk soil samples and anaerobic incubated soil samples in all soil layers were lower than the detection limit.

4.4. Discussion

4.4.1. Effects of elevated soil temperature and [CO_2] on SOC and TN contents

Many previous studies have indicated that elevated [CO_2] can lead to an increase of SOC content owing to stimulation of plant photosynthesis (Carrillo et al., 2011; Hofmockel et al., 2011; Wang et al., 2012). For example, Hofmockel et al. (2011) conducted a FACE experiment in forest ecosystem in Rhinelander, suggesting that elevated [CO_2] changed SOM cycling to favor C and N accumulation in less stable pools, with more rapid turnover. Due to the temporal and spatial variations in SOC contents, the response of SOC stocks to climate change such as elevated [CO_2] and soil temperature is still not well understood. Unexpectedly, in each treatment, there were huge variations of SOC and TN contents in different soil layers and soil sampling sites in this study. Therefore, ANOVA results were unavailable to correctly estimate the combined effects of elevated [CO_2] and soil temperature on SOC and TN contents in single rice paddy field in this study.

Before the T-FACE experiment, initial soil samples were collected from plow layer (approximately 15 cm). Similar variations in SOC and TN contents were also found in four replicate plots. The comparison of SOC and TN contents before and after 5-year T-FACE are shown in Fig. 4.10. Both SOC and TN contents were highest in Plot 1 and the lowest in Plot 3. The loss presents of SOC and TN contents in plots varied from 6.9% to 15.1% and from 7.6% to 17.8%, respectively. The inhomogeneity of paddy soil in soil layers and plots was the main reason for the large variations in parameters measured in this study. Despite this fact, as a whole, the increased SOC and TN contents by elevated [CO_2] and their decreased amounts by elevated soil temperature could be found in most of experimental plots and soil layers. Compared

with SOC and TN contents initial soil samples before the T-FACE experiment, SOC and TN contents decreased after 5-year T-FACE experiment, probably due to the yearly removal of rice straw after harvest.

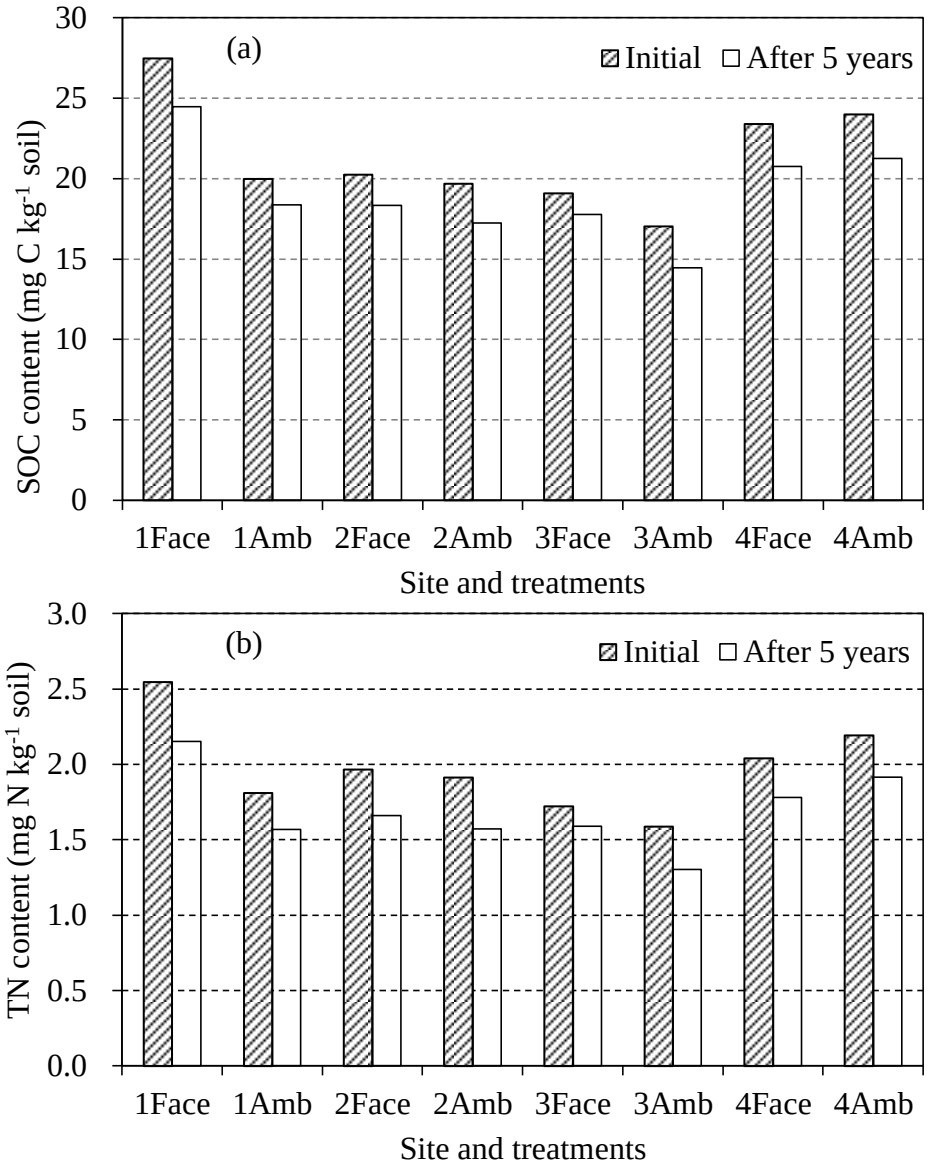

Fig.4.10. Amounts of SOC (a) and TN (b) in soil samples from 0-15 cm soil layer before and after 5-year elevated [CO_2] and soil temperature.

4.4.2. Effects of elevated soil temperature and [CO_2] on C decomposition

It is well known that soil C stocks are determined by the balance between plant growth and the subsequent input of plant detritus to soil, and C losses thorough

microbial decomposition. It has also been reported that elevated CO_2 can not only increase SOC stocks but also increase SOC decomposition by altering nutrient availabilities and microbial communities (Zak et al., 2000; Van Groenigen et al., 2014). For example, a meta-analysis which combined with data assimilation by Van Groenigen et al. (2014) indicated that atmospheric [CO_2] enrichment stimulated C input by 19.8% and the turnover of soil C by 16.5%. In some cases, the stimulation of SOC decomposition induced by elevated [CO_2] was greater than its input leading to the final decreased C stocks (Paterson et al., 2008).There was no doubt that elevation of soil temperature would promote SOC decomposition by stimulating microbial activities and altering substrate availability. However, more attention has been paid on the responses of rice growth and yield of different rice cultivars to elevated [CO_2] and soil warming (Cheng et al., 2009a; Cheng et al. ,2010a; Hasegawa et al., 2013; Usui et al., 2016; Wang et al., 2016). Few studies focused on the combined effects of elevated [CO_2] and soil temperature on SOC decomposition through anaerobic incubation.

The decomposed C as CO_2 and CH_4 productions are derived from labile components of SOC. In this study, we just conducted 4-week and 8-week anaerobic incubation experiments at 30 °C and under submerged conditions to measure CO_2 and CH_4 production potentials. Unfortunately, due to soil inhomogeneity in soil layers and experimental plots, the effects of elevated [CO_2] and soil temperature on SOC decomposition could not be statistically qualified. However, we can still get the general trends of the response of SOC decomposition to elevated [CO_2] and soil temperature. As expected, CH_4 production under elevated [CO_2] was higher than under ambient [CO_2] in the first soil layer. However, CO_2 production was similar in FT and AT in the first soil layer. The total 4-week and 8-week decomposed C

production in four treatments in three soil layers are shown in Fig. 4.11. The trends of 4-week and 8-week decomposed C productions with soil layers were similar to corresponding CO_2 production since CH_4 production was lower than CO_2 production. Decomposed C production also decreased with soil layers and it was in the order followed by FC> FT> AC> AT in the first and third soil layers. Our results showed that elevated [CO_2] had the trend to promote decomposed C production and elevated soil temperature had the trend to decrease decomposed C production.

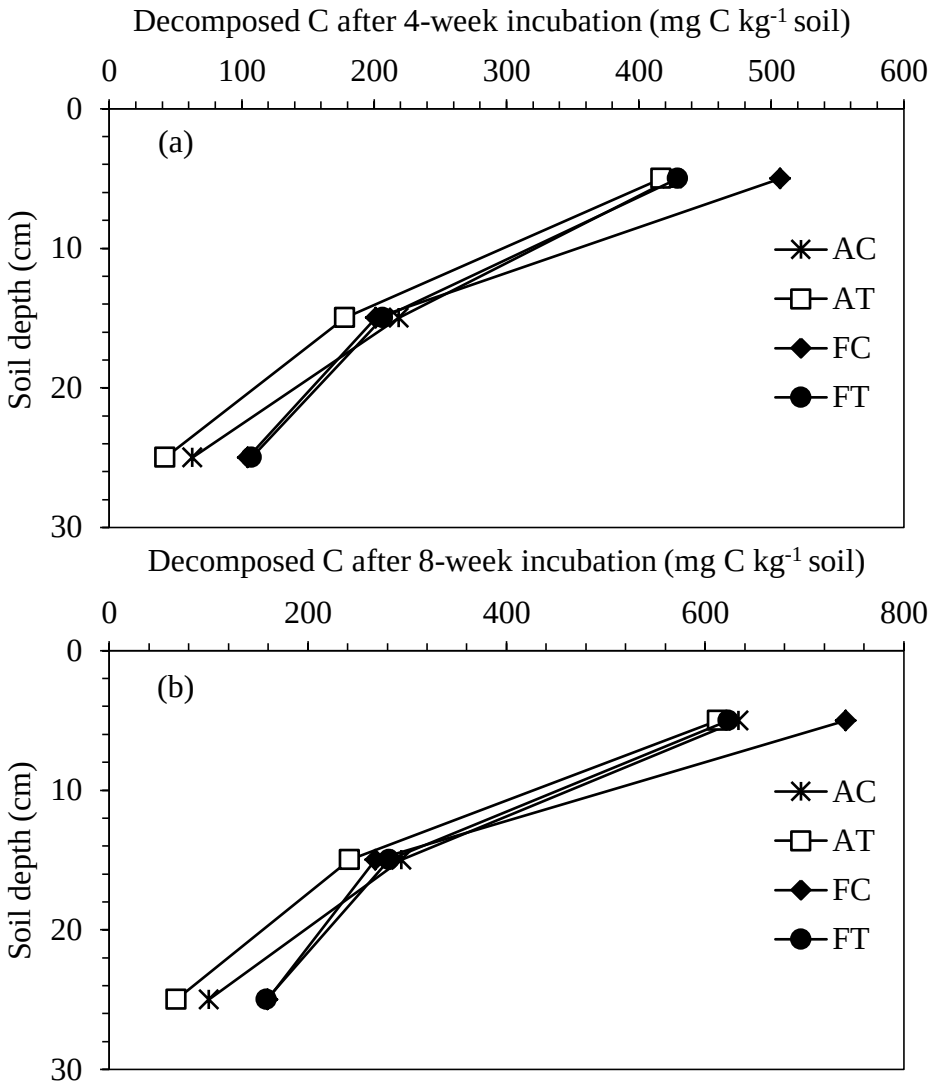

Fig.4.11. Decomposed C potentials obtained from 4-week (a) and 8-week (b) anaerobic incubation of soil samples in 0-30 cm soil layer under elevated [CO_2] and soil temperature conditions.

4.4.3. Effects of elevated soil temperature and [CO_2] on N mineralization

Surprisingly, NO_3^--N concentrations in initial soil samples before 5-year T-FACE experiment and after 4-week and 8-week anaerobic incubation were lower the detection limit. The probable reasons could be ascribed to low rate of nitrification in this single rice paddy field after rice harvest. During the anaerobic incubation at 30°C and under submerged condition, NH_4^+-N production could be considered as mineralized N which was also regarded as an index of soil fertility (Cheng et al., 2016). Our results showed that NH_4^+-N production in four treatments decreased in first two plow layers (0-10 cm and 10-20 cm) and increased in the third layer (Fig. 4.6). In top soil layer form 0-10 cm, the average of NH_4^+-N production was in the order followed by FC> AT> AC> FT. This result indicated that the stimulated of NH_4^+-N production caused by elevated [CO_2] was only evident in soil samples under control soil temperature. Under elevated [CO_2] condition, soil warming trended to decrease N mineralization observed by lower NH_4^+-N production. The lowest NH_4^+-N production in two plow layers was observed in FT treatment. This result could indicate that the under control soil temperature condition, elevated [CO_2] can decrease NH_4^+-N production in this single rice paddy soil (Fig. 4.9).

4.4.4. The ratio of decomposed C and Mineralized N to SOC and TN after 4-week and 8-week anaerobic incubation

As shown in Fig.4.12, the ratio of decomposed C to mineralized N after 4-week and 8-week incubation of soil samples taken from 0-30 cm soil layer varied from 2.9 to 5.4 and from 3.4 to 6.7, respectively. The ratios of decomposed C to mineralized N during 4-week incubation were slightly lower than those obtained from 8-week incubation. The reason could be attributed to the rapid decomposition of easily

decomposed SOM and slow decomposition of recalcitrant SOM. Undoubtedly, the ratios of decomposed C and mineralized N, SOC to TN decreased with soil layers. The ratio of SOC to TN in bulk soil samples ranged between 10.9 and 12.8, which was greatly larger than the ratio of decomposed C to mineralized N during 4-week and 8-week incubation. Moreover, there were no big changes in ratios of decomposed C to mineralized N, SOC to TN in four treatments. This result suggested that ratios of decomposed C to mineralized N were relative stable and not easily affected by elevated [CO_2] and soil temperature. It is well known that labile decomposed C and mineralized N are mainly derived from fresh plant tissues and microorganism. It cloud be regarded as an index of turnover rate of SOM decomposition.

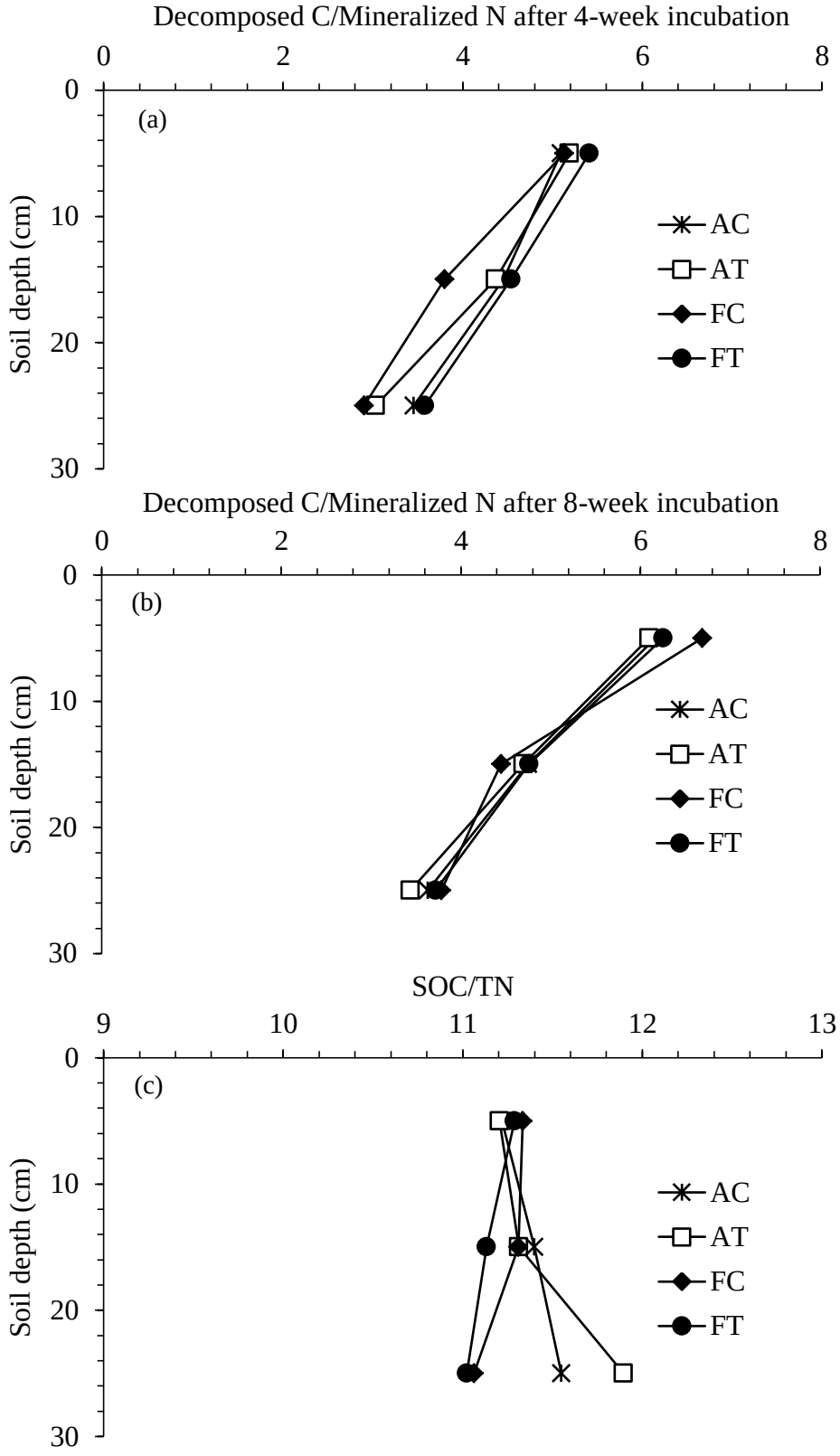

Fig.4.12. The ratios of decomposed C to mineralized N from 4-week (a) and 8-week (b) anaerobic incubation, and the ratio of SOC to TN (c) in soil samples from 0-30 cm soil layer under elevated [CO_2] and soil temperature conditions.

4.4.5. The fractions of C derived from rice plant calculated from $\delta^{13}C$ value in decomposed C potential

Paddy soil provides rice with various nutrients according to different rice growth stages. On the other hand, root exudates and dead tissues of rice can also increase soil C content which can be unutilized by microorganism. A part of fresh C derived from rice plant will be decomposed into CO_2 and CH_4 by microorganisms. Some parts of fresh C derived from rice plant can be assimilated into SOC to become the stable pool. It is well known that stable carbon isotope technique is a very powerful approach to trace the new C input from plant on the mechanism of ^{12}C and ^{13}C fractionation during SOC microbial decomposition. In this study, we calculated the fraction of new soil C input derived from rice plant (F_{plant}) with ^{13}C-labeling approach (Table 4.1). F_{plant} in first soil layer were 34.0% in C treatment and 34.1% in T treatment, respectively. Then it decreased to 18.1% in CT and 16.4% in ET in second soil layer. This result indicated that elevated soil temperature had no great impact on plant-derived C input into rice paddy soil. Unexpectedly, F_{plant} increased again in the third soil layer in two soil temperature treatments. Generally, the plow layer in rice paddy soil is in 0-20 cm in which soil and plant greatly interact with each other. Therefore, F_{plant} in two plow layers was highly reliable although large variation in decomposed C in experimental plots.

Table 4.1 The factions (%) of soil C input derived from rice plant calculated from $\delta^{13}C$ in decomposed C.

Soil layer (cm)	Control temperature (%)	Elevated temperature (%)
0-10	34.0	34.1
10-20	18.1	16.4
20-30	22.5	34.5

4.5. Conclusion

Due to high variations in soil parameters such as SOC, TN, NH_4^+-N in soil layers and replicate soil sampling sites, the really combined effects of elevated [CO_2] and soil temperature on C decomposition and N mineralization could not be statistically qualified in this study. However, the averaged data in four experimental replications showed that elevated [CO_2] had the trend to increase SOC and TN contents, thus promote C and N mineralization in 0-10 cm soil layer. Increased soil temperature had the trend to decrease SOC and TN contents in single rice paddy soil. The fraction of rice plant-derived C into paddy soil was not increased by soil warming in both the first and second soil layers. After 5-year T-FACE experiment, SOC and TN contents decreased with soil layers, mainly owing to the yearly removal of rice straw after rice harvest. Finally, soil homogeneity could be a noticeable factor to interrupt the real responses of SOM content and decomposition in rice paddy ecosystem to future climate change.

Chapter Ⅴ General discussion and conclusions

SOM represents one of the largest reservoirs of C on the global scale. Thus, the temperature sensitivities of bulk SOM and of different SOM fractions can be key factors determining the response of the terrestrial carbon balance to climatic warming. The change of SOC storage in response to climatic warming depends on how C inputs to soil by NPP and C outputs by SOM decomposition are balanced relative to each other (von Lützow and Kögel-Knabner, 2009). The impacts of climatic warming on dynamics of SOM decomposition have not been resolved due to apparently contradictory results from field and lab experiments, most of which have focused on labile C with short turnover times. But the majority of SOC are comprised of series of organic carbon with different turnover times from decades to centuries. Understanding the response of these C pools to climate change is essential for forecasting longer-term changes in soil C storage.

Rice paddy ecosystem plays a crucial role in soil C stocks and sequestration since it is one of the biggest sources of CH_4 and N_2O. Full understanding the feedbacks of rice growth and SOM decomposition to climate change is helpful to take effective measures to mitigate global warming and maintain food security. Paddy soil is mostly submerged during rice growth season and drained during fallow season. Even under the same water condition, SOC decomposition and N mineralization in paddy soils responses to soil temperature might vary with soil types. In this chapter, we discussed the feedbacks of rice growth, SOM amount, and decomposition, turnover in plant-paddy soil ecosystem to elevated soil temperature and [CO_2].

5.1. Rice growth and yield affected by elevated soil temperature and [CO_2]

The responses of rice growth and yield to global warming have been studied over several decades from the plot (chamber) to the open field in the world (Kim et al., 1996; Cheng et al. 2010, Hasegawa et al., 2013; Usui et al., 2016, Wang et al., 2016). Since CO_2 is the substrate of plant photosynthesis, elevated [CO_2] can definitely stimulate rice growth. A 3-year T-FACE experiment in Tsukuba showed that elevated [CO_2] significantly increased biomass and grain yield by approximately averaged 14% mainly due to the increased panicle and spikelet density (Usui et al., 2016). It has been reported that elevated [CO_2] can increase the rate of grain filling and the final weight of panicles as a result of increased panicle size (Liu et al., 2008; Yang et al., 2009). It is evident that rice cultivars respond differently to the elevated [CO_2] and the effect of elevated [CO_2] on the same rice cultivar growth also varies with its growth stages. For example, Hasegawa et al. (2013) conducted a comparative study on rice cultivar response to elevated [CO_2] at two FACE experiments in Shizukuishi and Tsukuba, Japan. They pointed out that the interaction of elevated [CO_2] and cultivar was significant for brown rice yield in both experiment sites. High-yielding cultivars with a large sink size had a great [CO_2] response. A wide range of yield from 3 to 36% under elevated [CO_2] was observed in Tsukuba site with eight cultivars. Moreover, a highly significant interaction CO_2 × cultivar interaction for percentage of ripened spikelet was only observed in Tsukuba trial and effect of elevated [CO_2] on percentage of ripened spikelet was highly dependent on rice cultivars from -3 to +17%.

Elevated air temperature has been projected to reduce rice yield and quality (Peng et al., 2004; Cheng et al., 2009; Usui et al., 2016). For example, Cheng et al. (2009) who conducted a controlled-environmental chamber experiment with two

levels of [CO_2] and night temperature (22 and 32 °C) suggested the whole plant and stem dry weight were significantly increased by elevated [CO_2] and high night temperature, while both the ear dry weight and brown rice yield were significantly increased by elevated [CO_2] but decreased by high night temperature. These results indicated that high night temperature would reduce the stimulatory effect of elevated [CO_2] on rice production in the future. Since this study was only conducted for one year in the chamber, the real effect of air temperature on rice yield could not be fully adapted to the open rice field. There are few studies on the effect of soil temperature on rice biomass in the field although soil temperature is quite closed with air temperature (Adachi et al., 2014; Usui et al., 2016). In our soil warming experiment, soil warming had a great impact on rice aboveground and root biomass of Koshihikari at maturity stage probably owing to decreased N availability observed as anaerobic NH_4^+-N production. We participated in T-FACE project in Tsukuba. The data about rice growth and yield was reported in the paper written by Usui et al. (2016). They found that combination of elevated [CO_2] and soil temperature significantly increased biomass but had not significant effect on the grain yield.

5.2. The SOC and TN contents affected by soil moisture, temperature, and elevated [CO_2] in rice paddy ecosystem

Since paddy soil is mostly submerged during rice growth season and drained during off-rice season, the changes in soil moisture will directly affect soil aeration and nutrient availabilities, thus influence SOC and TN by regulating their mineralization. Many previous studies have suggested flooded paddy is a major source of CH_4 emission (Kimura et al., 2004; Yang et al., 2010; Yuan et al., 2012; Zhang et al., 2015). However, under drainage condition, substantial produced CH_4

would be oxided into CO_2 by methanotrophs. Compared with upland, paddy soils have slow rate of SOM decomposition because of waterlogged condition. On the other hand, soil N could be lost as N_2O by denitrification under anxioc condition, and as NO_3^--N by leaching after nitrification under aerobic condition. In our first experiment, soil moisture had significant positive effect on SOC aerobic decomposition, leading to C loss. However, this significant stimulation of SOC decomposition induced by previous soil moisture was not observed under subsequently anaerobic incubation where soil moisture was kept at the same level. Increasing soil temperature showed a much larger effect on SOC and TN contents than soil moisture.

Early previous studies have suggested increased soil temperatures can accelerate SOM decomposition by increasing the activities of extra-cellular enzymes that degrade macromolecular organic matter into labile and low-molecular-weight organic compounds and by stimulating microbial activity and the uptake of soluble substrates ,resulting in increased microbial respiration rates (Wallenstein et al., 2011; Menichetti et al., 2015). The accelerated losses of SOC and TN contents induced by soil warming were observed in our three experiments. So far, the temperature sensitivity of SOM decomposition has not yet well understood in rice paddy ecosystems since SOM is the mixture of various organic compounds with different biodegradability.

Elevated [CO_2] can increase soil C and N inputs derived from stimulated plant photosynthesis. On the other hand, higher photosynthesis caused by elevated [CO_2] can also lead to greater rhizodeposition as root biomass (Tokida et al., 2010). In rice paddy ecosystems, rice straw can be incorporated into as organic manure to maintain soil fertility, hereby also increase SOC and TN contents. In our T-FACE experiment, rice straw was directly moved out after rice harvest which could be the reason for the

lower SOC and TN contents in soil samples after 5–year T-FACE experiment, compared with those in initial soil samples in 2010, spring. Despite no reliable statistical analysis, 5-year elevated [CO_2] had a trend to increase SOC and TN contents in this study.

5.3. C decomposition and N mineralization affected by soil temperature, moisture and elevated [CO_2] in rice paddy ecosystem

Paddy soils are different to upland soils because they are regularly flooded and intermittently irrigated. Many studies have suggested that C decomposition and N mineralization in rice paddy ecosystems were significantly affected by soil warming and/or elevated [CO_2] from controlled-environment chamber, indoor incubation to open-field condition (Cheng et al., 2001; Inubushi et al., 2003; Cheng et al., 2006; Tokida et al., 2010; Pereira et al., 2013). For example, Cheng et al. (2001) reported that elevated [CO_2] significantly accelerated biological N fixation, N mineralization and C decomposition in submerged rice paddy soil. The reason could be explained by more labile C and N inputs and accelerated soil microbial activates under elevated [CO_2] condition.

Due to more rapid global warming, there is strong concern that the increasing atmospheric [CO_2] and rising temperature are further stimulating CH_4 emission from flooded paddy field, given that CH_4 has much higher GWP than CO_2 (IPCC, 2013). An early previous study conducted in controlled-environment chambers showed that elevated [CO_2] significantly increased CH_4 emission by 58% but had no effect on N_2O emission compared with ambient [CO_2]. Pre-harvest drainage suppressed CH_4 emission but did not cause much N_2O emission from the rice-plant plots at both [CO_2]

(Cheng et al., 2006). A study conducted in an intermittent flooded paddy field in Portugal also suggested that elevated temperature with or without elevated atmospheric [CO_2] increased CH_4 emissions by 50% but had no significant effect on N_2O emission relative to the open-field treatment (Pereira et al., 2013). Tokida et al. (2010) reported that nearly 80% increase of total seasonal CH_4 could be attributed to elevated [CO_2] and soil warming. The enhancement of CH_4 emission could not be fully explained by the increase of rice biomass owing to soil warming. After stoichiometric evaluation, they concluded that despite little response to soil warming, in situ Fe reduction under flooded condition also greatly contributed to substantial CH_4 emission. We speculate that elevated [CO_2] had greater impact on CH_4 emission than soil warming because of large new soil C input from rice biomass at maturity stage and after rice harvest.

Pre-incubation in the first and second experiments in our study was designed to simulate the rewetting process of dry paddy soil after intermediated drainage. Interestingly, the significantly stimulated effect of soil warming on C and N mineralization was not found in soil samples after pre-incubation, probably owing to rapid depletion of labile substrates and alteration of soil community and activities. This result implied that the responses of C and N mineralization to future global warming might depend on moisture condition in the field. Although we did not measure the real CH_4 emission and N mineralization in rice paddy field, the evidences from anaerobic incubation in our three experiments showed that soil warming could accelerate SOC and N decomposition (observed by total CO_2 and CH_4 and anaerobic NH_4^+-N productions) in rice paddy soil.

Finally, since our studies on C decomposition and N mineralization responses to climate change were mainly conducted by indoor incubation experiments, more

attention should be paid on the in situ multi-location observations of C decomposition (e.g., CH_4 production, oxidation and emission) and N transformation (e.g., immobilization, nitrification and denitrification) responses in rice paddy ecosystem to future climate change at long-term scales.

5.4. Conclusions

From the sequence of studies, the responses of C and N contents and their mineralization to soil moisture, elevated soil temperature and elevated [CO_2] could be simply concluded as below.

1) Soil moisture (water regime) can affect SOC and TN contents mainly by regulating microbial community, enzyme activities, component availabilities and mineralization products.
2) Elevated soil temperature can significantly decrease SOC and TN contents in rice paddy ecosystems due to the accelerated their mineralization obtained from indoor incubation and in situ observation.
3) Elevated atmospheric [CO_2] would increase SOC and TN contents in rice paddy ecosystems due to stimulated photosynthesis, and it can also stimulate their mineralization due to increased labile substrates for microbial utilization. Overall, the increases of SOC and TN contents cause by elevated atmospheric [CO_2] may be greater than enhanced mineralization resulting in more C storage in ecosystems from the atmosphere.

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