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Contents

Preface to Symposium Papers on “Utilization of Resources and Environmental Protection”, 11th International Symposium of Integrated Field Science

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We, Field Science Center of Tohoku University, hold the international symposium for “Integrated Field Science” (IS-IFS) every year. This year’s symposium was held on August 1-2, 2013, jointly with the 12th Symposium on Animal Production Environmental Science Japan (S-APESJ) at Hotel Matsushima Taikanso. It was supported by the Project of Integrated Compost Science (PICS), the Innovative Research Center for Agricultural Science, the Graduate School of Agricultural Science and Miyagi Prefectural Government.

Among these projects, PICS, the Project of Integrated Compost Science, is the main organizer for this symposium. This project started in 2004. 16 laboratories in Tohoku University and 6 research institutes of Miyagi Prefectural Government worked on the project under the agreement of research collaboration between Tohoku University and Miyagi Prefecture. We have collaborated for the development of new composting systems such as Acidoro-compost, which is produced under acidic condition without emission of ammonia. We have also investigated the effects of the compost on plants, microbial changes in composting processes, functional microbes, life cycle assessments of the processes, and the remote sensing of the field application of composts.

In the symposium, we presented our achievements in almost 10 years, and exchanged opinions with participants , especially with distinguished invited researchers, Professor Hsia from Taiwan, Professor Lee from Korea and Professor Insam from Austria. Representative presentations are included in this issue of Journal of Integrated Field Science. Abstracts of all papers including poster presentations are presented in this collection as well.

I hope that various scientific works in this issue will contribute to advancing our research and developing technology for the utilization of resources to protect our environment.

Research and Development in Composting and Animal Waste Management in Taiwan

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Abstract

Animal waste was a resource for thousand years in Chinese society. Animal waste was made for compost and used as fertilizer. It was also used as feeds, directly or indirectly, for aquaculture animals. Unfortunately, the animal waste management in our country has not progressed as we expected because the size of animal farms is expanding. The result of this change caused animal waste became a contamination source, this included solid and liquid waste, air waste (odor) and greenhouse gases. In recent years, these wastes became resources again. The problem at present is how to use all these resources properly in an economical way. The solution depends on technical approaches but also requires government policy and consideration form the public. Waste management will include: management, nutrition, house, pig toilet, scraper under slatted floor, breeding, separator, anaerobic treatment, aerobic treatment, sludge treatment, deodorization, compost production, and greenhouse gases.

Introduction

Animal waste was a resource for thousand years in Chinese society. Animal waste was made for compost and used as fertilizer. It was also used as feeds, directly or indirectly, for aquaculture animals. Unfortunately, animal waste management in our country has not progressed as we expected because the size of animal farms is expanding. The result of this change caused animal waste became a contamination source, this included solid and liquid waste, air waste (odor) and greenhouse gases. In recent years, these wastes became resources again. The problem at present is how to use all these resources properly in an economical way. The solution not only depends on technical approaches but also requires government policy and consideration form the public.

There are two types of studies on waste management in Taiwan, as the whole world's research. Type 1 research depends on farms' need, whether it is a long or short term. Another type of research depends on researchers' personal view without field experience. This report will focus on Type 1 research. The whole idea will be based on Figure 1 which is a flow chart of animal waste management. The flow chart was drawn in 1997 and some modifications have been made.

Management

Good management on animal feeding resulted in less feed wastage spilled from the trough. Manure usually accounts for 35-25% of N and energy of feed due to digestibility efficiency. This is because the digestion is about 65-75%. Phase feeding is another good management on reducing feed and nutrient wastage in waste.

Nutrition

Ideal protein or amino acids- balanced feed can reduce N waste in manure, and also improve pig performance.

Balanced feed for animals can reduce feed wastage, e.g. Ca, P, and other minerals.

The reduction of common salt in feed is another way to reduce the salt in soil. Our result showed that 0.25% of salt for nursery pigs is sufficient to improve piglet performance.

Although the higher inclusion rate of Cu and Zn in pig diet can reduce diarrhea and improve piglet performance, it can cause metal contamination in soil, especially the accumulation of Cu and Zn in soil. Our government has a strict rule on the usage of Cu and Zn in pig diet.

Including phytase in pigs and poultry diet became a quite common operation in Taiwan.

It is important to use Ca and other micro minerals in animal diet properly. The procedure aims not only to reduce contamination but also to improve pig performance. Overdose of micro mineral also causes contamination of land.

House

Proper animal house design can reduce animal waste problems (solid, liquid and odor), reduce bacteria and improve animal welfare in the animal house, e.g. wet pad and forced ventilation system for animals.

Another important issue in designing an animal house is to separate rain water from waste water. This separation can not only reduce the quantity of waste water but also avoid the damage of anaerobic waste water treatment. The rain water has lower oxygen content. If all raining water pours in the anaerobic treatment system, anaerobic bacteria in the system will be killed. Since anaerobic bacteria need time to be built up, the whole waste treatment system will be wrecked by the rain water. Deep bedding system is appropriate for small farms, however, not practical for big pig farms Moreover, this system causes difficulty in field management because it cannot easily control diseases widely spread in the farms.

Pig toilet

The topic has become a very big issue in Taiwan. EPA Taiwan even believes this is the best way to solve waste management problems. It is true that pigs will urinate or defecate in a specific place when the group size in a pen is small. However, it creates problems when the group size is big and density of pigs in the pen becomes higher. The problem is that pigs will not follow the rule of toilet location when the group size becomes big and its density becomes higher.

Scraper under Slatted Floor

The scraper system should be accompanied with two systems. Firstly, the scraper system should separate solid from liquid; otherwise the waste will be too wet and cannot be handled easily. Secondly, if the farm uses the system, they should remove solid as soon as possible. They also should have a good ventilation system; otherwise the odor becomes quite heavy inside the animal house.

Breeding

The better the animal performance is, the more waste problems will be solved. Many politicians said we should reduce the total number of animals. On the basic calculation, domestic animal farms have reduced total waste over the last 40 years, but are still able to keep even more animals for human consumption.

Feed efficiency is 4.5 for G-F pigs.

Today, feed efficiency is 3.0, even less for G-F pigs.

In consequence, there is a 1.5 kg feed difference per kg body weight.

1.5 kg x (120 kg – 20 kg) = 150 kg feeds

Taiwan has 8,000,000 pigs / year; the total feed save per year is

150 kg x 8,000,000 = 1,200,000,000 kg feeds

1,200,000,000 kg feeds / (3.0 x 100 kg) = 4,000,000 pigs

This result showed that the whole Taiwan could raise 4,000,000 pigs or more.

Separator

Some time ago, there were five types of separators for animal waste treatment developed in Taiwan: round vibrated separator, square vibrated separator, stationary separator, water wheel separator, and centrifuge sedimentation. The stationary separator is attached with several accessories such as a screw dryer, a vacuum dryer, and cleaning filter components. At present, there are only two types: stationary separator and water wheel separator. This does not mean that other separators are not good. This is simply because the above two types sell well. We did several tests and found that there was a big difference in separation efficiency among separetors.

Below are several big arguments on separators.

Argument 1: If farmers use a separator, there is very little solid left for producing methane gas in anaerobic digester. The conclusion for this argument is farmers' demand. The farmers want more methane or they want more efficiency on waste treatment. If they want more methane, they also need to know how to deal with sludge. Since farmers need more solid to make compost, then they use separators, however they also need small quantity of less-odor compost for making plants.

Argument 2: The solid produced from a separator has less nitrogen. However, nitrogen in the separator product will also reduce during composting, and the final N content does not have a very big difference.

Argument 3: Do we really need a dryer for a separator? The first issue in this argument is where we put separator products, inside or outside the house. The second issue is how many separator products will be produced each day. If a farm only produces a limited

amount of products, they will have lower water content 3-5 days later.

Argument 4: Puting separator products on a special screen and letting water drop into the underneath tank in which waste water flows, accumulates, and is waiting for separation.

Argument 5: Whether or not the solid part of waste water accumulated in the bottom of the tank with a slatted floor can be separated by the separator. It seems that this kind of water is not easily separated by machine. It becomes small sticky particles within 3-5 days.

Anaerobic Treatment

Taiwan pig farmers, researchers and politicians are lucky because we promote anaerobic digesters to produce methane gas for family use. This project started in 1956 (JCRR, 1956). There were 4135 farms using this system from 1965 to 1970. During the period, farmers used an iron cover and later used a plastic cover to produce methane. In 1974, the first red mud plastic (RMP) bag in the world was developed by three researchers in Taiwan. Since then, there has been a rapid development in anaerobic digester and methane gas production.

The methane gas used for cooking, piglet heaters, cars, water pumps, and electricity became successful around 1975 (Hong, 1999). There were 1456 farms with piglet heaters from methane gas production. There were 717 farms using methane gas to produce electricity from 1990 to 1995 (Hong, 1999). The group also developed two major methods to reduce H2S and NH3 from methane gas. All these researches did not start using methane gas only for waste management. Based on this method, waste treatment for livestock was more actively promoted. This is why farmers are lucky. All these major development and researches were carried out before the law was enforced (1998).

There are also a lot of research in progress now; for example, how to use waste water from anaerobic treatment to cultivate algae.

Our group concentrated on how to improve the efficiency of anaerobic treatment. The first step was to use an economic biological membrane to improve the performance. The second step was to use a two-stage treatment to improve the anaerobic treatment.

Aerobic treatment

It is quite difficult to transform anaerobic treatment to aerobic treatment. There are two reasons. Firstly, we can use anaerobic treatment because we should transfer it to aerobic treatment. Secondly, aerobic treatment needs electricity. From today's point of view, some of these arguments are still true, and anaerobic treatment needs a big amount of investigation cost. However, if waste treatment needs to achieve government standard "consistency", farms need to use aerobic treatment. Usually, anaerobic and aerobic treatments use waste in high and low density, respectively. If we adopt the combination of the above separators, anaerobic and aerobic treatments may be still good methods.

In recent years, our research on aerobic treatment is focused on the following points: 1. how to improve the efficiency of aerobic treatment by a biological membrane, 2. how to reduce electricity cost by interval aeration. The result showed a significant reduction of N in waste water. This result requires more studies in the future because the issue of the issue of whether or not N becomes N_2 or N_2O needs further clarification.

Which kind of aeration can reach the maximum performance was tested by Hong's group many years ago. These methods include oxidation ditch, air jet surface aeration, sink air jet, air bubble. It seems that all methods can achieve some results, but they are not practical in animal waste treatment. We tried air pump in Pig Research Institute. Our results showed that the oxygen dissolving rate was not the best one, but the system was easy to maintain and inexpensive. The system also has another advantage; the system can stir waste water evenly and does not cause sediment.

Another crucial finding is that batch system is also better than continuous flow system. This is because the continuous flow system always causes a mixture of dirty and clean water.

Sludge

Aerobic and anaerobic waste water treatments always produce sludge. The sludge cannot be easily pumped out by water pump. On the other hand, it also needs sludge for waste treatment, so the best way is to pump 5 to 10 minutes of sludge and send it to sludge drying bed. The sludge used by Hong's group is too big. Our drying bed is divided into 7 layers.

Everyday a little sludge is pumped into each layer, and 7 days later they will become dry by evaporating water from the plastic cloth in natural and ventilation system.

Good dry sludge is dead bacteria. It is very hard itself, but will become soft when it is added with water.

Deodorization

Odor always offends your neighbor. There was an interesting method developed about 18 years ago in Taiwan. We measured and tested the method, and found that it was useful and efficient but needed to meet a few requirements. An ordinary black net is set 5-6 meters away from fans and it is about 1.5 to 2 meters higher than the top of the exhaust fan. The black net needs support on the back. The width of the net should be about 2 meters away from margins of the last fan on each side. The division on the back of black net is about 1 meter. The width should be about the same as the black net. If possible, the top of the net should have a slope toward fans. Both sides of the black net about 1 meter on each side should have a slope towards the house. The net is 5-6 meters away from the fans. If the distance between the fan and the net is too short, it may influence on the fan's speed. However, if the black net is too far away from the fan, the dust cannot be caught by the black net. The basic principle of this black net is that it can collect dust. According to the literature, it is proven that the odor and bacteria usually adhere to the dust. There is no need to clean the net unless the area has no rain.

If you do not use forced ventilation system to remove odor (tunnel ventilation system), you need to clean manure frequently and provide some feed additives for the animals, but problems still occur.

Compost

There are more than 186 compost plants in Taiwan in 1997. Most of them have an automatic control system. One organization will evaluate them once a year. Every year they will select A^+ and A class plants and present them certificates. The primary research on compost was centered from 1955 to 1965. During this period, Taiwan Sugar Company use pig manure as fertilizer for sugarcane. The second peak to make compost started in 1991. The government was willing to solve solid animal waste and other agriculture waste problems (e.g. saw dust from mushroom production, bagasse, rice hull and etc.). They encouraged people to invest in compost plants. At this stage, the most research was concentrated on the following points: 1. How to produce good quality of compost, 2. How to reduce odor, 3. How to evaluate the mature of compost, 4. How to sell compost to farms at the end of spring and fall, 5. How to make specific compost for special plants.

Over the last 10 years, compost plants were under two kinds of pressure: 1. How to produce nutrient-balanced compost, 2. How to produce compost which can inhibit bacteria growth in soil. The technology of organic farming causes some misunderstandings. It is important to use compost as fertilizer. Compost can improve the physical property of the soil. This is the primary contribution of compost to soil. It is also important to have balanced nutrients for plants. Today, Taiwan has several fertilizer additives which are not chemical bactericide and controlled by biological methods. There is a technology developed in Taiwan to kill spore and bacteria. We expect it will succeed.

Greenhouse Gases

Many researchers are working on the production of greenhouse gases. We also try several methods to reduce greenhouse gas production when we feed animals. We also try to reduce CH_4 and CO_2 from manure. Methane can be used for producing electricity and some other energy sources. After burning, they also produce CO_2. Our research may be the first to use CO_2 to cultivate algae and vegetables. It seems to have good results.

Conclusion

Good animal waste treatment and compost production can be conducted using different methods. These achievements also need government support and public understanding.

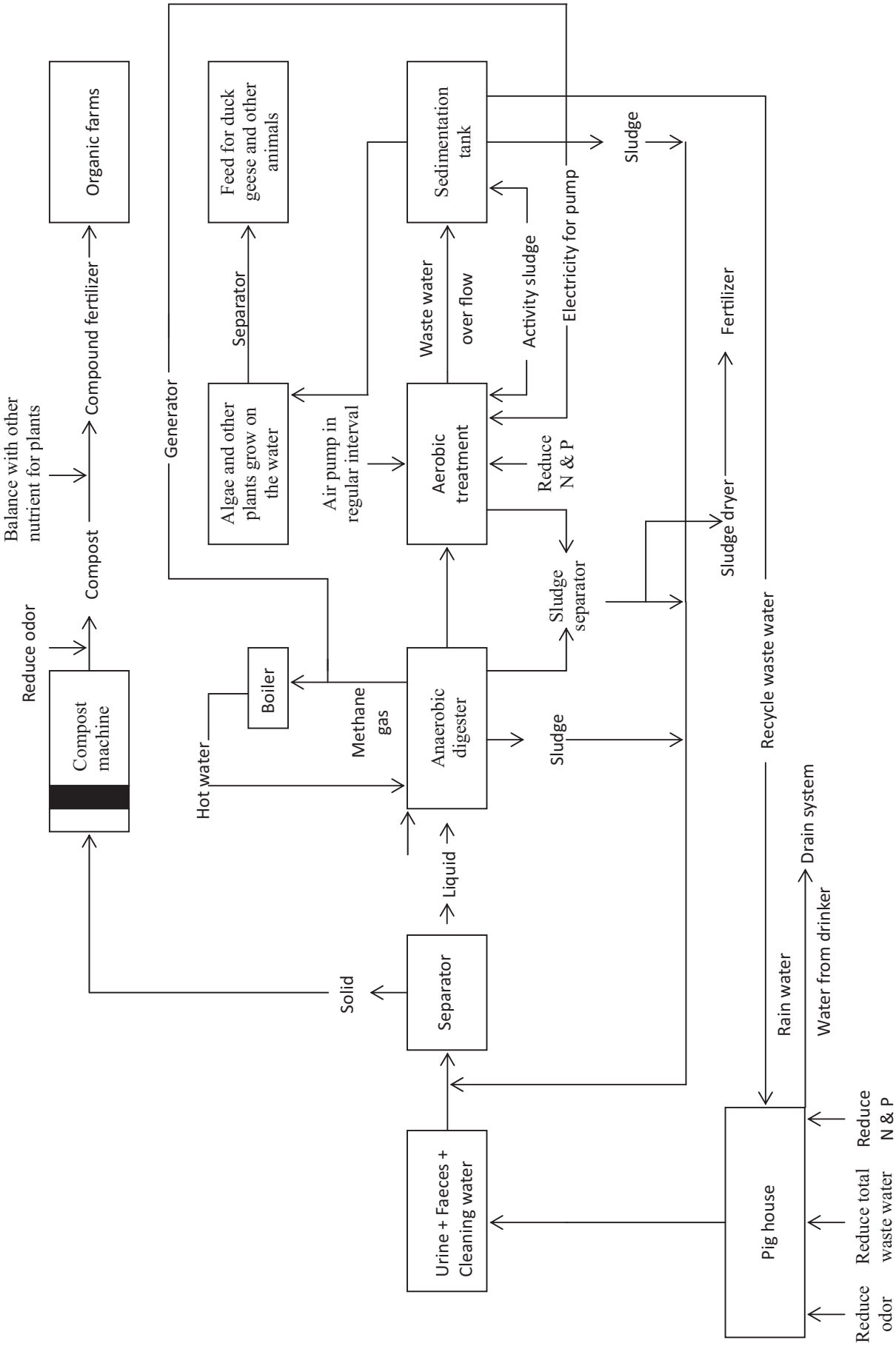

Figure 1. Waste Management Flow Chart (Hsia, 1997)

Research and Development in Composting and Animal Waste Management Including Wastewater Treatment, Renewable Energy and so on in Korea

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Keywords:sustainable livestock industry, livestock manure, quality of liquid fertilizer, LFQC(Liquid Fertilizer Quality Certification), MPAD (Multi Process of Aerobic Digestion)

Abstract

As a main structure of livestock farming in Korea has been shifted from small-scale diversified farming in the past to large-scale and full-time farming. Substantiality livestock farming has become the biggest issue in livestock industry these days. The total output of livestock waste in 2012 was 46.489 million ton. Among them, recycling (including treatment of composting and liquefying) rised to 88% and purification occupied 9%. The percentage of liquefaction has gradually increased since 2006, along with the practice of policy in reducing ocean disposal. However, based on field investigation of using manure as liquid fertilization in Korea, the quality of the fertilizer was not homogeneously managed. To encourage the use of liquid fertilizer, it is necessary to develop a system of Liquid Fertilizer Quality Certification (LFQC) that is appropriate for Korean circumstance. Technology for manufacturing liquid fertilizer consistent with the LFQC should be developed as well. So, this presentation introduces the six-step manufacturing procedure of liquid fertilizer, and a plan for LFQC, which can be applied to individual farms and Multi Process of Aerobic Digestion (MPAD), and a process of resource recovery by concentrating or solidifying a variety of high concentrate.

Introduction

1. Korean livestock industry and subsequent environmental problems

In order to establish environment-friendly livestock farming, Korean government and non-governmental research institutes have put a great deal of efforts on feed manufacture, management of livestock diseases, food safety management and recycling manure. Various kinds of manure treatment have been suggested. First, for cost efficiency, treatment using saw dust, operating inexpensive machine and reducing cost on management are considered. Second, in terms of environment, odor and purification treatment are required as a type of manure treatment. Third, prevention of disease occurence is also discussed related to hygiene. From the perspective of utilizing resource, recycling by composting and liquefaction, land application and treatment of swine slurry are discussed. Finally, in terms of public service, launching public resource management, professional consultant training and integrated treatment of liquefaction and purification are required as ideal alternatives for manure treatment.

This means that integrated technique and system that can solve the problems with limited agricultural land and time constraints in applying agricultural fertilizer in each region are necessary for livestock farms.

2. Problems of different manure treatment

The number of cattle and swine farm households in Korea was estimated at 186,105 in 2010. Additionally, in terms of the status in the different types of animal waste treatment, recycling and purification accounted for 88% and 9% respectively out of 46.489 million ton (total) in 2012, which showed that the most livestock waste was treated by recycling. The increase in the percentage of converting manure to liquid fertilizer since 2006 shows that of ocean disposal has been decreasing and simultaneously, the importance of manure liquefaction has been paying attention to.

Korean government has been promoting manure treatment as a public resource recovery treatment. Through a national government project, e.g., Live-

Table 1. The percentage of maturity degree of liquid fertilizer in LMPRCs and LFSCs.

facility of manufacturing liquid fertilizer	matured		semi-matured		immatured	
	number	percentage	number	percentage	number	percentage
LMPRCs[1]	18	49%	17	46%	2	5%
LFSCs[2]	37	33%	53	47%	23	20%

[1]Livestock Manure Public Resource Center
[2]Livestock Liquid Fertilizer Supply Center

stock Liquid Fertilizer Supply Center (LFSC), storage tanks for liquid fertilizer and Livestock Manure Public Resource Center(LMPRC) are being constructed now.

3. Case study on the quality of fertilizer liquefied in Korea

It examined the quality of Liquid fertilizer in 2012, especially fertilizer using swine manure collected mainly from LMPRCs and LFSCs. The results showed that concentration of the fertilizer manufactured from each center had significant discrepancy, which required a consistent manufacturing process of liquid fertilizer to be shared.

Study1. The percentage of maturity degree in LMPRCs and LFSCs

Specimens: 150 specimens of liquid fertilizer collected from LMPRCs and LFSCs

Results: 49% maturity in LMPRCs and 33% in LFSCs

Study2. Physiochemical comparison between the properties of liquid fertilizer from LMPRCs and LFSCs

In order to know the degree of uniformity of liquid fertilizer in LMPRCs and LFSCs, pH, EC, ORP, T-N, T-P, NH_4-N, NO_3-N, $SCOD_{Mn}$ and SS were surveyed. Each means showed significant deviation between each data and confirmed that the degree of uniformity was significantly low.

4. Necessity of mid and long-term plans of livestock manure and launching Liquid Fertilizer Quality Certification (LFQC)

In May 2013, Korean Ministry of Agriculture, Food and Rural Affairs announced a plan of manure management for the next 5 years. Strong administrative supports from the government for comprehensive manure management and schedule for implementation of related projects are listed below.

< Mid and long-term plan for manure management >

◦ Objectives: construction of sustainable environment-friendly livestock industry

◦ Four main projects

1. Expand manure treatment facility:
2. Establish manufacturing system for high-quality compost and liquid fertilizer:
3. Reinforcement of follow-up management:
4. System improvement, R&D expansion and etc:

The main projects include: recycling manure, high-quality process of composting and liquefaction, construction of more LMPRCs, transition to plant farming. In order to do this, following details are required; 1) manufacturing technology of composting and liquefying manure, 2) liquid fertilizer quality certification(LFQC), 3) building marketability of compost and liquid fertilizer, 4) consistency in administrative procedure and policy, 5) integrated local management, manpower development.

5. Necessity of Liquid Fertilizer Quality Certification (LFQC)

It is essential to establish a standard for quality assurance of liquid fertilizer to prevent complaints and promote its use. There is no exact standard for the products and grades in “Regulation of liquid fertilizer using livestock waste” under the fertilizer management law of Korea. In order to promote the use of liquid fertilizer, primary Main Level-Grading Factors(MLGFs) and Evaluation Standards(ESs) of Liquid Fertilizer Quality Certification (LFQC) are needed.

Liquid Fertilizer Quality Certification (LFQC), under research at present (2013), is a system where liquid fertilizer using manure is classified by four Main Level-Grading Factors (MLGFs: fertilizer efficiency,

environmental risks, stability and uniformity). The classified liquid fertilizer is scored according to the Evaluation Standards(ESs) in detail and graded into A, B and C so that the grading system can allow to develop high-quality products based on Grade A and enlarge marketability of commercial liquid fertilizer in the future.

Based on the system, it is required to develop a new strategic product in livestock environment industry by reviewing and gathering a wide variety of opinions with various experts.

6. The cases of research on liquefying technology in Korea

6.1 Introduction of six-step method for manufacturing liquid fertilizer

Most of Korean Swine farms do not fully equip with liquefaction technology so that a standardized process of liquid fertilizer is needed. Table 2 suggests a model of liquid fertilizer process necessary for Korean swine farms on the spots.

6.2 Introduction of MPAD

For fast transportation of liquid fertilizer from region to region, concentration method using membrane separation technology has been recently studied. Development of environment-friendly and future-oriented resource recovery techniques, which allows management of pathogenic microbe and manufacture of functional liquid fertilizer, is needed. MPAD (Multi Process of Aerobic Digestion)system introduced in this study is comprised of three different types of process; Thermophilic Aerobic Oxidation (TAO) system, lime solidification system, and membrane system with MF and Reverse Osmosis(R/O). Characteristics of each process are simply accounted for below.

1) TAO (Thermophilic Aerobic Oxidation) System
   The wastes in a manure tank were transferred to the biological process, the TAO (thermophilic aerobic oxidation) system by using a pump. Strong mixing and aeration were performed at the same time so self-heat (auto-thermophilic, 55ºC) reaction was induced by thermophilic bacteria.
2) Lime solidification system
   Floating particles in the livestock wastes after treatment of TAO system could be solidified. It is composed of a condensed sample control part, 1st - 2nd condensation part and a filter press. In the control part, to process thermophilic aerobic fermented liquor, lime was added to increase pH up to 9~12. The solidification of the liquid was maximized after adding Iron chloride, polymer coagulant. Then, it was filtered by the filter press to make cake.
3) Membrane system with MF and Reverse Osmosis
   Reverse osmosis is a selective penetration membrane system to remove dissolved solids like metal ion. It is composed of a pre-pump and a high pressure pump for osmotic pressure leading to MBR system with MF membrane.

Table 2. Features and target standards in each step of standardized six-step liquefying process.

6 steps		remarks	
1	step of swine slurry management	-inflow BOD: 30,000 ppm -Oxidation-Reduction Potential (ORP): less than -300mV	-outflow BOD: around 20,000 ppm
2	step of solid-liquid separation	-BOD after solid-liquid separation: 10,000 ppm	
3	1st step of fermentation	-inflow BOD: 10,000 ppm -processing period : 3- 20 days -ORP: maintain -50mV -volatile fatty acid: less than 300 ppm	-outflow BOD: below 5,000 ppm
4	step of returning fermented liquid fertilizer	-return 20 to 50% of fermented liquid fertilizer to swine slurry process	
5	2nd step of fermentation	-inflow BOD: less than 5,000 ppm -processing period: 30 days -air supply: 30 - 60 L/ m^3.min -ORP: maintain below -150 ~ -50mV -nitrogen concentration: maintain 2,500 ppm	-outflow BOD: below 1,000 ppm
6	step of applying to farmland	-operate program of applying liquid fertilizer in a year	

Table 3. Bio-chemical concentration in each step of MPAD system

Item	BOD_5 (mg/L)	CODMn (mg/L)	SS (mg/L)	T-N (mg/L)	T-P (mg/L)	E. coli (CFU/㎖)
Influent	34,540	25,877	40,927	4,864	1,082	47,837
TAO Fermentation liquid fertilizer	8,286	10,036	38,117	3,846	964	N.D*
1st Lime solidification	3,729	5,019	1,042	3,113	29	N.D*
2nd Lime solidification	1,119	2,510	150	2,959	7	N.D*
MF, R/O concentration liquid fertilizer	4,121	5,268	255	5,261	16	N.D*
R/O discharged water	15	25	3	43	0	N.D*
Efficiency(%)	99.9	99.9	99.9	99.1	99.9	100

*N.D : Not Detected

Table 3. shows the changes in bio-chemical concentration during each step of MPAD through which swine manure was treated. This pilot-scale process is operated on-farm with a level of a laboratory.

Conclusion

The main purpose of the Korean policy for livestock manure management is to promote utilizing manure as a high quality resource by composting and liquefying. This enables to increase the value of livestock manure in hygienic, economic and functional ways. Main policy of recycling manure is summarized into four below.

① Develop manure management as a new key industry by using the properties of manure
② Create high added value reflecting plant farms' point of view
③ Build integrated and cooperative technology system for high-quality resource recovery of manure
④ In order to optimize manure recovery management, economic, social and technological value and improvement of system should be considered together and the related technology should be developed as "an axis of growth in sustainable livestock farming."

Additionally, focus should be put on the development of social co-operatives and cooperative associations as a new business that can possibly deal with rural slumism resulted from aging, polarization and the elderly poverty.

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Agricultural Waste Management in Europe, with an Emphasis on Anaerobic Digestion

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Abstract

Traditionally, animal residues such as solid and liquid manures of cattle, pigs and chickens, have been used for fertilization purposes. However, with the onset of the industrialization of agriculture, animal production has become more and more centralized, and there is too little land for the application of such wastes within a short distance of the sources. Therefore, treatment technologies like the separation of the liquid and solid fractions of manures, their drying and composting have become popular.

Most recently, in response to climate and fuel crises, anaerobic digestion has been advocated and supported by political measures in many European countries. In 2010, the European Union made the commitment to reduce greenhouse gas emissions by 20% before 2020. Amongst other approaches, biomethane production from agricultural wastes has been proposed. In Germany alone, more than 7000 biogas plants are now in operation, and other countries in Europe are starting to follow this trend. Engineers and foremost microbiologists are constantly challenging to improve the technology in this field. Recent advances in microbiological and molecular techniques have made it possible to determine which microorganisms are present in the anaerobic digester environment, how active the microbes are, and how the microbial community as a whole responds to changes in substrate input and process conditions.

This paper gives a few examples of biomethanisation studies that have been carried out at the Institute of Microbiology in Innsbruck. There and elsewhere, Microbial Resource Management, a new attractive field for microbiologists, should help to mitigate climate change and to close biogeochemical cycles for a more sustainable future society.

Introduction

In the last few years, environmental policy in Europe has been reacting to various drivers in the energy sector. First of all, the notion was that non-renewable energy sources like natural gas and crude oil are limited, and that the world would soon be facing Peak oil. This upcoming shortfall in resources resulted in the soaring oil prices. Furthermore, political troubles showed that the dependency on the Middle East and Russia for oil and gas, respectively, was rising. Nuclear energy, which has been treated as a feasible alternative, is unpopular in many countries, and has become even more so after the Fukushima disaster. Renewable energies have thus been demanded by the people, and politics has responded adequately by heavily subsidizing research and application of energy sources including photovoltaics, wind, and biomass. Figure 1 summarizes the current situation for various European countries.

However, all these technologies have not remained undisputed. This mini-review will cover the bioenergy sector, which is currently being heavily debated by various sides. Wood is being increasingly used for heat production in many areas of Europe (Kuba et al., 2008). Energy crops like maize, rapeseed or sugar beet have long been in focus for the production of biodiesel or bioethanol. However, the ethical debate is very controversial, in particular concerning the increasing competition between the food and energy sector for valuable cropland (Leopoldina, 2012).

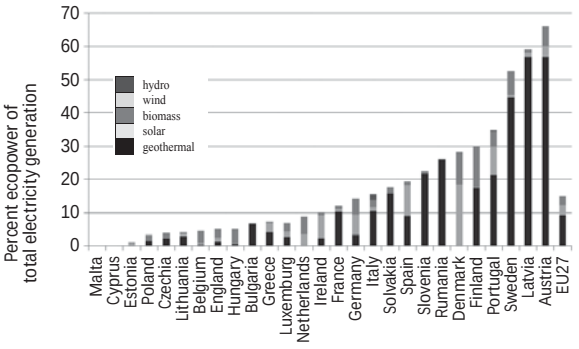

Figure 1. Percentage of ecopower technologies of the total electricity production in Europe.

Table 1. Selected sources of wastes as substrates for biogas production.
[1]http://www.biogas-renewable-energy.info; [2]Goberna et al., 2010;
[3]http://webapp.uibk.ac.at/biogas/results

Sector	Type of waste (fresh matter)	Methane potential [m^3 t^{-1}]
Agriculture	Liquid cattle manure	20[1]
	Potato pulps	50[1]
	Brewery waste	75[1]
	Lawn cuttings	125[1]
	Corn residues	150[1]
	Olive mill residues (6%VOC)	200[2]
Industry	Bread residues, cereal waste	300[1]
	Slaughterhouse wastes	180[1]
	Molasses	230[1]
Municipal	Biowaste (source-separated) (40% dm)	170[3]
	Used grease (95% dm)	800[3]

During the last few years, energy and greenhouse gas (GHG) balance analyses have shown that the savings achieved using energy crops compared to fossil fuels were disappointing, and some studies have even shown a negative greenhouse gas balance of biomass-based fuels. Biogas production from energy crops - it should rather be termed agrogas - became more and more popular for various reasons: huge subsidies of feed-in tariffs for electricity, and a better GHG balance than for biodiesel and bioethanol. However, recent life cycle analyses have shown that agrogas production is not at all as positive as had been hoped for. Thus, the only bioenergy option remaining is biogas production from wastes, be they of agricultural or domestic origin (Leopoldina, 2012).

Sources of wastes and their biogas potential

Biogas, or biomethane, may be produced from many sources of waste. In Table 1, a snapshot of possible sources is given.

Biogas plants

It is beyond this review to give a detailed overview of the many types of biogas plants that are in operation world-wide. While in several Asian countries like Thailand, China or Vietnam, small scale biogas plants run at the family level have successfully been used for years, Europe has focused on more centralized plants with capacities in the Megawatt range. Small scale plants have always been regarded as not financially viable. However, recent political measures, for example in Germany, are advocating such small scale solutions. Up to 28 Euro-cents are offered as a feed-in tariff to small scale biogas plant operators that only use animal wastes. Insam and Wett (2008) have shown how small-scale biogas plants could contribute to a reduction of greenhouse gas emissions. A program in the German state of Bavaria (approx. 10 million inhabitants) aims at constructing 6000 small scale farm-based biogas plants that will have the capacity to replace 2 nuclear power plants within the next few years.

Research

Until a few years ago research and development on biogas production was mainly driven by engineers. Control of biogas plants is primarily accomplished by quantifying the methane production. In the past, the microbiology of biogas plants was not considered important for process optimization although the process is largely driven by microorganisms (Figure 2). However, with the availability of novel methodological approaches based on molecular biology, this has changed during the past five years.

One example is syntrophic acetate oxidation (SAO) by *Clostridium* sp., *Syntrophaceticus schinkii*, *Tepidanaerobacter acetatoxydans* (Westerholm et al., 2010, 2011) and other bacteria followed by the subsequent conversion of H_2 to methane by hydrogenotrophic methanogens (Fig. 2, bottom). This pathway is reportedly favored by high temperatures (55ºC) (Zinder and Koch, 1984) and by low H_2 partial pressure (2.6 - 74 Pa, according to Hattori, 2008). Foremost, the presence of high concentrations of inhibiting ammonia, acetate or salinity (Sasaki et al., 2011; Schnurer and Nordberg, 2008; Westerholm et

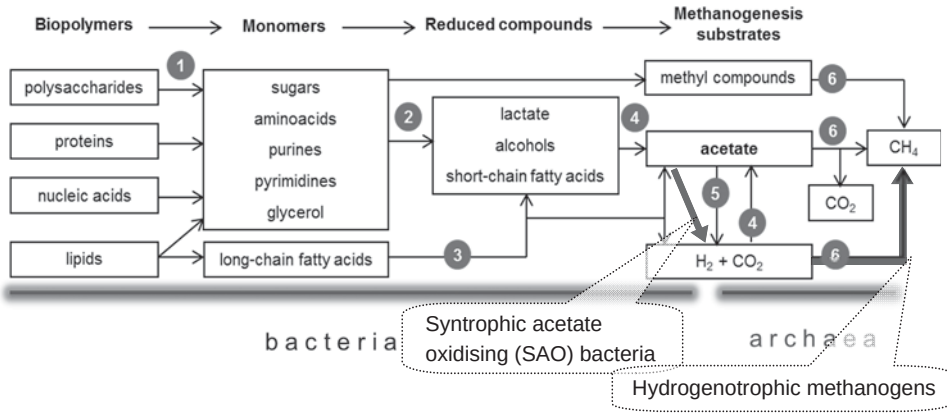

Figure 2. From complex compounds to methane (Insam et al., 2010, modified from Sousa, 2006); broad arrows: syntrophic acetate oxidation and subsequent hydrogenotrophic methanogenesis.

al., 2011) favors the SAO pathway. Methanogens of the genus *Methanosarcina* are able to rapidly adapt to increasing inhibitor concentrations since they are able to shift from the acetotrophic to the hydrogenotrophic pathway (Wang et al. 2011). A comparison of tolerance levels between *Methanosaeta* and *Methanosarcina* is given in Table 2, supporting the hypothesis that *Methanosarcina* is the workhorse in biomethanisation (Willy Verstraete, pers. comm.), a view that has been corroborated by Walter et al. (2012). Such knowledge not only confirms practical recommendations that are based on engineering experience (Laaber et al., 2007; Table 3), but this new understanding paves the way for process improvements.

Methods to analyse microbial communities

Classical isolation and cultivation techniques are laborious for anaerobic microorganisms, and particularly for archaea. The main reason is the long generation time and the specific requirements concerning oxygen partial pressure. For this reason, molecular tools have become popular for investigating microbial community dynamics in anaerobic digester environments. Such molecular tools include PCR-DGGE with follow-up sequencing, phylogenetic and functional microarrays (e.g., ANAEROCHIP and BACCHIP; Franke-Whittle 2009) and metagenome sequencing (e.g., Wirth et al. 2012). From such knowledge on the involved microbiota, benchmarks

Table 2. Characteristics of *Methanosaeta* and *Methanosarcina* (modified after De Vrieze et al., 2012, Fotidis et al. 2013).

Parameter	*Methanosaeta*	*Methanosarcina*
μ_{max} (d^{-1})	0.20	0.60
K_s (mg acetate/L)	10 - 50	200 - 280
Temp. range (ºC)	7 - 65	1 - 70
Acetate (mg/L)	< 3 000	< 15 000
NH_4^+ (mg/L)	< 3 000	< 7 000
Na^+ (mg/L)	< 10 000	< 18 000
pH-range	6.5 - 8.5	5 - 8

Table 3. Evaluation scheme for biogas plants used in Austria (Laaber et al., 2007, abbr.).

Parameter	Range of values		
	Green (safe)	yellow (caution)	red (avoid)
pH	7.5-8.1	7.1-7.5	<7.1; >8.2
Total volatile fatty acids	< 1500	1500-4500	>4500
Acetic acid	<1000	1000-3000	>3000
Propionic acid	<250	250-1000	>1000
Butyric acid	<50	50-100	>100
Valerianic acid	<50	50-100	>100
NH_4-N (mg/L)	<5000	>5000	-
TS	4-8	<4; 8-9	>9

Table 4. Microbiological evaluation scheme for biomethanisation processes (De Vrieze et al. 2012).

Advanced evaluation scheme	Benchmark
% Total SAO FTHFS genes / % Total bacteria 16S rRNA genes	≥ 10
% Total methanogens mcrA genes / % Total bacteria 16S rRNA genes	≥ 10
% Methanosaeta16S rRNA genes	Normal ≥ 10
% Methanosarcina 16S rRNA genes	Heavy duty ≥ 1

FTHFS = Formyltetrahydrofolate synthase; Methyl-coenzyme reductase

related to community composition and involved enzymes were established (De Vrieze et al. 2012; Table 4). These approaches need to be elaborated further in the future.

Outlook

Biomethane production from wastes is one of many options for the microbiologist to make use of an otherwise wasted resource. In Europe, and elsewhere, various approaches or combinations of approaches are being elaborated and tested, a few of them are named below:

- Methanogenic degradation of polycyclic aromatic hydrocarbons and other xenobiotics (Mogensen et al., 2003)
- Hydrogen producing bacteria (Bagi et al., 2007)
- *Syntrophomonas zehnderi* for oleate degradation (Palatsi et al., 2012)
- Long chain fatty acid degradation (Cirne et al., 2006)
- Genetically modified *Methanosarcina* (Lessner et al., 2010)
- Constructed ligno-cellulolytic cultures (Methanos®)
- Anaerobic cellulolytic fungi (Leis, 2013)
- Lactic acid production from biowaste (Probst et al., 2013)
- Bio-electrical systems (Logan et al., 2006)

Anaerobic digestion is currently driven by subsidies, this will change if processes are optimized and intermediate by-products are utilized. Life-cycle analysis tells that biomethane is at the forefront of the bioenergy sources. The involved microbiome needs to be further optimized, along with technical improvements. Altogether biogas from organic waste will have a bright future in the European and worldwide renewable energy context.

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Application of Airborne Remote Sensing to Biomass Estimation - A case study on Field Science Center, Tohoku University -

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Abstract

Remote sensing is one of the techniques to sample a large area at once. It is expected for biomass estimation in regional area. An approach to estimate biomass on Field Science Center, Tohoku University (FSC) using aerial remote sensing data is presented. FSC is an experimental study site to consider production and consumption in the compost science program. Hyperspectral remote sensing data were obtained in 2007. LiDAR (Light detection and ranging) measurements were performed in 2008. Hyperspectral sensor is a kind of optical sensor, and land cover classification method using spectral information was applied. LiDAR technology provides vertical information of high accuracy and fine spatial resolutions for the earth surface target.

Land cover map in 2007 is generated from aerial hyperspectral image. Land cover change from 1960's to 2007 is extracted. The most of the land cover change is recognized in the north region of the target area. Total 265 ha of grassland area transformed to broadleaf forest from 1960's to 2007.

Digital Canopy Height Model (DCHM) was calculated by subtracting the Digital Terrain Model (DTM) from the Digital Surface Model (DSM) generated from LiDAR data. The footprint interval of laser beams from observed data is larger for single tree mapping. Therefore, possibility of forest canopy density estimation by the acquired data is examined. Standard deviation of canopy height on each plot is affected by thinning rates except for one heavily thinning plot.

Evaluation of types and amounts of local resources is necessary for their utilization. For the assessment of larger areas such as a municipality, satellite remote sensing data would be valuable.

Introduction

Remote sensing technology has been widespread for mapping and monitoring land cover. Remote sensing means research of objects from afar without actually touching them by using sensors (Legg, 1992). Acquired data provide us basic information for biomass estimation on the target area (Lu, 2006). Image of large earth's surface area is obtained at once using airborne and spaceborne remote sensing technique.

Optical remote sensing sensor image contains spectral information of the targets. Land cover type can be uniquely identified by the optical remote sensing data. Fine spectral resolution image is obtained using hyper-spectral sensor, and it is expected to estimate precise land coverage, as mineral type and vegetation species.

LiDAR (Light detection and ranging) technology provides vertical information of high accuracy and fine spatial resolution for the earth surface target. Forest attributes such as canopy height can be directly retrieved from LiDAR data. Direct retrieval of canopy height provides opportunities to model aboveground biomass (AG biomass) and canopy volume (Lim et al., 2003). The use of small footprint airborne LiDAR systems is one of the main fields of research in the last decade (Koch, 2010).

We have been attempting to estimate biomass on Field Science Center, Tohoku University (FSC) using various remotely sensing data (Namiwa, 2009, Yabe, 2012, Yabe et al., 2013, Yonezwa et al., 2013). FSC is an experimental study site to consider production and consumption in the compost science program. We report result of land cover change estimation from 1960's to 2007 using aerial hyper remote sensing data. An attempt of LiDAR measurements on FSC is also reported.

Test Site

FSC is located in the hilly and mountainous area on the west side of Miyagi prefecture in Japan. Total area is about 2,200 ha. It has been used for experimental farm, grassland and forestry of the faculty of agriculture, Tohoku University since 1947. It was

used for war-horse training center until the end of world war II. Digital field science center system using GIS and remote sensing data has been developed since 2004 (Saito et al., 2009). There are records for crop, forest and livestock production by the university since the 1950s. The vegetation in the 1960s was recorded on 1:10,000 map. This map is estimated to be drawn in 1968 or 1969 (Nishiwaki, 1998). Change in the land use of this site until 1995 was summarized by Nishiwaki (1998).

Airborne Remote Sensing

Hyper-spectral imaging data

Aerial hyper-spectral imaging data were taken in the test site on 24 July, 11 August, 20 September, 21 September and 25 November in 2007. Observation sensor was Airborne Imaging Spectroradiometer for Applications (AISA) Eagle and Hawk owned by PASCO Co.. AISA Eagle operated across the VIS/NIR portion of the spectrum (400-1,000 nm), resolving spectral differences as 2.9 nm with 1.5 m spatial resolution. AISA Hawk obtained image of 8.5 nm spectral resolution in the spectral range of 1,000-2,400nm with 3 m spatial resolution. The observation swath width was 900 m and all of Kitayama area around FSC was covered by plural paths oriented by the approximate north-south direction. The observation was performed by the collaboration with Japan Space Systems.

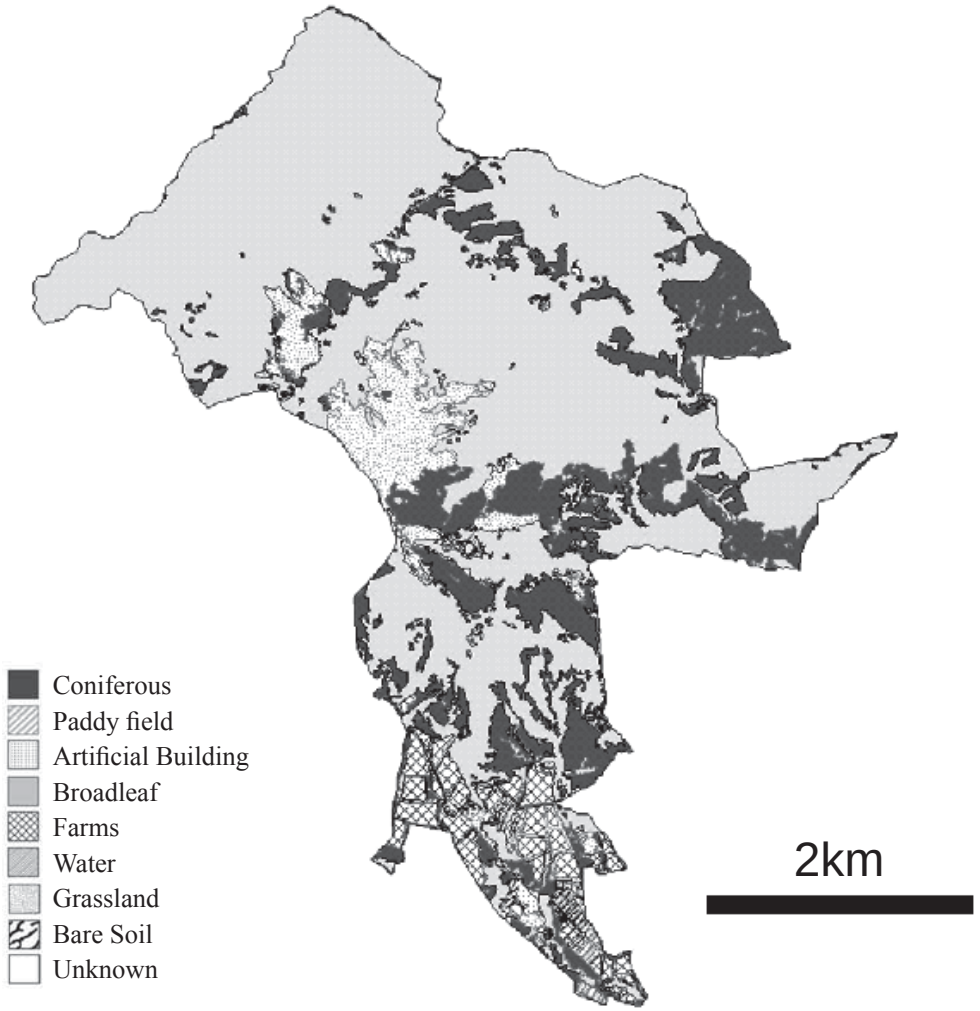

Figure 1. Land cover map in 2007 generated from aerial remote sensing data (Namiwa. 2009).

LiDAR data

The LiDAR dataset was acquired on 13, 14 and 26 November and 3 December 2008 by Ecosystem Adaptability Global COE, Tohoku University. All of Kitayama area around FSC was observed in the 14 flight courses, and one course was approximately northwest-southeast direction and other 13 flight courses were approximately north-south direction. In north-south direction measurements, footprint interval of laser beams was 2 m and swath width was ca. 900 m. The footprint interval of laser beams was 0.5 m in the measurement of the northwest-southeast flight course.

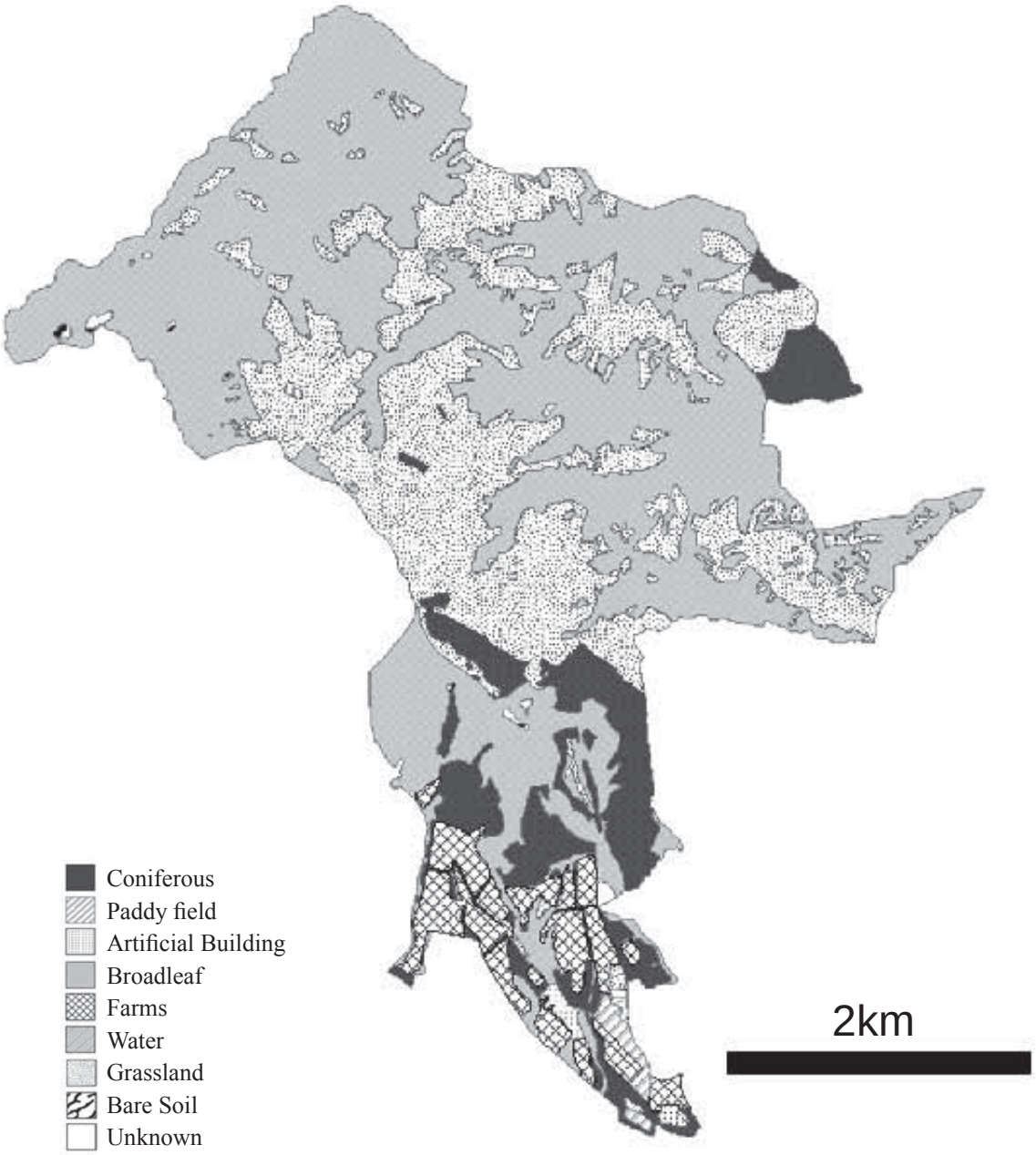

Figure 2. Land cover map in the 1960s (Namiwa. 2009).

Results and Discussions

2007 land cover

Land cover map in 2007 was generated from aerial hyper-spectral image and shown in Figure 1 (Namiwa, 2009). Most of the target area was covered by coniferous and broadleaf forests.

Land cover change from the 1960's to 2007

Land cover map in the 1960s is shown in Figure 2. This map is based on a paper vegetation map generated in the 1960s that was scanned and digitized by Namiwa (2009). It is obvious that the large grassland area was expanded in the north region of the target area.

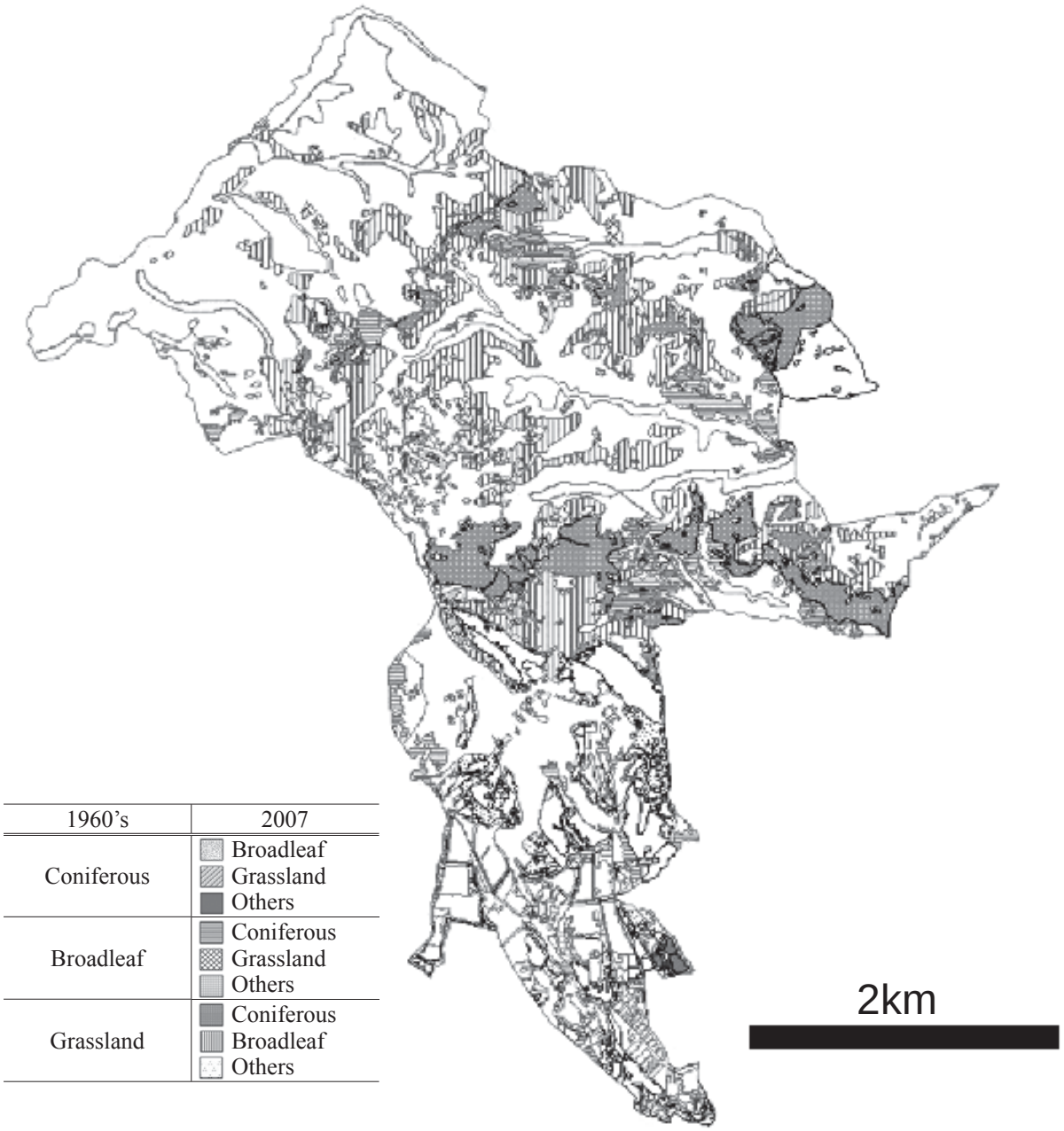

Figure 3. Land cover change from the 1960s to 2007 (Namiwa. 2009).

Figure 3 shows vegetation change from the 1960s to 2007. The landcover transformation is extracted and the transformed area is 32% of the total area. The largest change in landcover class is from grassland to broadleaf forest and it occupies 49 % of the transformed area. The change from grassland to coniferous forest, broadleaf forest to coniferous forest, and coniferous forest to broadleaf forest is 23%, 14 %, and 7 %, respectively.

DCHM and attempt for biomass estimation

Digital Surface Model (DSM) and Digital Terrain Model (DTM) were extracted from obtained LiDAR data. Digital Canopy Height Model (DCHM) was obtained by subtracting the DSM from the DTM.

To estimate AG-Biomass, it is necessary to estimate forest canopy height and density. It is pointed out that 3 hits per m^2 of the pulse density in the plots are necessary for the extraction of single tree information from DCHM (Nawamura et al. 2007). The footprint interval of laser beams in the observed data is larger for single tree mapping. Therefore, possibility of forest canopy density estimation by the acquired data is examined (Yonezawa et al., 2012). DCHM in the experimentally thinning plots in FSC were analyzed (figure 4). This plot includes heavy thinning plots, weak thinning plots and no thinning plots. Standard deviation of canopy height in each plot is affected by thinning rates except for one heavily thinning plot. Relative frequency of 0 m is larger than 1 m in the heavily and lightly thinning area except for one heavily thinning plot of which standard deviation is less than the lightly thinning areas.

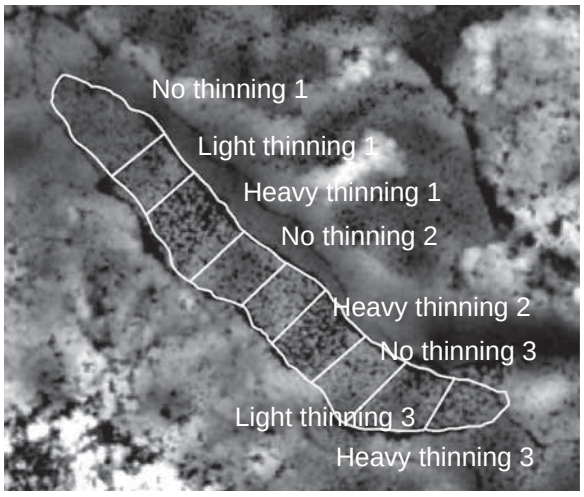

Figure 4. DCHM for experimentally thinned plots (Yonezawa et al., 2012).

Conclusion

An experimental study of biomass assessment using airborne remote sensing in a hilly and mountainous study site is presented. Land cover classification is one of the analysis methods by multispectral optical remote sensing data and the analysis result provides fundamental information for biomass estimation. Land cover change analysis is useful to evaluate transformation of local resources. Airborne LiDAR is powerful a tool to estimate AG-biomass if we do not have to think about measurement cost. The measurement data of FSC in 2008 has a large footprint interval of laser beams. Moreover, it is necessary to find effective method for biomass estimation to use this data.

Evaluation of types and amounts of local resources is necessary for their utilization. For the assessment of larger areas such as a municipality, satellite remote sensing data would be valuable.

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Life Cycle Impact Assessment of Compost, and Possibility of IT Solution

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Abstract

Life Cycle Assessment (LCA) is a method to estimate environmental load of products. The feature of this report is LCA applied to acidulocompost which is made by PICS project.

Since electric power is needed for warming, acidulocompost has a large environmental impact on a manufacturing process compared with the conventional compost,. LCA was applied to the compost which makes a fish raw material. Therefore, the performance of the compost as a product is equivalent to fish meal manure. Although fish meal manure has the high manure effect, it is expensive. Fish residue unsuitable for fish meal manure is incinerated by thermal disposal. In the case of composting without thermal disposal, carbon-dioxide emissions decrease in the whole process. (Dr. Ryosuke Tajima calculated and computed this LCA.)

There are some examples where LCA was applied to compost. However, there is no example of application to acidulocompost. LCA as environment assessment is going to evaluate commercial products. The one exit is a carbon footprint (CFP). Compost is the material for agriculture and is B2B goods. Therefore, it was not the main target of CFP. Evaluation of compost increases for an agricultural synthetic environmental impact assessment.

Microbial Community Dynamics during Composting Process of Animal Manure Analyzed by Molecular Biological Methods

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Keywords: 16S rRNA gene, animal manure, archaea, bacteria, cloning, compost

Abstract

Composting is a biological process involving stabilization of animal manure and transformation into organic fertilizer. Microorganisms such as bacteria and archaea participate in the composting process. Because bacteria form huge communities in compost, they are thought to play an important role as decomposers of organic substances. However, only few studies are tracking bacterial communities throughout the composting process. The role of archaeal communities in composting has not been also elucidated. To study bacterial and archaeal community dynamics, animal manure composts were analyzed by molecular biological methods.

A clone library constructed from bacterial 16S rRNA genes showed that the bacterial community structure dynamically changed with processing time. Based on phylum-level analysis, Firmicutes and Bacteroidetes were dominant at day 0. Phylum Firmicutes kept their abundance for 20 days, indicating that they may be active under high temperatures. In the final compost, the library consisted of various genes belonging to the phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria.

In contrast, the clone library of archaeal 16S rRNA genes was simply constructed by two groups, the methane-producing archaea (methanogens) and ammonia-oxidizing archaea (AOA). Thermophilic *Methanosarcina* spp.-like genes were constantly present, indicating that this methanogen may adapt to environmental changes such as high temperature. AOA-like sequences were first detected from compost and displayed a high similarity to cultured AOA originating from hot springs. Further study revealed that the abundance of AOA markedly varied because of raw material or composting process. In this study, we revealed the pattern of changes in the prokaryotic communities involved in the composting process.

Introduction

Cattle manure accounts for a large part of the total animal waste generated in Japan (MAFF, 2013) and can cause environmental problems such as soil contamination, air pollution, or offensive odor emission without appropriate treatment (Bernal et al., 2008). Composting is the most effective technique for mineralization of organic substances, microbial stabilization, removal of odors, and sanitization (Bernal et al., 2008; Haga, 1999). In addition, the final product can be applied to agricultural soil as high quality fertilizer (Haga, 1999). Throughout the process, various microorganisms such as bacteria, archaea, and fungi play an important role in degradation (Ryckeboer et al., 2003). Microbial community structure and diversity change dramatically during composting. These changes are affected by temperature, pH, moisture content, and aerobic/anaerobic conditions (Ryckeboer et al., 2003; Schloss et al., 2003). Because the bacterial population is more vast and complex than others (Ryckeboer et al., 2003), it has been considered that the bacterial community is the main decomposer in compost. To follow the dramatic change in structure and diversity of the microbial community during composting, researchers have recently used culture-independent techniques analyzing 16S ribosomal RNA genes (Chachkhiani et al., 2004; Peters et al., 2000; Tang et al., 2004). Using culture-dependent techniques, it is possible to detect only about 8.5% of total microbes (Gong et al., 2005). The changes in the bacterial community structure during the composting of animal manure have been analyzed in other studies (Cho et al., 2008; Green et al., 2004; Maeda et al.,

2010; Sasaki et al., 2009; Yamamoto et al., 2009); however, there are only few studies tracking the bacterial community in detail throughout the entire composting process. On the other hand, existing studies of the archaeal community are a minor component of the microbial community studies on composting because archaea mainly live in extreme environments such as thermophilic or anaerobic conditions (Insam and de Bertoldi, 2007). However, some reports have indicated the presence of methane-producing archaea (methanogens) in compost (Jäckel et al., 2005; Thummes et al., 2007). Moreover, a new archaeal group called ammonia-oxidizing archaea (AOA) was discovered living in moderate environments like seawater and soil and were found to be essential organisms in ammonia oxidation (Könneke et al., 2005; Leininger et al., 2006; Treusch et al., 2005). Therefore, archaea can be also considered as an essential component of the microbial community in compost. However, there is no existing study of the entire archaeal community in the composting of animal manure.

In this review, we investigated the bacterial and archaeal community structures and how they changed during the composting process.

Bacterial community structure during the composting process

It has been considered that bacteria play an important role throughout the process because of the existence of a wide variety of species and characteristics. Denaturant gradient gel electrophoresis (DGGE) has been widely used for the analysis of bacterial communities (Green et al., 2004; Maeda et al., 2010; Sasaki et al., 2009; Yamamoto et al., 2009). However, it is not sufficient to clarify the change in the dominant microbial species in bacterial communities during the composting process. The clone library method enables detection of a larger number of species within microbial communities than using DGGE analysis. However, only few studies are tracking bacterial communities throughout the composting process (Cho et al., 2008; Guo et al., 2007; Yamada et al., 2008). Here, we performed a composting experiment as a model case of field-scale composting and tracked the bacterial community using the cloning procedure. Cattle manure and sawdust were mixed and piled for about 30 days with aeration. To study the bacterial community, composting material was treated for 84 days more without daily mixing. Changes in physical and chemical parameters are shown in Fig. 1. The temperature reached 76.1 ºC within 5 days and remained at >50 ºC for 23 days. Moisture content decreased from about 70% to 41.8 % by the end of the process (Fig. 1).

We selected 5 samples at different time points for analysis. Total DNA was extracted and approximately 600 bp of bacterial 16S rRNA gene were amplified. PCR products with the correct DNA fragments were cloned and then selected for sequencing.

The clone library constructed from the sample collected on day 0 mainly consisted of members of the phyla Firmicutes and Bacteroidetes (Fig. 2), indicating that both members of bacteria originating from cattle manure (Ozutsumi et al., 2005). The number of clones grouped under the phylum Firmicutes, which can be active under high temperatures, maintained their relative abundance for 20 days. Especially on day 5 and day 20, the Phylum consists of the member of class Clostridiales and Bacillales, corresponding to other reports (Yamada et al., 2008). Thereafter,

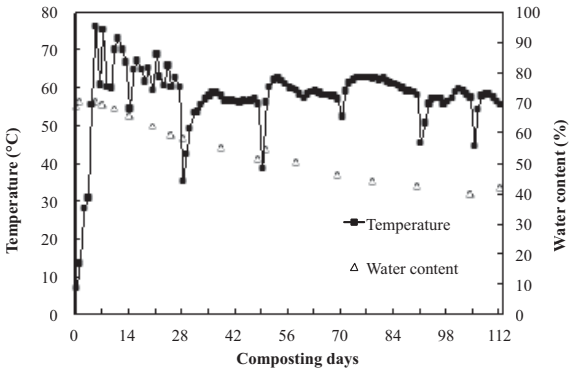

Fig. 1. Changes in temperature and water content during the composting process for analysis of bacterial community.

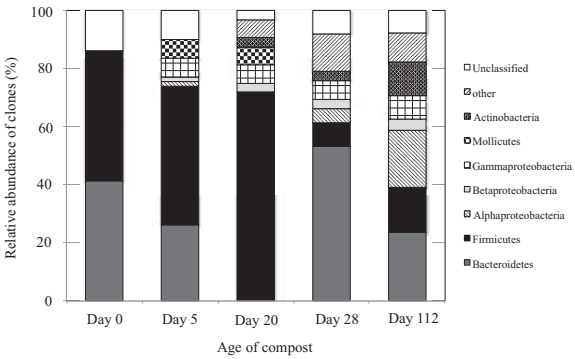

Fig. 2. Changes in bacterial community constructed from cloned 16S rRNA genes.

the ratio of the members of the phylum Firmicutes decreased, while those of the phyla Actinobacteria and Proteobacteria increased. By the end of the composting process, the library consisted of phyla Proteobacteria (32.7%), Bacteroidetes (26.5%), Firmicutes (18.4%) and Actinobacteria (12.2%), and others (10.2%), showing that various bacteria lived and forms complex community in the final compost. Bacteria sequences were classified into class Rhizobiales and Burkholderiales, that often observed in soil environment were also contained, suggesting that composting material reached stable phase.

We observed that the bacterial community reflected composting conditions and changed its structure accordingly. However, metabolic activity of bacterial community was not analyzed in this study. To determine dominant species in each stage of the composting process, RNA-based analysis is required. Some studies reported the community structure of one particular bacterial group diversity in compost with reverse-transcription PCR and DGGE (Halet et al., 2006; Kowalchuk et al., 1999). In addition, metagenomic analysis may be also the powerfull method for understanding whole microbial community in compost (Martins et al. 2013). It will cover all aspects of microbial ecology in composting in combination with PCR-based DNA analysis.

Archaeal community structure during the composting process

It has long been considered that the archaeal community has a low abundance in composting process because they are usually oligotrophic, thermophilic, or hyperthermophilic (Insam and de Bertoldi, 2007). However, the archaeal community has recently been considered as an essential component of the microbial community in compost. For instance, considerable methane emissions have been measured from compost, suggesting that methane-producing archaea (methanogens) live actively in compost (Beck-Friis et al., 2000; Fukumoto et al., 2003; Hao et al., 2001). In addition, some archaea of the phylum Crenarchaeota can oxidize ammonia under natural environments such as seawater, freshwater, various soil, and hot spring (Hatzenpichler et al., 2008; Könneke et al., 2005; Leininger et al., 2006; Treusch et al., 2005). AOA also was detected from artificial sites, like rice field (Fujii et al., 2010). However, no useful information about archaea in the compost of cattle manure was available, although methanogen-like sequences had been detected in cattle manure (Gattinger et al. 2007). Thummes et al. (2007) analyzed about 120 clone sequences using composting materials; however, no sequence belonging to AOA was detected.

Therefore, the composting experiment to analyze the archaeal community was performed using cattle manure and sawdust as described above (Yamamoto et al., 2011). Changes in physical and chemical parameters are shown in Fig. 1. The temperature reached 77.9ºC and was maintained at >60ºC for 18 days. The initial moisture content was about 67% (Fig. 3), and it continued to decline from day 8 and eventually reached its lowest value (~30%).

Compost samples for analysis were selected at 6 time points. In total, 14 OTUs were generated from sequenced clones, the number of which varied from 36 to 78. Almost all detected OTUs were related either to methanogens or to AOA (Fig. 4). Results indicated that both methanogens and AOA were dominant archaeal species during the composting process of cattle manure. The archaeal community

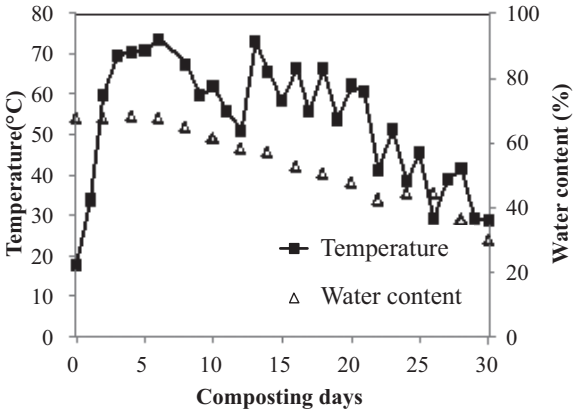

Fig. 3. Changes in temperature and water content during the composting process for analysis of archaeal community.

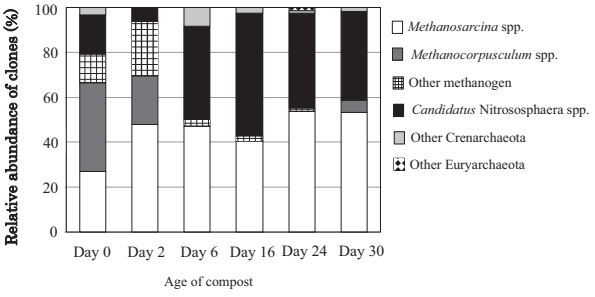

Fig. 4. Changes in archaeal community constructed from cloned 16S rRNA genes.

structure found in the present study differed in some respects from that found in previous reports analyzing composting materials. During the first two days, OTUs grouped into methanogens were dominant. We detected some OTUs, which were closely related with sequences from groundwater, animal rumen, or manure.

Another OTU formed the dominant group after day 2 and showed a high homology with uncultured thermophilic *Methanosarcina* spp. Thummes et al. (2007) also detected some clones grouped into this cluster from different composting materials. Thus, thermophilic *Methanosarcina*-like organisms appear to adapt to the composting environment and increase its detection rate after other methanogens originating from cattle manure have decreased in abundance because of high temperatures.

It is notable that AOA-like sequences were detected throughout the composting process. Our study indicated that AOA was an essential component of the archaeal community in compost, particularly from days 6 to 30. A large part of OTU was closely related to *Candidatus* Nitrososphaera gargensis with high homology (98%). This *Candidatus* Nitrososphaera gargensis-like AOA can grow under moderate thermophilic condition and may have the ability to oxidize ammonia to nitrite (Hatzenpichler et al., 2008). The AOA detected in the sample adapted to the temperature or ammonium concentration in cattle manure compost. Other OTUs were classified as soil crenarchaeota.

Diversity of AOA in various animal manure composts

Nitrification by ammonia oxidation occurs during composting process (Bernal et al., 2009; Vuorinen and Saharinen, 1997). Organic nitrogen in fresh manure is oxidized to nitrate through nitrification by ammonia oxidizing organisms. It has been reported that ammonia-oxidizing bacteria (AOB) may play an important role in nitrification during composting (Prosser and Nicol, 2008). However, previous researchers concluded that archaeal *amoA* gene was not detected from any stages of the composting process (Maeda et al., 2010; Yamada et al., 2007). Our study detected the archaeal *amoA* gene in compost for the first time (Yamamoto et al., 2010). It shows that AOA may have been more abundant than AOB during the cooling and maturation stages of composting (Yamamoto et al., 2010). In the samples collected from cattle manure compost, only one or two archaeal *amoA* bands were detected in the course of the composting process (Yamamoto et al., 2011). It was shown a useful information that the diversity of AOA community was low and AOA may have an ability of ammonia oxidation in composting. In addition, our group also revealed that an archaeon related to *Candidatus* Nitrososphaera gargensis was dominant in liquid cultures seeded with cattle manure compost (Oishi et al., 2012). However, there is no information available about AOA communities in other animal manure compost. To clearly understand the nitrification process in compost ecosystems, we researched AOA community structure in various animal composts (Yamamoto et al., 2012). For AOA community analysis, the bacterial *amoA* sequence was also amplified to compare their diversity and abundances.

Samples collected from other cattle manure compost were also able to amplify archaeal *amoA* sequences and show DGGE fingerprints. In contrast, a few *amoA* sequences were amplified using samples from fresh manure, swine manure compost, and chicken manure compost. The number of detected *amoA* sequences per sample varied from 1 to 4, except for the samples from one facility. All sequences were divided into about three groups: one is phylogenetically related to *Candidatus* Nitrososphaera gargensis (group NG), while the other two groups had *amoA* sequences from hot spring or wastewater as close relatives (Fig. 5). However, there were a few compost samples where AOA dominated over AOBs, indicating that the presence and abundance of ammonia oxidizers was determined by some unknown factors. We hypothesized that the difference of ammonia concentration between raw animal manure is the most influential factor in AOA presence and cattle manure was the best material to their growth in composting.

The number of AOA cells in comparison to ammonia-oxidizing bacteria (AOB) in the sample was estimated by real-time PCR (Fig. 6). In cattle manure compost, the abundance of archaeal *amoA* genes was clearly lower than that of bacterial genes. These results provided the finding that the abundance of AOA in compost was not influenced by the difference of animal species generating manure. Real-time PCR confirmed that archaeal *amoA* gene copy numbers were greater than bacterial gene copy numbers in the

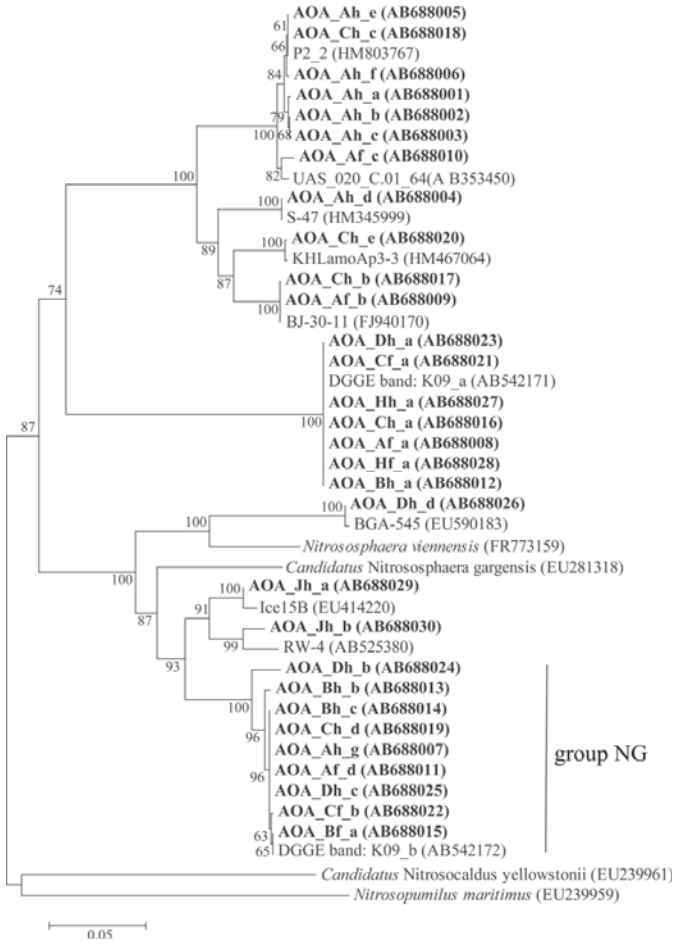

Fig. 5. Phylogenetic tree of the archaeal *amoA* sequences obtained from composting materials.

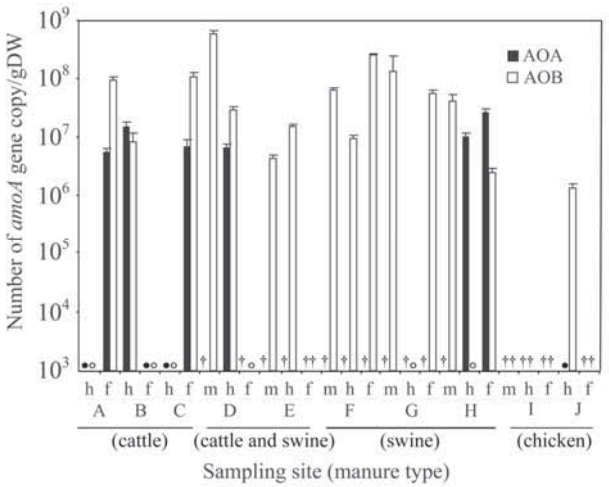

Fig. 6. *AmoA* gene copy numbers for AOA and AOB. Capital letter indicates sampling facilities. Samples were obtained from each facility at fresh manure (m), the high temperature stage (h), and the end of the composting (f). Samples with dagger did not perform real-time PCR. The sample with gene copy number below the detection limit were represented as closed circle (AOA) and opened circle (AOB).

end product at facility H. The AOA/AOB ratios varied from 0.06 to 10.54. This variation may have been caused by the operating conditions of the composting process, such as the addition of finished compost.

Our results so far suggest that the concentration of ammonium and the temperature are factors that control the AOA community. Further study is needed to show whether AOA are critical to nitrification in manure compost and influence the quality of the compost. To evaluate the contribution of AOA in nitrification, we need to collect and analyze more composting samples and measure various parameters to create meta-data for future research.

Conclusion

In this study, we revealed a part of the pattern of the changes in the bacterial community in the composting process by analyzing a large number of bacterial sequences. In addition, we detected AOA from compost for the first time. By studying various samples originating from different animal manure samples, we were also able to suggest that AOA could be involved in the nitrification of composting systems, although their abundance was lesser than that of AOBs. This study underlines the importance of investigating archaeal communities to understand the microbiology of compost.

However, metabolic activity of bacterial and archaeal community were not analyzed in this study. RNA-based analysis and metagenomics will cover all aspects of microbial ecology in composting by combining with PCR-based DNA analysis.

Acknowledgements

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Small Scale of Anaerobic Digestion for Decentralized Energy Production and Bioresource Recycling

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Abstract

In Japan, several different electric power companies produce energy and transmit power. A major disadvantage of this system is that when a fault arises at one electric power company an entire region may be without power, as occurred during the Great East Japan Earthquake. Small-scale methane fermentation reactors are being investigated as a source of energy. The advantages of small-scale methane fermentation include a small initial investment, few required materials, and reduced costs due to materials recovery. The development of small-scale methane fermentation in Japan may allow decentralized energy production for localized areas. The use of heat from hot springs or factory exhaust instead of kerosene boilers to warm methane fermenters would reduce the costs of energy production. Energy balance (production of biogas vs. energy consumed for running reactors) became plus although it was on a small scale of reactors. Replacing a single kerosene boiler with a hot spring could reduce CO_2 emissions by about 90 tCO_2/year. Now we research for applying a small methane fermentation using exhaust heat from a fishery factory, at Shiogama, the coast area of Tohoku, Japan. Digested liquid from the reactors could be used as liquid fertilizers for cultivating vegetable or microalgae as feeds for clams. Thus, this system may provide decentralized energy production with the added benefit of nutrient-rich digested liquid used as fertilizer, and it would also help towns to better survive disasters. Especially considering the limited resources available in Japan, the introduction and promotion of small methane fermentation reactors for energy production are vital.

Decentralized energy production

The Great East Japan Earthquake and Tsunami on March 11 2011, together with the subsequent accident at the Fukushima Nuclear Power Plant, revealed vulnerabilities in electrical power systems. In Japan, there is one power company in each region. The electricity supply was once centralized, and with this, damage to one power company may result in a loss of electricity supply to the entire region. In North America in 2003, a blackout affected regions from New York City to the Midwest state of Michigan, leaving as many as 50 million people in the dark. Decentralized energy production has significant advantages in terms of the resilience of the system, and can prevent such failures. In addition, energy production from biomass, including human waste and food waste, can be applied in both cities and local areas, and multiple small-scale methane fermentation systems can facilitate decentralized energy production.

Conventional methane fermentation

Methane fermentation systems currently exist in Japan; however, these systems are typically large, with a reactor capacity of around 500 to 1000 m^3. The benefits of large-scale reactors are that they can treat a large quantity of material at one time, and so have relatively small unit costs. However, the initial investment is large (500-1000M JPY), as are the maintenance costs (50-100M JPY per year). Some methane fermentation facilities in Japan have been suspended due to such large costs.

The surface area per unit volume of a small methane fermentation system is wider than that of a large system. For example, if the reactor is spherical, the surface area to unit volume ratio is $(4\pi r^2) \div 4/3\pi r^3 = 3 / r$. For this reason, in small-scale systems the heat dissipation per unit volume is large compared with large-scale systems. Therefore, small-scale systems require more energy for heating per unit volume. In addition, since the accepted quantity of material is small, there is a disadvantage in that comparatively little energy is produced. With a 50-m^3 methane fermentation facility, the energy consumed for heating (generally required only in winter) is 20 GJ per month. In addition, energy is required for stirring (Baba *et al*., 2013). Furthermore, the biological oxygen demand of the digested material resulting from

methane fermentation may reach 500-2500 mg/L. To reduce the cost of wastewater treatment, the digested material should be used as liquid fertilizer.

Small-scale methane fermentation with heating from a hot spring

We may overcome these problems of small-scale methane fermentation using the following methods:

1. By using natural energy for heating the reactor, the overall energy balance of the small methane fermentation system will be positive.
2. Little material is required for small-scale methane fermentation systems, which means that it can be sourced locally. This in turn reduces the cost of collecting the material.
3. Digested material from the fermenter can be used as liquid fertilizer to cultivate vegetables without requiring water treatment.

In this study, we used a hot spring to heat the fermentation vessel. Materials were collected and placed inside the fermenter manually. Digested liquid was used as liquid fertilizer for cultivation of vegetables. The study site was in the Naruko hot spring area, located in the northwest of Miyagi prefecture in Japan. The Naruko hot springs are artesian aquifers, and have a temperature of >90ºC. Hot water from the spring was pumped through a tube around the methane fermentation apparatus, by which means the reactor was maintained at 55ºC.

Fig. 1 shows the layout of the methane fermentation system. Two 350-L tanks were used, and because the materials were not crushed and stirring power was not required, the energy requirements for fermentation were minimal. The principal material used was leftover foods from hotels in the Naruko hot spring resort area. Leftover food was placed into paper bags, and dropped into the reactor directly. (Suzuki, et al. 2012)

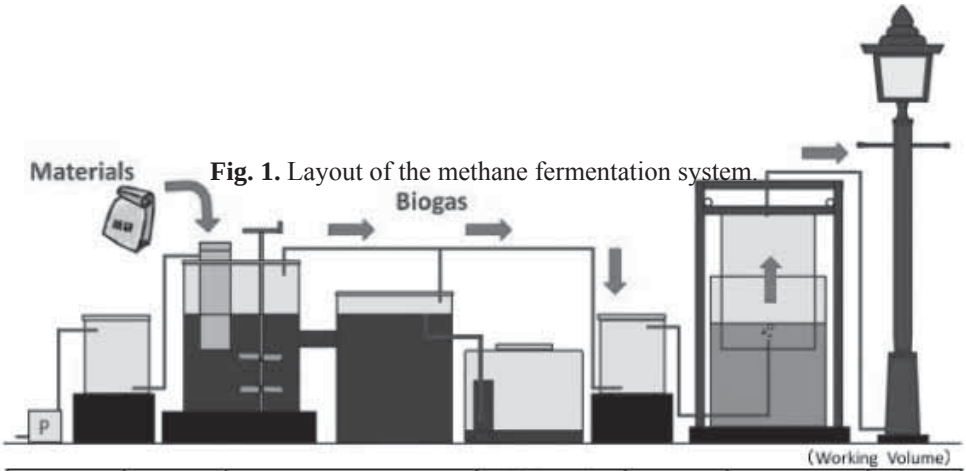
The average leftover food quantity from dinner and breakfast was ~300 g/day/person. Approximately half of this was rice. The carbon-to-nitrogen ratio was 20, which suggests that the leftover food was suitable for methane fermentation.

The effect of material loading rate on methane production was investigated. Volatile fatty acids accumulated at high loading rates. Based on this observation, microbial carriers were placed into the reactor. The biogas production rate was ~300 L/day, and the methane concentration in the biogas was 50% at a loading rate of 3 kgCOD/day/m^3(Suzuki, et al. 2013).

At the first stage of the operation, the pH was controlled using chemical additives. The liquid digested material from the second tank was returned to the first fermentation tank, so that additional pH control was not required.

Use of the methane gas

For electrical power generation using gas engine, high-quality gas-phase fuel is required, and the methane concentration should be ≥55%. The methane concentration of biogas generated from garbage



Equipment	Deodo -rizer	Fermentation tank		Digested slurry Storage tank	Desulfu -rizer	Gas Storage tank	Gas lamp
		1st tank	2nd tank				
Spec	50(42) L	350(230) L	350(305) L	300 L	50(42) L	200 L	6 MJ/h

Fig. 1. Layout of the methane fermentation system.

leftovers is typically 40-60%, and increases to >50% if the microbial flora is well controlled. In this case, we use biogas to light the gas lamp. Gas lamp could be lighted by more than 45% of methane gas. To use biogas for the gas lamp is easier than to make electricity with gas engine.

In addition, gas lamp is easy to understand for people to change biogas to light as energy. We could use this system for education about biomass energy.

Collection of raw materials

In this system, we adopted a raw material collection method whereby waste food is packed into a paper bag, and placed into the fermentation equipment. The waste accounted for 78% of leftovers, based on a survey of tourists. The waste food collection points were placed at the site 7-min walk from hotels on average, which was the ideal for the disposal of food waste.

Liquid fertilizer

Digested liquid material from the fermentation system contains nitrogen and phosphorus. There have been some reports on the use of digested liquid material as fertilizer (Hishinuma et al., 2008, Ishikawa et al. 2006, Collet et al. 2011). However, in cities, this material must undergo wastewater treatment.

Table 1 showed the composition of the digested liquid from the small methane fermentation system using hot spring. The principal material was leftover food (Tajima et al. 2013).

Experiments were carried out using the digested material as fertilizer in the hydroponic cultivation of *Brassica campestris*. The digested material inhibited crop growth; it is likely due to an increase in the electrical conductivity of the liquid due to addition of Na salts as a pH-controlling agent. Upon addition of the pH control agent, the electrical conductivity was 32 mS/cm. Typically, the electrical conductivity of the soil is less than 1 mS/cm. When fermentation was carried out without a pH control agent, the electrical conductivity of the digested material decreased to 6 mS/cm.

Table 1. Composition of digested liquids.

Digested liquids	NH_4-N (mg/L)	P_2O_2 (mg/L)	K_2O (mg/L)
leftover food	1.8	0.1	1.5
food garbage	1.0	0.5	3.0

Economic and environmental effects of the system

Carbon dioxide emissions can be reduced by using non-fossil-fuels and by heating using thermal springs. The installation cost of the system was 700,000 JPY. The waste food, which the system uses, would otherwise have a disposal cost of 30,000 JPY per ton. For these reasons, small-scale fermentation systems may be considered economically viable (Tajima et al. 2013).

Hot springs globally and in Japan

In Japan, there are many hot springs from north to south, the number of hot springs is reported 27532. 28 % of hot springs in Japan are artesian type. 50% of hot springs in Japan are more than 42 ºC. In addition, there are many hotels and inns around hot springs, and collection of food waste is easy there.

Hot springs in the world, such as the United state, Indonesia, Philippine, are famous for their geothermal power generation. but they are not used as hot springs, such as Japan. In Iceland, geothermal power is used not only as electricity but as the thermal energy.

In the mesophilic fermentation, as well as high temperature fermentation at 55ºC, using the waste heat or natural heat, energy balance is well maintained in the small-scale methane fermentation.

Waste heat utilization

We plan to construct small-scale methane fermentation systems using waste heat emitted from factories. The waste heat discharged from fish processing plants can be used to control the temperature of the fermentation system, as can waste heat from refrigerators and freezers be used. Heat from the cooling systems is currently discharged into the environment. When the air temperature is low, harnessing this waste heat will be problematic; however, heat recovery in the summertime is expected to be feasible. Furthermore, it may be possible to use solar energy for heating, even in winter, due to the large number of sunny days during this season.

Future development

Heat from hot springs is believed to be stable, and so it can be used to maintain the reaction tank at a constant temperature. Methane fermentation depends on temperature, and we investigated the effects of temperature variation on methane production. Methane fermentation for recovery of energy from domestic wastewater treatment has attracted recent interest(Kim et.al, 2011). The temperature used for fermentation of domestic wastewater is significantly lower than that in the waste food digester, typically 10-20ºC(Kim et al. 2013). In condominiums and apartment blocks, hot wastewater from showers and baths can be used as a heat source to encourage digestion of waste materials. F garbage from households may be fermented to methane using this waste heat. Furthermore, it may be possible to use digested material as liquid fertilizer for the gardens of the source apartments.

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Effect of Organic Matter Application on Soil Carbon Sequestration in Japanese Agricultural Land

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Abstract

Recently, in addition to improve soil quality or productivity, soil carbon sequestration for mitigating greenhouse gas emission has been highlighted. Huge amount of carbon is stored in soil and increase in it means decrease in atmospheric CO_2, hence it mitigates climate change. To increase SOC, two options would be possible: 1) increase inputs and/or 2) decrease outputs through decomposition. Application of organic matter such as compost or green manure is an option for increasing carbon inputs to soils. On the other hand, no-tillage can be considered as an option for decreasing outputs (decomposition) of SOM. Generally, changes in SOC through management changes need long time to be detected. Long-term field experiments are ,therefore, needed to detect changes in SOC. Long-term experiments revealed that organic matter application enhances soil carbon accumulation but the rate of changes in soil carbon is different among sites because many factors control organic matter decomposition and accumulation. SOM models are ,therefore, a useful tool for predicting changes in SOC through changes in management practices or by future climate change. We validated the Rothamsted carbon (RohC) model by using long-term field experiments in Japan and the model was modified for Andosols and paddy soils while it did not need any modification for other soils. By using the well validated model, we can estimate the potential of soil carbon sequestration in the future. Evaluation of greenhouse gas emission (or removal) together with other gases (CH_4 and N_2O) to calculate global warming potential (GWP), and evaluation of fossil fuel use in agricultural practices should be required, too.

Soil carbon and climate change

Soil organic matter (SOM) is one of important index of soil quality. SOM improves soil physical, chemical and biological properties and consequently improve soil productivity. Therefore, farmers have been making a great effort to increase SOM in their soils. Recently, in addition to improve soil quality or productivity, soil carbon sequestration for mitigating greenhouse gas emission has been highlighted (Lal, 2004). Huge amount of soil organic carbon (SOC) is stored in SOM, and carbon is cycling among soil, vegetation and atmosphere (Figure 1). Increase in SOC therefore means decrease in atmospheric CO_2, hence it mitigates climate change.

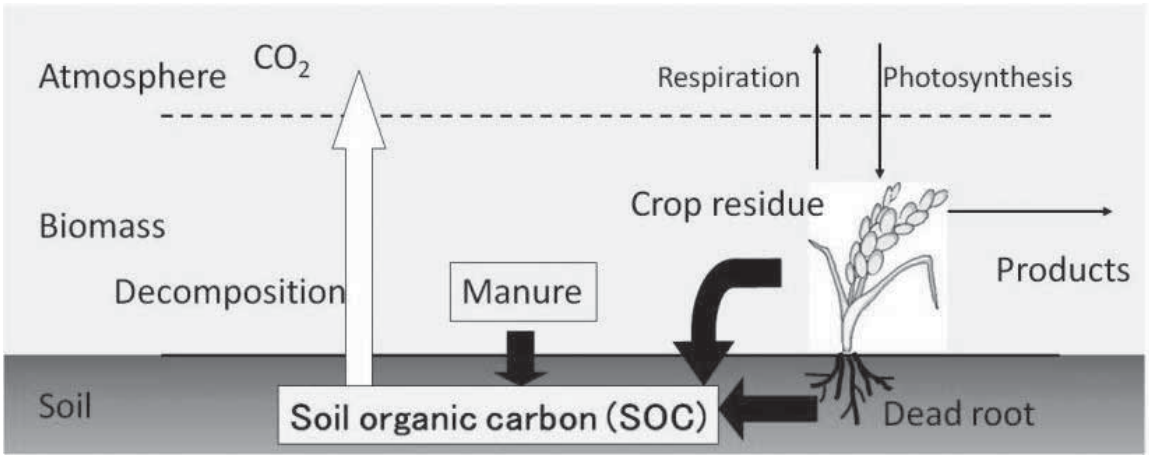

Figure 1. Carbon cycle around soil.

Management practices for increasing soil carbon

To increase SOC, two options would be possible: 1) increase inputs and/or 2) decrease outputs through decomposition. Application of organic matters such as compost or green manure is an option for increasing carbon inputs to soils. On the other hand, no-tillage or reduced tillage can be considered as an option for decreasing outputs (decomposition) of SOM.

Monitoring and modeling SOC

Generally, changes in SOC through management changes need long time to be detected. Long-term field experiments are, therefore, needed to detect changes in SOC. Long-term experiments revealed that organic matter application enhances soil carbon accumulation but the rate of changes in soil carbon is different among sites because many factors such as temperature, moisture, soil texture, tillage or organic matter application rate, etc. control organic matter decomposition and accumulation.

SOM models, which involve important factors of SOM turnover, are a useful tool for predicting changes in SOC through changes in management practices or by future climate change. Among published SOM models, we used the Rothamsted carbon (RohC) model (Coleman and Jenkinson, 1996; Figure 2) because it is one of the widely-used SOM models in the world having high reliability and needs fewer inputs than other models. We thus validated the RothC model by using long-term field experiments in Japan (Figure 3). The model was modified for Andosols (Shirato *et al*. 2004) and paddy soils (Shirato and Yokozawa 2005) so that changes in SOC with time can be well simulated on the plot scale while it did not need any modification for other soils (Shirato and Taniyama, 2003).

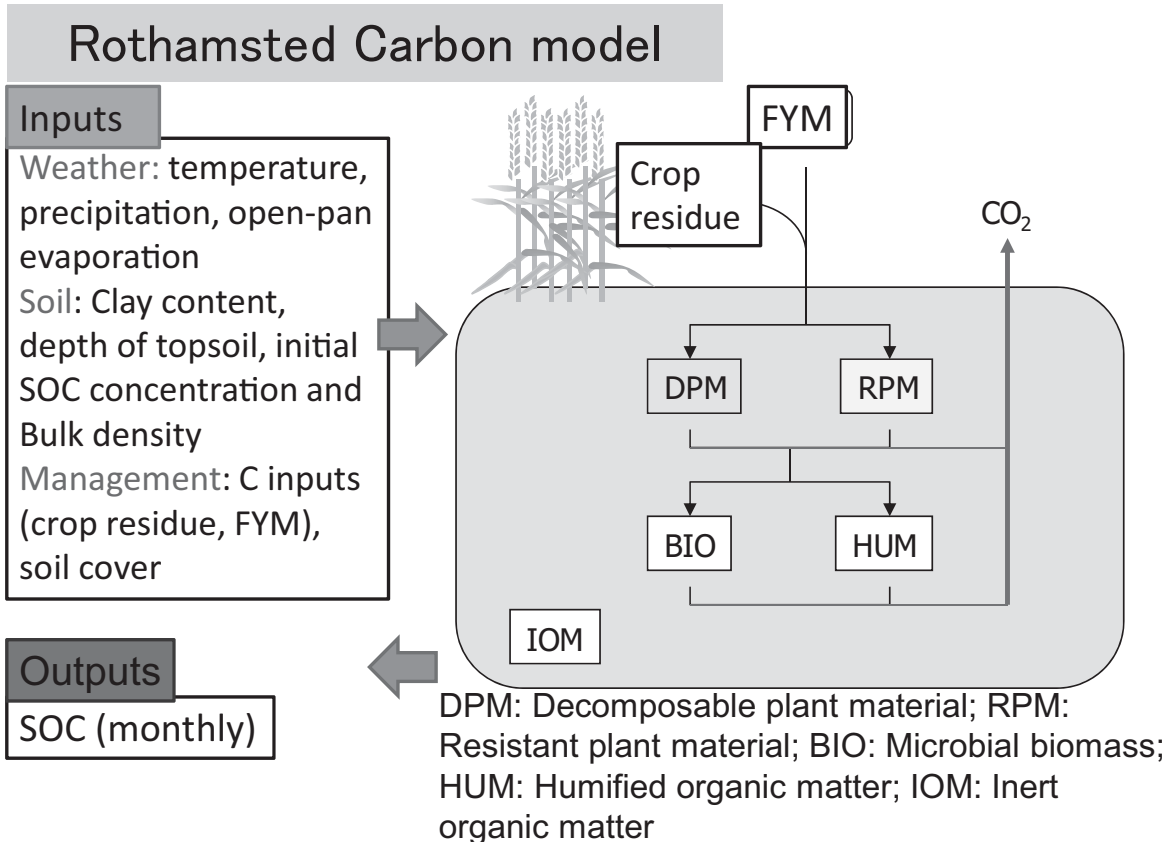

Figure 2. Structure, inputs and outputs of the Rothamsted Carbon (RothC) model.

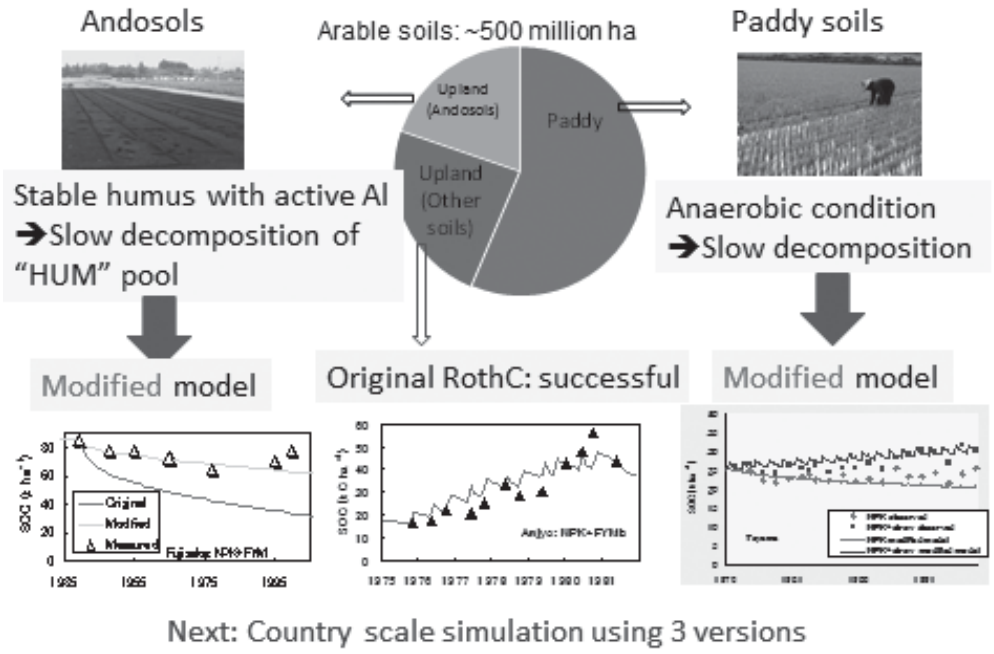

Figure 3. Validation and modification of the RothC model in Japanese arable soils.

Future target

By using the well validated model, we can estimate the potential of soil carbon sequestration in the future (Figure 4). We have developed a simulation system, by linking the RothC model and databases of weather, soil, land use and agriculture activity, for simulating changes in SOC with time by management and climate change.

Evaluation of greenhouse gas emission (or removal) together with other gases (CH_4 and N_2O) to calculate global warming potential (GWP), and evaluation of fossil fuel use in agricultural practices (e.g. fertilizers, pesticides, machinery use, etc.) should be required, too, because soil carbon sequestration and other gas emission often have a trade-off relationship.

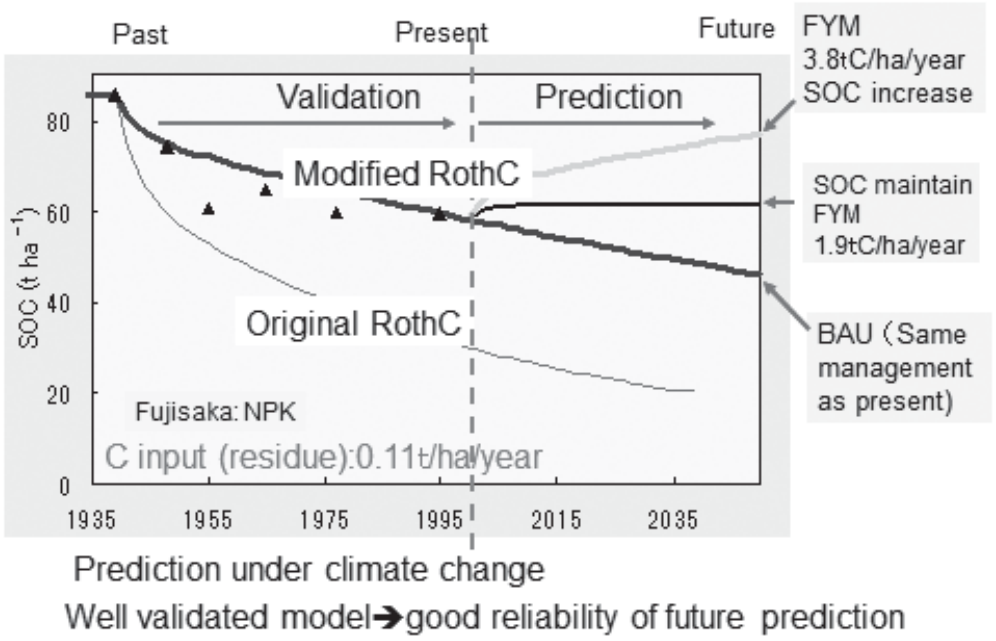

Figure 4. Example of future prediction by using the RothC model.

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New Function of Compost: Inhibitory Effect of Acidulo®compost on Weed Germination and Growth

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Abstract

Acidulo®compost (AC) is a type of compost derived from food waste processed under thermophilic and acidic conditions. Given this compost's high nitrogen content and potential to suppress weeds, its applicability in agriculture has been investigated. In this study, we evaluated the potential for AC to be used as a pre-emergent herbicide. Suppression of weed emergence was confirmed by applying AC to a cabbage field. Application of AC was particularly effective in suppressing the emergence of dicotyledonous weeds and had some effects on monocotyledonous weeds. The suppression was maintained until 30-40 days following transplanting, which is critical for weed control in cabbage cultivation. AC application to surface soil (5cm in depth) suppressed weed emergences more effectively than the application to the whole plow-layer of soil (15cm in depth). AC also adversely affected cabbage yields when applied in large amounts. To reduce the negative effects of AC on crop growth, an alternative method of AC application that replaces the AC-amended soil inside planting holes with AC-free soil was examined in a container experiment. AC application to the whole soil adversely affect the initial growth and the following cabbage head formation. The alternative AC application could mitigate adverse effects on initial growth, but partly affected the following stage. Therefore, AC has a potential to be used as an efficient biological weed control. However, further studies are necessary to determine the optimal AC application technique(s) and dose(s) for effectively suppressing weed growth while minimizing the effects on crop growth.

Introduction

A large amount of food waste is generated in home kitchens, restaurants, and school cafeterias. Food waste, typically, is treated by incineration or disposal in landfills, but it is increasingly being used as a renewable resource such as animal feed or fertilizer (compost). Following the enforcement and revision of the Food Waste Recycling Law, reuse as feed for animals could be the most effective method for processing food waste, but composting is also an important method, particularly in dealing with a mixture of various food wastes (Ministry of Agriculture, Forestry and Fisheries, 2012). An effective way to improve the low rate of household garbage recycling is on-site composting and agricultural use of the product (compost).

To process kitchen food waste (garbage), numerous composting apparatus have been developed that, typically, include heating and agitating devices to promote the decomposition of organic materials. However, several problems are associated with these devices, including foul odor emissions during composting and gradual decline in performance because of decrease in pH of the compost. To resolve these problems, Nishino et al. (2003) developed the Acidulo®composting method, whereby food wastes are treated under thermophilic and acidic conditions. This method enables continuous operation without periodic replacement of the substrate and also has the capacity to reduce odor emissions during composting. In the method, lactic acid bacteria comprise the majority of the microflora in the reactor (Asano et al., 2010; Hemmi et al., 2004). Greenhouse gas emissions from the process of Acidulo®composting from fish meal and the utilization of the compost were also evaluated (Tajima et al., 2013).

Previous studies evaluating the applicability of Acidulo®compost (AC; the product of the Acidulo®composting process) for agricultural purposes (Tatenai et al., 2006; Minamide et al., 2009)

showed that the nitrogen (N) content in AC is high because of the relatively low emissions of NH_3 gas during composting and that AC showed relatively high supply capacity of N. In a field study on a summer crop (potato), Ito et al. (2008) showed that AC application could suppress emergence and growth of summer weeds. These results suggest that AC can be used as a substitute for chemical herbicides in organic farming systems, and thus, can assist in minimizing environmental pollution risks.

In this context, we conducted field cultivation experiments to evaluate whether AC is effective in controlling weeds and whether it affects crop growth.

Materials and Methods

Star Engineering Co., Ltd. (Ibaraki Prefecture, Japan) provided the AC, which was made from the food wastes of a food service center using the AC apparatus (maximum daily load of food waste: 500 kg). In the composter, food waste (vegetable only) was added daily to the base material (sawdust inoculated with the starter microorganism *Alicyclobacillus sendaiensis* strain NTAP-1) and processed under heating and agitating conditions (Nishino et al., 2003). The chemical properties of the AC used in 2011 were as follows: total carbon, 458 g C kg^{-1}; total nitrogen, 24 g N kg^{-1}; and C/N ratio, 19. The properties of the AC in 2012 were as follows: pH, 4.6; EC, 9.8 dS•m^{-1}; total carbon, 459 g C kg^{-1}; total nitrogen, 30 g N kg^{-1}; C/N ratio, 15; and available P (Truog method), 2.5 g P_2O_5 kg^{-1}. Field studies were conducted in an arable field at the Field Science Center, Graduate School of Agricultural Science, Tohoku University. Soil (nonallophanic andosol) chemical properties were as follows: pH, 5.9; EC, 0.03 dS•m^{-1}; total carbon, 35 g C kg^{-1}; total nitrogen, 2.3 g N kg^{-1}; and available P (Truog method), 329 mg P_2O_5 kg^{-1}.

The effect of AC on weed emergence and growth was examined in field studies. We incorporated chemical fertilizer ($N:P_2O_5:K_2O$ = 20:20:20 g•m^{-2}) and lime into the entire plow layer of the soil (15 cm depth) over 2 years (Table 1). In 2011, we examined the effect of AC application rate on weed growth. AC was incorporated into the whole plow-layer of soil (15 cm in depth) at 0 (chemical fertilizer application, CF), 700 (AC700) and 1400 g•m^{-2} (AC1400) after making ridges. In 2012, we examined the effect of AC application methods (at a constant rate of 1000 g•m^{-2}) on weed and crop (cabbage) growth. The application methods involved four treatments (Figure 1): (1) mixing AC with the whole plow-layer of soil when making ridges (AC-W), (2) mixing AC with the whole plow-layer of soil when making ridges, but subsequently replacing the soil inside holes (63 mL) for transplanting cabbage seedlings with AC-free soils (AC-Wr), (3) mixing AC with surface soil (5 cm in depth) after making ridges (AC-S), and (4) without AC application (chemical fertilizer application: CF). Cabbage seedlings (*Brassica oleracea* var. *capitata*, cv. 'YR Seishun'; Watanabe Seed Co., Japan) were transplanted 30 cm apart in double lines (45 cm between lines) in rows with 180 cm between rows in September 2011 and June 2012. Growth and yield of four cabbages were determined with five replications for each treatment in both years, except for cabbage yield in 2012 where only two cabbages per treatment were used for the measurements. Weed populations of monocotyledonous and dicotyledonous weeds were measured at the center of the ridge (30 cm × 30 cm).

To develop an alternative application method of AC that mitigates negative effects on cabbage growth, a container experiment was conducted. Soil was collected from another arable field at the Field Science Center, air-dried, mixed with chemical fertilizer

Table 1. Application rates of AC and chemical fertilizers in field studies.

		Acidulocompost		Chemical fertilizer		
		dry weight	N	Urea	Superphosphate	Potassium sulfate
		g m^{-2}	g N m^{-2}	g N m^{-2}	g P_2O_5 m^{-2}	g K_2O m^{-2}
2011	CF			20	20	20
	AC700	700	16.5	20	20	20
	AC1400	1400	32.9	20	20	20
2012	CF			20		20
	AC-W	1000	29.8	20		20
	AC-Wr	1000	29.8	20		20
	AC-S	1000	29.8	20		20

AC, Acidulo®compost; AC700 (AC1400), AC application at 700 (1400) g•m^{-2}; AC-W, AC application to whole plow layer of soil; AC-Wr, AC application to whole plow layer of soil with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; AC-S; surface application (5-cm depth) of AC; CF, chemical fertilizer application (control).

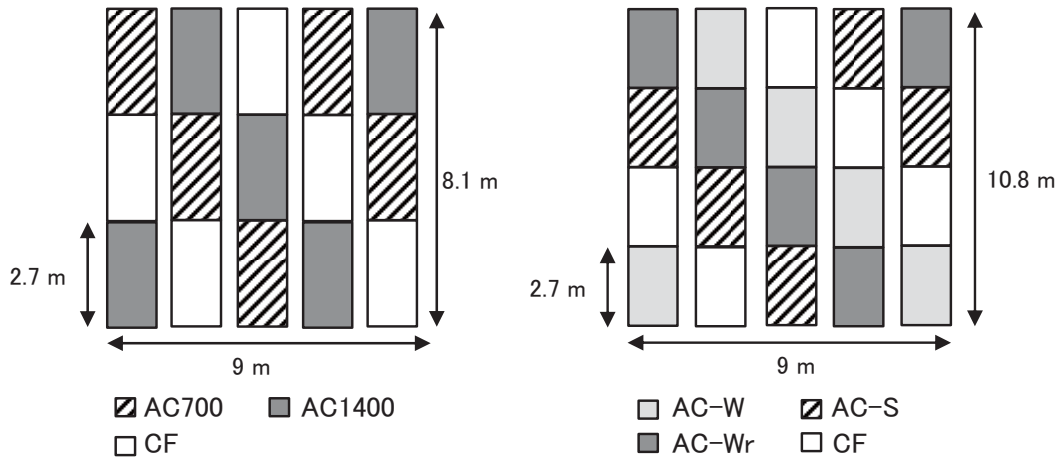

Figure 1. Designs of field studies conducted in 2011 (left) and 2012 (right).

AC, Acidulo®compost; AC700 (AC1400), AC application at 700 (1400) g•m^{-2}; AC-W, AC application to whole plow layer of soil; AC-Wr, AC application to whole plow layer of soil with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; AC-S; surface application (5-cm depth) of AC; CF, chemical fertilizer application (control)

($N:P_2O_5:K_2O$ = 0.22:0.22:0.22 g•kg^{-1}), and filled in a 1/5000 a Wagner pot (1830 g air-dried soil per pot). In the experiment, three AC application methods (at a constant rate of 15.3 g•kg^{-1}) were compared: AC application (AC), AC application with the soil in the vicinity of the planting hole (63 mL) replaced with AC-free soil (AC-r), and with no AC application (chemical fertilizer application, CF; see Figure 4). Three pots were used for each treatment. After the adjustment of water content to 40% of the water holding capacity, a cabbage seedling (*Brassica oleracea* var. *capitata*, cv. 'YR Seishun'; Watanabe Seed Co., Japan) was transplanted into each pot and cultivated in a greenhouse.

Experimental results were statistically verified using one-way analysis of variance (R ver. 3. 0. 2; R Development Core Team, 2008). Means were compared between treatments by Tukey's test at a confidence level of $P < 0.05$.

Results and Discussions

Weed emergence suppression by AC application

In the field studies, weed emergence was suppressed by AC application (Figure 2). In 2011, following AC application, the populations of monocotyledonous weeds decreased, albeit not significantly, to 56% (AC700) and 47% (AC1400) of the control (CF) treatment. Emergence and growth of dicotyledonous weeds were significantly suppressed to 48% (AC700) and 32% (AC1400) by AC application. The suppression of weed emergence by AC applications was maintained until 45 days after transplanting. Weed populations under cabbage leaves in the control (CF) also decreased 1 month after transplanting. In 2012, monocotyledonous weed populations were low (not significant) in three AC application methods 16 days after transplanting. The populations of dicotyledonous weeds were significantly suppressed in AC-W (43%), AC-Wr (57%), and AC-S (29%) compared to that in CF 16 days after transplanting and remained lower until 30 days after transplanting. These results suggest that the initial 30-40 days following transplanting are critical for weed control in cabbage crops and that AC application effectively controlled weed emergence in that period.

Among weed species, the emergence of the monocotyledonous weeds *Digitaria ciliaris* and *Echinochloa crus-galli*, and the dicotyledonous weeds *Chenopodium album*, *Stellaria neglecta*, and *Polygonum longisetum* were relatively (though not significantly) suppressed by AC applications in 2012 (Figure 3). Because these species accounted for a large proportion of weeds in summer at the study sites, it is unclear whether the reason for these results could be that AC application specifically affected the growth of these weed species or that the effects were nonspecific, but the AC application period was appropriate for suppressing the emergence of these species.

Compost is typically defined as the aerobically stabilized or matured organic matter and is used for crop

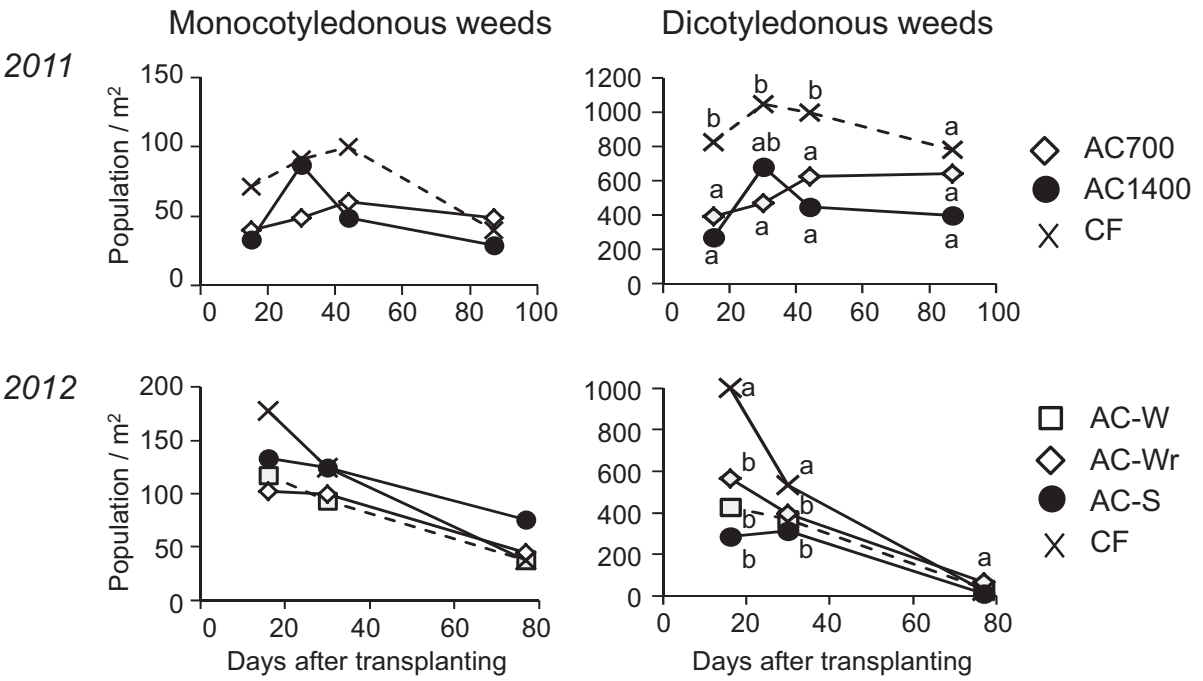

Figure 2. Monocotyledonous and dicotyledonous weed populations in cabbage cultivation.

Means followed by different letters are significantly different at $P < 0.05$ based on Tukey's test. AC, Acidulo®compost; AC700 (AC1400), AC application at 700 (1400) g•m^{-2}; AC-W, AC application to whole plow layer of soil; AC-Wr, AC application to whole plow layer of soil with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; AC-S; surface application (5-cm depth) of AC; CF, chemical fertilizer application (control)

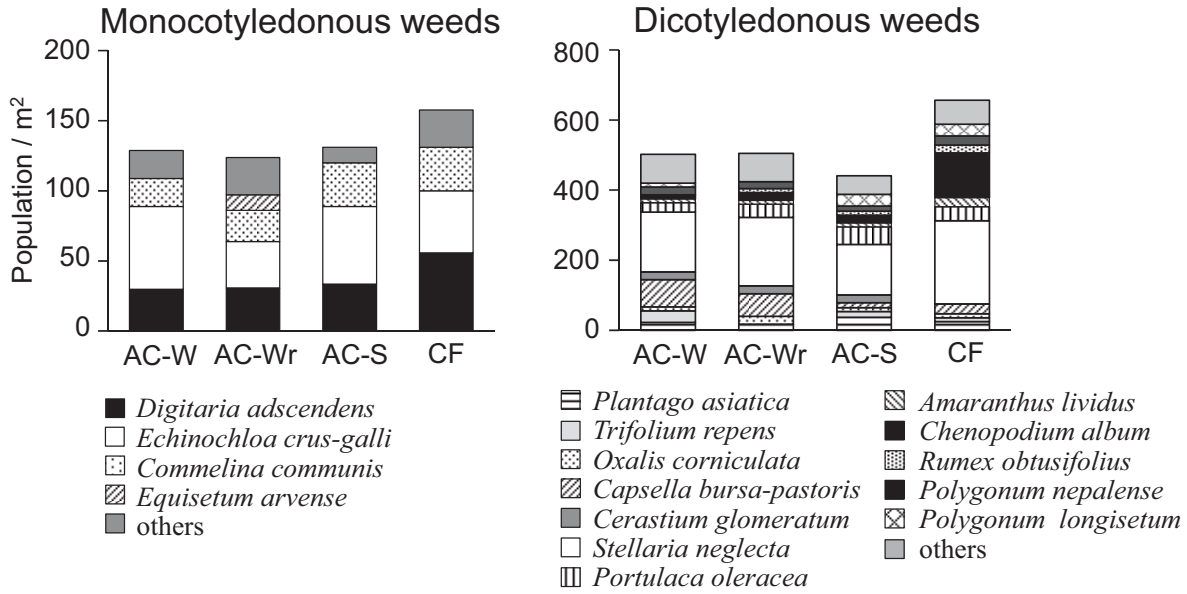

Figure 3. Populations of monocotyledonous and dicotyledonous weed species in a field study in 2012 (30 days after transplanting).

AC, Acidulo®compost; AC-W, AC application to whole plow layer of soil; AC-Wr, AC application to whole plow layer of soil with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; AC-S; surface application (5-cm depth) of AC; CF, chemical fertilizer application (control).

Table 2. Cabbage yields in field studies in 2011 and 2012.

		Head fresh weight g			Leaf fresh weight g		
2011	CF	387.5	(90.7)	a	483.8	(100.3)	a
	AC700	403.9	(160.3)	a	511.1	(93.1)	a
	AC1400	247.2	(88.5)	b	476.6	(83.0)	a
2012	CF	676.2	(48.2)	a	300.7	(138.9)	a
	AC-W	536.1	(112.5)	a	303.1	(202.6)	a
	AC-Wr	555.3	(67.1)	a	228.7	(299.2)	a
	AC-S	612.9	(129.3)	a	309.2	(257.4)	a

Numbers in parentheses indicate standard deviation. Means followed by different letters are significantly different at $P < 0.05$ based on Tukey's test. AC, Acidulo®compost; AC700 (AC1400), AC application at 700 (1400) g•m^{-2}; AC-W, AC application to whole plow layer of soil; AC-Wr, AC application to whole plow layer of soil with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; AC-S; surface application (5-cm depth) of AC; CF, chemical fertilizer application (control).

cultivation to enhance plant growth. Thus, mature composts commonly have almost no negative effects on crop growth. A lot of studies have been conducted for compost maturity (Hase and Kawamura, 2012; Jiménez and Garcia, 1989; Komilis and Tziouvaras, 2009). On the other hand, immature compost may be detrimental to plant growth by releasing high concentrations of acetic, butyric, propionic acids, or other organic compounds (Devleeschauwer et al., 1981; Marambe et al., 1993; Ozores-Hampton et al., 2001; Schuman and Mccalla, 1976). However, there is considerable potential for the phytotoxic ability of immature composts as a biocontrol agent against weeds. Ozores-Hampton et al. (2001) reported that the mulch of immature municipal solid waste-biosolids (MSW) compost could suppress weed germination and subsequent weed growth in greenhouse and field experiments. In their field study, the mulch at 7.5cm or greater thickness completely inhibited weed germination and growth for 8 months without affecting zucchini yield, due to the physical covering for the soil surface and/or the phytotoxic activity of the immature MSW compost. In our studies, AC applications significantly suppressed weed emergences. These findings suggest that phytotoxic ability of AC or immature composts can be available for weed control.

Effective AC application method to mitigate phytotoxic effects on cabbage growth

Since AC derived from garbage has phytotoxic potential, it is not only useful for weed control but is also potentially harmful to crop growth. In 2011, cabbage growth was not affected by the application of a relatively small amount of AC (AC700: 700 g•m^{-2}) but was significantly affected by the application of a larger amount in 2011 (AC1400: 1400 g•m^{-2}; Table 2). In 2012, we found no significant differences in cabbage yields, but the head weight was relatively low in AC-W (79%) and AC-Wr (82%), whereas that in AC-S (91% of CF) was similar to CF. A reason for lower effects on cabbage growth in the AC-S treatment could be that the surface application of AC (at 5 cm depth) scarcely affects root development below 5 cm. These results suggest that although AC application can adversely affect not only the growth of weeds but also the growth of cabbages, it is possible to minimize adverse effects of AC on cabbage growth by optimizing the dose and the method of application.

An alternative method of AC application to mitigate adverse effects on cabbage growth was examined by comparing the common treatment (AC: AC incorporation into the whole soil), the alternative treatment (AC-r: replacement of the soil inside seedling holes with AC-free soil), and control (CF; Figure 4). Cabbage growth was significantly suppressed in AC from 9 (for leaf length and width) or 14 days after transplanting (for shoot length), while the cabbage in AC-r grew well, similar to that in the CF until 22 days after transplanting (Table 3). This suggests that the replacement of the soil in the planting hole with AC-free soil can mitigate the adverse effects of AC on the initial growth of cabbage. However, cabbage growth in AC-r was significantly suppressed from 22 days

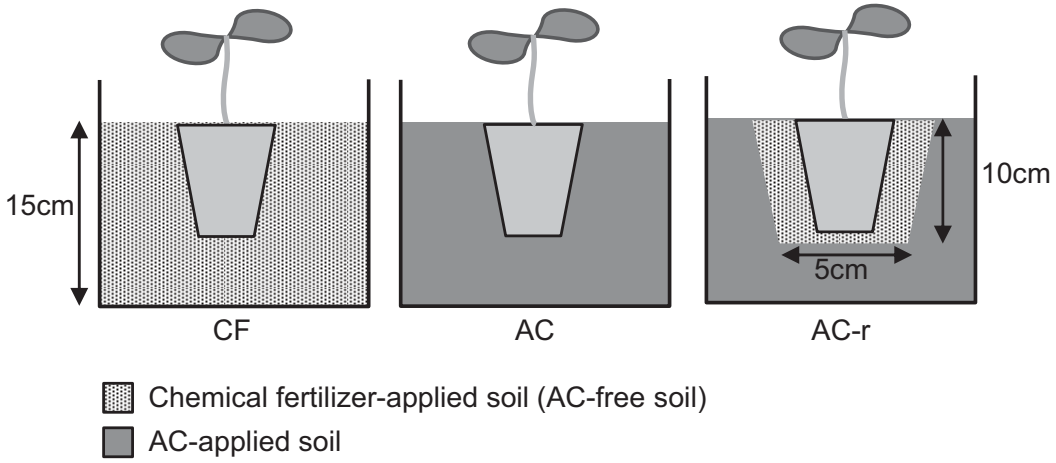

Figure 4. Acidulo®compost application methods in a container (1/5000 a Wagner pot) experiment in 2011.

AC, Acidulo®compost; AC, AC application; AC-r, AC application with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; CF, chemical fertilizer application (control).

Table 3. Cabbage growth in a container experiment in 2012.

		Days after transplanting						
		0	9	14	22	29	40	43
No. of leaves	CF	5.0 a	7.0 a	8.3 a	11.5 a	13.8 a	13.8 a	12.0 a
	AC	5.3 a	6.3 a	7.8 a	10.8 a	13.3 a	13.5 a	12.0 a
	AC-r	5.3 a	6.5 a	7.8 a	10.5 a	12.8 a	13.0 a	11.3 a
Shoot length (mm)	CF	16.5 a	17.9 a	20.9 b	25.3 b	26.8 a	28.8 b	27.9 b
	AC	15.1 a	16.4 a	16.6 a	22.1 a	24.9 a	26.1 a	25.6 a
	AC-r	14.9 a	16.4 a	18.9 ab	23.7 ab	25.4 a	26.3 a	25.8 a
Leaf length (mm)	CF	8.9 a	9.7 b	12.2 b	15.4 b	17.6 b	18.1 b	17.3 a
	AC	8.2 a	8.7 a	9.8 a	13.2 a	16.0 a	16.6 ab	16.8 a
	AC-r	7.9 a	9.4 ab	11.4 ab	14.8 b	15.4 a	16.0 a	16.3 a
Leaf width (mm)	CF	5.3 a	6.5 b	9.6 b	12.6 a	14.6 a	16.4 b	16.3 b
	AC	5.0 a	5.4 a	7.5 a	11.6 a	16.2 a	14.5 a	14.4 a
	AC-r	4.8 a	6.2 b	9.2 b	13.4 a	13.8 a	14.7 a	14.5 a
SPAD	CF		40.9 a	46.0 a	49.8 a	54.6 a	54.2 a	56.2 b
	AC		39.7 a	46.1 a	47.8 a	53.2 a	51.9 a	53.1 a
	AC-r		42.2 a	45.2 a	47.2 a	54.1 a	52.6 a	52.6 a

Means followed by different letters are significantly different at $P < 0.05$ based on Tukey's test. AC, Acidulo®compost; AC, AC application; AC-r, AC application with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; CF, chemical fertilizer application (control).

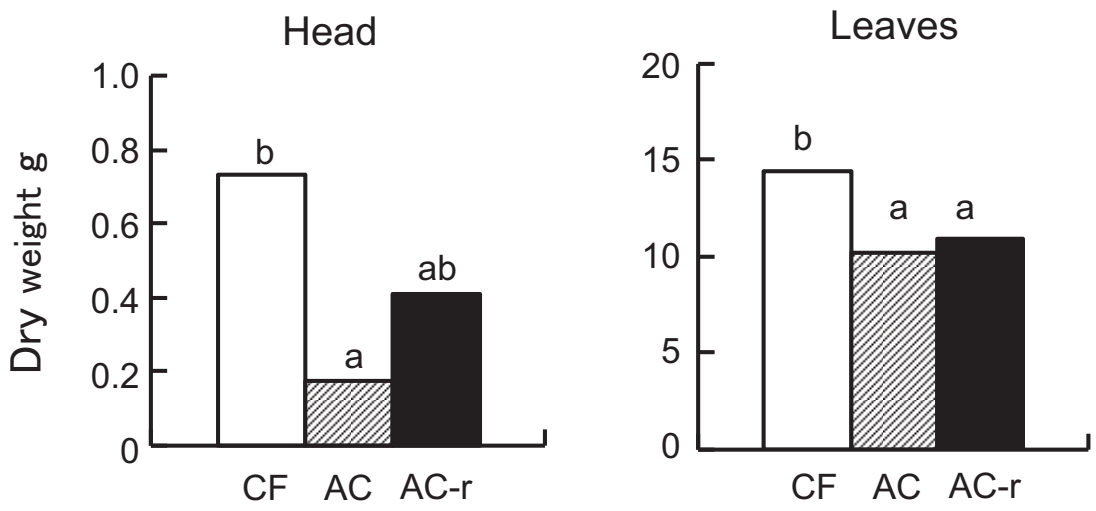

Figure 5. Weights of cabbage heads and leaves in a container experiment (45 days after transplanting) in 2012.

Means followed by different letters are significantly different at $P < 0.05$ based on Tukey's test. AC, Acidulo®compost; AC, AC application; AC-r, AC application with the replacement of the soil in the vicinity of the planting hole with an AC-free soil; CF, chemical fertilizer application (control).

after transplantation onward. The mean weight of cabbage heads was significantly lower in AC (but not in AC-r) than in CF (Figure 5) at 45 days after transplanting. As mentioned above, cabbage yields in the field experiment in 2012 (Table 2) were higher (not significant) when AC was surface-applied (AC-S) than that under other AC applications. These results suggest that it is important to avoid exposing cabbage roots to AC in the initial growth period. In addition to the application method, the dose of AC was also critical in suppressing weed growth while minimizing adverse effects on crop growth (Figure 2 and Table 2). Although there is controversy for applying phytotoxic organic materials to a cultivated field, AC has a potential to be used as an efficient biological weed control method, which can contribute to reduction of herbicide usage. Further studies are necessary to develop a more effective AC application technique that optimizes both the dose and the method of application for cabbage.

Conclusion

AC, a food waste material processed under thermophilic and acidic conditions, has the potential to be used in weed control. During cabbage cultivation, weed emergence was significantly suppressed by AC for 1 month after its application. Because the phytotoxic properties of AC may adversely affect crop yields, methods for the optimal application of AC must be developed. An alternative application method of AC that replaces the AC-amended soil inside planting holes with AC-free soil was effective in partly mitigating adverse effects on the growth of cabbage seedlings. Further studies are necessary to develop an appropriate AC application method that can suppress weed growth effectively without reducing crop yields.

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The 11th International Symposium on Integrated Field Science

“Utilization of Resources and Environmental Protection”

Date: August 1-2, 2013

Venue: Hotel Taikanso, Matsushima
10-76 Inuta, Matsushima, Matsushima-machi, Miyagi-gun, Miyagi, Japan

Organizer:
Field Science Center, Graduate School of Agricultural Science, Tohoku University
Project of Integrated Compost Science, Tohoku University
Innovative Research Center for Agricultural Sciences, Tohoku University
Animal Production Environment Society Japan

Co-Organizer:
Miyagi Prefectural Government, Agriculture, Forestry and Fisheries Department

Symposium Chair
Prof. Yutaka Nakai (Integrated Field Science Center, Tohoku University, Japan)

Organizer Committee:
Toyoaki Ito, Michiaki Omura, Fuyumi Tojo, Waka Fukunaga, Hideomi Minoshima
Office e-mail address: pics.symposium@gmail.com

Secretary General
Yutaka NAKAI
Professor,
Laboratory of Sustainable Environmental Biology,
Field Science Center,
Graduate School of Agricultural Science,
Tohoku University

Program

August 1

12:00-	Registration desk open
13:00-13:30	Opening Ceremony
13:30-17:30	Oral Presentation
	Chair Person: Yutaka Nakai (Tohoku University, Japan)
13:30-14:05	O-1. 3Research and development in composting and animal waste management in Taiwan Liang Chou Hsia (National Pingtung University of Science and Technology, ROC)
14:05-14:40	O-2. Research and development in composting and animal waste management including wastewater treatment, renewable energy and so on in Korea Myung Gyu Lee (Sangji University, Korea)
14:40-15:15	O-3. Agricultural waste management in Europe, with an emphasis on anaerobic digestion Heribert Insam (University of Innsbruck, Austria)
15:15-15:30-	------ Tea Break -------
	Chair Person: Masanori Saito (Tohoku University, Japan)
15:30-15:50	O-4. Application of airborne remote sensing to biomass estimation Chinatsu Yonezawa (Tohoku University, Japan)
15:50-16:10	O-5. Life Cycle Impact Assessment of Compost, and Possibility of IT solution Michiaki Omura (Tohoku University, Japan)
16:10-16:30	O-6. Microbial community dynamics during composting process of animal manure analyzed by molecular biological methods Nozomi Yamamoto (Tokyo Institute of Technology, Japan)
16:30-16:50	O-7. Small scale of anaerobic digestion for decentralized energy production and bioresource recycling Chika Tada (Tohoku University, Japan)
16:50-17:10	O-8. Effect of organic matter application on soil carbon sequestration in Japanese agricultural land Yasuhito Shirato (National Institute for Agro-Environmental Sciences, Japan)
17:10-17:30	O-9. New function of compost: Inhibitory effect of acidulocompost on weed germination and growth Takehiko Yamamoto (Tohoku Agricultural Research Center, Japan)
18:00-20:00	Banquet (Hotel Taikanso, Matsushima)

August 2

9:00-	Registration desk open
10:00-11:45	Poster Presentation
10:00-10:50	Odd number
10:55-11:45	Even number
11:45-12:00	Closing Ceremony

Poster Session

P-1	T. Takahashi H. Kanno M. Nanzyo	Tohoku University, Japan	Factors Affecting Organic Carbon Accumulation in Humus Horizons of Andosols from the Tohoku University World Andosol Database
P-2	G. Saito H. Seki K. Uto Y. Kosugi	Tokyo Institute of Technology, Japan	Development of a Hyperspectral Sensor on UAV for Biomass estimation at Costal Zone
P-3	H. Nasukawa T. Uno M. Saito R. Tajima T. Ito	Tohoku University, Japan	Effects of Bottom Sediment-Like Tsunami Deposit on Soil and Paddy Rice Growth
P-4	M. Saito	Tohoku University, Japan	Effect of Biochar on Crop Growth: Research in Japan
P-5	F. Takeda[1] K. Nakamura[2] K. Nakano[1]	[1]Nihon University, Japan., [2]Tohoku University, Japan	Contribution of Vegetation on Water Purification Performances in Constructed Wetlands
P-6	M. Nanzyo K. Ito	Tohoku University, Japan	Phosphates in the Air-dried Residues of Water or Dilute Acid Extracts from Compost
P-7	H. Kunishima M. Nanzyo H. Kanno T. Takahashi	Tohoku University, Japan	Improvement of P Recovery Rate in an Uncultivated Non-Allophanic Andisol Using Fermented Chicken Manure Pellets and P Foraging Root Growth of Japanese Radish
P-8	T. Ito[1] T. Yokota[1] N. Tanikawa[1,2] M. Saigusa[1,3]	[1]Tohoku University, Japan., [2]Aomori Prefectural Agriculture and Forestry Research Center, Japan., [3]Toyohashi University of Technology, Japan	Phosphate Bioavailability of Animal Manure Composts is Higher in Paddy Soil than in Upland Soil
P-9	R. Tajima[1] T. Yamamoto[2] M. Omura[1] Y. Nakai[1] T. Ito[1] M. Saito[1]	[1]Tohoku University, [2]Tohoku Agricultural Research Center, Japan	Assessment of Greenhouse Gas Emissions of the Production and Utilization of Acidulocompost from Fish Meal
P-10	S. Ogura T. Shishido S. Tanaka M. Tannai S. Sato K. Yusa	Tohoku University, Japan	Effect of Interannual Application of Cattle Manure Compost on Yield and Quality of Herbage and Soil Chemical Condition in a Temperate Grass Meadow
P-11	M. Nishida	NARO Tohoku Agricultural Research Center, Japan	Cattle Manure Compost Plays a Key Role in Keeping Soil Nitrogen Fertility in Paddy-Upland Rotation System

P-12	S. Ogaya[1] M. Nishida[2] K. Yasuda[1] Y. Kaneta[1] T. Sato[1] F. Takakai[1]	[1]Akita Prefectural University, Japan, [2]National Agricultural Research Center for Tohoku Region, Japan	Fate of Nitrogen Derived from Organic Materials Applied to Paddy and Converted Upland Fields -Results of the First Year Experiment-
P-13	W. Cheng Y. Okamoto S. Sato K. Kasahara K. Tawaraya H. Yasuda	Yamagata University, Japan	Combined Use of *Azolla* and Loach Suppressed Weed *Monochoria* vaginalis and Increased Organically Farmed Rice Yield
P-14	F. Sakurada T. Uno M. Saito R. Tajima T. Ito	Tohoku University, Japan	Positive Effects of Tubificid Worms on Rice Growth and Yield in Organic Farming System
P-15	S. Hara T. Shimizu T. Uno R. Tajima T. Ito M. Saito	Tohoku University, Japan	Phosphorous Uptake from Organic Matter via AM fungi - Possible Involvement of Phytate-Degrading Bacteria -
P-16	Y. Ito[1] F. Tojo[1] M. Watanabe[2] K. Watanabe[3] S. Hayashi[2] K. Seiwa[1] Y. Nakai[1] C. Tada[1]	[1]Tohoku University, Japan., [2]National Institute for Environmental Studies, Japan., [3]Center for Environmental Science in Saitama, Japan	Relationship between Nitrogen Concentration and Ammonia Oxidizing Microbes in the Japanese Cedar Forest Soils under Different Managements
P-17	F. Tojo Y. Ito C. Tada Y. Nakai	Tohoku University, Japan	Detection of Anammox Bacteria from Forest soils of Different Thinning Intensity
P-18	Y. Chiba Y. Fukuda C. Tada Y. Nakai	Tohoku University, Japan	Host Immune Response to Gastric *Cryptosporidium*
P-19	R. Sato[1] T. Takahashi[2] K. Numazu[2] T. Wakatsuki[3] Y. Morita[3] A. Komura[3] C. Tada[1] Y. Fukuda[1] Y. Nakai[1]	[1]Tohoku University, Japan., [2]NOSAI Miyagi, Japan., [3]NOSAI Okayama, Japan	Phylogenetic Analysis of *E. zuernii* and *E. bovis* with Nuclear 18S rRNA and Mitochondrial CO1 Genes

P-20	S. T. Nguyen[1, 2, 3] Y. Fukuda[2] C. Tada[2] V. V. Huynh[1] D. T. Nguyen[1] Y. Nakai[2]	[1]Central Vietnam Veterinary Institute, Vietnam., [2]Tohoku University, Japan., [3]Japan Society for the Promotion of Science, Japan	Prevalence and Molecular Characterization of *Cryptosporidium* in Domestic Animals in Central Vietnam
P-21	S. T. Nguyen[1, 2, 3] Y. Fukuda[2] C. Tada[2] V. V. Huynh[1] D. T. Nguyen[1] Y. Nakai[2]	[1]Central Vietnam Veterinary Institute, Vietnam., [2]Tohoku University, Japan., [3]Japan Society for the Promotion of Science, Japan	Prevalence and Molecular Identification of *Fasciola* in Cattle in Central Vietnam.
P-22	T. Kotogaoka Y. Fukuda C. Tada Y. Nakai	Tohoku University, Japan	Molecular Epidemiological Survey for Zoonotic Parasite Infection in Livestock in the Tuul Basin in Mongolia
P-23	C. G. Lee Y. Baba C. Tada Y. Nakai	Tohoku University, Japan	Investigation of Microbial Community Analysis during the Decomposition of Lignocellulosic Biomass Using Rumen Fluid
P-24	Y. Shimura[1] R. Asano[1] W. Kawada[1] Y. Hamano[1] T. Ohdan[1] J. Fukushima[1] Y. Baba[2] C. Tada[2] Y. Nakai[2] T. Inamoto[1]	[1]Akita Prefectural University, Japan., [2]Tohoku University, Japan	Comparison of Bacterial Flora in a Methane Fermentation Plant in Field Science Center at Tohoku University and Bovine Rumen Used as the Plant Microbial Resource by 16S rRNA Gene Sequencing
P-25	G. Yoshida K.Kimura C. Tada	Tohoku University, Japan	Improvement of the Anaerobic Digestion Performance for Fish Industry Waste Recycles
P-26	T. Suzuki C. Tada	Tohoku University, Japan	Effects of Carrier on the Performance of a Methane Fermentation System Using heat from a Hot Spring
P-27	Y. Baba[1,2] C. G. Lee[1] C. Tada[1] Y. Fukuda[1] Y. Nakai[1]	[1]Tohoku University, Japan., [2]Research Fellow of the Japanese Society for the Promotion of Science (JSPS), Japan	Pretreatment of Lignocellulosic Rapeseed Waste by Rumen Fluid for Methane Production

Factors Affecting Organic Carbon Accumulation in Humus Horizons of Andosols from the Tohoku University World Andosol Database

Tadashi Takahashi, Hitoshi Kanno and Masami Nanzyo

Tohoku University, Japan

Introduction Andosols or Andisols accumulate large amounts of soil organic matter (SOM). It is generally considered that SOM stabilization is related to the formation of organo-mineral (such as allophanic materials) complexes and/or organo-metallic (Al and Fe) complexes, physical protection of SOM by high porosity of the soils, and the low activity of soil microorganisms due to the low soil pH and the high level of toxic Al. Among the factors affecting SOM accumulation, the Al-humus complexes are considered to be the most important. Nanzyo et al. (1993) confirmed the close correlation (r = 0.84, P < 0.01) between pyrophosphate-extractable Al and organic carbon (OC) concentration in A horizon soils using data from the Andisol Tohoku University Database (Shoji et al. 1993). In this study, to assess the other factors including soil pH, aluminum toxicity, or short-range-ordered minerals for the accumulation of OC, we performed a path analysis using the database.

Materials and Methods

Database: We used the Tohoku University World Andosol Database (2002 version) that is the revised version of the Andisol Tohoku University Database (Shoji et al. 1993). From the database, we extracted 293 humus horizons (A or buried A horizons) of volcanic soils distributed in Chili, Ecuador, the California and Alaska States of the U.S.A., New Zealand, Indonesia, Taiwan and Japan.

Statistical Analysis: The path diagram in Fig. 1 was used to examine the causal path of selected soil properties to the OC content. The selected explanatory variables included the pH(H_2O), 1 M KCl-extractable aluminum (KCl-Al), sodium pyrophosphate-extractable Al and Fe (Al_p and Fe_p), and acid oxalate-extractable Si (Si_o). The direct effects of the soil properties on the OC are termed "path coefficient" and are standardized partial regression coefficients for each of the soil properties in the multiple linear regression versus the OC. The indirect effects of the soil properties on the OC were determined from the products of the simple correlation coefficient between the soil properties and the path coefficient.

Results The path analysis showed that a high correlative coefficient between the OC content and Al_p (r = 0.69, P < 0.01) was mainly explained by the direct effect of Al_p (0.52, P < 0.01) on the OC content. Strong correlations between OC and KCl-Al (r = 0.60, P < 0.01) or pH(H_2O) (r = -0.58, P < 0.01) were due not only to the direct effect (0.21 and -0.27, respectively, P < 0.01), but also to the indirect effects of other properties, especially that of Al_p. Thus, it is considered that, in the humus horizons of the Andosols, the Al-humus complexation mainly contribute to the OC accumulation, and the low soil pH values and Al toxicity partly relate to the humus reservation by depressing the microbial activity.

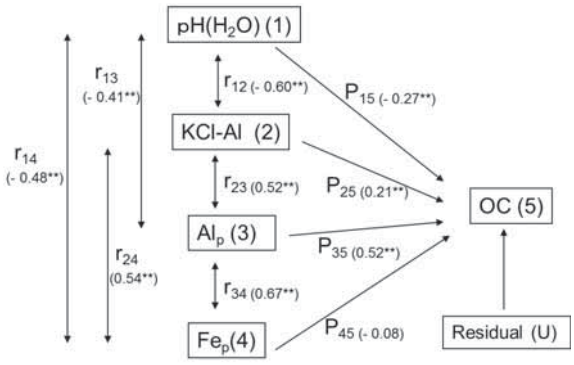

Fig. 1. Path analysis diagram for the relationships between organic carbon (OC) content and soil properties. The path coefficient (P_{ij}) of soil properties is represented by single-headed arrows, and the simple correlation coefficients (r_{ij}) between variables are represented by double headed arrows. **Significant at P < 0.01.

Development of a Hyperspectral Sensor on UAV for Biomass estimation at Costal Zone

Genya Saito, Haruyuki Seki, Kuniaki Uto and Yukio Kosugi

Tokyo Institute of Technology, Japan

In our previous studies, a hyperspectral array sensor system for an unmanned radio-controlled helicopter was developed. The specifications of the hyperspectral sensor were 400-1000 nm spectral range, 121 bands, 5 nm spectral resolution, and 11 kg weight. The system was controlled by note PC and total weight was about 20 kg. The unmanned radio-controlled helicopter was RMAX 18 (Yamaha Co.,), the mass of vehicle was 64 kg, and the payload was 30 kg. We used the system for agricultural studies about growth estimation and nutritional diagnosis at the paddy fields. The unmanned radio-controlled helicopter was operating by the special members belonging to Yamaha Co. and that makes limited opportunity at observing period. Recently new UAVs are progressing such as cycle body type with 4 propellers, and a small unmanned airplane. We want to use this kind of platform freely. However, the payloads of these new platforms are about below 15 kg. We determined to develop a low cost, small-sized and light weight hyperspectral sensor system for loading on small UAV platforms. We made a prototype measurement system last year and are modifying now. The system is composed of a hyperspectral sensor unit, GPS sensor unit, data logger unit, power supply, information display unit, and control switch, and total weight was 8 kg. Hyperspectral sensor unit has lens, spectrometer, AD converter. The spectrometer was Mini-spectrometer MS-C10988MA made by Hamamatsu Photonics Co., Ltd. (327-763nm wavelength range, wavelength resolution 14nm, 256band, 320μsec acquisition period, and 9g weight). At three paddy rice field in Yamagata Prefecture, on 12-14 hour of August 7, 2012, we conducted a spectral observation using the system. We set up the sheet of orange and white of reflectance known to each course. Observations were performed in the cloudy time and solar brightness was changing, but due to the reflectance calculation method with respect to the sheet measurements, and corresponded to sunlight change. We got the spectral reflectance of rice, white sheet, and orange sheet, and the spectral reflectance curves were accurately reflect the spectral characteristics of each target.

Effects of Bottom Sediment-Like Tsunami Deposit on Soil and Paddy Rice Growth

Hisashi Nasukawa, Toru Uno, Masanori Saito, Ryosuke Tajima and Toyoaki Ito

Tohoku University, Japan

Introduction

Tsunami caused by Tohoku Region Pacific Coast Earthquake damaged extremely huge area of farmlands (23,600 ha) in Tohoku region. Tsunami brought seawater and tsunami deposits in farmlands. Clayey tsunami deposits, partly composed of bottom sediments, has potential to increase soil salinity because they can hold much seawater and are not easily desalinated by rain or irrigation water. Although the deposit should be removed from farmlands, in fact they are incorporated into the surface soil when their thickness are less than 5 cm. Objective of this study is to investigate the effect of clayey tsunami deposits on soil and the rice growth when they are incorporated into soil.

Materials and Methods

Clayey tsunami deposit was collected from a tsunami-attacked field of Higashi-matsushima city, Miyagi in April, 2012. The deposit had a high content of salt with high electric conductivity (EC, material to water ratio of 1:5) of 3.7 dS m^{-1} and a part of the deposit was desalinized by fresh water into 0.28 dS m^{-1}. The original and desalinized deposits were mixed at the ratio of 1:5 (deposit: soil, v/v) with paddy soil (non tsunami-affected Alluvial soil) and packed into 1/2000a pot. A control treatment (no added deposit) was also prepared. We measured rice growth and yield, and silica concentrations in soil solutions and exchangeable cations of soils after harvesting.

Results

The desalinized deposit addition treatment (DD) enhanced rice growth (stem number per hill) and brown rice yield due to the increase of nitrogen mineralized from the tsunami deposit, while the original deposit addition treatment (OD) decreased rice growth and yield compared with the control treatment due to severe salt injury. Silica concentrations in soil solutions were kept higher in OD and DD treatments than the control soil. Exchangeable magnesium and sodium of soils extremely increased with the addition of original tsunami deposits and increased a little bit in the DD treatment compared with the control treatment. It was concluded that the clayey tsunami deposit gave decomposable organic nitrogen, soluble magnesium and silica to surface soils as nutrients and increased rice growth, but caused salt injury mainly due to the increase of soluble sodium in soil when it was incorporated into soil without desalinization.

Effect of Biochar on Crop Growth: Research in Japan

Masanori Saito

Tohoku University, Japan

Biochar is defined as carbonized biomass material (charcoal), especially when it is applied to soil as a soil amendment. Biochar has been traditionally used as a soil amendment in Japan as well as in other East and Southeast Asian countries. In Japan, biochar has been officially recognized as one of the Ministerial Ordinance-Designated soil amendments by MAFF, Japan, because of its improvement function of soil physical properties. In this poster I will discuss current research on biochar in Japan.

In 1980's, Dr. Makoto Ogawa shed light on "biochar" from the soil microbiological viewpoint (Ogawa & Okimori, 2010). Charcoal is a porous material with high water and air retention capacities and high alkalinity. He found that it stimulated root growth and enhanced the infection of various symbiotic microbes such as arbuscular mycorrhizal (AM) fungi. This finding promoted to use biochar to enhance indigenous AM fungi (i.e. Saito, 1990).

Recently, the findings of Amzonian Dark Earth Soil (*terra preta*), which may be formed by the addition of carbonized material from human waste, has promoted the research on biochar, because application of biochar to soil may contribute to long-term carbon storage, i.e., carbon sequestration to produce negative CO_2 emissions from soil (Lehman, 2006). So, it is now widely believed that biochar has the potential not only to enhance soil fertility but also to help mitigate climate change, via carbon sequestration.

Shindo et al. found that a large proportion of soil C in humus-rich Andisols is present as charcoal like materials, and that charcoal may be an important fraction in soil carbon storage (Miyazaki et al. 2010). In Japan, however, it is still not clear if biochar applied as soil amendment may contribute to long term C storage (Ueno et al. 2012).

Microbiological effect of biochar application has been suggested (Konno and Nishikawa 1993), while in most cases the effect of biochar on crop growth may be due to its improvement of soil chemical or physical properties. It should be stressed that, in spite of a lot of research on biochar application, soil chemical and physical properties are not well documented. Therefore, it is often difficult to clarify the mechanisms of biochar application on crop growth.

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Contribution of Vegetation on Water Purification Performances in Constructed Wetlands

Fumihiko Takeda[1], Kazunori Nakamura[2] and Kazunori Nakano[1]

[1]Nihon University, Japan
[2]Tohoku University, Japan

Objective To make clear the contribution of vegetation to water purification performance in constructed wetlands, the annual removal performances of BOD, TKN, TN and TP in planted and unplanted wetland for the first three years of operation were compared.

Methods A five-stage vertical flow constructed wetland system treating milking parlor wastewater derived from 30 milking cows was used in this study. The total area of constructed wetland was 111m^2. Average influent concentration of BOD, total nitrogen (TN), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) during three years of operation was 1490, 93, 93 and 19.7mg/l, respectively. Cumulative hydraulic loading during three years operation reached 2430m^3, resulting in the average daily hydraulic loading of 2.15m^3/d. The location of the constructed wetland has snowfall from December to March. The lowest daily average atmosphere temperature was below -6 ºC and the maximum snow lay was over 60cm deep. Even under such cold-climate conditions, the treatment had never stopped without any problems except 58 days of pause when the earthquake on March 11, 2011 made operation of the wetland impossible.

Results Removal performance of BOD, TN TKN and TP in planted and unplanted wetland was compared in Fig.1, Fig.2, Fig.3 and Fig.4, respectively. It was common for both planted and unplanted wetland in which the removal performance was improved in the 2nd year. There was no significant difference in BOD removal performances between planted and unplanted wetland. Difference in TN removal was, however, significant in the 2nd year, while that in the 3rd year was not. On the other hand, the effect of vegetation became significant in the 3rd year for TKN and TP removal. These differences in removal performance of TKN, TN and TP between planted and unplanted wetland were proved by statistical significant levels ($P<0.05$). The results of this study confirmed that presence of vegetation leads to a positive effect on TKN, TN and TP removal in constructed wetland even without plant harvesting process.

Acknowledgements This study was supported by the Global COE Program "Center for ecosystem management adapting to global change" and Tohoku Ecosystem-Associated Marine Sciences of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

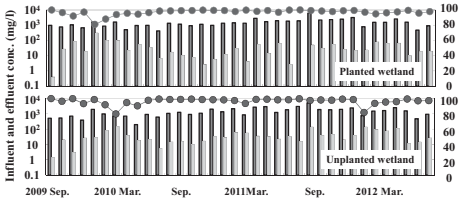

Fig.1 BOD removal performance for 3 years in planted and unplanted wetland.

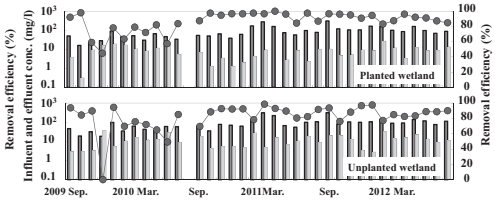

Fig.2 TN removal performance for 3 years in planted and unplanted wetland.

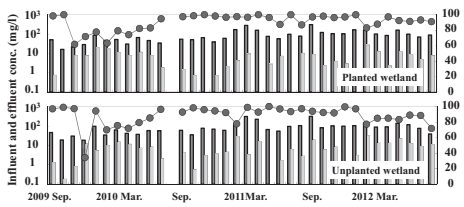

Fig.3 TKN removal performance for 3 years in planted and unplanted wetland.

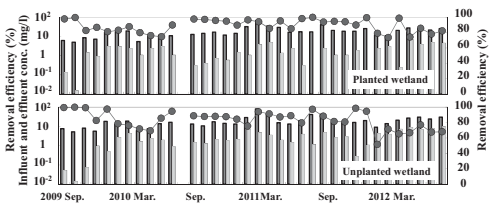

Fig.4 TP removal performance for 3 years in planted and unplanted wetland.

Phosphates in the Air-dried Residues of Water or Dilute Acid Extracts from Compost

Masami Nanzyo and Kumiko Ito

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The content of P in compost is reported to be 4.43-48.6 g kg^{-1} and P is mostly inorganic (Yokota *et al*., 2003). With a rise in prices of chemical P fertilizers, P in the composts is expected to play a role of P fertilizer. So far, chemical forms of P in the compost have been estimated to be $CaHPO_4.2H_2O$ or $MgNH_4PO_4.6H_2O$ (Tanahashi *et al*., 2010). However, it is not very facile to observe and detect these crystalline phosphates directly in cattle manure compost (CMC) using an electron microscope or X-ray diffraction. The objective of the present study was to examine chemical forms of P in the air-dried residue of water or dilute-acid extracts of compost.

Air-dried CMC, a product of Kawatabi Field Science Center, Graduate School of Agricultural Science, Tohoku University and commercially available fermented chicken manure pellets (FCMP) were ground to pass a 2 mm sieve. To 15 g each of these composts, 50, 49.25, 45, 40, 30 mL of pure water and 0, 0.75, 5, 10, 20 mL of 1 mol L^{-1} HCl, respectively, were added, and shaken by hand. Regarding FCMP, 20 mL of 2 mol L^{-1} HCl was used instead of 20 mL of 1 mol L^{-1} HCl because of its high carbonate content. Then, the mixtures were allowed to stand at room temperature for 24 h with occasional swirling. After measurement of suspension pH, solutions were separated by filtration using Toyo No.5C filter paper. After measuring electric conductivity of the solutions, Ca, Mg, Na and K were analyzed by atomic absorption spectrometry and P concentration was determined by the molybdenum blue method. The remaining solutions were air-dried. After removing highly soluble salts from the air-dried residues with small amount of water, the residual precipitates were examined by X-ray diffraction, scanning electron microscope observation and energy dispersive X-ray analysis.

The amounts of Ca, Mg, K and Na extracted from two composts were greater than those of P. Among these, the amounts of Ca, Mg and P increased with decreasing pH of the extracting solution, compared with those of K and Na. Crystalline phosphates found in the air-dried residues from CMC were $MgKPO_4.6H_2O$, $MgHPO_4.3H_2O$, and $CaHPO_4.2H_2O$, depending on extracting pH. Non-crystalline Ca phosphate was included in the precipitates from acidic extracts. In the air-dried residues from FCMP, non-crystalline phosphates were found as major components, and small amounts of $MgNH_4PO_4.6H_2O$ and $CaHPO_4.2H_2O$ were also detected.

Improvement of P Recovery Rate in an Uncultivated Non-Allophanic Andisol Using Fermented Chicken Manure Pellets and P Foraging Root Growth of Japanese Radish

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In Andisols, most of the fertilized phosphorus is sorbed by active Al and Fe, and P recovery rate is low in general. Because of this, P fertilizer was applied heavily and P has accumulated in the soils. There is concern about depletion of phosphate rock resources in the near future. Therefore, it is necessary to improve the P efficiency in the agricultural systems. The lateral roots of Brassica rapa nothovar. grow around P fertilizer and cover P fertilizer as if the roots are foraging P in P deficient soil (Nanzyo et al. 2004 Plant and Soil 265, 325-333). Japanese radish also shows this feature, and the radish shows higher P recovery rate than the average rate. The objective of this study was to examine the effect of fermented chicken manure pellets (FCMP) on growth of the radish and the P recovery rate in comparison with the alginate gel beads in which dicalcium phosphate dehydrate was suspended (DCPD-GB).

Japanese radish was planted in wooden frames, 120 cm long, 50 cm wide and 50 cm deep, filled with Kanuma pumice as subsoil and an uncultivated non-allophanic Andisol, collected from Integrated Terrestrial Field Station of Tohoku University, Ohsaki City, Miyagi Prefecture, Japan, as a plow layer soil. Three treatments were applied to the soil as large DCPD-GB (about 1.5 cm in diameter), small DCPD-GB (about 0.8 cm in diameter) and nil P in spring crop. The amount of applied P in the DCPD-GB treatments was 1.0 g P_2O_5 per plant. In autumn cropping, FCMP was applied 0, 0.25, 0.75, 1.5 g P_2O_5 per plant.

Lateral roots of radish completely covered DCPD-GB. But the diameter of taproot was less than 7 cm that is a typical taproot diameter of commercial Japanese radish. The P recovery rate obtained by the large GB and small GB treatment was 21% and 28%, respectively. These rates were higher than typical rate.

In the treatments with FCMP, lateral roots of radish covered FCMP. In addition, the lateral roots penetrated into the pellet. In the treatment with 1.5 g P_2O_5 FCMP, the diameter of taproot exceeded 7 cm. P recovery rate by the radish in the treatment with 0.25 g P_2O_5, 0.75 g P_2O_5 and 1.5 g P_2O_5 was 32%, 42% and 35%, respectively. Thus, the Japanese radish with FCMP showed higher P recovery rate than the rate shown with DCPD-GB.

Phosphate Bioavailability of Animal Manure Composts is Higher in Paddy Soil than in Upland Soil

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Introduction

Generally, compost application rates have been determined on the basis of the nitrogen (N) needs of crops. In most cases, this strategy has led to the accumulation of soil phosphate (P), due to the lower N/P ratios of animal manure composts than those of crop requirements. The excessive build-up of soil P increases the risk for P outflow from agricultural lands and eutrophication of surface waters. Phosphate-based compost application system based on the P fertilization recommendation and available P contents of composts, is effective for mitigating the soil P build-up. Estimating the available P of composts is inevitable for the P-based compost management but it has not been established. The objectives of this study were to evaluate the available P fraction of animal manure composts and compare those in upland soil and paddy soil by the combination of P composition analysis and a pot experiment.

Materials and Methods

Phosphate characterization analysis was conducted by the sequential extraction method of Frossard et al. (1996). Inorganic P was sequentially extracted by water, 0.5M $NaHCO_3$, 0.1M NaOH and 1M HCl with a sample/solution ratio of 1:200 for 16 hours. Cattle, swine and poultry manure composts with different P compositions were used for crop cultivation experiments. Twelve or 17 composts were added to the test pots so that the applied amounts of total phosphate were 0.32 or 0.25 g P_2O_5/pot, for growing tests of upland crop or paddy rice, respectively. Upland crop, corn (*Zea mays* L. cv. Pioneer 3352), was grown in a 2 L pot with 1.2 kg soil (subsurface soil of Fulvisol) for 32 days in a green house. Paddy rice (*Oryza sativa* L. cv. Hitomebore) was grown in a 4 L pot with 2.5 kg soil (subsurface soil of Andosol) for 54 days in a green house. The growing tests were conducted under sufficient nitrogen and potassium with 3 replications. After harvesting, P taken up by crops was determined. Relationships between P uptake of each crop and P fractions of composts were investigated by regression analysis.

Results

Phosphate taken up by corn ranged from 22 to 40 mg P_2O_5/pot and showed the closest correlation with the sum of water and 0.5M $NaHCO_3$ extractable P ($r=0.76^{**}$, n=12) among the various P fractions of the composts. Paddy rice absorbed P in a range of 2.4 to 33 mg P_2O_5/pot. Phosphate uptake of rice showed the highest correlation with the sum of water, 0.5M $NaHCO_3$, 0.1M NaOH and 1M HCl extractable P of the composts ($r=0.73^{***}$, n=17) . As shown by Ito et al. (2004, 2007), among the P fractions of animal manure composts, the sum of water and 0.5M $NaHCO_3$ extractable P was available for upland crops, on the other hand the sum of water, 0.5M $NaHCO_3$, 0.1M NaOH and 1M HCl extractable P was available for paddy rice. Availability of compost P was higher for paddy rice relative to upland crops. The reason is considered to be root-induced solubilization of P in the paddy rice rhizosphere by acidification due to ammonium ion uptake and proton release by rice roots (Saleque and Kirk, 1995).

Assessment of Greenhouse Gas Emissions of the Production and Utilization of Acidulocompost from Fish Meal

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It is necessary to recycle waste in fish processing and to evaluate its environmental impact in order to improve the sustainable marine product industry. Thus, we evaluated greenhouse gas (GHG) emissions, which is one of the important environmental impacts of recycling waste in fish processing from composting to the end-use steps. We evaluated the process of conventional composting: production of fish-meal fertilizer, disposal and acidulocomposting that is a novel eco-friendly composting system. In our evaluation, it was determined that acidulocomposting process needs a larger amount of energy for heating fermentation system than normal composting. The amount of energy required in acidulocomposting is almost same as that required in the manufacture of fish-meal fertilizer. The ammonia volatilization and CO_2 emissions in acidolocomposting process are much lower than those in the conventional composting process. The proportion of GHG emissions from transportation and spraying in the recycling processes was low in all cases. Acidulocompost has a much higher nitrogen content than the conventional compost because of low ammonia volatilization. Therefore, it can be used in much areas than conventional compost produced from equivalent amount of waste in fish processing. In addition, GHG emission in disposal is the largest of all steps. In conclusion, acidulocomposting needs a large amount of energy for heating fermentation system but has low GHG emissions from composting. Moreover, acidulocompost has a large nitrogen content. Acidulocomposting may be suitable for recycling waste from the sustainable marine product industry.

Effect of Interannual Application of Cattle Manure Compost on Yield and Quality of Herbage and Soil Chemical Condition in a Temperate Grass Meadow

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Introduction Application of animal manure compost to forage crop field has a profound significance in terms of resources recycling. However, there is scarce information on the effect of compost application on yield and quality of herbage under application to the surface of pasture as additional fertilizer. Thus, we conducted a three-year field experiment in a temperate grass meadow to assess the effects of interannual application of cattle manure compost on yield and quality of herbage and soil chemical condition. We also assessed residual effect of the cattle manure application.

Materials and Methods A mixed meadow of orchardgrass (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*) was established in the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan (38º 44' N, 140º 45' E) in 2005. Soil type is non-allophanic andosol. Six plots (200 m × 14 m each) were fixed in the meadow and subjected to the following six treatments in 2006-2008: 1) no fertilizer, 2) split application of chemical fertilizer (conventional application; N-P_2O_5-K_2O = 231-120-200 kg/ha/yr), 3) split application of cattle manure compost (equivalent to the amount of K_2O in treatment 2: 2,500-9,000 kg of compost/ha/yr) and chemical fertilizer to address N deficiencies 4) split application of cattle manure compost (equivalent to the amount of N in treatment 2: 27,000-81,000 kg of compost/ha/yr) 5) single application of cattle manure compost in spring (equivalent to the annual amount of manure in treatment 4), 6) split application of twice amount of the cattle manure in treatment 4. In 2009, we applied chemical fertilizer to all six plots, as in treatment 2, to assess residual effect of the cattle manure compost. In 2006-2009, herbage samples were collected at the height of 3cm from three locations (1 m × 1 m each) in each treatment in mid to late May (1st cut), late July to early August (2nd cut) and October (3rd cut), and the yield and chemical composition were measured. Four soil samples (0-10 cm in depth) were also collected from each treatment and their chemical composition were analyzed after the 3rd cut every year.

Results and Discussions There was no clear effect of compost application on forage yields during the experimental period. However, Ca content decreased and P content increased in 2007-2008 in three treatments (treatment 4-6) where only cattle manure compost was applied. The following residual effects were also observed in 2009 in those three treatments: low Ca content, low Ca/P ratios, high K content, high K/(Ca+Mg) ratio and high NO_3-N content. In the three treatments, a large increase of exchangeable K and Mg concentrations, a modest increase of exchangeable Ca, and a large increase of Mg/K and Ca/Mg equivalent ratio were observed in the soil surface in 2007-2008. In contrast, there was no clear effect of compost application on N and available P concentration. The following residual effect in 2009 was not observed in K concentration, but observed in Mg concentration in the three treatments. Such deterioration of soil mineral condition was rarely observed in the treatment 3 where the amount equivalent to that of K_2O in conventional treatments was applied.

Conclusions Interannual application of cattle manure compost reduces forage mineral balance in a few years due to deterioration of mineral condition of soil surface, if an amount equivalent to that of N in conventional treatments was applied. In contrast, application of the compost in the amount equivalent to that of K_2O in conventional treatments enables to sustainable forage production with well-balanced minerals, by reusing animal wastes.

Cattle Manure Compost Plays a Key Role in Keeping Soil Nitrogen Fertility in Paddy-Upland Rotation System

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Introduction

In Japan, rice supply has exceeded domestic demand and so rice production has been adjusted accordingly for about 40 years. A typical adjustment is the rotation of paddy rice (*Oryza sativa* L.) in paddy fields and upland crops in drained paddy fields, and this has been promoted to improve the self-sufficiency rates of upland crops. In the northeast of Japan, soybean (*Glycine max* (L.) Merr.) is a major crop in this rotation sequence. Because the yield of soybean reflects soil nitrogen (N) fertility, enhancement of soil N fertility in the crop rotation of paddy rice and upland soybean (paddy-upland rotation) is important. However, the quantitative relationship between soil N fertility and soil management including the application of organic materials and the paddy-upland rotation cycle is still vague. This study was performed to investigate the current soil N fertility of paddy soils affected by repeated application of cattle manure compost in paddy-upland rotation.

Materials and Methods

Topsoils of gray lowland soil (Fluvisols) were collected from 22 fields of five different farmers in Daisen, Akita prefecture, in northeast Japan, where paddy-upland rotation was conducted. Over five soil sample replicas were collected in each field. These soils were mixed well and air dried at room temperature. The air dried soil was passed through a 2-mm sieve and subjected to analysis. The collection of soil samples was performed after the crop season (October) of 2007. Available N of the soil was measured following submerged soil incubation for four weeks at 30ºC. Soil total N and carbon (C) were measured by the dry combustion method.

Results and Discussions

Regardless of cattle manure compost application, a significant negative correlation was found between available soil N and an increase in the proportions of upland seasons to total crop seasons after the initiation of paddy-upland rotation (upland frequency). Soil total N and total C also tended to decrease with an increase of upland frequency. In fields with repeated application of cattle manure compost, the soil available N was higher than in fields where only crop residue was applied. A significant negative correlation was also found between the soil available N: total N ratio and upland frequency. This indicates that the part of soil N related to available N was notably lost by the use of paddy fields as upland fields. In order to sustain available soil N over the minimum suitable level of 80 mg kg^{-1}, upland frequency should not exceed 65% when only crop residues and no other organic materials are applied. The upland frequency can be raised by the repeated application of cattle manure compost which maintains a higher level of available soil N. The results imply that care should be taken to maintain the N fertility of paddy soil at a suitable level in paddy-upland rotation and that upland frequency and organic materials applied are important factors to do this.

Fate of Nitrogen Derived from Organic Materials Applied to Paddy and Converted Upland Fields -Results of the First Year Experiment-

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Paddy-upland rotation, alternating between a rice paddy field and an upland field every few years, is one of the cultivation system practiced for sustainable agriculture in Japan. Recently, decrease in soybean yields due to decrease in soil fertility in converted upland fields have been reported in northern Japan. Organic materials application to fields is important to maintain the soil fertility of rotated paddy fields. Quantification of the distribution of nitrogen (N) derived from organic materials (plant uptake or remaining in soil) is required to determine the appropriate amount of organic materials application. The aim of this study is to evaluate the fate of N derived from organic materials in paddy-upland rotation for a long term by ^{15}N-tracer method. In the first year experiment, we considered the fate of N derived from organic materials applied to paddy and upland fields.

In 2012, microplot experiment was conducted in a paddy and a converted upland field at the Center of Field Education and Research, Akita Prefectural University, Ogata, Akita, Japan (N40º00', E139º50', -3 m a.s.l.). Polyvinyl chloride frames (17 cm × 30 cm, height 25 cm) were put into the both fields. Fresh soil previously collected from a plow layer in a paddy field (soil type: gley lowland soil) was well mixed with ^{15}N-labeled hairy vetch (HV: *Vicia Villosa* Roth cv. Kantaro) cattle manure compost (CMC) or chemical fertilizer (ammonium sulfate: AS) was put into the frame. The application rates of HV was 6 and 15 g N m^{-2} for the paddy and upland, respectively, CMC was 20 g N m^{-2} for both the paddy and upland, and AS was 6 and 2 g N m^{-2} for the paddy and upland, respectively. After puddling, three rice plant seedlings (*Oryza sativa* L., cv. Akitakomachi), were transplanted at the center of each paddy microplot. A soybean plant (*Glycine Max* (L.) Merr. cv. Ryuho) was cultivated in each upland microplot. Aboveground parts of the rice and soybean plants were harvested at 109 d after transplanting and 93 d after sowing, respectively. At the same time, the soils inside the frames were also collected. The plant and soil samples were ground into a fine powder after drying and then determine ^{15}N concentrations. The distribution of N derived from HV, CMC and AS were calculated on the basis of the ^{15}N results.

In the paddy field, the rate of N derived from the materials uptake by rice plants decreased in the order of AS (51%), HV (37%) and CMC (5%). The rate of N remained in the soil was the highest in CMC (66%) followed by HV (34%) and AS (27%) in the upland field. The rate of N uptake by soybean plants decreased in the order of HV (26%), AS (24%) and CMC (3%). The rate of N remained in the soil was highest in CMC (74%), followed by HV (66%) and AS (34%). From the results, although the rate of N uptake by soybean plants of HV were similar with AS, N derived from HV remained in the soil especially in the upland field. Therefore, HV application is expected to supply N efficiently for plants and soils especially in upland fields. CMC application is expected to maintain and increase the soil fertility in both paddy and upland fields.

Combined Use of *Azolla* and Loach Suppressed Weed *Monochoria vaginalis* and Increased Organically Farmed Rice Yield

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Abstract

The primary challenge in organic rice farming is controlling weeds without using herbicides. *Monochoria vaginalis* is one of the most common and troublesome annual broadleaved weeds in rice paddies, where it competes with rice for N uptake. *Azolla* is a genus of floating aquatic ferns, used for many centuries as a green manure in traditional rice production. Loach is a freshwater fish that was once widely spread in Asian rice paddies, but has disappeared in modern conventional rice paddies due to use of synthetic agricultural chemicals. We performed an in situ container experiment to study the effects of individual and combined use of *Azolla filiculoides* and loach (*Misgurnus anguillicaudatus*) to suppress *M. vaginalis* emergence and increase organically farmed rice yield. This study was designed with 4 treatments -control (with neither *Azolla* nor loach), *Azolla* (*Azolla* alone), loach (loach alone), and Az+Lo (combined *Azolla* and loach)- with 3 replications each. The results show that the use of *Azolla* alone and loach alone partially suppressed *M. vaginalis* emergence and improved rice yield due to the effects of both shading and N-fixation by *Azolla*, and aquatic bioturbation by loach. The combined use of *Azolla* and loach had a stronger effect, totally suppressing weed emergence and increasing rice yield to 131% that of control treatment, indicating that combined use of *Azolla* and loach may be a valuable approach in organic rice farming, especially in organically farmed rice paddies with high densities of *M. vaginalis* seeds.

Positive Effects of Tubificid Worms on Rice Growth and Yield in Organic Farming System

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Introduction

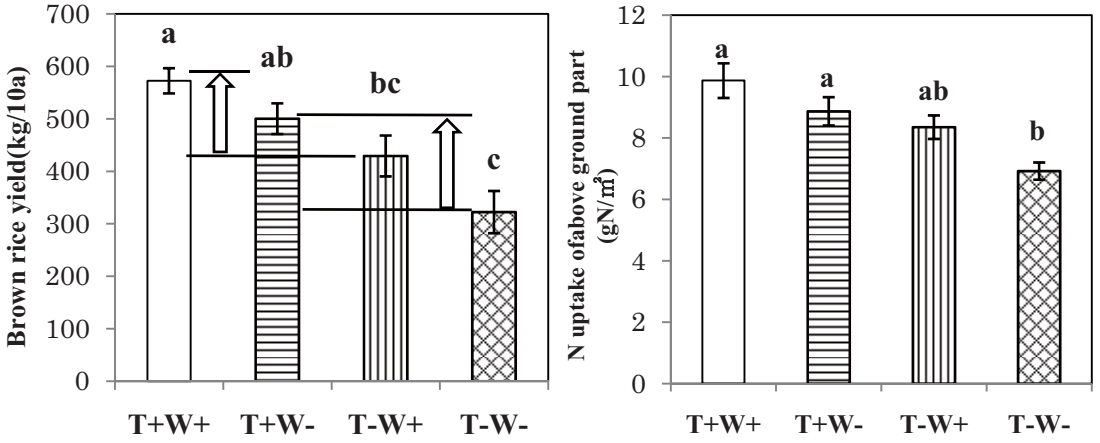
Tubificid worms are one of the important groups in benthic fauna of paddy field, and their population density increases in the paddy fields managed organically(Ito *et al*., 2011). Tubificid worms disturb soil layer with feeding and excreting soil on ground surface. The action of tubificid inhibits weed germination by burying seeds and promotes mineralization of soil organic nitrogen (Kikuchi and Kurihara, 1977). However, these effects have been investigated by small size experiments using beaker or cultivation pot and effects of tubificid worms in paddy fields are still unclear. We investigated the effects of tubificid worms on rice growth and soil nutrient change (nitrogen and phosphorus) in a paddy field managed organically.

Materials and Methods

We buried plastic frames with area of 0.11 m^2 to the depth of 0.15 m under the ground in the paddy field in FSC of Tohoku University, Japan. Two rice seedlings were planted in each frame. Organic fertilizer and rice bran were applied to each frame at the rate of 7 g /m^2 as the total nitrogen. We prepared four treatments with three replications in combination with tubificid worm addition and weed removal by hand: adding tubificid worms with weeding (T+W+), adding tubificid worms without weeding (T+W-), no tubificid worms with weeding (T-W+), no tubificid worms without weeding (T-W-). We added tubificid worms of 2178 ind. to each frame (20,000ind. / m^2) for the T+ treatment. Rice yields and ammonium-N and available phosphorus (P) extracted by Bray 2 method in the soils during rice growth period were measured.

Results

Brown rice yields significantly increased by 33 % and 55 % in T+W+ and T+W- treatments relative to the corresponding treatments (T-W+, T-W-), respectively. Numbers of grains per panicle and total numbers of panicle significantly increased with the addition of tubificid. Soil ammonium N and available P in soils tended to increase and N uptake of rice significantly increased with the addition of tubificid. Rice growth and yield increased probably due to the enhancement of soil N mineralization, which may be promoted by soil turbation with tubificid worms.



Phosphorous Uptake from Organic Matter via AM fungi - Possible Involvement of Phytate-Degrading Bacteria -

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Introduction

Plants uptake nitrogen from organic matter in soil via AM fungi (Hodge *et al*., 2001). Major comportments of phosphorous (P) in organic matter are phytate. Though phytate degrading-bacteria are frequently isolated from soil, AM fungi lack extracellular phytase. In this study, we investigated the possibility of P uptake from phytate in organic matter via AM fungi interacting with phytate-degrading bacteria.

Materials and Methods

Seedlings of Lotus (*Lotus japonicus* B-129 'Gifu') were cultivated in a sterilized loamy soil-sand mixture (ratio 1:1) with/or without inoculation of AM fungi (*Rhizophagus irregularis* or *Claroideoglomus etunicatum*). After 9 weeks, the young plant was transferred to plastic pot containing 300 g of sterilized loamy soil-sand mixture. A mesh bag (37-μm mesh) which contains soil as microorganism inoculum (farmland soil or forest soil, 4.0 g dry weight) with/or without phytate-rich organic matter (mixture of crushed soy bean seeds and crushed buck wheat seeds, total 0.8 g) was set under the plant in each pot. After 6 weeks, plants were harvested, and dry weight and P concentration were determined. Phytate-degrating bacteria were isolated from organic matter in mesh bag.

Results and Discussions

Plant growth and P uptake were increased in *R. irregularis* with organic matter treatment. This result suggested that organic P were degraded by soil organism and released inorganic P were uptaken by plant via AM fungi. Total 109 phytate-degrading bacteria were isolated and most of these were *Burkholderia* sp. or *Pseudomonas* sp., and these were isolated from all treatments. On the other hand, five strains of *Rhizobium* sp. were isolated from only *R. irregularis inoculated* treatment.

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Relationship between Nitrogen Concentration and Ammonia Oxidizing Microbes in the Japanese Cedar Forest Soils under Different Managements

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Introduction

Japanese cedar forest was commonly managed by 33% thinning (weak thinning); however, forestry management has generally been abandoned with the dwindling of the forestry industry, leaving forests unthinned. Recently, 66% thinning (intensive thinning) has been proposed as a new management strategy. A previous study revealed that the NO_3-N concentration of pore water in intensively thinned forest was much lower than that under other management methods. In this study, the relationship between thinning intensity and ammonia-oxidizing prokaryotes was investigated.

Methods

Cores were taken from unthinned and weakly and intensively thinned forests from May to November, 2012. All DNA from the soil samples was extracted, and ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) *amoA* genes in the soils were quantified by real-time PCR. Comparisons of the compositions of communities of ammonia-oxidizing microbes among thinning intensities were made using PCR-DGGE.

Results

In June, intensive thinning significantly increased the number of *amoA* gene copies of AOB, as compared to numbers in unthinned or weakly thinned forests, whereas in the other months numbers did not differ greatly despite thinning intensities. In May and July, the number of *amoA* gene copies of AOA was lowest in intensively thinned forests, and AOA/AOB ratios were much lower than in unthinned and weakly thinned forests. There was about four times more broad-leaf litter in the intensively thinned forest than in the weakly thinned forest, which may have affected the abundance of AOA and AOB.

Detection of Anammox Bacteria from Forest soils of Different Thinning Intensity

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Nitrogen gas was volatilized from livestock manure and is returned to soil by precipitation, it increases nitrogen load if the amount is large. In Japan, the forest accounts for 70% of the country, and most nitrogen compounds are absorbed by the forest soil, which performed removal and/or storage of nitrogen. In our previously study, we discussed the relationship of nitrogen removal and/or retention and forest management bycomparing the abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in forest soils of different thinning intensity. AOA and AOB are important microorganism responsible for part of the global nitrogen cycle, but in this study we focused on Anammox bacteria that intensifies ammonia oxidation activity more than AOA and AOB. Anammox bacteria have a reaction pathway that uses ammonia as an electron donor, nitrite as an electron acceptor, directly producing the nitrogen gas under anaerobic condition. Anammox bacteria is found in the environmental conditions with relatively high water content: ocean, paddy soil and manure pond, but the recently study reported it was detected from field soil. Compared to cultivated land, the large soil stirring does not occur in the forest, so we considered that there are likely to be anaerobic condition there and Anammox bacteria living there may contribute to nitrogen removal with AOA and AOB. In this study, cores were taken from unthinned, weakly and intensively thinned forests at the depth of 5cm and 20cm in June, August and November. All DNA from the soil samples was extracted and amplified by PCR with primers amx368F and amx820r. They are amplicons with the length of approximately 450 bp, which were confirmed from all the soil samples regardless of the depth and the thinning intensity. They were also analyzed by sequencing and homology search. Moreover, we investigated the seasonal change in the number of Anammox bacteria by real-time PCR.

Host Immune Response to Gastric *Cryptosporidium*

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Genus *Cryptosporidium* are intracellular protozoan parasites that infect primarily epithelial cells of gastrointestinal tract and cause diarrheal disease worldwide. Molecular mechanism of host immune response to the parasites has not been well understood, especially, knowledge of the mechanism to gastric *Cryptosporidium* is extremely less. This study analyzed changes of cytokine expression levels in gastric local immune response to gastric *Cryptosporidium* by using mice with different immune properties.

Here, we used *Cryptosporidium andersoni* Kawatabi types to conduct the experimental infection. Firstly, we orally inoculated 1.0×10^6 oocysts into BALB/c mice, which is immunocompetent, and counted oocysts shed in feces in certain intervals. According to the observed oocyst shedding shifts, three periods were determined as follows: period 1 was high oocyst shedding, period 2 was repressing of shedding, period 3 was no oocyst. In the next experiment, the same number of oocysts was orally inoculated into BALB/c mice (normal immune state) and SCID mice (immunological incompetence state). Their stomachs were collected in each period, and their total RNA was extracted. Then, expressions of four cytokines (IFN-γ, IL-4, IL-10, IL-12) were compared by quantitative RT-PCR.

Oocyst shedding peaked in 8 to 17 days post inoculation (DPI) in BALB/c mice, and this term was determined as period 1. Then, oocyst shedding gradually decreased and was not detectable after 41 DPI. Therefore, period 2 was from 18 to 40 DPI, and period 3 was after 41 DPI. In the comparison of IL-4 expression, significant difference was not observed in any analyzed periods nor in any species. By contrast, IFN-γ highly expressed in period 2 of BALB/c mice, while its increase was not shown in SCID mice during the period. Therefore, it was suggested that Th1 immune response including IFN-γ might eliminate gastric *Cryptosporidium*.

Phylogenetic Analysis of *E. zuernii* and *E. bovis* with Nuclear 18S rRNA and Mitochondrial CO1 Genes

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Intoroduction

Coccidiosis is an infectious disease caused by intestinal parasites of genus *Eimeria*, and 14 species are registered as bovine parasites in the world. Of these, *E. bovis* and *E. zuernii* have been regarded as highly pathogenic. In our recent epidemiological study, the two pathogenic species were frequently detected from calves without showing any clinical state. In this study, we collected *E. bovis* and *E. zuerni* from calves showing different symptoms and analyzed their genetic relationship and clinical states in their host.

Material and Methods

Fecal samples were collected from calves under 6 months old which showed clinical and subclinical of coccidiosis, and samples were collected in Tsuyama city, Okayama prefecture in 2012 and Osaki city, Miyagi prefecture in 2010-2011. Species of collected and purified oocysts were identified morphologically. Single oocysts identified as *E. bovis* and *E. zuernii* were were isolated and transferred into PCR tube. They were treated with freezing and thawing cycle in order to extract their genomic DNA. For genotyping analysis, nuclear 18S rRNA gene and 0.8 kbp of mitochondrial CO1 gene were amplified by nested-multiplex PCR. Nucleotide sequences of obtained PCR products were determined by direct sequencing. To analyze their genetic polymorphisms within the species, we compared molecular phylogenetic relationships in nucleotide sequences.

Results and Discussions

Oocysts of *E. bovis* and *E. zuernii* were collected from 23 calves, 11 of which shed bloody stool, and the rest showed no clinical features. The nested-multiplex PCR was successfully conducted in 40 oocysts, and we determined their nucleotide sequences. According to constructed phylogenetic trees with 18S rRNA gene, each two *Eimeria* species formed highly supported single clusters. Besides, no relationship implying host symptoms was observed in the tree. Similar trend was observed in a tree from CO1 gene. This result suggested that their molecular genetic diversity did not reflect on their pathogenicity and other factors such as infection sites might cause symptoms.

Prevalence and Molecular Characterization of *Cryptosporidium* in Domestic Animals in Central Vietnam

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Introduction

Cryptosporidiosis caused by *Cryptosporidium* parasite is a critical diarrheal disease in humans and livestock, and young and neonatal individuals sometimes show severe symptoms. In Vietnam, the government has recently promoted livestock production. However, the data of cryptosporidiosis have been still limited. Therefore, the aim of this study is prevalence and molecular characterization of *Cryptosporidium* in common domestic animals in central Vietnam.

Materials and Methods

Fecal samples were collected from cattle, pigs and ostriches in central Vietnam. *Cryptosporidium* oocysts were detected by modified Ziehl-Neelsen staining method, and species identification was conducted by using 18S rRNA, HSP70, and actin genes.

Results

In cattle, overall prevalence on samples and herd levels were 18.9% (44/232) and 50% (20/40), respectively. Genotyping based on 18S rRNA gene revealed the presence of the two non-zoonotic species *Cryptosporidium ryanae* and *C. bovis*, the former of which is a dominant species. In pigs, overall prevalence of 14.5% (28/193) was estimated. Genetic identification based on the 18S ribosomal RNA and 70 kDa heat shock protein genes revealed two species/genotypes *Cryptosporidium suis* and *Cryptosporidium* pig genotype II. In ostriches, overall prevalence was identified as 23.7% (110/464). Molecular analysis in the 18S rRNA, HSP70, and actin genes demonstrated the presence of only *Cryptosporidium* avian genotype II. The presence of these host- adapted species/genotypes suggests that domestic animal may not pose a significant public health risk in the study area.

Prevalence and Molecular Identification of *Fasciola* in Cattle in Central Vietnam.

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Introduction

Fasciolosis is a critical parasitic disease of cattle, causing significant economic losses. Fasciolosis also occurs in humans, resulting in diseases of liver. In this study, we investigated prevalence and determined molecular characterization of *Fasciola* in central Vietnam, a hyper-endemic area of human fasciolosis.

Materials and Methods

Fecal samples from calves and adult cattle were examined for *Fasciola* eggs by sedimentation method. A subset of fecal samples and blood samples was examined by sedimentation method and ELISA technique. *L. viridis* and *L. swinhoei* from paddy fields, lake banks, water fern ponds, sewerages were examined for *Fasciola* larvae. Sixteen *Fasciola* flukes from naturally infected cattle were used for molecular analysis. Species identification was conducted by using nuclear ITS1 and ITS2 regions, NDI gene, and mitochondrial CO1 gene.

Results

Overall prevalence of *Fasciola* was 45.3% (487/1075). From the subset of the animals (235), 46.3% were shedding *Fasciola* eggs while 87.2% were *Fasciola* seropositive. Prevalence of *Fasciola* in calves (37.6%) was low compared to that in adult cattle (53.7%). The prevalence in the rainy season (50.8%) was significantly different to that in the dry season (38.1%). Prevalence of *Fasciola* in *L. viridis* and *L. swinhoei* were 0.95% (31/3.269) and 0.62% (7/1.128), respectively. Analyses of sequences from ITS1 and ITS2 of the ribosomal RNA revealed that 13 out of 16 isolates were *F. gigantica* type, whereas three isolates presented a hybrid sequence from *F. gigantica* and *F. hepatica*. All the mitochondrial sequences (COI and NDI) were of *F. gigantica* type, suggesting that the maternal lineage of the hybrid form is from *F. gigantica*.

Molecular Epidemiological Survey for Zoonotic Parasite Infection in Livestock in the Tuul Basin in Mongolia

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Introduction

Mongolia, a landlocked country of the central Asian plateau, has undergone significant modifications in herding systems, and the number of reared livestock has been increasing. Livestock sometimes act as reservoirs for pathogenic parasites, therefore shed feces have brought water pollutions by waterborne parasites such as *Cryptosporidium*, *Giardia*, and diarrheagenic *Escherichia coli* (DEC) that cause self-threatening diarrhea and that sometimes lead severe symptoms in young, older and immunocompromised hosts. Therefore, epidemiological information in those livestock is necessary to control parasite. In this study, we analyzed parasite infection in fecal specimens and assessed the prevalence of enteric pathogen in livestock in the Tuul River basin.

Materials and Methods

Three sutes for sampling ware selected from upstream or downstream regions of the Tuul River. Another three sites for sampling, including ponds and groundwater, were selected as study area. Fecal samples of cattle, horse, goat, and sheep were collected in those sites. To detect parasite infection, we used molecular biological techniques. In brief, fecal DNA was extracted by using commercial kit, and infection was evaluated by PCR amplification with following marker genes. For *Cryptosporidium*, 18S rRNA gene was used, and 18S rRNA gene and *gdh* were targeted for decoction of Giardia. The DEC was evaluated with 10 genes (*eaeA*, *bfpA*, *stx*1, *stx*2, *estA*1, *estA*2-4, *eltB*1, and *ipaH*). In order to identify parasite species detected in feces, amplified PCR products were sequenced and applied to the BLAST search.

Results

In total, 59 fecal samples were collected in 6 designed sites, and fecal DNA extraction was performed in obtained samples. In *Cryptosporidium* detection, PCR product showing expected length was amplified in three samples. However, no sample was identified as *Cryptosporidium* sp. from nucleotide sequencing. After analyses for *Giardia* detection, five samples were pseudo positive. These results suggested that livestock were not likely to reserve zoonotic parasites.

Investigation of Microbial Community Analysis during the Decomposition of Lignocellulosic Biomass Using Rumen Fluid

Chol Gyu Lee, Yasunori Baba, Chika Tada and Yutaka Nakai

Tohoku University, Japan

Lignocellulosic biomass is potential raw material for producing several high-value products. This material was hydrolyzed to mainly volatile fatty acids, which are subsequently converted to acetate, CO_2 and CH_4 under anaerobic condition. The hydrolysis of plant cell wall to monosaccharide and several monomers were considered the important step in lignocellulosic biomass decomposition.

Then we tried to develop the effective decomposition method, which used rumen fluid to decompose plant cell wall prior to methane production. This method could increase volatile fatty acids production and methane production from lignocellulosic biomass by effective decomposition. Since rumen microorganisms play an important role to decompose lignocellulosic biomass, we examined microbial community structure during the pretreatment process.

Furthermore, acetic acid, which is often consumed by the substrate for methane fermentation, increased during the pretreatment process with rumen fluid. In general, acetate kinase, an enzyme widely distributed in the fermentative bacteria, catalyzes a reaction between acetyl-CoA to acetate. Therefore, analysis of the diversity of gene-encoding acetate kinase (*ack* gene) in environmental samples could provide an additional molecular marker for fermentative acetate-producing microbes.

The objective of this study was the evaluation of microbial communities during pretreatment process with rumen fluid. Waste paper was subjected to decomposition in rumen fluid under an anaerobic condition and microbial communities involved in cell wall decomposition and acetic acid production was analyzed using PCR-DGGE (denature gradient gel electrophoresis) method.

Comparison of Bacterial Flora in a Methane Fermentation Plant in Field Science Center at Tohoku University and Bovine Rumen Used as the Plant Microbial Resource by 16S rRNA Gene Sequencing

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Introduction

Grobal warming caused by greenhouse gas like CO_2 is an urgent environmental problem. Although methane is one of the greenhouse gases, it is the most important energy resource for natural gas-fired plant. Moreover, methane is recyclable energy resource and methane production using fecal waste from human or livestock is a low environmental load method . However the problem is that its production rate and volume are not stable. Therefore, we aim to find an effective bacterial flora for methane production from livestock waste. In this report, we analyzed and compared bacterial flora in the methane fermentation tank and bovine rumen used as the plant microbial resource by 16S rRNA gene sequencing with PCR-DGGE and PCR-pyrosequencing.

Materials & Methods

Bacterial flora of methane producing plant was gotten directly from methane fermentation tank in Field Science Center at Tohoku University (Osaki-shi, Miyagi). Bovine rumen were donated from a meet center and stored at -80ºC. Genome DNA was extracted from the bacterial flora and rumen by modified Venter method (Morita et al. 2009). Purified DNA was used as a template for polymerase chain reaction in PCR-DGGE and PCR-pyrosequencing analysis. PCR-DGGE was used denaturing gradient of 30-60% under 8% acrylamide gel and electrophoresis was running at 130V for 5 h at 60ºC. A 100% denaturant was the mixture of 7 M urea and 40% formamide. The Gel was stained by SYBR Gold (invitrogen) and band pattern was visualized by LAS-4000 (Fuji Film). PCR-pyrosequencing was carried out by GS Junior System (Roche). Sequencing results were analyzed by Pyrosequence Pipeline in Ribosomal Database Project (http://pyro.cme.msu.edu/).

Results & Discussions

PCR-DGGE analysis showed a different band pattern for major bacteria between methane fermented tank and bovine rumen, and the results indicated that bacterial flora from methane production was much different from that from bovine rumen. To know the result details of PCR-DGGE, PCR-pyrosequencing is now in progress.

Acknowledgements

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Improvement of the Anaerobic Digestion Performance for Fish Industry Waste Recycles

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Introduction

Marine products industry discharges large amounts of fish waste in Tohoku coastal area. Incineration is the general method of treating fish waste. But there is high energy loss because of the high water content of the waste. Fish waste can be used for anaerobic digestion because of its high chemical oxygen demand (COD). In general, fish waste is protein-rich substrates, and their degradation products (ammonium) can inhibit the process. Therefore, co-digestion assays of fish waste with active sludge were undertaken. Injections of active sludge lead to reduce protein concentration of raw materials. Additionally, the influence of oyster shell and carbon carrier for anaerobic digestion of fish waste was investigated. Main component of oyster shell is $CaCO_3$. Calcium ion improved methane production and counteracted ammonia inhibition.

Methods

Continuous operations of mesophilic anaerobic digestion were conducted. Reactor volume was 400 ml. HRT was shorten by gradation (HRT 70d, 40d, 20d). Substrates contained active sludge and crushed fish waste. Fish content of substrate was 10% or 20%. 25 g of oyster shell and 63 cm^3 of carbon carrier were put into reactors.

Results and Discussions

In mesophilic anaerobic co-digestion of fish waste with active sludge, it was observed that oyster shell contracted the inhibition and improved methane production. The methane production increased in anaerobic digestion of fish waste with oyster shell by carbon carrier. When fish content of the substrate was 20% and HRT was 20 days, methane yield was about 0.5 in the continuous anaerobic digestion of fish waste with oyster shell. To clarify the mechanism of anaerobic digestion with oyster shell, further studies about the microbiological characterization are required.

Acknowledgments

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Effects of Carrier on the Performance of a Methane Fermentation System Using Heat from a Hot Spring

Takashi Suzuki and Chika Tada

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A small-scale methane fermentation system was developed by hot spring water for warming the reactor. Materials were added directly into the first tank without grinding leftover food, including paper bags. The temperature of the fermentation tank was adjusted to ~55ºC by running hot spring water through a tube coiled around the tank. To improve the methane production efficiency, methane production and volatile fatty acid (VFA) concentrations in two operations were compared by different carriers (polyurethane and carbon fiber) in the reactors.

Methane produced during the operation with carbon fiber carrier was higher than that with polyurethane carrier. Concentrations of total volatile fatty acid (T-VFA) in the operation with carbon fiber carrier were 1/10 as high as in the operation with polyurethane carrier. Propionic acid concentration decreased by the addition of carbon carriers in the reactor. Methanogenic communities attached to each carrier were analyzed by PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. Methanogenic communities were different between those two carriers.

Pretreatment of Lignocellulosic Rapeseed Waste by Rumen Fluid for Methane Production

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Introduction and Objectives

Rapeseed is an oilseed crop that is cultivated around the world. Because rapeseed has a high tolerance to salinity, it is now being cultivated in Japan on agricultural lands affected by the tsunami disaster following the Great East Japan Earthquake of 2011. Byproducts of rapeseed oil production include thinned green growth, threshed stems, and pressed oilseed cakes. Typically, these byproducts are incinerated, but in this study they were used as substrates for methane production. The hydrolysis of lignocellulosic biomass such as plant stems is a rate-limiting step in methane production. We examined whether the efficiency of methane production from rapeseed waste could be improved by pretreatment with rumen fluid (from the first stomach compartment of a ruminant), slaughterhouse wastes.

Methods and Results

Rapeseed (*Brassica napus*) stems were dried and milled, and then used for methane production after pretreatment with rumen fluid. Rumen contents were collected from grass-fed cattle. The rapeseed stems were pretreated with rumen fluid at 37ºC for 24 h. The main metabolic products were acetate and propionate. In the following treatment, 200 ml of the rumen-treated rapeseed stems was added to 400 ml of seed sludge, and batch-type methane production was performed at 35ºC for 36 days. Methane production from untreated rapeseed stems was also performed for comparison. More methane was produced from pretreated stems than the untreated stems. These results suggest that the lignocellulosic biomass was well hydrolyzed during the rumen fluid pretreatment, allowing greater methane production. We are now analyzing the degradation rate of the lignocellulosic components (cellulose, hemicellulose, and lignin).

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